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<td>16</td>
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<td>23</td>
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<td>Wednesday</td>
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Simplicity

Genotropin MiniQuick®

The first preservative free, no waste, single use growth hormone

Pfizer Australia Pty Ltd ABN 50 008 422 348
38-42 Wharf Road West Ryde NSW 2114
## ESA OFFICE BEARERS 2005

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Professor Jeffrey Zajac

**Vice President**  
Professor Leon Bach

**Secretary**  
Dr Mark McLean

**Treasurer**  
Dr Vicki Clifton

**Councillors**  
Dr Catherine Choong  
Professor Evan Simpson  
Professor David Healy  
Dr David Torpy

**Newsletter Ed.**  
Dr David Phillips

## PAST ESA OFFICE BEARERS 1958-2006

<table>
<thead>
<tr>
<th>DATE</th>
<th>PRESIDENT</th>
<th>VICE PRESIDENT</th>
<th>SECRETARY</th>
<th>TREASURER</th>
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<tbody>
<tr>
<td>1958-60</td>
<td>E. Downie</td>
<td>P. Taft</td>
<td>K. Harrison</td>
<td>I. Jarrett</td>
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<tr>
<td>1962-64</td>
<td>K. Harrison</td>
<td>V. Trikojus</td>
<td>I. Thomas</td>
<td>I. Jarrett</td>
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<td>1966-68</td>
<td>B. Hudson</td>
<td>R. Melick</td>
<td>I. Jarrett</td>
<td>I. Jarrett</td>
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<td>1968-70</td>
<td>P. Taft</td>
<td>V. Trikojus</td>
<td>I. Jarrett</td>
<td>I. Jarrett</td>
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<td>1972-74</td>
<td>K. Ferguson</td>
<td>L. Lazarus</td>
<td>I. Jarrett</td>
<td>I. Jarrett</td>
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<tr>
<td>1976-78</td>
<td>S. Posen</td>
<td>J. P. Coghlan</td>
<td>I. Jarrett</td>
<td>I. Jarrett</td>
</tr>
<tr>
<td>1978-80</td>
<td>J. P. Coghlan</td>
<td>J. J. Eastman</td>
<td>I. Jarrett</td>
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<tr>
<td>1984-86</td>
<td>R. G. Larkins</td>
<td>D. P. Cameron</td>
<td>I. Jarrett</td>
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<td>1986-88</td>
<td>D. P. Cameron</td>
<td>R. C. Baxter</td>
<td>I. Jarrett</td>
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<td>1998-00</td>
<td>R. J. Rodgers</td>
<td>J. D. Zajac</td>
<td>I. Jarrett</td>
<td>I. Jarrett</td>
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<tr>
<td>2000-02</td>
<td>K. K. Y. Ho</td>
<td>B. J. Waddell</td>
<td>B. Canny</td>
<td>C. Coulter</td>
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<td>2002-03</td>
<td>B. Canny</td>
<td>J. D. Zajac</td>
<td>R. Cuneo</td>
<td>C. Coulter</td>
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<td>2004-06</td>
<td>J. Zajac</td>
<td>L. Bach</td>
<td>M. McLean</td>
<td>V. Clifton</td>
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</tbody>
</table>

## ESA INTERNATIONAL TRAVEL GRANT

- **2003**  
  Emma Ball

- **2004**  
  Gordon Howarth  
  Sophie Chan  
  Vincenzo Russo

- **2005**  
  Stuart Ellem
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![Servier](image2)

CORPORATE SPONSORS

![Lilly](image3)

![AstraZeneca](image4)

![Ipsen](image5)

![Pfizer](image6)

FUTURE MEETINGS

<table>
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**ESA Seminar 2006**
7th - 9th April 2006
Erskine on the Beach, Lorne VIC
[www.esaseminar.org.au](http://www.esaseminar.org.au)

**ESA Clinical Weekend 2006**
18th - 20th August 2006
The Royal Pines Resort, Gold Coast QLD
[www.esaclinicalweekend.org.au](http://www.esaclinicalweekend.org.au)

**Combined ESA/SRB Annual Scientific Meeting**
20th - 23rd August 2006
Gold Coast Convention Centre, QLD
[www.esa-srb.org.au](http://www.esa-srb.org.au)
**Control with confidence**

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

Diamicron MR (gliclazide modified release) 30mg Tablets. Before prescribing, please refer to Approved PI.

Indications: Type II diabetes, with dietary measures. Contraindications: Type I diabetes; diabetes keto-acidosis/coma, severe renal/hepatic impairment, pregnancy, lactation; concomitant microscopic hyperosmotic to sulphonylureas/sulphonamides; Precautions: hypoglycaemia, fever, trauma, infection, surgery. Patients may require insulin and stopping Diamicron MR therapy may be necessary. Age, poor nutrition increase sensitivity to anti-diabetic agents. Interactions: thiazide diuretics, barbiturates, chlorpromazine, dapsone, glucocorticoids, estrogens and contraceptive, salbutamol, tetracline should be used with caution. Caution with alcohol and drugs that potentiate hypoglycaemia, eg insulin, biguanides, sulphonamides, high dose salicylates, MAOIs, beta-blockers, omeprazole, clofibrate, ACE inhibitors, coumarin derivatives, chlorpromazine, isoniazid, fluoxetine and micrionef. Adverse Reactions: mild hypoglycaemia (severe hypoglycaemia uncommon). Other reactions - consult Approved PI.

Dosage and Administration: 30mg (1 tablet - 120mg of tablets) daily with breakfast in a single dose. No dosage adjustment required in patients aged ≥65 years or with mild to moderate renal impairment. Must not be broken or chewed. Date of Preparation: 16/12/2002. Approved by Therapeutics Advisory Committee. Date of Review: 20/04/2005. Approved by Therapeutics Advisory Committee. Date of Review: 20/04/2005.

Data from Reference 1


* Effective control. Less hypoglycaemia.

* than Amaryl, for equivalent glycaemic control.

58% fewer patients affected by hypoglycaemia

**p =0.003
We’ve been making a stand since 1923.
( you should come & see it )

Banting and Best first isolated insulin in the 1920s. Since then, process improvements have continuously raised the quality, allowed new types of insulin to be developed, and fundamentally changed the way insulin is made. To the point that we now have a once-daily basal insulin with a peakless profile which allows you to achieve glycaemic control without increasing the risk of hypoglycaemia.¹²

But we're not stopping here, in fact sanofi-aventis’ commitment to research and development of diabetes treatments dates back to 1923. And we won't stop until we can put this debilitating condition behind us.

If you'd like to know more please visit our stand in the exhibitor area.

### KEITH HARRISON MEMORIAL LECTURES

<table>
<thead>
<tr>
<th>Year</th>
<th>Speaker</th>
<th>Year</th>
<th>Speaker</th>
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<tbody>
<tr>
<td>1964</td>
<td>Kenneth Ferguson</td>
<td>1989</td>
<td>Hiroo Imura</td>
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<tr>
<td>1965</td>
<td>Geoffrey Harris</td>
<td>1990</td>
<td>Iain McIntyre</td>
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<tr>
<td>1973</td>
<td>Albert Renold</td>
<td>1991</td>
<td>Eli Adashi</td>
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<td>1974</td>
<td>Paul Franchimont</td>
<td>1992</td>
<td>Jan-Ake Gustafsson</td>
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<tr>
<td>1975</td>
<td>William Odell</td>
<td>1993</td>
<td>Eberhard Nieschlag</td>
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<tr>
<td>1976</td>
<td>John Landon</td>
<td>1994</td>
<td>Allen Speigel</td>
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<tr>
<td>1977</td>
<td>Hugh Niall</td>
<td>1995</td>
<td>Natalie Jossos</td>
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<td>1978</td>
<td>Samuel Yen</td>
<td>1996</td>
<td>Gregory Mundy</td>
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<td>1979</td>
<td>John Shine</td>
<td>1977</td>
<td>M.Geoffrey Rosenfeld</td>
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<td>1980</td>
<td>Ronald Swerdloff</td>
<td>1978</td>
<td>Ken Korach</td>
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<td>1981</td>
<td>Sidney Ingbar</td>
<td>1979</td>
<td>Henry Burger</td>
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<tr>
<td>1982</td>
<td>Jens Rehfeld</td>
<td>2000</td>
<td>Pierre Chambon</td>
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<td>1983</td>
<td>Philip Lowry</td>
<td>2001</td>
<td>Jack Martin</td>
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<td>1984</td>
<td>Fernand Labrie</td>
<td>2002</td>
<td>George Chrousos</td>
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<td>1985</td>
<td>Michael Berridge</td>
<td>2003</td>
<td>Derek LeRoith</td>
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<td>1986</td>
<td>Michael Thorner</td>
<td>2004</td>
<td>Bruce McEwen</td>
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<tr>
<td>1987</td>
<td>Lynn Loriaux</td>
<td>2005</td>
<td>Richard Pestell</td>
</tr>
<tr>
<td>1988</td>
<td>Axel Ulrich</td>
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</table>

### NOVARTIS JUNIOR AWARD

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

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<thead>
<tr>
<th>Year</th>
<th>Name</th>
<th>Year</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1976</td>
<td>Kathryn Rich &amp; Peter Fuller</td>
<td>1991</td>
<td>Marie-Christine Kightley &amp;</td>
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<tr>
<td>1977</td>
<td>David Kennaway</td>
<td></td>
<td>Helen Maclean</td>
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<td>1978</td>
<td>David Healy</td>
<td>1992</td>
<td>Fiona Young</td>
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<tr>
<td>1979</td>
<td>George Werther</td>
<td>1993</td>
<td>Emma Ball</td>
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<tr>
<td>1980</td>
<td>Rebecca Mason</td>
<td>1994</td>
<td>Vicki Clifton</td>
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<tr>
<td>1982</td>
<td>David Hurley</td>
<td>1996</td>
<td>John Walsh</td>
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<tr>
<td>1983</td>
<td>Carolyn Scott</td>
<td>1997</td>
<td>Bu Yeap</td>
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<tr>
<td>1984</td>
<td>David James</td>
<td>1998</td>
<td>Julie Joyner</td>
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<tr>
<td>1985</td>
<td>Guck Ooi</td>
<td>1999</td>
<td>Renea Jarred &amp; Helena Teede</td>
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<td>1986</td>
<td>Marie Ranson</td>
<td>2000</td>
<td>Jeremy Smith</td>
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<td>1987</td>
<td>Lora Hutchinson</td>
<td>2001</td>
<td>Stephen Heady</td>
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<td>1988</td>
<td>Vasilious Papadopoulos</td>
<td>2002</td>
<td>Patrick McManammy</td>
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<td>1989</td>
<td>David Phillips</td>
<td>2003</td>
<td>Sophie Chan</td>
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<td>1990</td>
<td>Sharon Gargosky</td>
<td>2004</td>
<td>Esme Hatchell</td>
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### 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.

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<th>Year</th>
<th>Name</th>
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<th>Name</th>
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<td>Sharon Gargosky</td>
<td>1998</td>
<td>Stephen Twigg</td>
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<td>Peter Stanton</td>
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<td>Dan Lee</td>
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<td>Janet Martin</td>
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<td>Fraser Rogerson</td>
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<td>1994</td>
<td>Chen Chen</td>
<td>2001</td>
<td>Karen Kroeger</td>
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<td>1995</td>
<td>Timothy Crowe</td>
<td>2002</td>
<td>Susan Fanayan</td>
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<tr>
<td>1996</td>
<td>Jun-Ping Lui</td>
<td>2003</td>
<td>Jenny Gunton</td>
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<tr>
<td>1997</td>
<td>Liza O'Donnell</td>
<td>2004</td>
<td>Peter Liu</td>
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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
Dr 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

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

CONFERENCE ORGANISING COMMITTEES

The Local Organising Committee
ESA: Bu Yeap (Chair), Catherine Choong, Esme Hatchell, Christin Down
SRB: Dominique Blache, Peter Mark

SRB Program Organising Committee
Sarah Robertson, Jon Hill, Eileen McLaughlin, Darryl Russell, Ann Drummond & 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.racp.edu.au/esa
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Mayne Pharma

Schering Pty Ltd

Novartis Pharmaceuticals

Meat Livestock Australia

CRC for Innovative Dairy Products

Serono
PRINCIPAL GUEST SPEAKERS FOR 2005

Professor David Dunger (United Kingdom)
David Dunger is Professor of Paediatrics at the University of Cambridge. He received his training in Paediatric Endocrinology at the Hospital for Sick Children Great Ormond Street, London. Between 1986 and 2000 he was Consultant Paediatric endocrinologist at the John Radcliffe Hospital in Oxford and between 1999 and 2000 he was Professor of Paediatric Endocrinology at the University of Oxford. His interests are in the GH/IGF-1 axis; early detection of diabetic complications and endocrine and genetic determinants of size at birth and early growth.

Professor John Eppig (United States)
John Eppig is a Senior Staff Scientist at The Jackson Laboratory. The focus of his research is on the development and function of the mammalian oocyte-granulosa cell complex. He achieved the first complete development of mammalian oocytes in vitro. He originated the concept of an oocyte-granulosa cell regulatory loop in which bidirectional communication between the oocyte and companion granulose cells is essential for both normal oocyte and follicular development. He was awarded the Gregor Mendel Honorary Medal of Merit in Biological Sciences by the Academy of Sciences of the Czech Republic in 2002, was President of the Society for the Study of Reproduction in 1999-2000, and became Co-Editor-in-Chief of Biology of Reproduction in July 2004.

Professor Ken McNatty (New Zealand)
Ken McNatty received his PhD on human ovarian function at the MRC Unit of Reproductive Biology at the University of Edinburgh. He undertook postdoctoral studies on human ovarian function at Harvard Medical School (1977-1979) and was Boerhaave Professor at the University of Leiden in 1980 and 1981. Since 1982, he has been a Science Programme Leader in Reproductive Biology at the Wallaceville Animal Research Centre, NZ. His main research activity is focussed on sheep with naturally-occurring genetic mutations affecting ovulation rate and fertility and the role that the oocyte plays in this process.

Associate Professor Hiroyuki Namba (Japan)
Associate Professor Namba is an Endocrinologist and Chief researcher at the department of Molecular Medicine, Atomic Bomb Disease Institute, Nagasaki University. He is also a clinician taking care of patients with thyroid disease at the International Hibakusha Medical Center in Nagasaki University Hospital. Dr. Namba serves on the Council Members of the Endocrine Society and the Thyroid Association of Japan. The focus of his research is on the investigation of pathogenesis and development of new diagnostic and therapeutic tools for thyroid cancer. He has also participated at the joint Chernobyl Humanitarian Aid and Research projects to screen for childhood thyroid cancer in the past 10 years. He has already published more than 100 peer-reviewed scientific articles. He was awarded the Tunoo price by Nagasaki University of Medicine in 1997.

Professor Richard Pestell (United States)
Dr Richard G. Pestell, (MBBS [UWA], MD [Melb U], PhD, FRACP) was born in Perth, Western Australia, trained in Hematology/Oncology and Endocrinology and continued clinical practice and research at Harvard University and Massachusetts General Hospital. He is Associate Vice President, Georgetown University Medical Center; Chairman, Department of Oncology; Director, Lombardi Comprehensive Cancer Center, Washington, DC. He has over 220 publications, including Cell, Science and Nature Medicine, and has patents for new cancer therapies. His laboratory first identified a novel mechanism of cellular metastasis, first showed hormone receptors are acetylated, first identified kinase-independent functions of cyclins, and developed light-activated gene therapy, for which he holds a patent. He serves on national and international medical and philanthropic boards.

Professor Roger Smith (Australia)
Roger Smith is Director of the Mothers and Babies Research Centre at the Endocrine Unit of the John Hunter Hospital. He is also Professor of the Faculty of Health in the School of Medical Practice & Population Health at The University of Newcastle, NSW. His primary focus is the endocrinology of pregnancy, especially his internationally leading research into the role and regulation of placental corticotrophin releasing hormone, which has featured in his over 130 scientific publications.
Rapid action

Tight control

NovoRapid

insulin aspart (rbs)

Speed. Control. Convenience

PBS Information: This product is listed on the PBS as a drug for the treatment of diabetes mellitus.
INFORMATION FOR DELEGATES & PRESENTERS

Venue Location
Perth Convention and Exhibition Centre
21 Mounts Bay Road
Perth, Western Australia 6000
Phone: 08 9338 0300 Fax: 08 9338 0309

The Perth Convention Exhibition Centre is situated in the heart of Perth, within easy walking distance of the city's major hotels, business, restaurant and retail districts and can be reached by a range of transport options.

Organiser’s Office and Registration Desk
The organiser’s office and registration desk will be located on level two in the main foyer area of the Perth Convention Centre. It is accessible by the escalators (or stairs) from the main foyer entrance. 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.

The Speaker Preparation Room
The speaker preparation room is Meeting Room 12, located on level two. 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. Should delegates be using 35mm slides, they must inform the secretariat as early as possible to ensure the equipment is booked for their session as these projectors are not standard in each room. Carousels will be available in the preparation room. Those using slides should collect them immediately at the end of their session. The organisers are not responsible for slides not claimed at the end of the session.

Session Locations
The Conference activities are spread out over two levels. Pavilion 1 Exhibition Hall, located on level one (ground floor), is where the exhibition is and all breaks are taken there. The posters are also displayed within the exhibition hall. The auditorium and scientific sessions are generally located on level two.
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 Pavilion 1. 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 days 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 Perth Convention Centre in the Ballroom pre-function foyer area on the third level 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 and is in Function Room 11 (located on the same level as the registration desk). 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 The WA Rowing Club. The WA Rowing Club is a white wooden building on the Swan River. It is located only 5 minutes from the city, a 5-10 minute walk from the Convention Centre, very close and next door to the WA Bell Tower. When you arrive at the Bell Tower, with the river in front of you and the city behind you, to your left on the river is a white wooden building. It is an independent building of 2 floors, and a large balcony. This is the building you want. As you walk left, away from the Bell Tower and towards the white building, the river will be on your right. When you arrive at the building, on the front of the building towards the left is a large door, and a set of stairs going upwards. The function is upstairs on the second level. 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 Perth Convention Centre in Functions Rooms 4 & 5 (located on the same level as the registration desk). Pre-dinner drinks will be served from 7:00pm for a 7:30pm start. Dress is neat casual. Entertainment for the night is provided by ‘Ormonde Waters’ a traditional Irish band. 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.

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.
**No other somatostatin analogue has been proven more effective in treating acromegaly.**

*Data is not from comparative studies.*

Before prescribing please refer to Full Product Information. Full Product Information is available on request.


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**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 on the first floor foyer.

**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.

**The Trade Passbook Competition -** Amongst delegate’s registration papers is a “Trade Pass Book” entry form. The form has spaces for the stamp or signature of each of the trade exhibitors. Visiting all of the trade sites, meeting their representatives and registering their ‘stamp’ in the designated space. Once you have collected 15 stamps or signatures, place your completed form in the entry box at the registration desk by the end of afternoon tea on the Tuesday. The prize for the first completed entry form is donated by ASN. *Trade representatives are not eligible to enter the competition.*
Sunday, 4 September 2005

Registration Desk opens
1:30 PM - 6:30 PM
Foyer, level 2

Session 1 - SRB Workshop - Writing Scientific Papers
3:00 PM - 4:00 PM
M2&3, level 2
Chairs: Mark Hedger and Janette Quennell
- David Lindsay
  Writing Scientific Papers  abs#001

Session 2 - SRB Symposium: Cell Fate Decisions - Driving Growth and Differentiation
4:00 PM - 6:00 PM
M2&3, level 2
Chairs: Kate Loveland and Lyn Kilpatrick
4:00pm  Sarah Meachem
  Sertoli Cell Terminal Differentiation: Doing a ‘U’ Turn on a One Way Street  abs#002
4:30pm  Arun Dharmarajan
  Expression of Secreted Frizzled Related Protein-4 (sFRP-4) And Associated Wnt Signalling In Cancer and Apoptosis  abs#003
5:00pm  Miranda Grounds
  Control of skeletal muscle cell proliferation and differentiation.  abs#004
5:30pm  David Jans
  Efficiency of nuclear import of the chromatin-remodelling factor SRY is critical for sex determination  abs#005

ESA-SRB Welcome Function
6:00 PM - 7:30 PM
Function sponsored by Sanofi-Aventis
Foyer, level 2

Women in Endocrinology Function
7:00 PM - 8:00 PM
Function sponsored by DSL Labs
M11, level 2

Proudly supporting the Endocrine Society of Australia Annual Scientific Meeting
Session 3 - SRB-ANZPRA orals: Placenta and Pregnancy
8:00 AM - 10:00 AM  M2, level 2
Chairs: Stephen Anderson and Arun Dharmarajan

8:00am  Jonathan McGuane
Effects of Relaxin Deficiency on Matrix Metalloproteinase Expression in the Cervix and Vagina of Pregnant Mice  abs#201

8:10am  Yin Lau Lee
Regulation of complement 3 in human and mouse oviduct  abs#202

8:20am  Kym Rae
A Differential Pattern of Follistatin Expression in the Placenta between Spontaneous, Induced and Non-Labouring Patient Groups.  abs#203

8:30am  Katy Freed
Isolation of CAG repeat containing genes from human placenta and decidua  abs#204

8:40am  Natalie Hannan
Fractalkine, HCC-1 & MIP-1β promote human trophoblast migration.  abs#205

8:50am  Cherise Fletcher
Effect of In Vitro Embryo Culture on Placental Gene Expression in the Sheep  abs#206

9:00am  Adrian Charles
The expression of caspase 14 in the human placenta.  abs#207

9:10am  Q Chen
Exposing necrotic trophoblasts to endothelial cells in vitro causes increased adhesion of monocytes.  abs#208

9:20am  Peter Mark
P-glycoprotein limits activation of the glucocorticoid receptor in placental BeWo cells.  abs#209

9:30am  Kirsty Pringle
Localisation of Insulin-Like Growth Factor-II (IGF-II) and its Receptor in Early Murine Pregnancy: A Role in Placentation and Angiogenesis in the Decidua?  abs#210

9:40am  Ying Li
Expression and cellular localization of HTRA3 protease during placental development in mice  abs#211

Session 4 - SRB orals - Sperm and Spermatogenesis
8:00 AM - 10:00 AM  M3, level 2
Chairs: Chris Scott and Julia Young

8:00am  Sirisha Mendis
Regulation of tgf β superfamily- related genes in the newborn mouse testis by activin  abs#213

8:10am  Duangporn Jamsai
The role of testis specific protein 1 (Tpx-1) and gametogenetin (Ggn) in mammalian spermatogenesis  abs#215

8:20am  Sridurga Mithra Prabhu
c-Kit Expression study: Timing of onset in rodent testis and irradiated rat testis model  abs#216

8:30am  Jennifer Ly
Interaction of CDYL (chromodomain y-chromosome like) with the nuclear transport protein importin α2  abs#217

8:40am  Mai Sarraj
Expression OF Wsb2 in the mouse testis  abs#218

8:50am  Kirstin Hengstberger
Prevalence of sperm chromatin instability amongst bulls in a subtropical environment: a preliminary investigation  abs#219

9:00am  Christopher Scott
Equine growth hormone enhances motility and extends longevity of ram spermatozoa in vitro  abs#220

9:10am  Angela Wagner
SPRASA, a sperm protein with a post-fertilization function?  abs#221

9:20am  Phillip Matson
Sperm morphology within the testis and cauda epididymis of a Tasmanian devil (Sarcophilus harrisii).  abs#222
9:30am    Russell Jones
The epididymis secretes proteins involved in sperm competition: evidence from the echidna abs#223

Session 5 - ESA Australia-Japan Lecture
Session sponsored by Schering
8:30 AM - 9:30 AM  
Chair: Stephen Twigg
Hiroyuki Namba
Molecular Diagnosis and Novel Therapies for Advanced Thyroid Carcinomas abs#006

Session 6 - ESA Servier Award
Session sponsored by Servier
9:30 AM - 9:45 AM  
Chair: Peter Fuller
Simon Chu
Transrepression of Estrogen Receptor β Signaling by Nuclear Factor-κB in ovarian Granulosa Cells abs#101

Morning Tea  ESA breaks 9:45am, SRB at 10am
9:45 AM - 10:30 AM  
Break sponsored by Mayne Pharma
Pavillion 1, level 1, trade exhibition

Session 7 - ESA Novartis Junior Investigator Award Finalists
Session sponsored by Novartis
10:15 AM - 12:00 PM  
Chair: Esme Hatchell and Jeffrey Zajac
10:15am  Agnes Kovacic
Functional interactions between LRH-1 and CREB regulate aromatase expression in breast cancer abs#102
10:30am  Amy Au
PAX8-PPARγ alters normal thyroid transcriptional regulation abs#103
10:45am  Katie Dixon
In vivo photoprotection by a rapid response, low calcemic analog of 1,25-dihydroxyvitamin D3 abs#104
11:00am  Todor Arsov
Fat Aussie Mouse – a Rodent Model of Alström Syndrome abs#105
11:15am  Caitlin Wywoll
Fetal programming of adult hypertension and hyperleptinaemia: Prevention by postnatal dietary omega-3 fatty acids abs#106
11:30am  Theresa Hickey
Differential patterns of X-inactivation among sisters in family groups with polycystic ovary syndrome. abs#107
11:45am  Kirsten McTavish
Ovarian phenotype in female transgenic mice expressing pituitary-independent FSH during initial hyperfertility followed by premature infertility abs#108

Session 8 - SRB-ANZPRA Symposium: Healthy Start to Life: Pregnancy and Parturition
10:30 AM - 12:00 PM  
Chair: Brendan Waddell and Sarah Robertson
10:30am  John Newnham
Recent Advances in Understanding and Preventing Pre-term Birth - the Oral Health Connection abs#007
11:00am  Jeffrey Keelan
Placental inflammation and preterm labour: Studies of pathophysiology and intervention using human ex-vivo models abs#008
11:30am  Vicki Clifton
The effect of maternal asthma during pregnancy on placental function, fetal growth and childhood development abs#009
Session 9 - SRB Founders Lecture
12:00 PM - 1:00 PM
Chair: Lois Salamonsen
John Eppig
Oocyte control of granulosa cell development and function  abs#010

Session 10 - ESA Clinical Meet the Expert
12:00 PM - 1:00 PM
John Walsh, Subclinical Thyroid Disease  abs#011

Lunch
1:00 PM - 2:00 PM
Break sponsored by Eli Lilly

Session 11 - SRB orals: Oocyte and Follicle Development
1:30 PM - 3:30 PM
Chairs: Jeff Keelan and Peter Mark
1:30pm  Leanne Sleer
Platelet derived growth factors and receptors in the rat ovary contribute towards preantral follicle growth  abs#224
1:40pm  Hannah Brown
Early ovarian follicle dysgenesis in a disintegrin and metalloproteinase with thrombospondin motifs type 1 (ADAMTS-1) null mice  abs#225
1:50pm  Lyn Harland
Expression of Matrix Metalloproteinases in Bovine Thecal Cells  abs#226
2:00pm  Janette Quennell
FSH receptor expression in small human ovarian follicles  abs#227
2:10pm  Pradeep Tanwar
In vivo evidence for a role of Bone Morphogenetic Protein-4 in ovarian function.  abs#228
2:20pm  Almudena Veiga-Lopez
Effect of follicular status on sheep embryo yields is mediated by changes in the preovulatory LH surge  abs#229
2:30pm  Kylie Dunning
Altered Matrix Composition of Cumulus Oocyte Complexes Following in vitro Maturation  abs#230
2:40pm  Rebecca Dragovic
Mouse oocyte paracrine signalling to cumulus cells by TGF-β superfamily molecules is indispensable for cumulus expansion.  abs#231
2:50pm  Tamer Hussein
Oocytes prevent bovine cumulus cell apoptosis by maintaining a morphogenic paracrine gradient of bone morphogenetic proteins  abs#232
3:00pm  Kelly Banwell
Oxygen concentration during in vitro maturation of murine oocytes affects blastocyst cell lineage.  abs#233
3:10pm  Paul Edgecumbe
The ubiquitin-proteasome pathway in bovine and murine oocytes undergoing maturation.  abs#234
3:20pm  Cheryl Schelbach
Abberant murine embryonic development following glucosamine exposure during IVM or embryo culture  abs#235

Session 12 - SRB orals: Uterus and Implantation
1:30 PM - 3:30 PM
Chairs: Jane Girling and Naomi Morison
1:30pm  Caroline Gargett
Characterising the Stem Cell Activity of Human Endometrial Epithelial and Stromal Cells  abs#236
1:40pm Laura Lindsay
Aquaporins in rat uterine epithelial cells during early pregnancy and in response to progesterone abs#237

1:50pm Megan Nicholson
Claudins and occludin in the rat endometrium abs#238

2:00pm Jane Girling
Hormonal control of vascular mural cell recruitment in the mouse endometrium abs#239

2:10pm Lynette Kilpatrick
Spatial and Temporal Expression Pattern of Furin in the Human Endometrium abs#240

2:20pm Anna Ponnampalam
Changes in the expression of Annexin IV mRNA and protein in human endometrium abs#241

2:30pm Melinda Jasper
LIF expression is induced in the mouse oviduct following activation by seminal factors abs#242

2:40pm Marina Zaitseva
In vitro culture significantly reduces differences in gene expression profiles between Myometrial and Fibroid Smooth Muscle Cells. abs#243

2:50pm Tu‘hevaha Kaitu‘u
Effect of batimastat, a specific inhibitor of matrix metalloproteinases, on endometrial breakdown and repair in a mouse model abs#244

3:00pm Naomi Morison
Endometrial changes in a mouse model for long-term progestin exposure: Implications for break-through bleeding. abs#245

3:10pm Ray Garry
A new look at menstrual repair: The role of CD34⁺ cells and CD56⁻ uterine NK cells abs#246

Session 13 - ESA Poster viewing #1: Metabolism, Pituitary, Reproduction, others
2:00 PM - 3:00 PM Poster area, adjacent pavillion 1, level 1
See listing at end of day’s program

Session 14 - ESA Basic Symposium: Endocrine Tumorigenesis
3:00 PM - 5:00 PM Auditorium, level 2
Chairs: Anne Nelson and Charles Allen
Session sponsored by Novartis

3:00pm Peter Leedman
Coregulators and hormone action in breast cancer abs#012

3:30pm Grant Buchanan
Chaperones and regulators: mediators of androgen receptor function in prostate cancer abs#013

4:00pm Qihan Dong
Novel Therapeutic Targets In Prostate Cancer abs#014

4:30pm Christine Clarke
Ovarian Hormones And Breast Cancer abs#015

Session 15 - ESA Clinical Symposium: When the Adrenal Goes Awry
3:00 PM - 5:00 PM M2, level 2
Chair: Shaun McGrath and David Hurley
Session sponsored by Pfizer

3:00pm Peter Pullan
Addison’s disease abs#016

3:30pm David Torpy
Adrenal Insufficiency: Absolute, Transient and Relative Forms abs#017

4:00pm Peter Fuller
When the Adrenal Goes Awry : Hyperaldosteronism abs#018

4:30pm Hieu Nguyen
Adrenal Surgery - The considering issues abs#019
Session 16 - ESA orals: Reproduction/Pregnancy/Parturition 1
3:00 PM - 5:00 PM  
M10, level 2
3:00pm  Catherine Coulter  
Developmental expression of angiotensin receptors in the sheep adrenal gland - a role for angiotensin-II in regulating adrenal growth and steroidogenesis before birth  abs#109
3:15pm  Timothy Cole  
Analysis of glucocorticoid and cAMP signaling during lung development using glucocorticoid receptor and CREB null mice  abs#110
3:30pm  Renée Johnson  
Influence of Labour and Fetal Sex on Glucocorticoid Receptor mRNA Transcript Expression in the Human Placenta  abs#111
3:45pm  Carolyn Mitchell  
The Role of NFκβ in the Regulation of Prostaglandin H-2 Synthase in Term Human Amnion in vivo.  abs#112
4:00pm  Damien Hewitt  
Changes in placental expression of PPARγ in rat pregnancy are associated with altered expression of Muc1 and VEGF  abs#113
4:15pm  Kathryn Gatford  
Acute maternal alcohol treatment restricts fetal growth and reduces fetal plasma IGF-II but not IGF-I.  abs#114
4:30pm  Adrian Charles  
The regulation of apoptotic genes during spontaneous apoptosis using an in-vitro explant culture model.  abs#115

Session 17 - ESA-SRB joint orals: Male Reproduction
3:45 PM - 5:45 PM  
M4, level 2
Chair: David Aridi
3:45pm  Mark McCabe  
Contribution of claudin-11 to the inter-Sertoli cell tight junction, in vitro  abs#116
4:00pm  Anette Szczepny  
Expression of components of the hedgehog signalling pathway during murine spermatogenesis  abs#247
4:15pm  Claire Kennedy  
Meiosis arrest in a new animal model--a mutation on chromosome 5  abs#117
4:30pm  Maree Gould  
Oestrogen receptor beta is involved in the regulation of leydig cell number in the mouse.  abs#248
4:45pm  Ruth Escalona  
Regulation of inhibin binding and action via betaglycan expression in mouse leydig-like TM3 cells  abs#118
5:00pm  Moira O’Bryan  
Fibroblast growth factor receptor-1 (FGFR-1) is essential for spermiogenesis, capacitation and male fertility  abs#249
5:15pm  Elspeth Gold  
Over-expression of activin βC in vivo reveals a role in male fertility  abs#119
5:30pm  Amy Glover  
Adult Exposure to Dietary Phytoestrogens Reduces Fertility of Male Rats  abs#250

Session 18 - SRB orals: Ovary and Corpus Luteum
3:45 PM - 5:45 PM  
M3, level 2
Chairs: Darryl Russell and Guela Gibori
3:30pm  Rob Gilchrist  
Growth differentiation factor 9 signalling systems regulate marmoset monkey granulosa cell proliferation.  abs#251
3:40pm  Elisabeth Feary  
Morphometric and histological analysis of ovaries from sheep heterozygous for the prolific Woodlands allele  abs#252
3:50pm  Christopher Grupen  
Calcium ionophore induction of marmoset oocyte activation  abs#253
4:00pm  Melanie Bagg  
Effect of donor age and follicle size on oocyte developmental competence in the pig  abs#254

4:10pm  Andrew French  
In vitro maturation of bovine oocytes in serum-free media  abs#255

4:20pm  R. Tecirlioglu  
Bovine oocyte vitrification in sodium free medium  abs#256

4:30pm  Kara Cashman  
Addition of Glycine to Vitrification Solutions Protects Oocyte and Embryo Physiology and Health  abs#257

4:40pm  Douglas Eckery  
Characterisation of ovarian follicular growth in the brushtail possum  abs#258

4:50pm  Jason Liew  
Reproductive phenotype of the female aromatase overexpressing mouse  abs#259

5:00pm  Cadence Minge  
Effects of Diet-Induced Obesity on Ovarian Function and Female Fertility  abs#260

5:10pm  Stephen Anderson  
Resistance to GH signalling through stat5 is an early event in PGF2α induced luteolysis in the ewe.  abs#261

5:20pm  K Ford  
Follistatin Secretion by the Ovary is Not Directly Related to PGF2α Induced Luteolysis in the Ewe  abs#212

Session 19 - ESA AGM
5:00 PM - 6:00 PM  Auditorium, level 2

ESA-SRB Student Function
7:00 PM - 10:00 PM  Perth Rowing Club

Monday Poster listing
Leon Brownrigg  
9-HODE-induced apoptosis in U937 monocytes is not inhibited by blockade of PPARγ, and is enhanced by activation of PPARδ.  abs#401

Soelaiman Ima Nirwana  
Heated Palm Oil Is Not Detrimental To Bone Metabolism In Estrogen Deficient Rats  abs#402

Kamsiah Jaarin  
Aortic histomorphometric finding in ovariectomized rats fed with heated vegetable oils  abs#403

Norhayati Moktar  
Effect of Heated Soya and Palm Oil on Serum Homocysteine, Interleukin and MDA in Ovariectomized Rats  abs#405

Nor Umar  
Changes in Serum Lipid Profiles in Estrogen Deficient Rats fed with Soya and Palm Oil Diet  abs#406

Yue Chen  
Effects of Androgens on Myoblast Proliferation  abs#407

Greg Anderson  
Gonadotrophin-inhibitory hormone (GnIH) suppresses LH secretion in the rat  abs#408

Matthew Dalrymple  
Orexin receptor subtypes-1 and -2 exhibit distinct beta-arrestin profiles determined using bioluminescence resonance energy transfer (BRET) and confocal microscopy  abs#409

Shaofu Li  
Prenatal betamethasone exposure significantly alters fetal adrenal steroidogenic enzyme P450c17 gene expression in sheep  abs#410

Hamish Russell  
Use of a Diurnal Cortisol Blood Spot Profile for Adjustment of Hydrocortisone Dose.  abs#411

Mohd Fahami Nur Azlina  
Effects of tocotrienol and tocopherol on corticosterone level and gastrointestinal changes in rats exposed to stress  abs#412
Morton Burt
A Comparison Of Body Composition In Cushing's Syndrome And Growth Hormone Deficiency  

David Phillips
Activin as a novel marker in clinical inflammatory processes: elevations in burns and traumatic brain injury patients.  

Anna-Maree Axell
Testosterone Administration Prevents Skeletal Muscle Atrophy and Enhances Resistance to Fatigue in Orchidectomised Male Mice  

Jenny Spaliviero
Effects of low dose estradiol silastic implants in aromatase deficient (ArKO) mice.  

Preetika Balanathan
Inhibin-alpha in prostate cancer: tumor suppressor and pro-metastatic factor  

Reece Wells
Testicular hypertrophy after hemicastration in the neonatal boar requires gonadotrophin but not testosterone support  

Almudena Veiga-Lopez
Ovarian status and embryo yields in superovulated ewes: the equilibrium between absence of dominant follicles and presence of size-adequate follicles  

Carmel Cluning
Expression Profiles of Steroid Receptor-Associated Immunophilins in Human Endometrium During the Menstrual Cycle  

Alexandra Umbers
A Pharmacogenomic Approach to the Treatment of Infertility in Polycystic Ovarian Syndrome  

Penelope Hawken
The endocrine response of maiden ewes to the ram effect is not dependent on prior experience with rams  

A Dhali
Secretion patterns of LH and FSH around estrus in mithun (Bos frontalis)  

Yao Wang
Retinoic acid (RA) regulation of type II receptors for the TGF-β superfamily in a mouse adrenocortical cell line
Tuesday, 6 September 2005

Session 22 - SRB Meat Livestock Australia Plenary Lecture
8:15 AM - 9:30 AM
Auditorium, level 2
Chair: Graeme Martin
Session sponsored by Meat Livestock Australia

Ken McNatty
Oocyte signalling molecules and their effects on reproduction in ruminants abs#020

Jock Findlay
An update from the Chairperson of the Embryo Research Licensing Committee of the NHMRC

Session 23 - ESA Mayne Pharma Bryan Hudson Award Finalists
Session sponsored by MaynePharma
8:30 AM - 9:30 AM
M2, level 2
Chair: Jeffrey Zajac

8:30am Jui Ho
Septic shock and Sepsis: A Comparison of Total and Free Plasma Cortisol Levels abs#120

8:45am Cherie Chiang
The value of synacthen during adrenal vein sampling in differentiating aldosterone producing adrenal adenoma from bilateral adrenal hyperplasia. abs#121

9:00am Carolyn Allan
The Effects of Physiological Testosterone Therapy on Body Composition and Markers of Cardiovascular Risk in Non-Obese Ageing Men: A 12-month Placebo Controlled Trial. abs#122

9:15am Morton Burt
How Is Protein Metabolism Perturbed In Cushing's Syndrome? abs#123

Morning Tea
9:30 AM - 10:00 AM
Break sponsored by Schering
Pavillion 1, level 1, trade exhibition

Session 24 - SRB Symposium: New Frontiers in Reproductive Biotechnology and Cloning
10:00 AM - 11:30 AM
Auditorium, level 2
Chair: Michael Holland and Andrew French
Session sponsored by CRC for Innovative Dairy Products

10:00am Jon Hill
Current progress into testis cell transfer between cattle breeds. abs#021

10:30am Paul Verma
Isolation of stem cells from embryos and adult bovine tissues abs#022

11:00am Graeme Martin
Biotechnology and reproduction in mainstream animal industry - a perspective abs#023

Session 25 - ESA-SRB joint orals: Female Reproduction
10:00 AM - 12:00 PM
M4, level 2
Chair: Caroline Gargett

10:00am Johanna Barclay
 Regulation of SOCS3 expression by prostaglandin, prolactin and growth hormone: challenging the Jak/STAT signalling dogma. abs#124

10:15am Rebecca Kelley
 Gonadotrophic hormones affect the uterus, implantation and fetal development in mice abs#265

10:30am Craig Harrison
 Identification of a functional binding site on betaglycan for inhibin and TGFβ abs#125

10:45am Evdokia Dimitriadis
 Supressor of cytokine signalling 3 regulates IL-11 induced human endometrial stromal cell decidualization. abs#266

11:00am Norfilza M. Mokhtar
 Progesterone regulates CXCL14 (macrophage inflammatory protein 2γ) mRNA in human endometrium. abs#126
11:15am  Sarah Robertson  
Interleukin-10 inhibits TNFα synthesis and protects against LPS-induced miscarriage and preterm labour  abs#267

11:30am  Leanne Sleer  
Platelet derived growth factors and receptors contribute toward development of the corpus luteum  abs#127

11:45am  Susanne Meier  
Experimentally induced hypoglycemia: a model to examine the effects of lactation on reproductive function in dairy cows?  abs#268

Session 26 - ESA orals: Cancer  
10:00 AM - 12:00 PM  
M2, level 2

10:00am  Linda Dawson  
Characterisation of FLJ22318 in prostate cancer cells  abs#128

10:15am  Esme Hatchell  
Investigation of the cellular function and interactions of SLIRP, a novel nuclear corepressor of the estrogen receptor pathway.  abs#129

10:30am  Shane Colley  
SRA is a target for both SLTM and SAFB  abs#130

10:45am  Andrew Redfern  
Contrasting roles for novel SRA-binding proteins in nuclear receptor pathway co-regulation between different malignant cell lines.  abs#131

11:00am  Niroshani Pathirage  
Molecular basis of aromatase over-expression in Ovarian Granulosa Cell Tumours  abs#132

11:15am  Jian Ang  
CD151 Promotes the Migration and Invasion Properties of the Prostate Cancer Cell Line LNCap.  abs#133

11:30am  Diana Azzam  
Interactions between the Androgen Receptor and Mitogen Activated Protein Kinase Pathways in Breast Cancer Cells  abs#134

11:45am  Simon Mahoney  
X-Linked Inhibitor of Apoptosis Protein Expression and Chemoresistance in Ovarian Cancer  abs#135

Session 27 - ESA orals: Pituitary/Neuroendocrine  
10:00 AM - 12:00 PM  
M3, level 2

10:00am  Jasmin Dromey  
Differential kinetics and affinities of ligand-induced G-protein coupled receptor interactions with beta-arrestins as detected by extended bioluminescence resonance energy transfer.  abs#136

10:15am  Kin-chuen Leung  
Mapping of functional domains of oestrogen receptor-α that mediate regulation of growth hormone signalling by oestrogen and SERMs  abs#137

10:30am  Udo Meinhardt  
Direct Comparison of the Effects of Raloxifene and Oestrogen During GH Therapy on Body Composition in Hypopituitary Women  abs#138

10:45am  Christina Jang  
Ovarian Hyperstimulation and Amenorrhoea secondary to Pituitary Adenoma cosecreting FSH and Prolactin  abs#139

11:00am  Warrick Inder  
Acromegaly secondary to intra-thecal morphine administration for chronic pain  abs#140

11:15am  Anne Nelson  
Effect of sporting type on growth hormone-responsive markers in elite athletes.  abs#141

11:30am  Joey Kaye  
Aberrant stress responses in autonomic failure syndromes: Mechanisms and potential clinical implications.  abs#142

11:45am  Ben Canny  
The coupling of ACTH and cortisol secretion is greater in female than male sheep under basal conditions  abs#143
Session 28 - ESA orals: Androgens and Androgen Action
10:00 AM - 12:00 PM

10:00am  David de Kretser
Reference intervals for reproductive hormones in healthy fertile young men: evaluation of automated platform assays.  abs#144

10:15am  Bu Yeap
Interaction between testosterone and APOE ε4 on cognition in normal older men: The Fremantle Endocrinology of Ageing Research Study.  abs#145

10:30am  Helen MacLean
Androgens Act Directly Through the Androgen Receptor in Skeletal Muscle to Maintain Muscle Mass  abs#146

10:45am  Helen MacLean
Decreased Skeletal Muscle Mass and Strength in Male but Not Female Universal Androgen Receptor Knockout Mice  abs#147

11:00am  Kerry McNiness
Aromatase-overexpressing mice (AROM⁺) gain excess adipose tissue.  abs#148

11:15am  Kerry McNiness
Dihydrotestosterone inhibits activation of ampK in adipose tissue of female mice  abs#149

11:30am  Christin Down
Role of the Hu proteins, HuR and HuD, in the regulation of androgen receptor expression and activity in prostate cancer cells.  abs#150

Session 29 - SRB orals: Reproductive Biotechnology
11:30 AM - 12:00 PM  Auditorium, level 2
Chair: Michael Holland and Andrew French

11:30am  Julia Young
Towards derivation of primordial germ cells from murine embryonic stem cells  abs#262

11:40am  Jeanette Olejnik
The successful use of busulfan to deplete endogenous spermatogonia in ram testes  abs#263

11:50am  George Riding
Proteomic approaches to the study of conceptus fluids from first trimester bovine pregnancies  abs#264

Session 30 - ESA Harrison Lecture
12:00 PM - 1:00 PM  Auditorium, level 2
Chair: Jeffrey Zajac

Richard Pestell
Nuclear receptors and cyclins in hormone signaling  abs#024

Lunch
1:00 PM - 2:00 PM  Lunch sponsored by Novartis
Pavilion 1, level 1, trade exhibition

Session 31 - SRB Symposium: Molecular Communication in the Ovary and Testes
1:00 PM - 3:00 PM  Auditorium, level 2
Chairs: Sarah Meacham and Rob Gilchrist

1:00pm  Geula Gibori
Prolactin signaling through the short form of its cognate receptor causes severe ovarian defect.  abs#025

1:30pm  Darryl Russell
The Cumulus Matrix in Ovulation; Inert Packaging or Active Delivery Vehicle for the Oocyte?  abs#026

2:00pm  Michelle Lane
In vitro growth and maturation: How does this technology fit for clinical application?  abs#027
2:30pm  Mark Hedger  
Cytokine networks and regulation of spermatogenesis - what should we really believe?  abs#028

Session 32 - ESA poster session #2: Pregnancy/Clinical/Cancer  
2:00 PM - 3:00 PM  
Poster area, adjacent pavillion 1, level 1  
See listing at end of day's program

Afternoon Tea  
2:45 PM - 3:15 PM  
SRB breaks at 3pm

Session 33 - ESA Symposium: Biological Clocks in Neuroendocrinology  
3:00 PM - 5:00 PM  
Auditorium, level 2  
Chair: Brian Oldfield  
Session sponsored by Mayne Pharma

3:00pm  Sato Honma  
The circadian system composed of the central and peripheral clocks: Monitoring clockworks by bioluminescent reporters  abs#029

3:40pm  Gary Pickard  
Serotonergic Regulation of Circadian Rhythms  abs#030

4:20pm  David Kennaway  
Hypothalamic and peripheral tissue circadian rhythms and the endocrine system  abs#031

Session 34 - ESA orals: Reproduction/Pregnancy/Parturition 2  
3:00 PM - 5:00 PM  
M2, level 2

3:00pm  Amanda Beardsley  
Hormonal regulation of phosphorylated proteins in seminiferous tubules; possible involvement in spermiation.  abs#151

3:15pm  Patrick Lim  
Estradiol induction of mouse spermatogenesis requires a functional androgen receptor  abs#152

3:30pm  Rebecca Craythorn  
Isoform Specific Follistatin Mouse Models  abs#153

3:45pm  Marelyn Wintour  
Differential effects of early pregnancy treatment with natural and synthetic glucocorticoids in sheep  abs#154

4:00pm  David MacIntyre  
Peptide profile changes between labouring and non labouring human myometrium identified using SELDI-TOF MS  abs#155

4:15pm  Tan Erdonmez  
Progestosterone Receptor Protein Expression in Human Fetal Membranes and Myometrium with the Onset of Labour:  abs#156

4:30pm  Roger Smith  
Modulation of Progesterone Receptor Expression by Corticotrophin Releasing Hormone in Human Myometrial Cells  abs#157

4:45pm  Claire Morbey  
Human Myometrial Histology at Term  abs#158

Session 35 - ESA orals: Clinical  
3:00 PM - 5:00 PM  
M3, level 2

3:00pm  Richard Prince  
Endogenous Estrogen Predicts Mortality Due to Coronary Heart Disease in Elderly Women  abs#159

3:15pm  Pratibha Saini  
A new approach of recombinant FSH doses in sub-fertility patients with polycystic ovarian syndrome.  abs#160
3:30pm  John Walsh  
Parity and the risk of autoimmune thyroid disease: a community-based study  abs#161

3:45pm  Mathis Grossmann  
Isolated ACTH deficiency presenting as severe hypercalcaemia  abs#162

4:00pm  Ashish Gargya  
The evolutionary enigma of hereditary carotid body tumours  abs#163

4:15pm  Vijay Panicker  
Change in Bone Density over Time in Adults with Cystic Fibrosis.  abs#164

4:30pm  Malgorzata Brzozowska  
An association between Non Alcoholic Steatohepatitis and Polycystic Ovarian Syndrome  abs#165

Session 36 - SRB New Investigator Award Finalists
3:15 PM - 5:00 PM  
Chair: Lois Salamonsen  
Session sponsored by Serono and Meat Livestock Australia  
M4, level 2

3:15pm  Muren Herrid  
Optimal testicular size of donor and recipient for testicular germ cell transplantation in the bovine  abs#269

3:30pm  Theresa Hickey  
Androgens augment the mitogenic effects of oocyte-secreted factors and growth differentiation factor 9 on porcine granulosa cells.  abs#270

3:45pm  Kjiana Schwab  
Prospective isolation of human endometrial mesenchymal stem cells using CD146 and platelet-derived growth factor receptor-β  abs#271

4:00pm  Lisa Walter  
Progesterone stimulates endothelial cell proliferation, but not stromal cell proliferation, in mouse endometrium.  abs#272

4:15pm  John Bromfield  
Seminal plasma influences pregnancy outcome through effects on both uterine receptivity and the pre-implantation embryo.  abs#273

4:30pm  Damien Hewitt  
Placental expression of secreted frizzled related protein (sFRP4) in the rat: association with β-catenin localization and regulation by glucocorticoids  abs#274

4:45pm  Ambika Singh  
Neuronal regeneration peptide (NRP): A novel trophoblast migration and survival enhancing factor  abs#275

Session 37 - ESA Taft Lecture
5:00 PM - 6:00 PM  
Chair: Catherine Choong  
Session sponsored by Sanofi-Aventis  
Auditorium, level 2

David Dunger  
Developmental Origins of the Metabolic Syndrome  abs#032

ESA-SRB Dinner  
7:00 PM - 11:00 PM  
Function sponsored by Pfizer  
M4&M5, level 2

Tuesday poster listing

Elisa Tyson  
Effects of Corticotrophin Releasing Hormone and Salbutamol on Term Pregnant Human Myometrial Contractile Activity in Vitro  abs#425

Thilee Sivananthan  
A pregnancy with hydatidiform mole, co-existing twin and severe thyrotoxicosis: A case report  abs#426

Naomi Scott
Sex differences in placental cytokine expression and their relationship to fetal cortisol  abs#427
Annette Osei-Kumah
Maternal and cord plasma cytokine / chemokine profiles in pregnancies complicated by asthma  abs#428
Hayley Wyper
Identification and characterisation of macrophages in placentae from pregnancies complicated by asthma  abs#429
Hayley Wyper
Placental glucocorticoid receptor expression in pregnancies complicated by asthma  abs#430
Cynthia Ong
Diabetic Pregnancy & Congenital Malformations: When should we stop worrying?  abs#431
Cynthia Ong
Oncogenic Osteomalacia: A Case of Diagnostic Dilemma and Management Challenge  abs#432
Mridula Lewis
Case Presentation:Widespread Osteitis Fibrosa Cystica and Primary Hyperparathyroidism in a 41 year old woman.  abs#433
Ann McCormack
The monitoring of vitamin D in Ostelin-treated patients  abs#434
Shirley Elkassaby
Impact of a hospital-based intervention on the outcome of minimal trauma fractures  abs#435
Melissa Gillett
Assessment of the clinical utility of urinary NTX in osteoporosis- an audit  abs#436
Caroline Meyer
Cardiovascular Effects of Medical Therapies in Polycystic Ovary Syndrome  abs#437
Michael Hooper
Clinical experience with 2 years of zoledronic acid in osteoporosis  abs#438
Channa Perera
Thyrotoxic Hypokalaemic Periodic Paralysis in a young Australian Caucasian man: A case report  abs#439
Erik Helmerhorst
Insulin Like Growth Factor-2 Occupancy of the Insulin Receptor Isoform A: Significance in Normal and Malignant Tissues.  abs#440
Kristy Shipman
The Multifunctional Protein CREAP is a Nuclear Protein  abs#442
Lauren Miles
Identification of renin and regulatory role of the renin mRNA-binding proteins HuR, HADHB and CP1 in human breast cancer cells.  abs#443
Jeremy Drake
Expression of sFRP-4 and β-catenin in Serous Ovarian Carcinoma  abs#444
Danny Mok
Two Structurally-Related Coumarin Antibiotics Exert Different Effects On Disruption Of Hsp90 Dimerization  abs#445
Rudi Allan
The Heat Shock Protein 90-binding Coumarin Novobiocin Inhibits Steroid Receptor Activity without the Stress  abs#446
Wednesday, 7 September 2005

Session 39 - ESA Plenary  
8:30 AM - 9:30 AM  
Chair: Mark McLean

Roger Smith  
New Roles for Old Hormones and the Gordian Knot of Human Birth  abs#033

Session 40 - SRB orals: Reproductive Immunology  
8:30 AM - 9:40 AM  
Chairs: Peter Rodgers and Sean O’Leary

8:30am  Kylie Van der Hoek  
Regulated expression of the c-fms receptor characterises distinct ovarian macrophage populations  abs#283

8:40am  Sean O’Leary  
Leukocyte trafficking in the ovary of mice immunised with recombinant murine cyclomagalovirus expressing murine zona pellucida 3  abs#284

8:50am  Jacqui Donoghue  
Endometrial lymphatics are reduced in the functionalis compared to basalis and myometrium  abs#285

9:00am  Julie Crewe  
The role of uterine natural killer cells in causing irregular bleeding in HT users  abs#286

9:10am  Danielle Glynn  
LPS introduced at mating induces KC production in the murine uterus during early pregnancy  abs#287

9:20am  Jenni Scott  
The Post-insemination Inflammatory Response in the Ewe  abs#288

9:30am  David Aridi  
Comparison of lymphocyte subsets and function in the rat and mouse testis  abs#289

Session 41 - SRB orals: Neuroendocrine Regulation of Reproductive Processes  
8:30 AM - 9:40 AM  
Chairs: Dominique Blache and Alan Tilbrook

8:30am  Alan Tilbrook  
Sex difference in the effect of cortisol on the LH response of the pituitary to exogenous GnRH in hypothalamo-pituitary disconnected gonadectomised sheep  abs#276

8:40am  Julia Young  
Is the high level of circulating FSH in Booroola ewes due to the BMP R1b mutation?  abs#277

8:50am  Penelope Hawken  
Repeated 24-hour exposure of ewes to rams during the transition into breeding season compacts the mating of the flock  abs#278

9:00am  Teuku Ferasyi  
A dynamic model of the control of pulsatile luteininizing hormone secretion by gonadotrophin-releasing hormone  abs#279

9:10am  Gemma Graham  
Photo inhibited heat shock protein 108 gene expression in the chicken hypothalamus  abs#280

9:20am  Karense Menzies  
The role of insulin in milk protein synthesis  abs#281

9:30am  Melissa Berg  
Anti-apoptotic effects of glucocorticoids and progesterone in a human mammary epithelial cell line  abs#282
Morning Tea
9:30 AM - 10:00 AM Pavillion 1, level 1, trade exhibition

Session 42 - ESA-SRB Symposium: The Genome-Environment Interaction in Determining Life Course
10:00 AM - 12:00 PM M2&3, level 2
Chairs: Cathie Coulter and Jeremy Thompson

10:00am Marie Pantaleon
Nutrient sensing by the early mouse embryo: Hexosamine biosynthesis and glucose signalling during preimplantation development abs#034

10:30am David Kennaway
Circadian rhythms and the early life programming of adult physiological systems abs#035

11:00am Caroline McMillen
Nutritional programming, fetal growth and competence for life after birth abs#036

11:30am Brendan Waddell
Impact of glucocorticoids on fetal-placental growth and the postnatal phenotype abs#037

Session 43 - ESA-ADS Symposium: Glycation and diabetes - from Monitoring to Pathogenesis
10:00 AM - 12:00 PM M4, level 2
Chairs: Glenn Ward and George Jerums Session sponsored by GlaxoSmithKline

10:00am Peter Colman
Using HbA1c in the Clinic - why worry about standardization? abs#038

10:30am Ian Goodall
HbA1c from a laboratory perspective abs#039

11:00am Josephine Forbes
Advanced glycation and interventions in the laboratory abs#040

11:30am Merlin Thomas
Diabetic complications: Is it time to start worrying about AGEing? abs#041

Session 44 - ESA-ANZBMS Symposium: Osteoporosis Therapeutic Update
10:00 AM - 12:00 PM M5, level 2
Chairs: Ego Seeman and Jeffrey Zajac Session sponsored by Servier

10:00am T J Martin
Mechanisms Of Action In Current And Emerging Osteoporosis Therapies abs#042

10:30am Richard Prince
Calcium Supplementation And/Or Vitamin D In Osteoporosis - Myth Or Reality? abs#043

11:00am John Eisman
Sex Hormone Replacement And SERMS abs#044

11:30am Richard Eastell
Osteoporosis: Whom To Treat? What Drug? abs#045

Lunch
12:00 PM - 1:00 PM Pavillion 1, level 1, trade exhibition
Session 45 - SRB orals: Embryo and Assisted Reproduction
1:00 PM - 3:00 PM  M2, level 2
Chairs: Karen Kind and Keith Jones

1:00pm  Keith Jones  
Calmodulin-dependent protein kinase II, and not Protein Kinase C, transduces the Ca\textsuperscript{2+} signal at fertilization.  abs#290

1:10pm  Gudrun Keck  
Potential role of Glycodelin for fertilization success in ART  abs#291

1:20pm  Neil Borg  
Influence of the in vitro environment on rat gametes and pre-implantation embryos  abs#292

1:30pm  A Fazeli  
Gametes Alter the Oviductal Secretory Proteome in vivo  abs#293

1:40pm  Fan-chin (Emmy) Hung  
Insulin receptor internalization in mouse preimplantation embryos  abs#294

1:50pm  Sarah Jansen  
Peroxisome proliferator activated receptor-alpha is involved in H\textsuperscript{+}-monocarboxylate transporter 2 and catalase protein expression in cultured preimplantation mouse embryos.  abs#295

2:00pm  Erica Little  
Characterization of E74 Like Factor 3 in the Murine Pre-Implantation Embryo  abs#296

2:10pm  Michael Boden  
Reproduction in the Arrhythmic Bmal1 Knockout Mouse  abs#297

2:20pm  Megan Mitchell  
Dietary protein does not influence mitochondrial distribution in the 2-cell mouse embryo  abs#298

2:30pm  Deirdre Zander  
Sensitivity of embryos to an environmental stressor, ammonium, is dependent on stage of temporal exposure.  abs#299

2:40pm  Ralf Moser  
Differential expression patterns of genes with immune and developmental relevance in individual bovine preimplantation embryos produced by nuclear transfer.  abs#300

Session 46 - SRB orals: Male Reproductive Tract
1:00 PM - 3:00 PM  M3, level 2
Chairs: Michael D’Occhio and Duangporn Jamsai

1:00pm  Leanne McGrath  
Effect of exogenous transforming growth factor beta 1 on reproductive performance in male TGF\textbeta\textsc{1} null mice  abs#301

1:10pm  Wendy Winnall  
Characterisation of lipopolysaccharide (LPS) receptor expression and the inflammatory response of the rat testis  abs#302

1:20pm  Megan Crane  
Further characterisation of lymphocyte-suppressing activity in gonadal fluids  abs#303

1:30pm  Esther Camp-Dotlic  
Murine HIF-1\alpha localisation by immunohistochemistry in a mouse reproductive tissue  abs#304

1:40pm  Brian Setchell  
Effects of moderate spinal cord injury on the expression of a barrier marker in endothelial cells of the testis and in the prostate of rats  abs#305

1:50pm  Reece Wells  
Testicular growth factor expression after hemicastration in the neonatal boar  abs#306

2:00pm  Karole Hogarth  
The Effect of Oxytocin on Cell Growth and Steroid Production in Normal Human Prostate Cells in vitro  abs#307

2:10pm  Miranda Shehu-Xhilaga  
Characterization of SIV infection in the male genital tract of juvenile macaques.  abs#308
Luciene Lomas Santiago  
Nutrition, insulin, leptin and puberty in Merino ram lambs  
abs#309

Jo Fink  
Oestrogen receptor alpha and beta in the prostate of the brushtail possum (Trichosurus Vulpecula)  
abs#310

Gerard Tarulli  
Adult Sertoli Cells Proliferate in Response to Exogenous Follicle Stimulating Hormone in the Adult Photo-Inhibited Djungarian Hamster  
abs#311

Michael D'Occhio  
Testosterone secretion in the Australian sea lion.  
abs#312

Session 47 - ADS Eli Lilly Plenary  
Session sponsored by Eli Lilly  
Auditorium. level 2

Steven Shoelsen  
Nucelar Factor kappa beta (NF-κβ): The master regulator of inflammation central to the aetiology of insulin resistance.  
of interest to ESA members

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Thyroid Cancer Myths

Myth: WBS is the gold standard diagnostic test.

Fact: 40-49% of patients with proven metastatic disease failed to be diagnosed on a TSH-stimulated Diagnostic WBS.¹

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Percent of False Negative Studies in Patients with Metastases on RxWBS

<table>
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<tr>
<th>Condition</th>
<th>Negative 4 mCi DxWBS</th>
<th>Negative Serum Tg (&lt;2μg/L)</th>
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<td>T4 Withdrawal DxWBS</td>
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<td>rHTSH DxWBS</td>
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<td>Tg on L-T4 Therapy</td>
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False negative rate of studies in patients with metastases seen on RxWBS. This includes all 35 patients with complete Tg data and metastatic disease confirmed by RxWBS.¹²

- "Ten studies comprising 1599 patients demonstrate that a TSH-stimulated Tg test using a Tg cutoff of 2 μg/l (either after thyroid hormone withdrawal or 72 h after rHTSH) is sufficiently sensitive to be used as the principal test in the follow-up management of low-risk patients with DTC and that the routine use of diagnostic whole body scanning in follow-up should be discouraged."¹³

- "Little information is added by performing a DxWBS in the evaluation of patients at low risk of having persistent disease."¹³


Genzyme Australasia Pty Ltd ABN 24830420526
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WRITING SCIENTIFIC PAPERS

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If you haven't written about your scientific work you might as well not have done it. But, if you haven't written it well, you won't have done much better because your scientific colleagues probably won't read it. This short workshop illustrates an approach to structuring scientific papers that encourages readers to find and retain the important information that you wish them to read. It demonstrates that logical structure is even more important than style and shows how to organize the structure of a paper around the scientific hypothesis on which the work was based. The hypothesis is a statement of what might plausibly have been expected from the experiment before it was done and that expectation can be used to describe the experiment in a way that induces readers to anticipate what they are about to read. This anticipation not only persuades them to read on but enhances their chances of retaining the information that they pick up when they do.

SERTOLI CELL TERMINAL DIFFERENTIATION: DOING A ‘U’ TURN ON A ONE WAY STREET

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The concept of terminal differentiation of Sertoli cells has been challenged and this new information has important implications for male fertility. The mammalian Sertoli cell has two distinct functions, i) formation of the seminiferous cords and ii) provision of nutritional and structural support to the developing germ cells. For these to occur successfully, Sertoli cells must undergo numerous maturational changes between foetal and adult life, the main switches occur around the onset of puberty, coincident with the rise in serum gonadotrophins. These switches include the loss of proliferative activity and the formation of the blood testis barrier. Follicle stimulating hormone (FSH) plays a key role in supporting Sertoli cell proliferation in early postnatal life and thus is critical in establishing sperm output in adulthood. After puberty, the size of the Sertoli cell population is considered to be stable and unmodifiable by hormones. This accepted view has been contested as data shows that the size of the adult Sertoli cell population is modifiable by hormone suppression, and that Sertoli cells can regain proliferative activity when stimulated by FSH in the Djungarian hamster\(^1\). The molecular mechanism(s) by which Sertoli cells re-enter proliferation are not known in this model however a study demonstrated that Helix-loop-inhibitor of differentiation proteins can induce terminally differentiated Sertoli cells to re-enter the cell cycle and proliferate\(^2\). Thyroid hormone and testosterone maybe involved in the cessation of Sertoli cell proliferation. Gonadotrophin suppression in the adult Djungarian hamster also results in the disruption of the blood testis barrier and spatial organisation of the inter Sertoli cell tight junction proteins and as a consequence the loss of all germ cells that reside inside the blood testis barrier. FSH restores the organisation of these tight junction proteins which is associated with the appearance of more mature germ cells. It is expected that the integrity of the blood testis barrier is also re-established. It is suggested that this demonstrated plasticity of the adult Sertoli cell may be relevant in clinical settings, particularly to some types of infertility and testicular malignancies where Sertoli cells have failed to undergo these important maturational switches.

\(^{(1)}\) Chaudhary et al 2005, Biol Reprod 72:1205

\(^{(2)}\) Meachem et al 2005, Biol Reprod. 72: 1187
EXPRESSION OF SECRETED FRIZZLED RELATED PROTEIN-4 (SFRP-4) AND ASSOCIATED WNT SIGNALLING IN CANCER AND APOPTOSIS

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We examined the interplay between Wnt and Secreted Frizzled Related Protein-4 (sFRP4) in estradiol induced cell growth in breast cancer cells (MCF-7), and also determined the in vivo distribution of sFRP-4 in human breast cancer. MCF-7 Cells were treated with estradiol, sFRP-4 conditioned media and a combination of the two. Real-time RT-PCR and Western blot analysis were used to determine the expression of the sFRP-4 and its associated Wnt signalling molecules following treatment. Immunohistochemistry was performed to examine sFRP-4 expression patterns in human breast cancers. Estradiol treatment up-regulated the expression of the Wnt signalling genes Wnt-10b, beta-catenin and fz-4 (p<0.001 for all genes). This up-regulation was not associated with an increase in the Wnt signalling pathway as measured by the levels of active beta-catenin. sFRP-4 conditioned media reduced MCF-7 cell proliferation, down regulated the Wnt signalling genes beta-catenin and fz-4 as well as down-regulating wnt signalling activity. sFRP-4 was able to reduce the proliferation of estradiol stimulated MCF-7 cells. Cytoplasmic sFRP-4 protein was expressed in all breast tumours examined, with intense staining evident in the lobular carcinoma in situ and the ductal carcinoma. These data demonstrate that sFRP-4 is a potent inhibitor of the Wnt signalling pathway in MCF-7 cells, acting not only to down-regulate the activity of the wnt signalling pathway, but also down-regulate the transcription of Wnt signalling genes. The results of these in vitro and immunohistochemical experiments warrant further investigation as to whether sFRP-4 expression can be indicative of prognosis in human breast cancer. In addition to breast cancer, we have also examined the role of sFRP-4 in other cancers such as ovarian and prostate.

CONTROL OF SKELETAL MUSCLE CELL PROLIFERATION AND DIFFERENTIATION.

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Skeletal muscle is formed by mononucleated precursor cells (myoblasts) that cease cell proliferation to start differentiation; this results in fusion between the myoblasts to form multinucleated cells (myotubes) that continue to differentiate (and fuse with more muscle cells) and mature into myofibres. Myogenesis has been widely used as a model to study in vitro factors controlling cell proliferation and differentiation. Condition in vitro may not reflect what happens in the more complex in vivo environment. Some of the key issues are what activates quiescent myoblasts in mature skeletal muscle in vivo, and what controls the switch between proliferation and differentiation? The role of the matrix, and molecules such as MyoD, p53, NFAT and IGF-1 will be considered.
EFFICIENCY OF NUCLEAR IMPORT OF THE CHROMATIN-REMODELLING FACTOR SRY IS CRITICAL FOR SEX DETERMINATION

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15% of cases of human XY sex reversal are due to mutations in SRY (sex determining region on the Y chromosome), many of which map to one of SRY’s two independently acting nuclear localization signals (NLSs) flanking its DNA binding domain. The C-terminal NLS (C-NLS) targets SRY to the nucleus through a “conventional” pathway dependent on the nuclear import receptor importin-β (Imp-β). No importin has been shown to bind the N-terminal NLS (N-NLS), but it is known to interact with the Ca\(^{2+}\)-binding protein calmodulin (CaM). We examined seven distinct missense mutations in the SRY NLSs from XY sex-reversed human females for effects on nuclear import and ability to interact with CaM/Imp-β1. All mutations were found to result in reduced nuclear localization in transfected testicular cells compared to wild type. The CaM antagonist, calmidazolium chloride (CDZ), was found to significantly reduce SRY nuclear accumulation, indicating a dependence of SRY nuclear import on CaM. Intriguingly, N-NLS mutants were resistant to CDZ’s effects, implying a loss of interaction with CaM; this was confirmed directly by in vitro binding experiments using recombinantly expressed protein. Either impaired CaM or Imp-β1 binding can thus be the basis of sex-reversal in human patients. Our results 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.

MOLECULAR DIAGNOSIS AND NOVEL THERAPIES FOR ADVANCED THYROID CARCINOMAS

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Final diagnosis of thyroid nodule is usually made by aspiration needle biopsy. However, 5-15% of cytological examinations are diagnosed as suspicious. Based on the recent molecular studies, BRAF mutations are the most common genetic abnormalities in papillary thyroid carcinomas. We show that BRAF analysis is a useful diagnostic tool for assistance of cytological examination. Concerning the therapy, most differentiated thyroid carcinomas have a fairly good prognosis after conventional therapy including surgery and following radioactive iodine therapy. However, undifferentiated thyroid carcinomas, so called anaplastic thyroid carcinomas (ATC) are very aggressive and eventually all fatal despite of the conventional therapy. To overcome the limitation of the conventional therapy for the ATCs, we have firstly attempted to establish p53 gene therapy because mutated p53 is a molecular hallmark in ATCs. As a candidate of targeted gene therapy, p53 restoration is essentially effective to induce apoptosis, particularly when combined with exposure to the histone deacetylase inhibitor. Although our findings clearly indicate that theoretically p53 gene therapy is applicable to clinical trial for ATCs, several technical problems still exist until now on utilization of viral vectors. Then we tried to molecular target therapy that is based on the universal features observed in ATCs. Immunohistochemical analysis showed the parallel overexpression of NF-κB in human ATC tissues, suggesting the therapeutic possibility of selective inhibitors of intracellular signal transduction molecules. A novel NF-κB inhibitor, Dehydroxy methylepoxyquinomicin (DHMEQ) was tried for treatment of ATC cell lines at first. The results strongly suggest that the use of DHMEQ is a clinically potential, selective anticancer modality for human ATCs. Treatment with DHMEQ markedly inhibited the translocation to the nucleus and DNA-binding activity of p65/p50 NF-κB heterodimer. Growth inhibition and apoptosis by DHMEQ was induced in all thyroid cancer cell lines. Administration of DHMEQ to transplanted ATCs tumor-bearing mice significantly inhibited the tumor growth without observable side effects. In conclusion, based on our recent data and results of other investigations, identification of pathways involved in thyroid carcinogenesis offers specific opportunities of development of new and comprehensive molecular diagnosis and targeting therapy for the patients with advanced thyroid carcinomas.
Recent Advances in Understanding and Preventing Pre-term Birth - The Oral Health Connection

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Pre-term birth remains one of the major unsolved problems in human health. The incidence is increasing in many Western countries, despite several decades of research studies aimed at findings ways that early birth may be prevented. Nearly two thirds of very early pre-term births are associated with features of inflammation in the newborn, suggesting that infection may be the origin. Many studies have focussed on the possibility that pre-labour rupture of membranes or the early onset of uterine activity may result from infection spreading upward from the vagina. Unfortunately, trials designed to identify potential pathogens in the genital tract followed by appropriate use of antibiotics have failed to prevent prematurity.

The strong association between features of intra-uterine inflammation and pre-term birth, and the ineffectiveness of antibiotics to prevent the problem, suggest that the source of inflammation may be from a distant site. We are addressing the possibility that the site may be the gums. Periodontal disease affects 15% of the adult Australian population, is often undiagnosed and is not responsive to systemic antibiotic therapy. In our pregnant population, we have shown that periodontal disease is strongly associated with low birth weight. Our sheep studies have taught us that the lipopolysaccharides (LPS) from periodontal pathogens, when injected into the amniotic cavity, have much greater lethality that enteric LPS, and similar effects in inducing inflammation. We are now investigating the effects of treating periodontal disease during mid-pregnancy in a randomised controlled trial which aims to screen approximately 5000 pregnant women and allocates those with periodontal disease to treatment during pregnancy or soon after. This study is known as the Smile Study and commenced in Feb 2005. Improving oral health is an exciting, but yet unproven, strategy by which a major health problem may be prevented by a relatively simple and community-based intervention.

Placental Inflammation and Preterm Labour: Studies of Pathophysiology and Intervention Using Human Ex-vivo Models

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Inflammatory processes and mediators are an integral aspect of the mechanics of parturition. Leukocyte infiltration/activation of the extraplacental membranes, cervix and uterus occurs prior to term labour and is accompanied by increased cytokine expression, eicosanoid production, and extracellular matrix degradation. These processes are propagated during labour to ensure progression through to delivery, and in normal parturition they occur in concert in a timely fashion. In contrast, pathological intrauterine inflammation appears to be a causative factor in a significant proportion of preterm births. While the presence of an infective organism in the amniotic cavity is confirmed in about half of pregnancies delivered with chorioamnionitis, in the remainder the cause of the excessive inflammatory response remains undetermined.

We have established and applied various models employing human placental tissues to study the processes involved in triggering intrauterine inflammatory activation and potential pharmacological approaches for intervention. Of these, an ex-vivo fetal membrane perfusion model has been the most powerful, allowing multi-aspect analysis of membrane gene expression, inflammatory mediator production, histological and structural integrity in response to maternal challenge with endotoxin (lipopolysaccharide). Using a variety of techniques including oligonucleotide and protein arrays we have determined that a robust and rapid inflammatory response is manifested in both the maternal and fetal compartments after maternal (decidual) exposure to lipopolysaccharide; this is accompanied by a marked increase in apoptosis in the chorionic membrane, but no detectable changes in membrane integrity.

We have evaluated in this model several anti-inflammatory drugs that inhibit the NF-κB pathway, as potential pharmacologics for treating inflammation-induced preterm labour. Surprisingly, most were completely ineffective in suppressing lipopolysaccharide-induced cytokine production. Sulfasalazine, however, administered to the maternal face, effectively and rapidly abrogated the lipopolysaccharide response in both maternal and fetal compartments, with modest membrane transfer of drug. However, chorionic apoptosis was doubled in sulfasalazine-treated membranes, raising concerns over possible toxicity. Studies using these models are continuing to further evaluate the potential of novel and existing pharmacotherapies for the prevention of inflammation-associated preterm birth.
THE EFFECT OF MATERNAL ASTHMA DURING PREGNANCY ON PLACENTAL FUNCTION, FETAL GROWTH AND CHILDHOOD DEVELOPMENT

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Fetal growth and neonatal birth weight are significant contributing factors to the development of adult disease states in later life. In human pregnancy, we have identified sexually dimorphic differences in fetal growth with the female fetus reducing growth in response to maternal asthma and the male fetus continuing to grow at a normal rate but being at an increased risk of _in utero_ death. The physiological mechanisms that confer sex-specific differences in the fetal response to maternal asthma are unknown. However our research has identified differences in mechanisms associated with fetal glucocorticoid regulation which are also associated with changes in childhood growth patterns. Asthmatic and control pregnant women were recruited at their first antenatal visit and followed through to delivery. Subjects were assessed for severity of asthma and their use of medication, including glucocorticoid therapy was recorded. In addition to routine antenatal care, fetal growth was determined using Doppler ultrasound. Following delivery placentas and cord blood were collected. The children of the women followed during the study were examined by a paediatrician at 6 months of age and every 12 months after that initial visit. Our data shows that in response to maternal asthma, the female fetus has an increase in cortisol which down regulates placental GR expression, immune and hypothalamic-pituitary-adrenal function and is associated with decreased growth. The male fetus responds to increased cortisol with an increase in GR expression and no change in HPA or immune function or growth. These data indicate that the male and female fetus have different strategies to control growth and in their response to a maternal stress, such as asthma.

OOCYTE CONTROL OF GRANULOSA CELL DEVELOPMENT AND FUNCTION

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Oocytes orchestrate the rate of follicular development and the patterns of gene expression by granulosa cells (GCs). There are two populations of GCs in large antral follicles: mural granulosa cells (MGCs) that line the ovarian follicle wall, and cumulus cells (CCs) closely associated with the oocyte. Subtraction hybridization was used to find transcripts more highly expressed in CCs than MGCs. Among the genes expressed more highly in CCs was one encoding an amino acid transporter (Slc38a3). Slc38a3 mRNA was not detected in oocytes. Expression of Slc38a3 mRNA was reduced in the CCs after removal of the oocyte and restored by co-culturing CCs with fully-grown oocytes. Alanine is one of the amino acids transported by SLC38A3. This amino acid is poorly transported across the oocyte plasma membrane, but gains access to the oocyte from the cumulus cells via gap junctional communication. Alanine transport into cumulus cells was promoted by paracrine factors secreted by fully-grown oocytes (FGOs), but not by growing oocytes (GOs) from preantral follicles. Thus FGOs promote the transport of alanine into CCs, and this amino acid is then passed on to the oocyte via gap junctions. Transcripts encoding enzymes in the glycolytic pathway were also more highly expressed in CCs than MGCs. FGOs, but not GOs, promote elevated expression of some of these transcripts. Likewise, FGOs promote both glycolysis and oxidative phosphorylation by isolated CCs and MGCs. Oocytes do not effectively utilize glucose as an energy source, and oocytes require the presence of CCs to resume meiosis when glucose is the only energy source present. In contrast, oocytes can resume meiosis in the absence of CCs when pyruvate is the sole energy source. Thus oocytes apparently promote glycolysis by their companion granulosa cells to provide energy for their own development. In addition, this may be one way that oocytes coordinate their development with that of follicular somatic components. (Supported by Grants HD23839 and HD44416 from the NICHD)
SUBCLINICAL THYROID DISEASE

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Subclinical thyroid disease is common: in the 1981 Busselton Study, subclinical hypothyroidism was present in 5.6% of participants and subclinical hyperthyroidism in 1.8%. The management of these disorders is remarkably contentious, particularly in the United States, where three prominent professional societies (the American Thyroid Association, the American Association of Clinical Endocrinologists and the Endocrine Society) recently sponsored the establishment of a consensus development panel on subclinical thyroid disease, then disowned the management guidelines which emerged. (1)

This interactive session will explore what is and is not known about the natural history and treatment of subclinical thyroid disease, with the aim of putting management of these disorders (as far as possible) on a rational footing.

(1) Surks MI, Ortiz E, Daniels GH et al. Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. JAMA. 2004;291:228-38

COREGULATORS AND HORMONE ACTION IN BREAST CANCER

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Discovery of over 100 nuclear receptor (NR) coregulators (coactivators and corepressors) in the past decade has provided considerable insight into the complexity of NR signaling as well as an array of potential new therapeutic targets. Interestingly, some coregulators have multiple functions eg. SRC-3/AIB1 is a coactivator that is also an independent breast cancer (BCa) biomarker and when co-overexpressed with HER2 confers a particularly poor prognosis. Of the many NR coregulators, one stands alone, SRA (Steroid Receptor RNA Activator)1, as it is an RNA coactivator. SRA plays an important role in transactivation of the estrogen receptor (ER), and is aberrantly expressed in many human BCas, suggesting a potential role in pathogenesis. Several key stem-loops within SRA, contribute to its transactivation capacity2. In an attempt to elucidate the function of SRA and its mechanism of action in BCa, we have isolated several novel SRA-binding proteins from a BCa library that have potent coregulation activity. Three of these proteins are members of a family of double-stranded RNA-binding proteins, PACT, PKR and TAR-binding protein (TAR-BP). Previously recognized for their roles in antiviral responses and some aspects of NFkB signaling, PACT and TAR-BP are NR coactivators, whilst PKR is a powerful corepressor. These effects are dependent upon a functioning RNA-binding domain, and for PKR, an active kinase domain. As expected, inhibition of PACT expression with siRNA results in ↓ endogenous estrogen regulated gene expression. Interestingly, all of these proteins are expressed in BCa and co-overexpression of PACT with SRA abolishes the repressive effect of Tamoxifen, suggesting a potential role in hormone resistance. We identified a further protein, which we termed SLIRP (SRA stem-Loop Interacting RNA-binding Protein), that targets a different stem-loop of SRA. SLIRP is expressed widely in normal tissue and also in BCa, and acts as a NR corepressor, requiring an intact RNA-binding domain. All of these SRA-binding proteins are recruited to endogenous estrogen-regulated promoters in BCa cells. Remarkably, however, SLIRP is predominantly located in the mitochondria, suggesting it may play a role in NR signaling in both nuclear and mitochondrial compartments. Our studies suggest a model in which SRA is a target for multiple RNA-binding proteins, each of which is expressed in BCa and has the capacity to significantly modify ER signaling. Furthermore, some of these proteins may serve as biomarkers in BCa and have a role in regulating NR signaling within the mitochondria.

CHAPERONES AND REGULATORS: MEDIATORS OF ANDROGEN RECEPTOR FUNCTION IN PROSTATE CANCER

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Studies of androgen signalling in prostate cancer have revealed that the cellular mediator of androgen action, the androgen receptor (AR), remains the major determinant of disease progression despite aggressive androgen ablation therapies. However, the mechanisms leading to castrate-resistant signalling by the AR have only been partially defined. Recent studies indicate that an altered cellular profile of molecular chaperones and known AR transcriptional coregulators (cofactors) may play a major role in disease progression and androgen-resistance. This is clearly evident in our new mouse model, where prostate-specific expression of an AR mutant with altered capacity to interact with its normal complement of cofactors results in spontaneously prostate cancer. However, more than 120 molecular chaperones and transcriptional regulators have been reported to affect the function of the AR, and little is currently known about the expression of the majority in prostate cells or their relationship with cancer progression. We therefore assessed the expression of 107 putative AR coregulators in primary and metastatic prostate cancer samples using microarray technology. The expression of 26/107 (24%) cofactors was significantly different (p<0.05) in primary and metastatic disease, with 14 (0 corepressors, 9 coactivators, 5 cochaperones) increased and 12 (6 corepressors, 6 coactivators, 0 chaperones) decreased in metastatic samples. Significantly, LNCaP cells derived from a metastatic human prostate tumour, have retained 24/26 (92%) of these changes (R²=0.93). We present immunohistochemical analysis of these cofactors during the progression of prostate cancer in the transgenic adenocarcinoma of the mouse prostate (TRAMP) and LNCaP xenograft models of the disease. In addition, we detail how a novel molecular chaperone, SGT, can modulate AR sensitivity, nuclear localisation and promiscuity for ligand activation, and demonstrate the impact of the Hsp90 inhibitor, 17-allylamino-geldanamycin, on AR function. These studies represent a significant step in defining how AR chaperones and regulators contribute to prostate cancer progression.

NOVEL THERAPEUTIC TARGETS IN PROSTATE CANCER

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Eicosanoid products of both the cyclooxygenase (COX) and lipoxygenase (LOX) pathways are important mediators of the proliferation and progression of prostate cancer cells. We found that the secreted phospholipase A2 (sPLA2) enzymes, which regulate the provision of arachidonic acid to both COX- and LOX-derived eicosanoids, also regulate the growth of prostate cancer. Since Annexin A1 and A2, known inhibitors of cytosolic phospholipase A2 (cPLA2) activity, are absent in prostate cancer tissues, we aim in this study to determine the role of cPLA2 and its relationship with sPLA2 in prostate cancer. Anti-phospho-cPLA2-α antibody was used for immunohistochemistry of human tissues. MTS and flow cytometry were used to determine cell growth, proliferation and apoptosis of prostate cancer cells in the presence or absence of cPLA2-α inhibitor (pyrrolidine) for 72 hours in culture media (RPMI with 10% FCS). Angiogenesis was measured based on tube formation of HUVEC cells. Phosphorylated cPLA2-α (the active form) is significantly increased in human prostate cancer tissue. Inhibition of cPLA2-α causes a decrease in prostate cancer cell growth (IC50 ~ 3 uM) and proliferation in prostate cancer cells, but has no effect on cell apoptosis. Angiogenesis is decreased in the presence of cPLA2-α inhibitor. The growth-promoting effect of sPLA2-IIA on prostate cancer cells is also abolished in the presence of cPLA2-α inhibitor. The dysregulated PLA2 enzyme function by both induction of sPLA2-IIA and activation of cPLA2-α may contribute to the pathogenesis of prostate cancer. The increased sPLA2-IIA has an oncogenic action through cPLA2-α. We propose that, in addition to COX and LOX enzymes, PLA2 enzymes represent important targets for the treatment of prostate cancer.
OVARIAN HORMONES AND BREAST CANCER
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Oestrogen and progesterone regulate the growth, metabolic activity and differentiation of normal breast epithelium and are strongly implicated in development and progression of breast cancer. Recent findings implicate progestins in increasing risk of breast cancer, and highlight both the lack of knowledge of progesterone signalling in the normal breast and the importance of endocrine research in breast cancer. The complex physiological roles of progesterone are reflected in the number of cellular pathways it regulates via its nuclear receptor (PR). PR is expressed as two proteins, PRA and PRB, which have different in vitro activity. PRA and PRB are co-expressed, at similar levels, in the normal breast, but progression to malignancy is accompanied by unequal expression of PRA and PRB, and a significant proportion of carcinomas express a predominance of one isoform, usually PRA. This is associated with poor clinical features: PRA predominance is more common in tumours of higher grade. In clinical cohorts in which response to treatment is known, we have confirmed the importance of aberrant PR in hormone-dependent cancers. In laboratory studies we have identified molecular targets that become activated only when PR expression is aberrant. These include specific functional groups of genes, notably metabolism and cell shape/adhesion, which are not progestin targets in cells with co-ordinated PRA:PRB levels. Taken together, these studies suggest that maintenance of equivalent cellular levels of PR isoforms is an important feature of normal epithelial responsiveness to progesterone, and, more broadly, that changed ovarian hormone signalling may contribute to the early stages of breast cancer development.

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ADDISON'S DISEASE
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The finding of low plasma cortisols in patients with non-specific symptoms may lead to an incorrect diagnosis of Addison's disease, particularly in patients taking oral, inhaled or intra-articular glucocorticoid. A healthy scepticism regarding this diagnosis in patients already commenced on replacement therapy may therefore be warranted. Measurement of early morning plasma ACTH and a short synthetic ACTH stimulation test may be illuminating. Aetiology is usually autoimmune/idiopathic but adrenal haemorrhage and adrenomyeloneuropathy (in males) are more common in our community than tuberculosis.

The pharmacokinetics of glucocorticoid therapy are such that measurement of plasma cortisol and ACTH is not helpful in judging the adequacy of replacement. Plasma renin is sometimes useful in judging the adequacy of fludrocortisone replacement although measurement of creatinine and electrolytes often suffices. Clinical judgment is of primary importance here -- thin-skin and ecchymoses suggest glucocorticoid excess, hyper-pigmentation suggests inadequate glucocorticoid (although it may be difficult to completely eradicate hyperpigmentation), hypertension suggests fludrocortisone (or glucocorticoid) excess and postural hypotension suggests inadequate replacement of either. Subtle over-replacement with glucocorticoid can lead to insidious bone loss so bone densitometry should be undertaken periodically. Cortisone acetate (and its metabolite hydrocortisone) are physiological glucocorticoids and are generally preferred to prednisolone and dexamethasone -- the last is a very potent pure glucocorticoid with a long half-life and it is difficult to titrate the dose adequately. Controversy persists regarding the usefulness or otherwise of DHEA replacement (worth considering in women with Addison's disease). Hepatic enzyme inducers can lead to larger replacement requirements.

Secondary hypoadrenalism is unaccompanied by mineralocorticoid deficiency so fludrocortisone replacement is not required. The hyponatraemia of this condition is due to SIADH and the treatment is fluid restriction and glucocorticoid whereas the hyponatraemia of primary hypoadrenalism (Addison's disease) is due to salt loss because of mineralocorticoid deficiency and requires intravenous saline and glucocorticoid.
ADRENAL INSUFFICIENCY: ABSOLUTE, TRANSIENT AND RELATIVE FORMS
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Absolute: Addison's observations (1855) of adrenal insufficiency followed autopsy findings of adrenal damage in the context of known autoimmune or tuberculous disease. It was not until the 1950s, after identification and synthesis of corticosterone that steroid administration routinely saved the lives of Addisonian patients. Past glucocorticoid dose recommendations were excessive leading to many cases of excessive glucocorticoid effect. Modern doses limit Cushingoid effects but many patients report suboptimal well-being suggesting alternatives may still be required. Transient: Widespread use of anti-inflammatory dose glucocorticoids is a leading cause of secondary adrenal insufficiency. Experiments after cure of pituitary Cushing's suggest that the site of defect in HPA function after steroid withdrawal is the hypothalamus/brain rather than the pituitary or adrenal cortex. Theoretically, agents which stimulate the CRH neuron or noradrenergic drive to the CRH neuron may accelerate recovery of HPA suppression but there are no clinical studies. In the absence of clinical studies comparing glucocorticoid withdrawal schedules, a reasonable strategy would involve hydrocortisone 12mg/m² body surface area, and intermittent (1-2 monthly) 8-9AM cortisol levels – endogenous cortisol levels in the range 200-400 nmol/L could be met with glucocorticoid withdrawal at 20-50% decrements. Clinical monitoring of postural BP and fatigue helps exclude cortisol deficiency. Relative/idiopathic: Mild reductions in cortisol concentrations have been described in association with a range of common idiopathic disorders such as fibromyalgia and chronic fatigue syndrome (CFS). The neuroendocrine hypothesis for these disorders is of a deficiency in hypothalamic CRH release. Cortisol levels are not diagnostic in individual cases, most studies are small and reporting bias is likely. Studies of glucocorticoid supplementation have produced inconsistent effects and can not be widely recommended. Findings of novel mutations producing low CBG or reduced cortisol binding in isolated families led us to screen patients with CFS for CBG mutations – these mutations were not found and a weak possible effect of a CBG polymorphism was noted. Relative/septic shock: Recently, low cortisol responses to synacthen have been detected in a subset (30-60%) of patients with septic shock – such a response appears to predict response to hydrocortisone supplementation in terms of mortality and time-to-withdrawal of vasopressor support. There are theoretical reasons to expect that free cortisol, given sepsis induced falls in CBG and albumin, will be a better guide to circulating cortisol levels. Our data show that free cortisol correlates more closely to sepsis severity than total cortisol. Outcome based studies, perhaps based on a tissue marker of cortisol action in sepsis are needed – such a marker may guide glucocorticoid dose and produce optimal results from this inexpensive treatment. Conclusion: Although adrenal hormone replacement is highly successful, careful clinical judgement is needed to optimise results. Steroid withdrawal also requires careful judgement guided by intermittent biochemical testing. New horizons have included the possibility of an adrenal component to common undiagnosed syndromes, although the adrenal role may represent an epiphenomenon of central processes. The new outcome-driven definition of adrenal relative adrenal insufficiency in septic shock requires further validation with better cortisol measures and perhaps a tissue marker of cortisol effect.

WHEN THE ADRENAL GOES AWRY: HYPERALDOSTERONISM
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Adrenal disease as a cause of hypertension is well recognised; the importance of corticosteroids in heart disease however has only recently attracted attention. Aldosterone is the principal antinatriuretic steroid produced by the adrenal, tumours of the adrenal glomerulosa, Conn's syndrome, may result in hyperaldosteronism with surgically-remediable hypertension. Mineralocorticoid-induced hypertension is characterised by a suppressed plasma renin, hypokalaemia and sodium-dependence. Recent insights into the mechanisms underlying mineralocorticoid hypertension have come from studies of monogenetic syndromes of hypertension. Thus, glucocorticoid-remediable hypertension, the syndrome of apparent mineralocorticoid excess, pregnancy-associated hypertension and Liddle's syndrome (pseudaldosteronism) each result from mutations at specific points in the pathway from aldosterone to sodium retention [1]. The incidence of milder non-classical forms of Conn's syndrome has been re-evaluated as a result of the work of Richard Gordon and Michael Stowasser [2]. Although the incidence of surgically remediable disease remains controversial, the greater attention to this possibility through diagnostic screens such as the aldosterone:renin ratio provides valuable therapeutic information. Therapy of hyperaldosteronism, beyond removing the cause, relies on either antagonists at the mineralocorticoid receptor (MR) or the epithelial sodium channel. Recent large clinical trials in cardiac failure using spironolactone or the related MR-selective anti-mineralocorticoid, eplerenone, indicate a role for aldosterone in cardiac disease. The molecular and cellular basis of the response is the subject of intense study with a strong focus on the MR [3]. This therapy is however limited by hypokalaemia, suggesting the need for tissue-selective MR antagonists.
ADRENAL SURGERY – THE CONSIDERING ISSUES.
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The wide spread use of better imaging procedures and laparoscopic surgery have resulted in an increasing number of adrenalectomies. All adrenal incidentalomas should have Pheochromocytoma excluded and hormonal activities selectively evaluated. All hormonally active tumours should be removed if possible. For hormonally inactive tumours, lesions less than 4cm are generally monitored whereas those bigger than 4cm resection should be considered depending on age, interval growth and clinical circumstance. There are many adrenal imaging procedures available; yet many limitations remain. Hence their uses should be selective to be cost effective. Laparoscopic adrenalectomy is the procedure of choice in most cases except for primary adrenal carcinoma, which not only requires an open approach but also needs wide margins of clearance for better result. Laparoscopic “cortical sparing” or partial adrenalectomy is technically possible; its liberal use needs further careful evaluation. An experienced surgeon is important in providing good surgical results; however multidisciplinary care is essential in the overall delivery of an optimal outcome.

OOCYTE SIGNALLING MOLECULES AND THEIR EFFECTS ON REPRODUCTION IN RUMINANTS
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Sheep (Ovis aries) are a highly diverse species with more than 900 different breeds that vary significantly in their physiological characteristics including ovulation rate and fecundity. From examination of inherited patterns of ovulation rate in sheep, several breeds have been identified with point mutations in two growth factor genes that are expressed in oocytes. Currently, five different point mutations have been identified in the BMP15 (GDF9b) gene and one in GDF9. Animals heterozygous for the GDF9 and/or the BMP15 mutations have higher ovulation rates (i.e. +0.6 to +5.0) than their wild-type contemporaries. In contrast, those homozygous for any of the aforementioned BMP15 or GDF9 mutations are sterile due to abnormal follicular development from the primary stage of growth. In bovine and ovine ovaries, GDF9 is expressed exclusively in oocytes throughout follicular growth from the primordial stage of development, whereas in sheep BMP15 is expressed exclusively in oocytes from the primary stage: no data for BMP15 are available for the cow. In vitro, ovine GDF9 (oGDF9) has no effect on 3H-thymidine incorporation by either bovine or ovine granulosa cells, whereas oBMP15 has modest (1.2 to 1.6-fold; p<0.05) stimulatory effects. GDF9 or BMP15 alone inhibited progesterone production by bovine granulosa cells, whereas with ovine cells only GDF9 was inhibitory. The effects of GDF9 and BMP15 together were often cooperative and not always the same as those observed for each factor alone. Active immunisation of ewes with BMP15 and/or GDF9 peptides affected ovarian follicular development and ovulation rate. Depending on the GDF9 and/or BMP15 vaccine formulation, ovulation rate was either increased or suppressed. For example, immunisation of ewes with a BMP15 peptide in a water based adjuvant has led to a 25% increase in lambs born per ewe lambing. Collectively the evidence suggests that oocyte signalling molecules have profound effects on reproduction in mammals including rodents, humans and ruminants. Moreover, that in vivo manipulation of these oocyte signalling molecules provides a new approach to managing the fertility of ruminants.
CURRENT PROGRESS INTO TESTIS CELL TRANSFER BETWEEN CATTLE BREEDS.

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Male germline cell transfer has produced offspring in mice (Brinster and Zimmermann 1994). Recently the first livestock animal, a goat, was produced (Honaramooz et al. 2003), while early results in cattle are promising (Oatley et al. 2002; Izadyar et al. 2003). There is an opportunity to develop this technology for the beef industry by transferring male germ line stem cells between breeds to improve the genetics of extensive Australian beef herds. This project is a part of the CSIRO National Research Flagship program that combines expertise and facilities in divisions with complementary expertise at Monash University and the University of Sydney. The environmental constraints of Northern Australia dictate that Brahman type animals show far better survival than Bos taurus cattle, although the carcass value of Brahman is lower than Bos Taurus animals. Artificial insemination is impractical in Northern Australia and thus we aim to develop testis cell transfer technique in cattle to permit Brahman bulls to deliver semen from elite Bos taurus or composite bulls, thereby significantly increasing the growth rate, yield and meat quality of the northern beef herd. Experiments using cattle were performed to determine the applicability of techniques used in the mouse. Initial proof of concept has been achieved that germ cell transfer can result in the donor cells successfully colonizing areas of recipient testis. The viability of isolated testis cells following short term (24 hour) culture has been demonstrated through transfer into recipient calves. We have completed >50 male germ cell transfers into recipient calves, using ultrasonographic guided injection into the rete testis. Success of this procedure has been demonstrated by persistence of PKH26 dyed donor cells in the seminiferous tubules of a majority of recipients > 2 months after transfer. These recipient male calves have not been depleted of their endogenous spermatogonial populations and we thus expect the efficiency of the procedure to increase as depletion procedures (ongoing) are established. Concurrent with these developments has been research into large scale culture of male germ line stem cells to provide large numbers of stem cells for transplant. Culture of testis cell suspensions has demonstrated survival of enriched testis cells under varying media and culture conditions. Initial passaging of testis cell colonies has revealed mixed cell populations (immunohistochemistry positive for spermatogonia and somatic cells). Further studies will aim to demonstrate that these cultured donor cells are able to undergo spermatogenesis in the recipient animals.

ISOLATION OF STEM CELLS FROM EMBRYOS AND ADULT BOVINE TISSUES

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The isolation of stem cells has become an area of increasing interest due to their potential uses in animal reproduction, somatic cell nuclear transfer and cell therapies. The most attractive options are the isolation of stem cells from individual embryos or adult somatic tissues. In addition, for cell therapy, the use of autologous stem cells is considered to have an advantage over heterologous cell based therapies in that immune rejection issues would be circumvented. Here we report on our attempts to isolate stem cells from both these sources in a bovine model.

1) Bovine ES-like (bES) cells were successfully isolated from embryos and maintained in vitro for up to 6 passages. These cells retained the morphology characteristic of bES cells; small cytoplasmic/nuclear ratio, nuclei with multiple nucleoli, and multiple lipid inclusions in cytoplasm. bES cell colonies grew as monolayers, as islands of ES cells surrounded by trophoderm (TE) cells. Immuno-histochemical detection of SSEA-1 and SSEA-4 demonstrated expression of these markers in bES cells but not in TE cells. Further, the expression of the pluripotent markers Oct-4, Rex-1 and SSEA-1 by RT-PCR was also detected in bES cells but not in TE cells. On spontaneous differentiation, these cells were able to form a variety of cell types including beating muscle with the cells displaying a propensity to differentiate in a manner reminiscent of human ES cells. 2) We also report the isolation of putative stem cells from adult bovine skin biopsies, which express the stem cell markers Oct-4 and SSEA-1 analysed by RT-PCR and are capable of forming 3 dimensional colonies. These cells are obtained from a skin biopsy, a relatively non-invasive technique which makes them useful as donors for therapeutic applications. In summary, we have identified populations of stem cells from embryonic and adult bovine tissues, which are readily isolated. Further characterization of the differentiation potential of these cells is needed to identify the suitability of this population for use in autologous stem cell therapies.
BIOTECHNOLOGY AND REPRODUCTION IN MAINSTREAM ANIMAL INDUSTRY – A PERSPECTIVE

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This paper considers strategies to improve the reproductive performance of small ruminants in ways that lead to production systems that are “clean, green and ethical”. This view arises from feedback from consumers, particularly in attractive export markets, and from a need to refocus on the needs of Australian producers, most of whom operate large, extensive enterprises. These people cannot use ‘high-tech’ systems but need low-cost, low-labour solutions to their problems. First, to control of the timing of reproductive events, they can use the socio-sexual inputs of the “male effect” to induce synchronised ovulation in females that would otherwise be anovulatory (seasonal, lactational, prepubertal). Second, they can use nutritional stimuli for “focus feeding”, in which short periods of nutritional supplements are precisely timed and specifically designed for individual events in the reproductive process: gamete production, embryo survival, ‘fetal programming’, and colostrum production. Third, they can use simple behavioural observations to genetically select for temperament – this will maximize offspring survival, product quality and animal welfare. All of these approaches involve non-pharmacological manipulation of the endogenous control systems of the animals and complement the detailed information from ultrasound that is now becoming available (1). The use of such clean, green and ethical tools in the management of our animals can be cost-effective, increase productivity and, at the same time, greatly improve the image of meat and milk industries in society and the marketplace. This does not mean, however, that they will not benefit from the opportunities that evolve from breakthroughs in reproductive technology or gene research. On the contrary, if this “high-tech” research is done within the context of the needs of a “clean, green and ethical” industry, first class science can have very direct and immediate benefits to our livestock industries.


NUCLEAR RECEPTORS AND CYCLINS IN HORMONE SIGNALING

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The estrogen PPARγ and androgen receptor are members of the nuclear receptor (NR) superfamily that consists of conserved modular transcriptional regulators. These receptors play essential roles in human development, physiology, and cancer. The nuclear receptor (NR) superfamily consist of conserved modular transcriptional regulators. The overall functional domain structure common to “classical” receptor subclass comprise an N-terminal region (Activation Function 1 (AF1), a well conserved central DNA binding domain with two zinc finger structures and a C-terminal region which includes the hinge and ligand-binding domain (LBD).

Distinct types of functional interactions between nuclear receptors and cyclins coordinate metabolism, cellular differentiation and proliferation. The expression and abundance of cyclins are regulated by NR, and NR, in turn, regulate expression and activity of cyclins. Studies of cyclin D1 knockout mice, and tissue-specific inducible transgenic mice, suggest an important role for cyclin D1 in NR function and metabolism in vivo. Cyclin-dependent kinases phosphorylate NRs, and kinase-independent functions of cyclins regulate the activity of several nuclear receptors (i.e. androgen receptor (AR), estrogen receptor α (ER) and peroxisome proliferator activator receptor γ (PPARγ). The kinase-independent function of cyclin D1 are at least in part mediated by the recruitment of histone acetylases (HATs) and histone deacetylases.

HATs modify histones, coactivators, nuclear transport proteins, structural proteins, cell cycle components and transcription factors including nuclear receptors. ERα, the AR and several other NRs, are acetylated at a motif that is conserved between species and other NR. Acetylation of the AR and ERα occurs in cultured cells and point mutations at the acetylation site have been identified in breast and prostate cancer. The NR acetylation site governs ligand sensitivity, hormone antagonist responses and cellular growth, thus representing an ideal target for drug therapies.
PROLACTIN SIGNALING THROUGH THE SHORT FORM OF ITS COGNATE RECEPTOR CAUSES SEVERE OVARIAN DEFECT.

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Extensive investigations from our laboratory have clarified the action and interaction of estradiol (E) and prolactin (PRL) on corpus luteum (CL) function. Our research has led us to discover and isolate a CL specific gene that encodes a protein we named PRAP, that associates with the intracellular domain of the short form (PRLRS) but not the long form (PRLRL) and whose expression is tightly regulated by E. Our laboratory and others have established that this protein, expressed in CL of every species investigated, is a novel 17 beta hydroxysteroid dehydrogenase (17bHSD-7) whose function is to catalyze the transformation of estrone to E. Our results with cells expressing only PRLRS revealed that PRL acting through PRLRS leads to phosphorylation of PRAP/17bHSD-7 (PRAP/17b) by JAK2 establishing for the first time that a steroidogenic enzyme can be phosphorylated through its association with a membrane bound protein. The association of PRAP/17b with the PRLRS and its phosphorylation leads to its stabilization. To further investigate the role of PRL signaling through PRLRS, we used PRLR(-/-) mice expressing the PRLRS as a transgene. The results obtained were totally unexpected and of great interest. The follicles of the ovaries, expressing only PRLRS, underwent premature development followed by severe granulosa and oocyte death leaving holes surrounding collapsed zona pellucida and premature ovarian failure. The observations that: 1) the expression of PRLRS in the ovaries of PRL null mice leads to inhibition in Foxo3a and of GALT two proteins whose deletion/mutation causes similar premature ovarian failure and, 2) that GALT promoter activity is stimulated by Foxo3a transcription factor led us to hypothesize that PRL acting through PRLRS prevents the expression of Foxo3a which normally stimulates GALT transcriptional activity. Absence of Foxo3a then leads to inhibition of GALT and increases in galactose and its metabolites, causing galactose toxicity and granulosa as well as oocyte cell death.

THE CUMULUS MATRIX IN OVULATION; INERT PACKAGING OR ACTIVE DELIVERY VEHICLE FOR THE OOCYTE?

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Preovulatory follicles respond to the LH-surge with a cascade of molecular events. The ovulatory signal initially impinges on the mural granulosa layers triggering rapid tissue morphogenesis and ultimately terminal differentiation of these cells. Mural granulosa cells transiently produce a suite of transcriptional regulators, EGF-like ligands as well the extracellular matrix (ECM) proteoglycan, versican and the protease ADAMTS-1. These act in concert with permissive oocyte signals to induce and organise a complex hyaluronan (HA) rich ECM surrounding the cumulus cells and oocyte. This expanded cumulus matrix is analogous in composition to an extensive form of pericellular matrices actively associated with cell migration. During ovulation the cumulus matrix becomes anti-adhesive to the intra-follicular environment but is strongly pro-adhesive for the oviductal fimbria. When, the follicle apex is perforated the COC is released binds to the fimbria and transports into the oviduct where fertilisation occurs. Success of ovulation and fertilisation is sensitive to the appropriate production and assembly of cumulus matrix components that are in turn dependent on an appropriate balance of oocyte and granulosa derived signals. Production of these cumulus matrix components is thus a potential checkpoint that assures ovulation of competent oocytes. The HA matrix is cross-linked by organiser molecules and also is enriched in proteases ADAMTS-1, 4, 5. Although these have potentially redundant functionality, ADAMTS-1 null female mice are profoundly sub-fertile and have reduced ovulation rate. Specific components of the cumulus matrix are disorganised in ADAMTS-1 null mice and cleavage of versican in these cumulus complexes is reduced. Thus ADAMTS-1 and versican have unique roles in normal cumulus matrix expansion that is important for ovulation. Altered interaction of the cumulus complex with neighbouring tissues alters transport through the oviduct, while abnormal persistence of COC matrix structure after ovulation is also likely to impair sperm interaction and penetration. Evidence thus indicates that the expanded cumulus matrix plays several active roles in oocyte release, transport and sperm interaction.
IN VITRO GROWTH AND MATURATION: HOW DOES THIS TECHNOLOGY FIT FOR CLINICAL APPLICATION?

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Superovulation protocols used in IVF result in multiple eggs that can be fertilized and grown in the laboratory to allow for selection of the best embryo for return to the mother, thereby increasing the chances for a successful pregnancy. However, there are many side effects of these superovulation drug protocols, such as deep vein thrombosis and hyperstimulation. The latter is of particular concern for women with polycystic ovary syndrome. Furthermore, the use of gonadotrophins has been reported to compromise both oocyte quality and the uterine environment and may contribute to the low success rates of IVF. Therefore the ability to collect large numbers of oocytes from women and mature them in vitro is an attractive alternative. However, although there are reports in the literature on extended maturation/culture periods of human oocytes the pregnancy rates are significantly lower than that observed after in vivo maturation. The ability to offer such technology is currently limited by the lack of understanding of how the conditions for in vitro maturation affect the quality of the oocyte and the resulting embryo. Our research is concentrated on establishing the role of metabolic balance in the oocyte for the maintenance of subsequent viability. We have determined that disruptions to the balance between mitochondrial and cytoplasmic metabolism in animal oocytes have significant adverse consequences for the resultant embryo. Changing conditions for in vitro maturation were also found to alter the establishment of the metabolic settings of the oocyte. The ability to determine the role of such parameters in maturing human oocytes will be important for the prospect of adoption of this technology for routine clinical practice.

CYTOKINE NETWORKS AND REGULATION OF SPERMATOGENESIS – WHAT SHOULD WE REALLY BELIEVE?

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Spermatogenesis is a complex yet highly organised process involving intimate interactions between the supporting Sertoli cells and germ cells at various stages of development. The repeating pattern of the cycle of the seminiferous epithelium is due to the fact that spermatogonia enter spermatogenesis at regularly spaced intervals and proceed through the process at a species-specific rate. How this degree of coordination is maintained remains poorly understood, but recent evidence has focussed attention on the role of growth factors produced by the Sertoli cells and germ cells. Several of these growth factors, such as interleukin-1α (IL-1α), IL-6, tumour necrosis factor (TNFα) and activin A, are also inflammatory cytokines. This has led some researchers to question the physiological significance of these data with respect to normal testicular function. For example, in spite of the fact that IL-1α is produced by the Sertoli cell and regulates spermatogonial proliferation and development \textit{in vitro}, mice lacking the IL-1R and hence unresponsive to IL-1α, possess relatively normal fertility. So what role, if any, do these cytokines play in the normal testis, or are they only important during inflammation? It is quite evident that these cytokines have stimulatory and/or inhibitory effects on spermatogonial and spermatocyte development. These cytokines also interact at multiple levels within each others signalling pathways and have considerable redundancy of action. Moreover, expression of these cytokines varies across the cycle of the seminiferous epithelium, with major changes in production coinciding with two key events within the cycle: the release of sperm from the epithelium, and the major peaks of DNA synthesis by spermatogonia and preleptotene spermatocytes. It is therefore possible to hypothesise that release of sperm and resorption of the residual cytoplasm triggers a self-regulating inflammatory cascade within the epithelium which initiates and then modulates the next round of spermatogenic development, ensuring that spermatogonia enter the process at the appropriately spaced intervals.
THE CIRCADIAN SYSTEM COMPOSED OF THE CENTRAL AND PERIPHERAL CLOCKS: MONITORING CLOCKWORKS BY BIOLUMINESCENT REPORTERS

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The master circadian clock of mammals resides in the suprachiasmatic nucleus of the hypothalamus (SCN). Many SCN neurons exhibit circadian firing rhythms with individually different period in dispersed cell culture on a multi-electrode array dish while a synchronized firing rhythm only with an intact neuronal assemblage of the SCN slice, indicating that cellular clocks synchronize and express a single periodicity from the SCN. The intracellular molecular machinery is considered to base on interlocked autoregulatory feedback loops associated with expression of several clock genes, such as Per, Clock, Bmal1 and Cry, and their protein products. On the other hand, most peripheral organs also have their own clocks which generate circadian rhythms in clock and clock-controlled gene expressions. We have constructed two transgenic mouse lines expressing firefly luciferase under the control of clock gene Bmal1 promoter and Per1 promoter. And we examined oscillatory mechanisms of the central and peripheral clocks by continuously monitoring bioluminescence of cultured SCN and liver. The oscillation mechanisms are also examined in Rat-1 fibroblasts using the luciferase reporter construct. In the peripheral clock model of Rat-1 fibroblasts we showed a type 0 phase response not only to a brief exposure of dexamethasone but also to vehicle treatment, suggesting that desynchronized cellular oscillators are resynchronized to each other by a single perturbation. Synchronization of a number of peripheral clocks by this method seems to be advantageous for the orchestration of circadian rhythms in physiology and behavior. However, a single peripheral clock responds to multiple mediators with different sensitivities, which may cause internal desynchronization among organs and cells.

SEROTONERGIC REGULATION OF CIRCADIAN RHYTHMS

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The hypothalamic suprachiasmatic (SCN) is a circadian oscillator that derives functional utility from its ability to be synchronized or entrained to the 24 h environmental day/night cycle. Entrainment provides for stable and appropriate phasing of rhythmic behavior and neuroendocrine rhythms with the environment, thereby, in effect, enabling recognition of local time. The SCN circadian oscillator is thus said to function as a biological clock. Photic signals that re-set the circadian clock are transmitted from the retina to the SCN via the retinohypothalamic tract (RHT). The retinal ganglion cells that contribute to the RHT use glutamate as a neurotransmitter and the majority of these neurons are intrinsically photosensitive and express the opsin, melanopsin. The SCN also receives serotonergic afferents from the midbrain that modulate photic input.

Several of the 14 currently identified serotonin (5-HT) receptor subtypes are found in the SCN and the 5-HT1B receptor subtype is located on RHT terminals. Activation of these presynaptic 5-HT1B receptors in vivo attenuates light-induced: 1) behavioral phase shifts; 2) suppression of pineal melatonin; and 3) SCN gene expression. 5-HT1B receptor agonists inhibit optic nerve evoked glutamatergic excitatory postsynaptic currents (EPSCs) in the SCN in vitro and these responses are eliminated in 5-HT1B receptor knockout (5-HT1B KO) mice. It might be predicted that functional removal of the 5-HT1B receptor would augment the response of the circadian system to light (i.e., dis-inhibition). However, the response of 5-HT1B KO mice to maintenance in non-24 h light:dark cycles and to light-induced phase shifts is attenuated and the circadian rhythm of corticosterone secretion is blunted. In an attempt to resolve this apparent paradox, we searched for and found 5-HT1B receptors on GABAergic terminals in the SCN. Excessive GABA activity in the SCN may provide a physiological mechanism responsible for the attenuated response to light in the 5-HT1B KO mouse.
HYPOTHALAMIC AND PERIPHERAL TISSUE CIRCADIAN RHYTHMS AND THE ENDOCRINE SYSTEM
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Our increasing 24/7 society is placing heavy demands on our physiology with an increased incidence of sleep, cardiovascular and metabolic disorders in shift workers. Accepting that this demand will continue rather than decrease, there is a need to understand both how the circadian system is linked to the environment and how physiological functions are affected by rhythmicity.

The discovery of the genes that generate and entrain cellular circadian rhythms to the environment is beginning to have an impact on how we view many physiological systems. The key clock transcription factors *Bmal1* and *Clock* have been shown to rhythmically induce many functional genes not previously considered to be rhythmic. Further, by driving rhythmicity of other transcription factors, *Bmal1* and *Clock* indirectly impose rhythmicity on a wider range of genes in a range of cells. As a consequence the previously accepted dominant role of the hypothalamic suprachiasmatic nucleus (SCN) in circadian rhythm control must be considered in tandem with endogenous peripheral tissue rhythmicity.

The SCN provides access to the external environment via the retina and as such ensures rhythms are tightly entrained to the light/dark cycle across the seasons. The neurotransmitters involved in the daily adjustments of SCN function include both excitatory amino acids and serotonin. Studies in our laboratory have implicated SCN 5-HT$_2C$ receptors in entrainment and there is the possibility of altering SCN function in humans through administration of specific agonists for this receptor.

At the peripheral tissue level it has become clear that orderly rhythmic cell function is required for the maintenance of metabolic homeostasis. Disrupted rhythmicity through mutations or rapid phase shifting as experienced by shift workers results in the breakdown of normal glucose control. The challenge is to integrate the diverse fields and develop an understanding of and appropriate strategies to help people with disturbances to their internal rhythms.

DEVELOPMENTAL ORIGINS OF THE METABOLIC SYNDROME
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Both insulin-like growth factor 1 (IGF-1) and growth hormone (GH) have important roles in the regulation of glucose metabolism and low IGF-1 levels could explain reported links between small size at birth and the risk of developing type 2 diabetes and cardiovascular disease in adult life. Low birthweight and rapid post-natal weight gain have been linked to the risk for the development of insulin resistance and obesity but reduced insulin secretion and a lower disposition index during childhood are related to reduced IGF-1 levels and statural growth. Common genetic polymorphisms in the IGF-1 gene have been linked to small birth size, post-natal growth and future diabetes risk but the results of these studies have been inconsistent.

Recent adult studies have demonstrated that lower baseline IGF-1 levels predict the subsequent development of impaired glucose tolerance, type 2 diabetes and cardiovascular disease. Adult GH deficiency shares many features in common with the metabolic syndrome and GH treatment in these subjects leads to reductions in central adiposity, but this is not always accompanied by improvements in insulin sensitivity. Recently it has been shown that the administration of a very low dose of GH therapy, which is able to increase IGF-1 levels, without inducing lipolysis, can lead to improved insulin sensitivity in young healthy adults and in GH deficient adults. GH treatment has also been explored in subjects with impaired glucose tolerance and visceral obesity. Again higher doses do not necessarily improve insulin sensitivity consistently, but ultra low doses of GH have been shown to improve post-oral glucose load insulin sensitivity. It is yet to be determined whether low dose GH, recombinant IGF-1 or combined IGF-1 with IGF binding protein 3 therapy could be used as preventative strategies in subjects with impaired glucose tolerance or type 2 diabetes.
NEW ROLES FOR OLD HORMONES AND THE GORDIAN KNOT OF HUMAN BIRTH
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While some aspects of endocrine function have been extraordinarily conserved across hundreds of millions of years of evolution the biology of pregnancy is extremely varied. As examples, parturition in goats is precipitated by luteolysis while in the sheep fetal mechanism predominate. This diversity is thought to be due to maternal-paternal genetic conflict as proposed by David Haig. From a medical perspective this diversity means that extrapolation from animal studies to the human are unwise. Experiments in pregnant women are necessarily restricted. For these reasons we remain substantially ignorant of the processes that regulate human birth and observational studies predominate. Surprisingly the hypothalamic stress hormone CRH is made in the placenta of primates and in the human increases in maternal blood in an exponential pattern peaking at delivery. In a prospective cohort study we have shown that the timing of birth is related to the rate of the exponential rise in this placental hormone. Effectively a biological clock exists in the placenta that determines the timing of birth. As labour approaches the uterus is transformed into an actively contracting pump for the expulsion of the fetus, in most mammals this event is signalled by a fall in circulating progesterone, but not in humans. In humans we have produced evidence for a functional progesterone withdrawal generated by changing expression of progesterone receptor isoforms. The stimulus to this change in receptors has been explored using novel mathematical approaches which allow cause and effect relationships between variables to be deduced without the need for experimental intervention. The approach uses Directed Graphs together with statistical testing of the likelihood that particular graphs are consistent with the data set on the variables. Using this approach strong evidence has been produced that inflammatory pathways are activated early in the pathway to human birth. Understanding the physiology of normal human birth provides a foundation upon which predictors of preterm birth can be developed and will also allow the identification of appropriate targets for novel therapeutics to arrest preterm labour.

NUTRIENT SENSING BY THE EARLY MOUSE EMBRYO: HEXOSAMINE BIOSYNTHESIS AND GLUCOSE SIGNALLING DURING PREIMPLANTATION DEVELOPMENT
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Adequate nutrient supply prior to implantation is not only essential to early embryonic growth and development but has also been implicated in metabolic programming events that influence later stage development and the onset of adult disease. The molecular mechanisms involved in early embryonic nutrient sensing and subsequent programming however have not yet been determined.

Glucose can act as an essential molecular signal for metabolic differentiation and blastocyst formation (1, 2). Our work demonstrates that propagation of this nutrient signal involves glucose metabolism through the hexosamine biosynthetic pathway, whose end-product, uridine 5’-diphospho-N-acetylg glucosamine (UDP-Glc-Nac) acts as a donor substrate adding a single O-linked b -N-acetylg glucosamine (O-GlcNAc) unit to serine and threonine residues of nucleocytoplasmic proteins. The number of proteins modified by this O-linked glycosylation is large and includes transcription factors, cytoskeletal components, metabolic enzymes and other cellular signaling components. This tightly regulated and dynamic modification operates in a functionally reciprocal relationship to the more familiar phosphorylation at the same sites hence altering the activity and/or stability of targeted proteins and providing a mechanism for modulating cellular physiology in response to nutrient availability.

We show that early embryonic glucose exposure, whilst not essential for energy generation during cleavage development, is nonetheless critical for the maintenance of cellular homeostasis with perturbations in glucose levels during early development leading to decreased levels of cell survival. Furthermore, using antisera specific for O-GlcNAc we have examined levels of O-glycosylated proteins in early mouse embryos in response to the presence or absence of glucose and find dramatically reduced global levels of O-linked glycosylation as well as altered nuclear levels of key transcription factors in embryos deprived of glucose.

We believe that this is the first demonstration of a nutrient effect on levels of transcriptional regulators in early development. Elucidation of the mechanisms by which the nutrient environment influences embryonic development is of fundamental importance to our understanding of the origins of adult disease.

CIRCADIAN RHYTHMS AND THE EARLY LIFE PROGRAMMING OF ADULT PHYSIOLOGICAL SYSTEMS
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We are all familiar with the idea that the external environment influences many diverse physiological systems. For example, the level of nutrition can not only influence adult health directly, but also fetal development and subsequently many adult functions in the offspring. Maternal stress can affect fetal outcomes as can the administration of drugs during pregnancy. Until recently, however, the daily changes in environmental light have been considered to really only influence the time that we sleep and in many other species the optimal time to mate. The impact of circadian rhythms on life trajectory has had little attention. In the last five years it has become clear that circadian rhythmicity is entrenched in virtually every cell of our bodies. A suite of clock gene transcription factors that include Clock, Bmal1 and the period and cryptochrome genes, generate a robust daily cycle of transcription and translation of hundreds of proteins. This cellular clock system is synchronised with the external photoperiod through retinal light perception, the hypothalamic suprachiasmatic nucleus (SCN) and neural and hormonal pathways. Most importantly when the clock system in peripheral tissues is disrupted, a growing list of detrimental consequences are being uncovered. As an example, mice with mutations in either Clock or Bmal1 have non-rhythmic peripheral tissues and exhibit mild to severe reproductive failure and metabolic dysfunction. Null per2 mice have a higher incidence of salivary gland hyperplasia, teratomas and increased susceptibility to radiation induced lymphomas. It is also apparent that intrauterine insults (e.g., cocaine administration, poor nutrition and stress) can have long term effects on the central circadian timing system in the SCN. Whether this involves alterations in neural development or gene function is not known. Nevertheless it is time we paid more attention to the temporal nature of our environment as a possible contributor to lifetime disorders and diseases.

NUTRITIONAL PROGRAMMING, FETAL GROWTH AND COMPETENCE FOR LIFE AFTER BIRTH
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During the past decade there has been a world-wide series of epidemiological and clinical studies which have demonstrated that there are associations between prenatal growth restriction and the risk of insulin resistance, central obesity, hypertension, type 2 diabetes, and cardiovascular disease in adult life. More recently there has also been increasing interest in the consequences of exposure of the fetus to increased levels of maternal nutrition and whether maternal ‘overnutrition’ may program an ‘intergenerational cycle of obesity’. In this presentation, we review recent experimental evidence that highlights the impact of varying levels of fetal nutrition on the structural and functional development of the adipocyte, and on expression of a range of appetite regulatory peptides within the developing brain. The importance of the timing of nutritional perturbations and the different consequences of fetal undernutrition and overnutrition on subsequent gene expression within different fat depots and on the expression of appetite stimulatory and inhibitory neuropeptides will be reviewed. The importance of defining those critical windows during an individuals lifespan when nutritional or other intervention strategies will have the maximum benefit in preventing the development of obesity and cardiovascular disease will also be considered.

IMPACT OF GLUCOCORTICOIDS ON FETAL-PLACENTAL GROWTH AND THE POSTNATAL PHENOTYPE
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Glucocorticoids are recognised as a key fetal programming signal, with excess glucocorticoid exposure in utero linked to various adverse outcomes in offspring including delayed puberty onset, hyperleptinemia and hypertension. Fetal glucocorticoid exposure is controlled by the placental glucocorticoid barrier, whereby two 11β-hydroxysteroid dehydrogenase enzymes regulate transplacental passage of active glucocorticoids (cortisol and corticosterone). Fetal programming by glucocorticoids is likely due to their actions in several fetal tissues, but may also be mediated via effects exerted within the placenta. Indeed, in our model of fetal programming, treatment of pregnant rats with dexamethasone inhibits both fetal and placental growth, and dose-response experiments suggest that the placenta is more susceptible than the fetus to this growth inhibition. Moreover, glucocorticoid treatment stimulates placental apoptosis and reduces expression of several placental gene products, including PPARγ, Muc1 and VEGF. This down-regulation of gene expression occurs specifically within the labyrinth zone, the region of maternal-fetal exchange, and is associated with a marked reduction in placental vascularity. These data indicate that excess
placental glucocorticoid exposure is likely to compromise fetal nutrient supply which in turn could result in adverse fetal programming effects. Subsequent, long-term effects of fetal programming in offspring can either be amplified or attenuated by the postnatal environment. Thus, while programmed hyperphagia and adiposity are exacerbated by a high-energy diet in postnatal life, we have demonstrated that programmed hyperleptinemia and hypertension are prevented by a postnatal diet enriched with omega-3 fatty acids. These effects are mediated, in part, by changes in the adipocyte phenotype, most notably in relation to leptin mRNA expression. In conclusion, fetal programming by glucocorticoids is likely to be mediated, in part, by their detrimental effects on placental growth and vascularity. Postnatally, adverse outcomes of glucocorticoid-induced fetal programming can be prevented by dietary manipulations, thus raising the possibility of preventative, therapeutic interventions.

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USING HBA1C IN THE CLINIC – WHY WORRY ABOUT STANDARDIZATION?
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HbA1c is one of if not the most important measure in assessment of glycemic control in diabetes. The landmark DCCT and UKPDS trials have related both the level of HbA1c and change over time to development of microvascular complications. In Australia, in addition to its use in day to day diabetes management, the level of HbA1c is used to determine qualification for some subsidized medications. In addition there is increasing suggestion that, given its reflection of mean glucose concentration over the previous 2 to 3 months, HbA1c would logically be used for diagnosis of diabetes. Reproducibility, that is long-term comparability of the values within an individual patient, is thus an absolute necessity. Clinicians need to have confidence that the result is ‘accurate’ to within 0.5% HbA1c units and that the results of tests can be directly compared between different pathology laboratories and different states and countries. Unfortunately, more than 20 different methods are used, based on three different assay principles and all yielding different HbA1c results and standardization of HbA1c, although a long held goal, is not complete. Although several standardization schemes have been established, until lately, there has not been a ‘reference standard’ for HbA1c. With the development of an International Federation of Clinical Chemistry (IFCC) International reference system (based on pure reference standards of HbA1c) there is an opportunity to achieve standardization. Should the IFCC reference method become the global reference standard, the reported A1c numbers would be 1-2% less than those currently reported. Thus the ‘cut-points’ for what is normal, good/poor control would shift downward. This has created wide discussion. Theoretically, the current values could be maintained, but this would involve a major conversion from the IFCC value to DCCT HbA1c units. It is argued that adopting a new IFCC range would, in addition to reporting actual values, also provide an opportunity to reeducate patients and health professionals about the test and redefine HbA1c. Another option which has been discussed is to change the name of the assay to ‘mean blood glucose’ and actually report mean blood glucose through the relationship which was obtained using the DCCT data (Mean Blood glucose = 1.84 x IFCC A1c). While this has the advantage of becoming a more understandable measure for patients there is still concern regarding the possibility that the simple proportionality, or even a straight linear relationship between HbA1c and mean blood glucose may not apply to all populations or to extremes of mean blood glucose or HbA1c. Whatever the final decision, clinicians should prepare themselves for imminent change in the reporting of HbA1c.

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HBA1C FROM A LABORATORY PERSPECTIVE
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Glycohaemoglobinins (HbA1c) are products of glucose and HbA and reflect mean glycaemia over the prior 1-3 months. The American Diabetes Association has established a target of 7.0% HbA1c, and a change of therapy recommended at 8.0% HbA1c. Such levels require a precision of <3%CV, and imprecision of <2%CV has recently been recommended and is clinically desirable. Different National Standardisation Schemes in the USA (NGSP), Japan and Sweden have been established in the past 10-12 years and have resulted in significantly improved inter laboratory variability. The IFCC formed a Working Group on International Standardization of HbA1c in 1994 and this group has prepared purified HbA1c standards and developed two Reference Methods, and established an International Network of Primary and Secondary IFCC Reference Laboratories. More than ten comparison studies (currently two per year) using pooled samples of whole blood have been performed by the IFCC and NGSP, Japanese and Swedish reference laboratories and all major manufacturers in the USA, Europe and Japan. Master Equations have been delineated to link the IFCC system to all previous National schemes and diabetes studies. The reporting unit is currently under intense discussion. The IFCC and IUPAC have proposed and recommended the SI Unit (mmol/mol) as the new unit (mmol HbA1c/mmol Hb). This would result in the current NGSP/DCCT
upper limit of normal of 6.0% HbA1c converting to 42 mmol/mol, the target of 7.0% to 53 mmol/L and the Change of Therapy level of 8.0% to 64 mmol/L. The expression of HbA1c as Mean Blood Glucose is also possible, but now considered unlikely. Expression of HbA1c as a percentage (%HbA1c IFCC) is extremely unlikely. The choice of the HbA1c assay and instrumentation in each laboratory is crucial. Analytical needs in terms of accuracy, precision and interferences will be outlined.

**ADVANCED GLYCATION AND INTERVENTIONS IN THE LABORATORY.**

J. Forbes

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Advanced glycation is the irreversible attachment of reducing sugars via the “Maillard reaction” onto the free amino groups of proteins, in particular at lysine and arginine residues. Its physiological roles are thought to include the identification of senescent proteins and hence there is a time dependent accumulation of advanced glycation end products (AGEs) as we grow older. When a protein is labelled with AGEs, it is catabolized by cells into peptides and amino acids and excreted via the kidneys. This process appears to be tightly controlled by AGE clearance receptors such as AGE-R1, AGE-R3 and scavenger receptors such as CD36, SR-AII and SR-BL. Evolution has also provided protection against excess AGE formation by utilisation of glucose as the major metabolic sugar, as it is a slowly reacting reducing sugar. Conditions such as diabetes, however, which have a metabolic overload of a number of reducing sugars, rapidly accelerate AGE formation. In addition, advanced glycation is facilitated by oxidative stress even in the absence of increases in reducing sugar concentrations. Both of these processes contribute to the increased AGE pool seen in diabetes. Food chemists have seen the value of advanced glycation in foodstuff manufacture by utilising heat in processes such as roasting, brewing, frying and cooking. As part of our western diet, we ingest AGEs of which approximately 70-80% are absorbed, catabolized and excreted over a period of two days. This is a third major source of AGEs and becomes an increasingly important contributor as the clearance of AGEs by the kidneys decreases during diabetes-induced renal impairment. As AGE levels rise during diabetes, interruption of normal function occurs via three distinct mechanisms. The first involves AGE induced cross-linking of extracellular matrices, which stiffens elastic fibres, disturbs cellular adhesion and prevents renewal of “old” proteins causing structural defects. The second is by intracellular formation of AGEs, which causes generalised cellular dysfunction by interference in enzyme/substrate interactions, signalling and by steric hindrance including conformational changes within proteins and DNA. The third involves the binding of circulating AGEs to specific receptors such as RAGE, the receptor for advanced glycation end products, TOLL-like pattern recognition receptor involved in activation of inflammatory pathways which when chronically activated contributes to excesses in inflammatory molecule production. Due to the range of dysfunction produced by the accumulation of AGEs in diabetes, there is a growing need for intervention in this process. Indeed, advanced glycation has been implicated as a major therapeutic target in both micro- and macrovascular complications of diabetes and the race is on to produce an inhibitor capable of addressing the many actions of AGEs.

**DIABETIC COMPLICATIONS: IS IT TIME TO START WORRYING ABOUT AGEING?**

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Prolonged hyperglycaemia, dyslipidaemia and oxidative stress in diabetes result in the production and accumulation of AGEs. The level of this AGE accumulation is strongly correlated with adverse outcomes in patients with diabetes, even more so than conventional risk factors such as HbA1c. However, more than being a simple marker of these pathogenic processes, it is now clear that AGEs independently contribute to the development and progression of cardiovascular disease in diabetes, as well as other complications including nephropathy and retinopathy. AGEs are thought to act through receptor-independent and dependent mechanisms to promote vascular damage, fibrosis and inflammation associated with microvascular damage. In addition, receptor-independent actions of AGE-modification leads to proteins that are stiff, less soluble and, more importantly, dysfunctional. As a result, novel therapeutic agents to reduce the accumulation of AGEs in diabetes have gained interest as potential approaches for preventing diabetic complications. These include aminoguanidine, pyridoxamine, benfotiamine, OPB-9195, alagebrium chloride and LR-90. In addition, it has been demonstrated that a number of established therapies have the ability to reduce the accumulation of AGEs in diabetes including ACE inhibitors, angiotensin receptor antagonists, metformin, PPARs, and some antioxidants. The fact that many of these inhibitors of AGEs are effective in experimental models, despite their disparate mechanisms of action, supports the keystone role of AGEs in end-organ damage associated with diabetes. While the clinical utility of AGE inhibition remains to be firmly established, upcoming trails will likely provide some important clues for this developing field.
MECHANISMS OF ACTION IN CURRENT AND EMERGING OSTEOPOROSIS THERAPIES

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Until recently osteoporosis treatment has relied upon drugs that inhibit bone resorption, with successful trials of bisphosphonates, SERM's and a strontium salt. Current novel treatments are aimed at interfering with osteoclast development (e.g. anti-RANKL), as well as inhibiting osteoclast activity (e.g. V_ATPase, cathepsin K and chloride-7 channel inhibitors).

The only proven anabolic therapy for bone is PTH. The anabolic effect of PTH is dependent upon intermittent administration, but when an elevated PTH level is maintained even for a few hours it initiates processes leading to new osteoclast formation, and the consequent resorption over-rides the effects of activating genes that direct bone formation. The observation that concurrent treatment with bisphosphonates impairs the anabolic response to PTH, adds to other clues that osteoclast activity is necessary to complement the direct effect that PTH has in promoting differentiation of committed osteoblast precursors. This might involve the generation of a coupling factor from osteoclasts that are transiently activated by RANKL in response to PTH. Both human and mouse genetics provide evidence supporting the view that osteoclasts, despite in some circumstances being unable to resorb bone, e.g. failure of acidification, can nevertheless be associated with normal, or even increased bone formation. An implication is that it may be possible to design resorption inhibitors that do not block PTH anabolic action when given simultaneously.

New approaches to anabolic therapies come from the discovery that an activating mutation in the LRP5 gene is responsible for an inherited high bone mass syndrome, and the fact that this can be recapitulated in transgenic mice, whereas inactivating mutations result in severe bone loss. This has focused attention on the Wnt/frizzled/β catenin pathway as an important one in bone formation, and provides intriguing choices of any of a number of drug targets in this pathway.

CALCIUM SUPPLEMENTATION AND/OR VITAMIN D IN OSTEOPOROSIS - MYTH OR REALITY?

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One of the central problems in nutrition science is to determine the optimal intake of nutrients to prevent disease in an individual taking into account their genetic, environmental and disease status. In the first part of the 20th century the principal nutritional focus was on the prevention and treatment of rickets in children, still a substantial problem. With increasing longevity the focus now includes the other end of life where fragility fracture presents a major social, health and financial threat to millions of individuals who have survived other threats because of much improved public and personal health services. The aetiology of the threat includes the combined and independent effects of estrogen, calcium and vitamin D deficiency.

We have shown effects in humans of high endogenous estrogen to reduce fracture propensity, increase DXA bone density and reduce renal calcium and phosphate excretion, effects mediated in part by both aromatase and estrogen receptor gene polymorphisms. These data are supported by in-vitro and in-vivo animal and cell culture studies showing stimulation of calcium transport by estrogen. We and others have shown that this bone loss can be prevented by increased dietary calcium intake.

However the size of the treatment effect on fracture remains uncertain. We have recently completed a public health based, prospective, double blind, randomised trial of 1200 mg calcium compared to placebo in women mean age 75. After 5 years of treatment the intention to treat analysis showed a hazard ratio for clinical fracture of 0.86 95% CI 0.67-1.11. In those consuming 75% of tablets the HR was 0.48 95% CI 0.24-0.97. Thus calcium therapy may be effective if compliance is high, vitamin D and calcium remain indicated as a first step those with marginal vitamin D intake.
SEX HORMONE REPLACEMENT AND SERMS

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Postmenopausal bone loss is characterised by increased remodelling at different skeletal sites and in different bone compartments. Oestrogen sex hormone replacement, which can block this excessive ‘turnover’, had been the mainstay of postmenopausal osteoporosis prevention. The Women’s Health Initiative demonstrated HRT efficacy in reducing fragility fractures but increased cardiovascular adverse events in a double blind RCT. The women were older and largely overweight women at relatively low risk for osteoporosis and high risk for vascular adverse events. Despite the limited relevance to younger postmenopausal woman, this reported adverse effect profile led to calls to restrict HRT to 1-2 years postmenopause and only for relief of menopausal symptoms.

Bone density improvements following HRT gradually disappear over about 5 years after cessation. Although oestrogen-only therapy appears to have a better safety profile, this could only be recommended post-hysterectomy. Tibolone may prevent postmenopausal bone loss with less breast or uterine effects. However a large scale RCT of its safety and efficacy on fragility fractures is still in progress.

Raloxifene, the best-studied SERM, has been shown to reduce bone loss and reduce vertebral but perhaps not peripheral fragility fractures with a good safety profile with fewer breast cancer diagnoses. Studies are continuing on its cardiovascular safety profile and more potent SERMs are under evaluation.

Despite some concerns about their longer-term safety and efficacy, HRT and SERMs offer useful alternatives in the armamentarium for the prevention and treatment of the earlier stages of postmenopausal bone loss.

Conflicts of Interest (all 3 and/or 4):
Eli Lilly, Merck Sharp and Dohme, NPS Pharmaceuticals, Novartis, Organon, Roche, Sanofi-Aventis, Servier

OSTEOPOROSIS: WHOM TO TREAT? WHAT DRUG?

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The drugs for osteoporosis are best used in those in whom they have been clearly shown to reduce fractures – patients with vertebral fractures or with a low spine or hip BMD (at or below a T-score of -2.5). The targeting of these treatments may also be done on health economic grounds. In the UK, such an approach has been applied by the National Institute of Clinical Excellence. The treatments for osteoporosis that are effective in reducing the risk of fracture have been recently renamed ‘anti-catabolic’ and ‘anabolic’. The anti-catabolic treatments work by reducing the activation frequency and possibly restoring remodelling balance. This class includes potent agents such as alendronate, risedronate, and hormone replacement therapy, and weaker agents such as raloxifene and calcitonin. The anabolic treatments work by increasing the activation frequency and by causing a positive remodelling balance. This class includes parathyroid hormone (teriparatide). Some treatments, such as strontium ranelate, don’t fit easily into this classification. The choice of treatment is based on the evidence for efficacy, its cost-effectiveness, and side effect profile. The choice of treatment is also dictated by the patient’s history and the efficacy of treatment in the individual may be monitored by serial measurements of bone mineral density or bone turnover markers.
TRANSGENER RECEPTOR β SIGNALING BY NUCLEAR FACTOR-κB IN OVARIAN GRANULOSA CELLS

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ERβ is the predominant ER in granulosa cells of the ovary, and is expressed at high levels in granulosa cell tumours (GCT). The functional significance of ERβ expression is not known. To gain insight into ERβ function in granulosa cells and in GCT, we are using two GCT-derived cell lines, COV434 and KGN cell lines, which express ERβ mRNA and protein but do not express ERα. Despite having demonstrable estradiol (E2) binding, transcriptional activation of a transfected estrogen-responsive reporter vector (ERE2-luc) by E2 is not observed. Transactivation was also not observed with co-transfected ERα or ERβ. Additionally, co-transfection of the glucocorticoid receptor (GR) with a GRE reporter plasmid also failed to demonstrate ligand-dependent transactivation. This transcriptional resistance is specific to steroid receptor transactivation; reporter plasmds that are activated by the transcription factors activator protein 1 (AP-1) and nuclear factor-κB (NF-κB) demonstrate both constitutive and inducible transactivation. These transcription factors are known to cause transrepression of both ERα and GR-mediated transcription, hence we explored the possibility that activation of either of these pathways might cause transrepression of ERβ-mediated transactivation. In the presence of inhibitors to the AP-1 or NF-κB signalling pathways, the response of E2 activity on the ERE2-luc reporter in both cell lines was monitored. The AP-1 inhibitors alone had no effect, however, inhibition of NF-κB signalling allowed a 3- to 4-fold E2-mediated induction of ERE2-luc. This response was both ligand and ER dependent as addition of the ER antagonist ICI 182,780 in the presence of E2 blocked this induction. Inhibiting the NF-κB signalling pathway also restored GR-mediated transactivation. The relevance of these observations to GCT in vivo as well as normal ovarian maturation is unclear. There are several lines of evidence suggesting that the role of ERβ in granulosa cells may be antiproliferative and proproliferative effects but also by its repression of ERβ signalling.

FUNCTIONAL INTERACTIONS BETWEEN LRH-1 AND CREB REGULATE AROMATASE EXPRESSION IN BREAST CANCER

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Aromatase is over-expressed in adipose tissue of breast cancer patients due to stimulation by tumour-derived prostaglandin E2 (PGE2). PGE2 induces transcription of the CYP19 gene (the gene that encodes aromatase) via an alternative cAMP-responsive promoter (promoter II), thereby increasing the availability of estrogen for tumour growth. We have previously shown that promoter II activity in adipose tissue requires the action of the orphan nuclear receptor LRH-1, and that LRH-1 synergises with cAMP in activating promoter II. The aim of the present study was to identify the molecular basis of this synergism. Initial studies aimed to map the promoter regions within promoter II that mediate the synergistic effect. 3T3-L1 preadipocytes were transfected with luciferase reporter genes driven by 516 bp of promoter II upstream sequence, either wild type or containing mutations within cis elements previously shown to be essential for PGE2-induced transcription (2 cyclic AMP response element (CRE)-like sequences and 1 LRH-1 binding site). As expected, mutation of the LRH-1 binding site abrogated the ability of LRH-1 (but not cAMP) to activate promoter II. However, when the most proximal CRE was mutated neither cAMP nor LRH-1 stimulated promoter activity, indicating that LRH-1 action requires a functional CRE, in addition to its own response element on promoter II. To address the possibility that LRH-1 might physically interact with CRE-binding protein (CREB), a mammalian 2-hybrid approach was adopted using GAL4/CREB/LRH-1 fusion constructs and a GAL4-responsive reporter. This analysis showed that LRH-1 and CREB display a strong physical interaction when transfected into 3T3-L1 cells, which is independent of the phosphorylation status of CREB. We are currently verifying this interaction by independent means. In conclusion, aromatase expression in breast adipose requires functional interactions between CREB and LRH-1. Therefore, targeting this interaction might allow for the development of new aromatase inhibitors for breast cancer therapy.
PAX8-PPARγ ALTERS NORMAL THYROID TRANSCRIPTIONAL REGULATION

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The PAX8-PPARγ fusion gene is the result of a chromosomal translocation t(2;3)(q13;p25) that fuses the thyroid-specific transcription factor PAX8 with the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ). Initially demonstrated in follicular thyroid carcinomas (FTCs) by Kroll et al. (Science 2000; 289:1357-1360), this fusion gene has also been reported in adenomas; however its function in thyroid regulation is still relatively unknown. In cell model experiments using FRTL-5 cells, our laboratory has showed that PAX8-PPARγ over-expression increases cell proliferation assessed by thymidine uptake and colony formation in soft agar. Here we have investigated the mechanisms by which PAX8-PPARγ may disrupt normal follicular thyroid cell growth. In transfection studies, we found that PAX8-PPARγ stimulated expression from PAX8-responsive thyperoxidase and sodium-iodide symporter gene promoters. However PAX8-PPARγ failed to stimulate transcription from another PAX8-responsive element in the thyroglobulin promoter (Tg-Luc). PAX8 and thyroid transcription factor-1 (TTF-1) are known to synergistically activate the Tg promoter, however PAX8-PPARγ not only failed to co-operate with TTF-1 but also inhibited TTF-1 and TTF-1/PAX8-mediated Tg-Luc expression in a dominant negative manner. Moreover, we found that PAX8-PPARγ transcriptional function of a PPARγ-response element (3x acyl-CoA oxidase element, PPRE) was cell-type specific. In heterologous HeLa cells we confirmed previous findings that PAX8-PPARγ was transcriptionally silent on a PPRE, however in rat thyroid (FRTL-5) cells, the fusion protein stimulated this promoter to levels of activation seen with wild-type PPARγ. In preliminary transfection studies, we also show PAX8-PPARγ stimulation of another PPRE in the Aquaporin7 promoter (a gene over-expressed in PAX8-PPARγ-positive FTCs identified by micro-array) to levels similar to wild-type PPARγ activation. In conclusion we suggest that PAX8-PPARγ stimulates some thyroid-specific genes but inhibits transcription of others, the net effect of which is likely to result in dysregulated thyroid cell growth and/or loss of differentiation.

IN VIVO PHOTOPROTECTION BY A RAPID RESPONSE, LOW CALCEMIC ANALOG OF 1,25-DIHYDOXYVITAMIN D₃

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Vitamin D is produced by exposure of 7-dehydrocholesterol in the skin to UV irradiation (UVR) and can be further converted in the skin to the biologically active hormone, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃). UVR also results in DNA damage producing cyclobutane pyrimidine dimers (CPD), signature mutations in UVR-induced carcinogenesis. We previously reported that 1,25(OH)₂D₃ protects human skin cells from UVR-induced apoptosis, and decreases CPD in surviving cells. 1,25(OH)₂D₃ has been shown to generate biological responses via 2 pathways – the genomic pathway and a rapid, non-genomic pathway mediated by a putative membrane receptor. A cis-locked, low calcemic, rapid-acting agonist 1,25(OH)₂lumisterol₁ (JN), reduced keratinocyte loss and CPD damage after UVR, to an extent similar to that of 1,25(OH)₂D₃. Its effects were abolished by a rapid-acting antagonist 1β,25(OH)₂D₃ (HL) , but not by a genomic antagonist. Furthermore, the protective effects of 1,25(OH)₂D₃ were abolished by a neutralizing antibody to the 1,25(OH)₂D₃-membrane-associated, rapid response steroid-binding protein. Studies in Skh:HR1 mice exposed to 3 times the minimal erythema dose of solar-simulated UVR and treated topically with 1,25(OH)₂D₃ or JN immediately after UVR showed a reduction in UVR-induced oedema, erythema and peeling compared with vehicle-treated mice. Topical 1,25(OH)₂D₃ reduced the level of CPD measured 3 and 24h post UVR from 22 ± 6% to 9 ± 3% (p<0.05), and preliminary studies showed similar reductions in CPD by JN. 1,25(OH)₂D₃ and JN significantly reduced systemic UV-induced immunosuppression in Skh:HR1 mice from 23 ± 1% to 5 ± 1% (p<0.001) and -3 ± 2% (p<0.001) respectively. These results show for the first time an in vivo biological response mediated by a rapid-acting analog of the vitamin D system. The data support the hypothesis that 1,25(OH)₂D₃ exerts its photoprotective effects via the rapid response pathway and that the intrinsic photoprotective properties of the skin may be enhanced by low calcemic analogs of 1,25(OH)₂D₃.
FAT AUSSIE MOUSE – A RODENT MODEL OF ALSTRÖM SYNDROME

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Obesity is emerging as one of the most serious public health problems in the modern world: up to 60% of people in the Western world are obese or overweight. It is the strongest single risk factor for type 2 diabetes (T2D) and cardiovascular disease, and causes significant morbidity and mortality posing a serious financial burden on the health-care systems worldwide. Still, the molecular mechanisms underlying obesity and T2D remain poorly understood.

Here we describe the discovery of the NOD Fat Aussie Mouse (FAM), an exciting new mouse model of obesity, T2D and hypercholesterolemia. These traits are inherited in a fully penetrant autosomal recessive fashion. Using an outcross strategy of NOD Fat Aussie mice to the mapping C57Bl/10 strain, we identified linkage to a single 1.3 cM interval on chromosome 6. A good candidate gene within this interval was Alms1, a gene that when mutated causes Alström syndrome in humans (early onset obesity, T2D, retinal dystrophy, sensorineural deafness, cardiomyopathy, kidney and liver dysfunction). We sequenced the Alms1 transcript in FAM and found an 11 bp deletion that shifts the reading frame creating a stop codon.

We have been able to study the pathogenesis of obesity and T2D in this unique mouse model. Obesity in FAM is secondary to hyperphagia pointing to a defect in the satiety axis. Obesity is accelerated when mice are fed with a high fat diet. FAM also develop severe liver disease with steatosis and elevated liver enzymes. Both male and female FAM mice are sterile due to an arrest at the round spermatid cell stage of spermatogenesis and absence of both primary and secondary follicles in the ovaries. Elucidation of the mechanisms that underpin weight gain and T2D in the presence of ALMS1 mutations may lead to the discovery of new molecular therapeutic targets.

FETAL PROGRAMMING OF ADULT HYPERTENSION AND HYPERLEPTINAEMIA: PREVENTION BY POSTNATAL DIETARY OMEGA-3 FATTY ACIDS

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Fetal programming is now recognised as a key determinant of the adult phenotype, with major implications for adult-onset diseases including hypertension. Two mediators of fetal programming are maternal nutrition and fetal glucocorticoid exposure and recent studies show that postnatal dietary manipulations can exacerbate programming effects. Whether programming effects can be attenuated by postnatal dietary manipulations, and thus provide a possible therapeutic strategy, is unknown. Therefore, we tested the hypothesis that postnatal ingestion of omega-3 fatty acids attenuates glucocorticoid-induced programming of hypertension and hyperleptinaemia. Dexamethasone (0.75 µg/ml in drinking water) was administered to pregnant rats 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. Systolic blood pressure was measured by tail-cuff plethysmography, plasma leptin by radioimmunoassay and leptin mRNA expression in retroperitoneal and epididymal fat by real time RT-PCR. Maternal dexamethasone treatment reduced birthweight by 27% and delayed the onset of puberty in offspring. Hypertension was evident in offspring by 6 months of age in dexamethasone-exposed animals consuming a standard omega-3 diet, but these effects were completely blocked by a high omega-3 diet, with systolic blood pressure 13.2 and 19.3 mmHg lower in male and female offspring respectively. Similarly, hyperleptinaemia was evident in offspring by 6 months of age in dexamethasone-exposed animals on a standard omega-3 diet, but these effects were blocked by a high omega-3 diet, which reduced plasma leptin by 27% in males and 40% in females. These patterns of plasma leptin were paralleled by changes in leptin mRNA in retroperitoneal fat. These results demonstrate for the first time that manipulation of postnatal diet can attenuate fetal programming effects, with the postnatal ingestion of omega-3 fatty acids completely preventing programmed hypertension and hyperleptinaemia induced by fetal dexamethasone exposure.
DIFFERENTIAL PATTERNS OF X-INACTIVATION AMONG SISTERS IN FAMILY GROUPS WITH POLYCYSTIC OVARY SYNDROME.

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The genetic determinants of polycystic ovary syndrome (PCOS) are unknown, however some studies have suggested the involvement of X-linked genes. Such linkage would presumably be confounded by non-random X-inactivation. We explored this possibility with respect to PCOS by analysing X-inactivation patterns among sisters within 40 families in which at least one sister was diagnosed with PCOS, defined by unexplained hyperandrogenic chronic anovulation. Family members were genotyped for the (CAG)n repeat polymorphism within the androgen receptor (AR) gene, which is methylated during X-inactivation. Quantitative comparison of fluorescently labelled PCR products from a DNA sample before and after digestion with the methylation-sensitive enzyme HpaII was used to determine relative allele expression patterns. Patterns were considered different between sisters with the same genotype if: 1) there was preferential inactivation of opposite alleles, or 2) if PCR products from a DNA sample before and after digestion and rapidly deplete follicle reserves. We have examined UPS WITH...
DEVELOPMENTAL EXPRESSION OF ANGIOTENSIN RECEPTORS IN THE SHEEP ADRENAL GLAND – A ROLE FOR ANGIOTENSIN-II IN REGULATING ADRENAL GROWTH AND STEROIDOGENESIS BEFORE BIRTH

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The period of adrenal suppression in mid-gestation, is essential to limit the exposure of fetal organs and tissues to the actions of cortisol, which potently inhibits growth and promotes differentiation of function. The mechanism regulating the period of adrenal suppression in mid-gestation is not known. Studies have shown that administration of angiotensin-II (AII) to fetal sheep adrenal cells in vitro, inhibits ACTH-induced expression of the steroidogenic enzyme CYP17 and suppresses cortisol secretion in mid-gestation. The actions of AII are mediated via 2 receptors the angiotensin type 1 receptor (AT1R) and type 2 receptor (AT2R), thus the aim of this study was to determine the developmental expression of the angiotensin receptors in the sheep adrenal gland. Adrenal glands (n=41) were collected from fetal sheep between 63rd gestation and post-natal day 1, and frozen for determination of the mRNA expression the AT1R and AT2R using Real Time PCR and expressed relative to ribosomal protein mRNA. The relative expression of AT1RmRNA was significantly greater at 81d gestation (1694.5 ± 304.3; i.e. the onset of the period of adrenal quiescence) when compared to earlier in gestation (63-67d; 760.9 ± 90.4) and near term (140-141d; 702.9 ± 158.1; ie periods of adrenal growth and steroidogenic activity). In contrast, the relative expression of AT2RmRNA was significantly greatest at 63-67d gestation (5.93 ± 1.02), after which AT2R expression declined and was decreased significantly by 102-104d (1.01 ± 0.31). Therefore, it maybe that the high expression of AT1R, which is coincident with timing of the decrease in CYP17 mRNA expression, maybe the mechanism by which cortisol synthesis is suppressed in the fetal sheep adrenal gland in mid-gestation. The highest expression of AT2R mRNA in early gestation, confirms it as a candidate for regulating adrenal growth and function at this time. 

ANALYSIS OF GLUCOCORTICOID AND cAMP SIGNALING DURING LUNG DEVELOPMENT USING GLUCOCORTICOID RECEPTOR AND CREB NULL MICE

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Glucocorticoids regulate many physiological processes including aspects of embryonic and fetal development. In particular, they provide important signals for fetal lung maturation and, as a result, antenatal glucocorticoids are routinely used to reduce the respiratory insufficiency suffered by infants born very preterm. The cAMP intracellular signaling pathway is also important for promoting lung development yet little is known of its specific role in the developing lung. To further understand the role of glucocorticoids and cAMP in fetal lung development, we have analyzed mice with a targeted null mutation for either the glucocorticoid receptor (GR) or the cAMP responsive element binding (CREB) protein gene. In the absence of glucocorticoid signaling via GR, lung development is severely retarded. The lungs of fetal GR null mice are hypercellular with reduced septal thinning. Surfactant protein gene expression is relatively unaffected as is the production and release of pulmonary surfactant. Surprisingly, electron microscopy revealed increased proportions of differentiated type II cells and greatly reduced proportions of differentiated type-I alveolar epithelial cells (1). These findings indicate that the beneficial effects of glucocorticoids on fetal lung maturation are not mediated via effects on type-II epithelial cells, but rather on lung structure and hypercellularity within the peri-alveolar parenchyma. Increased cell proliferation in the GR-null fetal lung was confirmed by immunostaining with Ki67, a marker of cell proliferation. CREB null mice also do not survive birth and display a severe retardation in lung development. There is marked hypercellularity, but also an increase in the level of apoptosis, leading to elevated tissue volumes and large blood/gas diffusion barriers. This demonstrates that during murine embryonic development, glucocorticoids and cAMP signaling are essential for the proper structural development and differentiation of the terminal gas exchange regions of the lung which allows efficient gas exchange to occur after birth.

INFLUENCE OF LABOUR AND FETAL SEX ON GLUCOCORTICOID RECEPTOR mRNA TRANSCRIPT EXPRESSION IN THE HUMAN PLACENTA

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Glucocorticoids (GCs) regulate the expression of many proteins with important roles in human pregnancy, including CRH and IGF-B1. GC actions are mediated primarily through the glucocorticoid receptor (GR). Differential promoter use and alternative splicing generate a variety of GR mRNA transcripts, which may potentially alter the GC responsiveness of the gestational tissues during pregnancy and parturition. Here we have examined GR mRNA transcript expression in the placenta with labour onset and in association with fetal sex.

RNA was extracted from the placentas of women (14 with male babies and 14 with female babies) delivering following elective Caesarean section (CS, n=10) or spontaneous vaginal delivery at term (SL, n=18). The abundance of the GR mRNA splice variants GR-α, GR-β, GR-P and GR-γ and the untranslated exon one mRNA variants 1A1, 1A2, 1A3, 1B and 1C, which indicate differential promoter usage, were determined by quantitative real-time RT-PCR. Data were normalized to 18s rRNA abundance.

All GR mRNA variants were detected in the human placenta. The relative abundance of GR splice variants was in the order of GR-α>GR-P>GR-β>GR-γ mRNA. Abundance of the untranslated exon one variants was in the order of 1C>1B>1A>1A2>1A1. Only GR-P mRNA abundance was altered by labour, with a decrease following SL (p<0.01, Mann-Whitney U Test). Analysis of fetal sex differences between the GR transcripts showed that only GR 1A3 mRNA was different, exhibiting a decrease in placentae from women pregnant with a female fetus (p<0.05, Mann-Whitney U Test).

Our results suggest that the GR-α mRNA variant transcribed from the 1B and 1C promoters accounts for the majority of GR produced in the placenta. Alterations in promoter usage between male and female fetuses, particularly the GR 1A3 variant, may confer differential responses to glucocorticoids between women carrying male or female fetuses.

THE ROLE OF NFKB IN THE REGULATION OF PROSTAGLANDIN H-2 SYNTHASE IN TERM HUMAN AMNION IN VIVO.

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Prostaglandins are involved in the initiation and maintenance of labor with increasing prostaglandin H-2 synthase (PGHS-2) expression in the amnion playing a crucial role. The promoter region of the PGHS-2 gene contains two consensus NFkB-binding motifs (Site-1, -213/-222; Site-2, -438/-447) which bind the NFkB subunits p65 and p50. In vitro experiments with amnion have implicated NFkB in the regulation of the PGHS-2 gene. We examined NFkB subunit binding to NFkB sites-1 and -2 on the PGHS-2 promoter of amnion in vitro, before and during labour. Amnion tissue was collected from women within 30 minutes of delivery by spontaneous labour or elective Caesarean section at term. Women showing signs of intrauterine infection or inflammation were excluded. DNA-protein complexes were detected with chromatin immunoprecipitation (ChIP) using antibodies specific for TATA-binding protein (TBP) and the p65 and p50 subunits of NFkB. The antibodies were verified by examining NFkB binding at the promoter region of the IkB gene. Real time RT-PCR primers were designed to the TATA-box region and NFkB Sites-1 and -2 of the human PGHS-2 promoter. PGHS-2 gene activity was determined by measuring PGHS-2 mRNA and hnRNA with real time RT-PCR. The PGHS-2 gene was active in all tissues. Immunoprecipitation showed a significant enrichment of the PGHS-2 sequence in the TATA-binding region (p=0.0011, t-test), compared to the mock-immunoprecipitated control. The p65 and p50 antibodies, showed no enrichment of Site-1 or Site-2 compared to the mock-immunoprecipitated control. Regulation of the PGHS-2 gene through binding of the NFkB transcription factors p65 and p50 at Site-1 and -2 on the PGHS-2 promoter in term amnion in vivo, was not supported by our data.
CHANGES IN PLACENTAL EXPRESSION OF PPARγ IN RAT PREGNANCY ARE ASSOCIATED WITH ALTERED EXPRESSON OF MUC1 AND VEGF

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Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily that act as ligand-activated transcription factors. Recent gene deletion studies indicate that PPARγ plays a critical role in mouse development, including effects on placental vascularisation. Previously, we have demonstrated changes in the expression of PPARγ in the labyrinth zone of the rat placenta, increasing near term in association with the maximal rate of fetal and placental growth (Proceedings of the ESA 2004). In this study we investigated the expression of the PPARγ regulated genes, Muc1 and VEGF, in the two functionally and morphologically distinct zones of the rat placenta during normal gestation and after glucocorticoid-induced fetal and placental growth restriction.

Real-time RT-PCR analysis demonstrated markedly higher expression of Muc1 (3-fold; \( P < 0.01 \)) and VEGF (4-fold; \( P < 0.001 \)) in the labyrinth zone over the final third of pregnancy in association with the intense vascular development observed during this period. Glucocorticoid-induced fetal and placental growth restriction (1\( \mu \)g/ml dexamethasone acetate in drinking water; days 13-22) was associated with reduced Muc1 (51%, \( P < 0.01 \)) and VEGF (64%, \( P < 0.001 \)) expression in the labyrinth zone at day 22 (term = 23 days). Detailed histological analysis of the labyrinth zone revealed an impaired vasculature in placentas of dexamethasone-treated mothers, with an apparent reduction in total surface area for fetal-maternal exchange.

These data suggest that PPARγ:RXRα heterodimers play important roles in labyrinth zone development late in pregnancy, possibly supporting vascular development via the expression of VEGF. Moreover, it appears that glucocorticoid-regulated inhibition of fetal and placental growth may be mediated, in part, via a labyrinth zone specific suppression of PPARγ and subsequent inhibition of VEGF expression. Further studies are required to determine whether PPARγ may provide a suitable therapeutic target for growth restricted pregnancies.

### ACUTE MATERNAL ALCOHOL TREATMENT RESTRICTS FETAL GROWTH AND REDUCES FETAL PLASMA IGF-II BUT NOT IGF-I.

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Repeated acute maternal ethanol (EtOH) treatment, mimicking ‘binge drinking’, causes a near complete cessation of growth in the fetal sheep. Insulin-like growth factor-I and -II (IGF-I and IGF-II) are important regulators of fetal growth, and circulating levels are reduced in other models of fetal growth retardation. We therefore hypothesised that decreased fetal circulating IGFs might mediate some of the effects of acute alcohol exposure on reduced fetal growth.

EtOH (1 g/kg maternal weight, n=8) or saline (n=6) was administered to twin-bearing, chronically catheterised ewes for 1 h on 3 consecutive days at 0.8 of gestation. Fetal and maternal plasma were collected prior to and 6 h after infusions each day, and also 1, 2, 3 and 4 h after infusion on the first day. Fetuses were weighed at postmortem two days after the final infusion. Plasma was subjected to size-exclusion high pressure liquid chromatography at pH 2.5. IGFs and IGF-binding capacity were measured by RIA in fractions containing free IGFs and IGFBPs respectively, and analysed by repeated measures ANOVA. Maternal alcohol treatment reduced fetal body weight (Control: 2.66 ± 0.16 kg; EtOH: 2.13 ± 0.12 kg; \( p=0.02 \)), but did not change fetal plasma concentrations of circulating IGF-I or IGF-binding capacity. There was tendency for a treatment x time interaction (\( P=0.082 \)) for fetal plasma IGF-II, which tended to be reduced in the EtOH group 54 h after the first alcohol infusion (\( P=0.099 \)), although not at other times. Maternal and hence fetal alcohol exposure reduced fetal IGF-II by ~26% after 3 days, but this may not be sufficient to account for the extent of fetal growth restriction observed, suggesting that other mechanisms are also involved.

<table>
<thead>
<tr>
<th>Table 1. LIH characteristics of RE and RN ewes before and after ram introduction. Values differ within treatment: *P&lt;0.01, **P&lt;0.005, ***P&lt;0.001.</th>
<th>RN</th>
<th>RE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse per 6 hours</td>
<td>Before infusion</td>
<td>1.28 ± 0.50</td>
</tr>
<tr>
<td>After infusion</td>
<td>3.20 ± 0.37***</td>
<td>4.49 ± 0.81***</td>
</tr>
<tr>
<td>Mean concentration (ng/ml)</td>
<td>Before infusion</td>
<td>0.27 ± 0.03</td>
</tr>
<tr>
<td>After infusion</td>
<td>1.67 ± 0.49***</td>
<td>1.40 ± 0.50***</td>
</tr>
<tr>
<td>Pulse amplitude (ng/ml)</td>
<td>Before infusion</td>
<td>1.61 ± 0.09</td>
</tr>
<tr>
<td>After infusion</td>
<td>1.71 ± 0.46</td>
<td>2.18 ± 0.96</td>
</tr>
<tr>
<td>Basal concentration (ng/ml)</td>
<td>Before infusion</td>
<td>0.31 ± 0.08</td>
</tr>
<tr>
<td>After infusion</td>
<td>0.55 ± 0.23***</td>
<td>0.64 ± 0.351***</td>
</tr>
</tbody>
</table>
THE REGULATION OF APOPTOTIC GENES DURING SPONTANEOUS APOPTOSIS USING AN IN-VITRO EXPLANT CULTURE MODEL.

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Apoptosis has a key role in placental development and disease. Previous studies have observed that apoptosis occurred rapidly following incubation of placental villi without trophic support and the free radical scavenger superoxide dismutase (SOD) suppressed apoptosis in the placenta. The SOD model enables the regulatory genes in apoptosis to be explored in an in-vitro situation in the first trimester human placenta. The objective of this study was to identify genes that are up-regulated using the well established in-vitro explant culture model. The expression of various genes related to apoptosis including bcl-x, bax, caspase -3, PARP, Secreted Frizzled-related protein 4 (SFRP-4), Frizzled-4 and β -catenin were studied using Real time RT-PCR and Western blots. The activation of Caspase-3 and PARP was identified with fluorescent substrate analyses. As reported previously, an increase in DNA internucleosomal fragmentation, a hallmark feature of apoptosis was evident in placental cultures in serum free conditions. Interestingly, the apoptosis was associated with an increase of caspase-3 and PARP activity. The real time PCR data showed no significant difference between the bax and bcl-x genes. mRNA for a secreted frizzled related protein 4 (sFRP-4) a novel gene known to be associated with apoptosis was expressed particularly in the first trimester placenta. SFRP-4 did not appear to change in incubation with or without SOD. Frizzled 4 showed an inverse pattern of expression. β -catenin is expressed more in the term than in the first trimester placenta. The in-vitro explant culture model appears to be an excellent model to examine the genetic changes associated with apoptosis in the human placenta.

CONTRIBUTION OF CLAUDIN-11 TO THE INTER-SERTOLI CELL TIGHT JUNCTION, IN VITRO

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The inter-Sertoli cell tight junction (TJ) forms the blood testis barrier (BTB) between Sertoli cells and is composed of three major transmembrane proteins claudin-11, occludin and junctional adhesion molecule. Formation of the BTB occurs during puberty associating with an increase in circulating gonadotrophins. Claudin-11 and occludin are hormonally regulated in vitro although their importance to the function of the TJ is unknown. The aim of this study was to investigate the contribution of claudin-11 to the inter-Sertoli cell TJ in vitro by blocking gene expression using RNA interference. Two claudin-11 specific siRNA fragments were designed for this purpose. Sertoli cells in primary culture formed stable TJs within 5 days as measured by transepithelial electrical resistance (TER). The addition of siRNA for 2 days resulted in a significant (p<0.01) 55% (mean, SD, n = 4 cultures) decrease in TER along with a major reduction in claudin-11 localisation to the TJ as assessed by immunocytochemistry. The specificity of the siRNA was shown by the presence of extensive immunostaining of occludin and of the adherens junction protein β -catenin in the same treatments. Similarly, claudin-11 mRNA expression significantly (p<0.01) decreased by 71% (mean, SD, n = 3 cultures) in response to both claudin-11 siRNA fragments. Occludin mRNA expression was not affected. It is concluded that claudin-11 contributes at least 55% to the function of the rat Sertoli cell TJ in vitro. It is hypothesised that the remaining 45% of TJ function can be attributed to other integral proteins, such as occludin and junctional adhesion molecule. It is expected that claudin-11 and other TJ proteins play a pivotal role in the function of the BTB in vivo with potential implications in fertility and contraception.
MEIOSIS ARREST IN A NEW ANIMAL MODEL--A MUTATION ON CHROMOSOME 5

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Many causes of male infertility are currently unknown and there is a great need for the identification of new genes involved in spermatogenesis. The chemical mutagen, N-ethyl-N-nitrosourea (ENU) was utilized to induce random point mutations in the germline of C57BI-6J mice in order to generate models of male infertility. 3 generation breeding programs produced mice homozygous for ~3-4 point mutations. Following a phenotype-based screen of 1650 G3 males from 155 pedigree's, 15 lines were identified with abnormal male fertility parameters consistent with a recessive mutation. One infertile line, ENU23, exhibited a complete meiosis arrest with no germ cells ever proceeding to become spermatozoa. No infertile females have been observed. Light microscopic examination of Bouin's fixed paraffin embedded testis sections showed that the arrest occurred post-prophase and prior to the completion of metaphase I, since normal metaphase was not observed. In cells beyond the prophase stage of development, spermatocytes appeared condensed, became enlarged and subsequently underwent cell death by apoptosis confirmed by TUNEL analysis. Electron microscopy visualized normal synaptonemal complexes. The degenerating primary spermatocytes exhibited condensed chromosomes irregularly arranged on a poorly formed microtubular spindle, indicating an abortive attempt to complete metaphase. The percentage of tubules with diplote/metaphase-like cells was calculated for 3 abnormal and 3 normal ENU23 mice and showed 10.8% compared to 1.37% respectively, indicating that prior to cell death the cells undergo a lag period. To identify the ENU23 causal mutation, the mutation was bred onto a mixed C57BI-6J/CBA background and linkage analysis was performed, identifying a region on chromosome 5 between microsatellite markers D5Mit7 and D5Mit32. Candidate genes within this region are currently being sequenced in the search for the ENU-induced mutation causing the ENU23 phenotype. We believe this model of metaphase arrest is unique and will provide insights into the male specific events of meiosis.

REGULATION OF INHIBIN BINDING AND ACTION VIA BETAGLYCAN EXPRESSION IN MOUSE LEYDIG-LIKE TM3 CELLS

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The actions of inhibin and transforming growth factor (TGF)-β2 are enhanced when their respective target cells express the TGF-β/inhibin co-receptor, betaglycan. In the present studies, we investigated the effects of multiple members of the TGF-β superfamily on betaglycan expression, and examined the consequences of such regulation for inhibin binding, and inhibin and TGF-β actions in mouse Leydig-like TM3 cells.

Isoforms of activin (A and B), TGF-β (1 and 2), and BMP (2, 6 and 7) each suppressed the level of betaglycan mRNA in TM3 cells to 43-46%, 26-39%, and 50-71% of control, respectively, during overnight treatment. Subsequent inhibin A binding was suppressed to 72-77%, 35-36%, and 66-70% of control, respectively, with IC50s of 0.07-0.7, 0.05-0.5, and 0.4-0.6 nM, respectively. The effects of inhibiting betaglycan expression by TM3 cells on their responses to inhibin and TGF-β2 were examined by transfecting cells with a promoter construct that contains three copies of the activin-responsive sequence of the GnRHR promoter (3XpGRAS-PRL-lux) either alone or in the presence of small (21 bp) duplex siRNAs corresponding to the betaglycan gene. Activin A (0.5 nM) stimulated 3XpGRAS-PRL-lux expression 3-4 fold over control in TM3 cells, and inhibin dose-dependently abolished this stimulation, with no interference from the control siRNA (against BF-1 forkhead-like protein). However, inhibin suppression of activin-stimulated activity was antagonized in cells co-transfected with betaglycan siRNA. TGF-β (1 and 2) stimulated 3XpGRAS-PRL-lux expression 5-8 fold over control, and the action of TGF-β2, but not TGF-β1, was attenuated by the betaglycan siRNA.

In summary, activin, TGF-β and BMP isoforms inhibit betaglycan expression by Leydig-like TM3 cells, and inhibit A binding is commensurately reduced. The “knock-down” of betaglycan expression by specific siRNA inhibits TM3 responses to inhibit and TGF-β2. Whether the inhibition of betaglycan expression by activin, TGF-β and BMP has similar consequences for inhibin and/or TGF-β2 action is yet to be determined. These studies raise the possibility that multiple members of the TGF-β superfamily participate in cross-talk via the inhibin/TGF-β co-receptor, betaglycan.

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OVER-EXPRESSION OF ACTIVIN βC IN VIVO REVEALS A ROLE IN MALE FERTILITY

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Introduction: Activin βC subunit antagonises the formation and bioactivity of activin A via intracellular heterodimerisation and decreases activation of the activin signalling pathway (Mellor et al, 2003). Therefore the activin βC subunit heterodimers provide a new mechanism of regulating activin levels. Vedja and colleagues over-expressed the activin βC subunit in malignant liver cell lines which subsequently displayed inhibition of cell proliferation and induction of apoptosis (Vedja et al. 2003). Conversely, Wada et al. demonstrated that treatment with hr-activin C stimulates growth of a liver cell line (Wada et al., 2004). These recent (and contradictory) reports about the in vitro activity of activin βC have prompted us to examine the in vivo role of activin βC by creating a transgenic mouse over-expressing the βC activin subunit.

Methods: The full length human cDNA under the control of a CMV promoter was incorporated into the genome of three founder C57/B6 mice. Genotyping was performed by both Southern and PCR. Mice were monitored weekly and culled at 14-16 weeks (adult). Blood was collected by cardiac puncture, organs were weighed and a portion fixed in Bouin's or frozen for subsequent RNA and protein extraction. Daily sperm production (DSP) was determined by standard methods. Sertoli and germ cell number will be determined using the optical dissector (sic) stereological technique in Bouin's fixed resin sections. Proliferation and apoptosis will be examined using PCNA and TUNEL respectively. Activin A was assessed by ELISA, while FSH, LH, follistatin and total inhibin were determined by RIA.

Results and conclusions: Over-expression of activin-βC resulted in decreased circulating activin A (p < 0.005, TG1, p < 0.05 TG2 and p = 0.08 TG3), a progressive age related decrease in litter sizes (9.3 WT vs 6.3 TG1, 5.8 TG2 and 4.5 TG3; p < 0.005 vs WT) and testicular DSP (p < 0.05). These data support the hypothesis that βC is a novel in vivo regulator and is the first indication of a role for activin-βC in male fertility. This novel mouse model will significantly advance our understanding of the in vivo role of activin-βC.

(1) Mellor et al., Endocrinology 2003, 144, 4410-4419
(2) Vedja et al., Carcinogenesis 2003, 24, 1801-1809

SEPTIC SHOCK AND SEPSIS: A COMPARISON OF TOTAL AND FREE PLASMA CORTISOL LEVELS

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Relative adrenal insufficiency (RAI), defined as failure to maintain an adequate cortisol secretion in the setting of severe illness, is associated with rapid clinical and haemodynamic improvement following low dose glucocorticoid therapy. Decreased cortisol-binding proteins rather than unbound (free) cortisol may account for diminished cortisol in critical illness. The purpose of this study was to examine the relationship between total and free cortisol and cortisol-binding proteins in septic shock (with and without RAI) and sepsis. We measured total and free cortisol and the cortisol-binding proteins, corticosteroid-binding globulin (CBG) and albumin, before and after tetracosactrin in septic shock (SS, n=45), sepsis (S, n=19) and healthy controls (HC, n=10). One-third of septic shock patients (15/45) had RAI but none in sepsis patients. RAI patients had higher basal total cortisol (1157 vs 756 nmol/L, P=0.028) and basal free cortisol (287 vs 140 nmol/L, P=0.013) than non-RAI patients. Mean cortisol increments in RAI were lower than non-RAI patients had higher basal total cortisol (SS 880 vs S 417 vs HC 352 nmol/L, P<0.001). Stimulated free cortisol increments were more significant in SS than S and HC (SS 474 vs S 576 vs HC 524 nmol/L, P=0.013). These differences in cortisol increments between patient groups may be due to altered levels of cortisol-binding proteins. Our findings suggest that total cortisol may not be a reliable guide to bioavailable cortisol in systemic infection, as the septic state is associated with marked changes in levels of cortisol-binding proteins. However, the attenuated cortisol increment in response to ACTH seen in RAI is not due to altered levels of binding proteins. Overall, free cortisol appears to be a better guide to circulating glucocorticoid activity than total cortisol in systemic infection.
THE VALUE OF SYNACTHEN DURING ADRENAL VEIN SAMPLING IN DIFFERENTIATING ALDOSTERONE PRODUCING ADRENAL ADENOMA FROM BILATERAL ADRENAL HYPERPLASIA.
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Background: Whilst adrenal vein sampling (AVS) is accepted as the “gold standard” for determining lateralization in primary hyperaldosteronism, the usefulness of synacthen is unclear and a consensus on interpretative criteria is unavailable. This study aims to ascertain the value of synacthen use during AVS in the differentiation of aldosterone producing adrenal adenoma (APA) and bilateral adrenal hyperplasia (BAH). Method: Of the 30 consecutive AVS performed, 10 had incomplete collection. AVS involves a peripheral, right and left adrenal vein sample drawn before and 15 minutes after an intravenous bolus of 250 mcg synacthen. Lateralization was defined by: a) A/C ratio of affected side > 4 times the unaffected side [i], plus b) A/C ratio in the unaffected side < periphery A/C ratio. [ii] Results: 20 patients were hypertensive, 6 had serum potassium < 3.5mmol/L. Twelve patients had pre and post synacthen results which concurred on the presence/absence of lateralization. One patient had a 6cm adrenal mass and AVS results did not alter surgical management. In the remaining 7 patients, synacthen clarified the results by: 1) lateralizing APA with low pre-synacthen A/C ratio differential with improved hypertension post adenoma removal (n = 1), 2) distinguishing BAH with a dominant gland which equalized post synacthen (n = 3), 3) confirming APA by suppression of the unaffected A/C ratio compared to periphery (n = 1), 4) alerting clinicians of spurious samplings (n = 2), one of whom later had an APA resected. Of the 11 patients with APA diagnosed post synacthen testing (Refer Table 1), 10 proceeded to adrenalectomy with subsequent adenoma on histology. All had normokalemia post surgery and 7 had improved hypertension. Conclusion: Synacthen assisted interpretation of borderline results and prompted the decision to successful adrenalectomies in an additional 3 patients with APA while alerting clinicians to spurious results in 2 patients.

(1) Young WF et al. Surgery 1996;120:913-920

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THE EFFECTS OF PHYSIOLOGICAL TESTOSTERONE THERAPY ON BODY COMPOSITION AND MARKERS OF CARDIOVASCULAR RISK IN NON-OBESE AGEING MEN: A 12-MONTH PLACEBO CONTROLLED TRIAL.
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Background: Male ageing is associated with a declining serum testosterone (T), increasing fat mass (FM) and decreasing fat free mass (FFM). These changes are partially reversible with testosterone replacement therapy (TRT) but there is no data specifically regarding non-obese men. The influence of these changes on cardiovascular (CV) risk is also uncertain. Aim: To describe the effect of TRT on body composition and CV risk markers in non-obese ageing men. Methods: Men ≥55yrs with androgen deficiency symptoms participated in a double blind RCT of TRT (Androderm® 5mg patch/day) or placebo; baseline BMI<30kg/m², waist circumference (WC)<102cm and mean serum T<15nM. Body composition (DEXA and MRI) and CV markers (lipids, insulin resistance and vascular function) were measured at 0 and 52 weeks. Results: 60 men aged 62.9±1.1yrs (mean±SEM) (BMI 26.1±0.5 kg/m² and WC 94.8±1.6 cm) entered and 42 completed the study (TRT n=17, placebo n=25; delayed hypersensitivity in 30% TRT). Serum T rose by 30% with TRT (13.6±0.5 to 17.7±1.2nM; p=0.01) but fell with placebo (14.5±0.6 to 13.3±0.5nM; p=0.04). LH fell by 50% with TRT. Relative to placebo visceral fat decreased with TRT (-6.8±4.8 vs. +16.6±5.0%; P=0.002) although abdominal subcutaneous fat and total FM did not change; FFM was increased (+1.3±0.8 vs. -0.53±0.4%; P=0.03). Total and LDL-cholesterol increased by 10% with placebo (P=0.01) although between group change was not significant; insulin resistance and vascular function did not change. Conclusion: Physiological TRT, relative to placebo, prevents the modest gain of visceral fat and loss of FFM in a symptomatic non-obese male cohort with low-normal T levels. Obese men (with their greater baseline visceral fat and lower T levels) are likely to experience a more pronounced loss of visceral fat which in turn may improve their CV risk profile.

Acknowledgements: NHMRC #169020, National Heart Foundation and Mayne Pharma Australia Pty Ltd
**HOW IS PROTEIN METABOLISM PERTURBED IN CUSHING’S SYNDROME?**

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Cushing’s Syndrome (CS) causes marked diminution of body protein. Body protein status is maintained by a dynamic equilibrium between proteolysis, oxidation and synthesis. To understand the mechanisms by which glucocorticoid excess causes protein loss, we studied whole body protein metabolism in 18 subjects with CS and 18 normal subjects matched for age (42±3 vs 47±3yrs) and weight (75±4 vs 72±4kg). Mean UFC in CS was 1471±329 nmol/d, N<300). LBM and fat mass (FM) were assessed by DXA. Whole body protein turnover was studied with a 3h primed constant infusion of 1-[13C] leucine, from which rates of leucine appearance (LRa, index of protein breakdown), leucine oxidation (Lox, index of protein oxidation) and leucine incorporation into protein (LIP, index of protein synthesis) were estimated. FM was significantly higher and LBM lower in subjects with CS. LBM was significantly related (r>0.65, p<0.005) to LRa, Lox and LIP in both groups. FM significantly correlated (r>0.39, p<0.02) to LRa and LIP, but not Lox. In multiple regression, LBM and FM independently correlated with LRa and LIP, while LBM, but not FM, correlated with Lox.

<table>
<thead>
<tr>
<th></th>
<th>FM (kg)</th>
<th>LBM (kg)</th>
<th>LRa$^a$ (umol/min)</th>
<th>Lox$^b$ (umol/min)</th>
<th>LIP$^c$ (umol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>31.7±2.3</td>
<td>37.1±1.7</td>
<td>130.4±4.7</td>
<td>28.3±1.5</td>
<td>102.0±4.1</td>
</tr>
<tr>
<td>Normal</td>
<td>24.2±2.6</td>
<td>43.1±2.4</td>
<td>119.5±4.7</td>
<td>23.7±1.5</td>
<td>95.8±4.1</td>
</tr>
</tbody>
</table>

After correcting for LBM and FM, Lox was greater in CS, while no significant differences in LRa and LIP were present between groups. In summary, FM is increased and LBM reduced in CS. Both FM and LBM significantly and independently influence indices of protein metabolism. After correcting for differences in body composition, Lox, but not LRa or LIP, is increased in CS. As oxidation represents irreversible protein loss, sustained enhancement of protein oxidation explains reduced protein mass and progressive protein loss in CS.

**REGULATION OF SOCS3 EXPRESSION BY PROSTAGLANDIN, PROLACTIN AND GROWTH HORMONE: CHALLENGING THE JAK/STAT SIGNALLING DOGMA.**

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SOCS3 is an inhibitor of various cytokine-receptor signalling pathways and is therefore involved in suppression of cellular responsiveness to these critical regulators. SOCS3 expression is thought to be regulated by a STAT Responsive Element (SRE). However, our research suggests the involvement of other signalling pathways. In T-47D breast cancer cells, we found that PGE2 induces a 3-5 fold increase in SOCS3 mRNA, as determined by Real-Time PCR. This effect was not due to phosphorylation of STATs, or inhibited by the Jak2 inhibitor, AG490, but was inhibited by the PI3Kinase inhibitor, LY294002, Akt Inhibitor IV and partially inhibited by the PKA inhibitor, H89. It was not affected by inhibitors of MEK, PDK1, mTOR or p38-MAPK. We concurrently examined PRL-induced SOCS3 expression, and found that although STAT1 and 5 phosphorylation was increased, SOCS3 expression was again inhibited by Akt Inhibitor IV and H89 but unaffected by AG-490. To explore this further, we used a model of GH signalling, BaF3 cells stably expressing GH receptor. GH induced a 15 – 20 fold increase in SOCS3 mRNA, which was accompanied by increased STAT5 phosphorylation. However the SOCS3 response was not inhibited by AG-490 or H89, but was diminished by Akt Inhibitor IV. Analysis of the SOCS3 promoter revealed a FOXO binding site. When we mutated this site in a mouse SOCS3 promoter-luciferase construct, basal and GH-induced promoter activity was significantly increased. These results are consistent with FOXO acting as a repressor, which is inactivated by Akt. We propose that in T-47D cells, SOCS3 expression involves cross-talk between PI3K/Akt and cAMP/PKA, whereas in BaF3 cells, expression is enhanced by Akt phosphorylation and subsequent FOXO inactivation. These findings contrast with the accepted Jak/STAT regulation of SOCS3 expression. This work is supported by the Australian Research Council.
IDENTIFICATION OF A FUNCTIONAL BINDING SITE ON BETAGLYCAN FOR INHIBIN AND TGFβ

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Betaglycan is a co-receptor that modulates signaling by TGFβ superfamily members including TGFβ's and inhibins. Loss of betaglycan expression, or blocking of betaglycan function, has been implicated in several human diseases and in animal disease models. However, characterization of the superfamily ligands and receptors involved in these disease states is complicated because of the pleiotropic nature of betaglycan co-receptor action. Here we report the identification and characterization of a domain within the extracellular region of betaglycan that binds inhibin-A and TGFβ-1. We show that both ligands bind to the membrane proximal region (amino acids 591-760) of the betaglycan extracellular domain. This inhibin/TGFβ-binding region is within the ZP-domain of betaglycan, but is not integral to the conserved ZP motif. Using deletion studies and site-directed mutagenesis, we show that the inhibin and TGFβ binding sites on betaglycan overlap and identify individual amino acids essential for the binding of both ligands. In particular, point mutant V614Y abolishes inhibin and TGFβ binding to the membrane proximal domain of the betaglycan ECD. A full-length betaglycan construct containing this point mutation can still bind TGFβ-1 via a separate N-terminal binding site, but is unable to bind inhibin-A. This mutant is incapable of mediating inhibin's antagonism of activin or BMP signalling. Mutation of betaglycan V614Y thus separates the co-receptor actions of betaglycan for inhibin and TGFβ. This will allow the clarification of the role of betaglycan in human disease states such as renal cell carcinoma and endometrial adenocarcinoma.

PROGESTERONE REGULATES CXCL14 (MACROPHAGE INFLAMMATORY PROTEIN 2γ) mRNA IN HUMAN ENDOMETRIUM.

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The emergence of microarray technology has enabled a thorough study of the level of transcripts in the human body. A high density cDNA microarray analysis revealed a comprehensive list of transcripts, which were significantly different between mid-proliferative and mid-secretory phase endometrium (Borthwick et al., 2003). An EST was identified from the HG_U95B chip is identical to the 3'UTR of CXCL14 or macrophage inflammatory protein 2g (MIP 2γ). The level is 19-fold higher in the mid-secretory compared to the mid-proliferative phase of menstrual cycle. This has suggested the transcript level of CXCL14 may be directly regulated by progesterone. Northern hybridisation and in situ hybridisation confirmed the transcript level of CXCL14 (MIP 2γ) was high in the mid to late secretory endometrium and its mRNA was localised in the glandular epithelium of this tissue (Mokhtar et al., 2003). In silico analysis has predicted 6 progesterone response elements (PREs) within 2040 bp upstream from the ATG site. To investigate the possible functions of these PREs, a dual luciferase assay was performed on the Ishikawa cell line transfected with 5 deletion constructs of the gene promoter. Cells were co-transfected with progesterone receptor B (PRB) and treated with 10⁻⁶ M progesterone. Luciferase activities of these constructs have localised 2 fragments that were most likely to contain the active PREs, i.e. PRE1 and PRE2. An electrophoretic mobility shift assay showed PRE oligonucleotides within these 2 regions were able to bind with PRB that was synthesised in vitro, although there was a stronger signal seen in the PRE2 region. A dose competition study revealed PRE1/PRB and PRE2/PRB protein binding could be competed with different concentrations of cold wild type competitor oligonucleotides. Mutagenesis of PRE 1 and PRE2 analysed by luciferase reporter assay reduced the inductive effect of progesterone treatment. This study presents indicates that progesterone induced transcript encoding a chemokine in the human endometrium may likely act as a chemoattractant for leucocytes during secretory phase of menstrual cycle.

PLATELET DERIVED GROWTH FACTORS AND RECEPTORS CONTRIBUTE TOWARD DEVELOPMENT OF THE CORPUS LUTEUM

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In this study the expression of the family of platelet derived growth factors (PDGF) and receptors in the ovarian corpus luteum was identified and characterized, and an effect of their activity on development of the corpus luteum revealed. Gonadotropin-stimulated immature rats were utilized as a model of induced ovulation, luteogenesis and pseudopregnancy, and levels of mRNA for platelet derived growth factors (PDGF-A, PDGF-B, PDGF-C and PDGF-D) and receptors (PDGF-Rα and PDGF-Rβ) in response to gonadotropins were investigated. Intraovarian injection of immature rats with pregnant mare's serum gonadotropin (PMSG) followed 54 hours later with human chorionic gonadotropin (hCG) resulted in a significant increase in ovarian mRNA levels for PDGF-Rβ and its ligands, PDGF-B and PDGF-D, as early as 4 hours after hCG injection. Gonadotropin regulation of PDGF-B was confirmed by in vitro promoter-reporter assays, which showed a 2-3 fold increase in PDGF-B promoter activity in response to luteinising hormone (LH), and inhibition studies implicated protein kinase A, phosphatidylinositol 3-kinase and mitogen activated protein kinase signaling pathways in the LH-induced upregulation. In the corpus luteum, PDGF-Rα was localized to a subset of luteal steroidogenic cells, and PDGF-Rβ to cells of the luteal microvasculature. PDGF-A, PDGF-B and PDGF-C were also identified in a population of luteal steroidogenic cells. Intraovarian injection of an inhibitor of PDGF receptor activity, the tyrphostin AG1295, prior to injection of hCG in PMSG-primed immature rats resulted in a significant 22.85 +/- 10.7% decrease in corpora lutea per treated ovary in comparison to the contralateral vehicle-injected control ovary. In addition, the treated ovary of 3 of 12 rats showed widespread hemorrhage throughout the entire ovary, indicating a possible role for PDGF receptor activity in maintenance of the ovarian vasculature. In summary, these data identify expression of members of the family of platelet derived growth factors and receptors in cells within the corpus luteum and reveal a role during development of the corpus luteum.

CHARACTERISATION OF FLJ22318 IN PROSTATE CANCER CELLS

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FLJ22318 is a novel protein that was shown in a yeast two-hybrid screen to bind to NNX3.1, a largely prostate-specific homeodomain protein that is involved in controlling proliferation and maintaining the differentiated state of adult prostatic epithelium. Loss of NNX3.1 expression in prostate cancer is strongly associated with androgen independent disease and advanced tumour stage. FLJ22318 expression has been reported in cDNA libraries derived from a range of different tissues, suggesting that it is widely expressed and potentially interacts with a number of proteins. Analysis of the FLJ22318 amino acid sequence identified a lissencephaly type-1-like homology domain (LisH), a C-terminal to LisH domain, three putative nuclear receptor boxes and a newly reported CRA domain. As FLJ22318 has not been studied previously, its binding partners, function and the effects of its interaction on NNX3.1 activity in prostate cancer cells is unknown. Following cloning of full-length FLJ22318 cDNA, interaction between FLJ22318 and NNX3.1 was confirmed by GST pull-down and co-immunoprecipitation analyses carried out using V5-tagged NNX3.1 and GFP-tagged FLJ22318 expressed in DU145 and LNCaP prostate cancer cells. In addition, the cellular localisation of FLJ22318 was examined by cloning FLJ22318 into the pCMV-Myc mammalian expression vector, with FLJ22318-Myc fusion proteins detected using antibodies directed against the Myc epitopes. Confocal microscopy indicated co-localisation of FLJ22318 and NNX3.1 in the nucleus and perinuclear region of LNCaP cells and in DU145 cells luciferase assays demonstrated FLJ22318 increased the transcriptional repressor activity of NNX3.1 on a consensus NNX3.1 response element. Using a yeast two-hybrid screen and full length FLJ22318 as bait, 33 prospective binding partners were identified including NDK2 a reputed tumour metastasis suppressor. Ongoing studies will characterise the FLJ22318 interaction with NDK2, providing new insights into the biological activity of FLJ22318 in prostate cancer cells.
INVESTIGATION OF THE CELLULAR FUNCTION AND INTERACTIONS OF SLIRP, A NOVEL NUCLEAR COREPRESSOR OF THE ESTROGEN RECEPTOR PATHWAY.

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SRA (Steroid Receptor RNA Activator)\(^1\), the only known RNA coactivator, plays an important role in transactivation of the estrogen receptor (ER). SRA expression is aberrant in many human breast tumours suggesting a potential role in pathogenesis. The structure of SRA is complex, containing multiple stem-loops, some of which contribute to transactivation capacity\(^2\). We have previously reported the discovery of SLIRP\(^3\) as a novel RNA Recognition Motif (RRM) containing SRA-binding protein and nuclear receptor corepressor. We isolated a previous uncharacterised molecule referred to as SAF like Transcription Repressor (STR7) of SRA via a yeast three-hybrid screen of a human primary breast cancer library, and confirmed this binding via IP-RT-PCR and REMSA assays. Although we found SLIRP is a predominantly mitochondrial protein, with chromatin immunoprecipitation (ChIP) assays we have also shown that it can be recruited to endogenous estrogen response elements within target genes in the nucleus. Here we further detail the cellular function and protein interactions of SLIRP. We generated several mutants of SLIRP, including mutations in the RRM domain and mitochondrial localisation sequence. These mutants each reduce SLIRP's repressive ability. In addition, a SRA STR7 mutant also abolishes SLIRP's repressive effect on SRA, suggesting that the specific interaction with STR7 is important for SLIRP's repressive function. We found that cotransfection of SHARP\(^4\), another ER corepressor that has been shown to bind SRA, augments the repressive effects of SLIRP. In addition, SHARP binds to the same SRA stem-loop, STR7, in vitro. In preliminary siRNA experiments targeting SLIRP, we have observed an effect on SRA coactivation in the GR and ER signaling pathways. In summary, these studies demonstrate both the importance of the RRM and mitochondrial signal sequences for SLIRP's repressive activity, and a new set of interactions with SHARP. These data suggest a broad functional role for SLIRP as a regulator of nuclear gene expression.


SRA IS A TARGET FOR BOTH SLTM AND SAFB

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The actions of nuclear receptors (NR) are modulated by an array of positive and negative acting co-factors. While the majority of these are proteins a notable exception is Steroid Receptor RNA Activator (SRA) which functions at least in part as a non-coding RNA. Over expression of SRA augments NR transactivation and is itself the target of RNA Recognition Motif (RRM) domain containing coregulators. We isolated a previously uncharacterised molecule referred to as SAF like Transcription Modulator (SLTM) that contains a SAP DNA binding domain, an RRM and overall has a high degree of homology with the estrogen receptor interacting molecule SAFB. Over expression of SAFB represses estrogen signalling and its deregulated expression has been associated breast cancer progression. SLTM also represses both steroid dependent and independent reporter constructs. To characterise these molecules further we initially expressed wild type, SAP and RRM deletion mutant constructs in Hela and MCF-7 cells. Loss of the SAP DNA binding or RRM domains did not affect the nuclear localisation of SLTM or SAFB proteins. In subsequent ERE-luc reporter assays, transcriptional repression by wild type or SAP deletion mutant SAF and SLTM constructs were similar however preliminary studies suggested that loss of the RRM domain may compromise the repressive activities of SLTM. Binding studies demonstrated that SLTM and SAFB RRM/GST fusion proteins both bound endogenous SRA while regions lacking this motif did not. IP-RT-PCR analysis comparing wild type and RRM deletion proteins further confirmed this association in whole cells. These data show that the RRMs of SLTM and SAFB are functional and define them as new members of the SRA binding protein family. SRA binding by SLTM and SAFB is consistent with their regulation of steroid dependent pathways and may therefore influence the growth and ultimately disease progression in steroid dependent tissues.
CONTRASTING ROLES FOR NOVEL SRA-BINDING PROTEINS IN NUCLEAR RECEPTOR PATHWAY CO-REGULATION BETWEEN DIFFERENT MALIGNANT CELL LINES.

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SRA (steroid receptor RNA co-activator) (1) is the only described RNA co-activator for nuclear receptor (NR)-mediated signaling. We have identified a family of double stranded RNA-binding proteins (dsRNABP) - PACT, TARBP and PKR – which bind SRA ‘in vitro’ and ‘in vivo’, associate with estrogen response elements of endogenous estrogen controlled genes in Chromatin immunoprecipitation (ChIP) assays and bi-directionally modify SRA-mediated co-activation of estrogen action in transfection. Furthermore, SRA and PACT synergize, both with each other and with SRC3, but not SRC 1 and 2, in the context of estrogen regulated transactivation, and combine to overcome tamoxifen and ICI182780 inhibition of estrogen signaling. Subsequent studies have shown SRA and the dsRNABPs co-regulate a range of Type I (glucocorticoid (GR), androgen (AR)) and Type II (thyroid (TR), Vitamin D (VDR)) receptors in Hela cells in a similar pattern to that seen for estrogen signaling. Synergy between SRA and PACT was observed in all Type I but no Type II NR interactions. Expanding these studies across a number of cell lines, SRA effects ranged from a 300% stimulation of estrogen signaling in Hela and prostate cancer PC3 cells through to a 25% inhibition in MCF7 cells. Interestingly, endogenous concentration of PKR, a dsRNABP we have shown to inhibit estrogen signaling, predicted the SRA effect, with the highest levels recorded in MCF7 cells and lowest in PC3 and Hela cells. This suggests variance of SRA-dependant PKR recruitment may be a regulatory mechanism for estrogen signaling control. In contrast PACT-dependant transactivation was most potent in MCF7 cells, which display the highest levels of SRC3. In summary, co-regulatory actions of SRA and dsRNABP are constant across a range of promoters within a cell line but show significant variations between cell lines. Differing levels of co-regulators, and their recruitment to target promoters, are implicated as a mechanism.


MOLECULAR BASIS OF AROMATASE OVER-EXPRESSION IN OVARIAN GRANULOSA CELL TUMOURS

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Adult granulosa cell tumours (GCT) of the ovary possess characteristics of FSH stimulated pre-ovulatory granulosa cells, and exhibit elevated aromatase expression1. The molecular basis of this remains unknown. The aims of this study were to quantify mRNA expression of aromatase and its putative transcriptional regulators in GCT and normal ovaries and to identify the transcriptional mechanisms driving aromatase over-expression in GCTs. Transcripts levels were quantified in a panel of GCT (n=8), normal premenopausal ovaries (n=9) and GCT-derived KGN cell line using real time RT-PCR. Transfections and electrophoretic mobility shift assays (EMSA) were conducted to analyse the transcriptional regulation of aromatase. Aromatase mRNA expression was approximately 17-fold higher in GCT than in normal ovaries. Aromatase transcripts were derived from the gonadal type promoter, promoter II (PII) in both normal and GCT tissues. Since PII activity can be stimulated by members of the NR5A family of nuclear receptors Steriodogenic Factor-1 (SF-1) and/or Liver Receptor Homologue-1 (LRH-1), mRNA levels of these receptors were quantified. Whereas SF-1 expression was unchanged in normal and GCT tissues, LRH-1 mRNA was ~30-fold higher in GCT than in normal ovary, suggesting that aromatase over-expression is driven by LRH-1 in GCT. However, in individual tumours, aromatase expression correlated significantly with SF-1 (r=0.64, p<0.005) rather than with LRH-1 (r=0.15, p=0.3). KGN cells exhibited a similar profile to GCT: high aromatase and LRH-1, low SF-1 expression. Transfections showed both recombinant SF-1 and LRH-1 can stimulate PII transcription. However, the binding activity in KGN cell nuclear extracts was predominantly SF-1, not LRH-1. Our results indicate that although GCTs over-express aromatase and LRH-1, LRH-1 does not appear to be responsible for aromatase expression in GCTs. Rather; our observations support a direct role for SF-1 in the induction of estrogen biosynthesis during GC tumourgenesis. The role of LRH-1 over expression in GCT remains to be determined.

CD151 PROMOTES THE MIGRATION AND INVASION PROPERTIES OF THE PROSTATE CANCER CELL LINE LNCAP.

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Introduction: Prostate cancer is the second commonest cancer in the world. However, the molecular mechanisms underlying prostate cancer development and progression remain poorly understood. Tetraspanin family member CD151 has been reported as an “adaptor” between integrins and signal pathways. In this study, we created a mutant (QRD194-196 to INF) CD151 cDNA. The CD151 cDNA and mutated CD151 cDNA were transfected into prostate cancer cell line LNCap. The changes in proliferation, migration and invasion properties were evaluated after transfection. Methods: The expression of CD151 in wide-type and transfected LNCap cells was confirmed by Western Blot. The CD151-QRD194-196 to INF mutant was generated using QuickChange 2 site directed Mutagenesis Kit (Stratagene). LNCap cells were transfected with wide type CD151 cDNA, mutated CD151 cDNA and vector (negative control) using FuGENE transfection reagent (Roche). Cell proliferation was measured by Trypan blue dye exclusion assay and anchorage independent growth assay. Cell migration and invasion assays were carried out with a cell invasion kit (BD). Results: There was no difference in proliferation between wild-type and CD151 transfected LNCap cells (P>0.05). The LNCap cell transfected with the CD151-mutant grows more slowly compared to the other cell lines (P<0.01). The same trend was found in an anchorage independent growth assay (P>0.05, P<0.01). There were more CD151-transfected cells vs. control cells observed on both control migration assay and matrigel-coated invasion assay membranes (P<0.01, P<0.01). Fewer numbers of mutant-transfected cells were found on the membranes for both migration and invasion studies (p<0.01, p<0.01). Conclusion: CD151 overexpression does not change the proliferative properties of the prostate cancer cell line LNCap, but does promote migration and invasion. These data suggest that CD151 plays a specific role in promoting prostate cancer cell motility.

INTERACTIONS BETWEEN THE ANDROGEN RECEPTOR AND MITOGEN ACTIVATED PROTEIN KINASE PATHWAYS IN BREAST CANCER CELLS

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Androgen receptor (AR) expression is upregulated in human breast tumours with androgens such as 5α-dihydrotestosterone (DHT) inhibiting the growth of breast cancer cells. This study has investigated the effects of the mitogen-activated protein kinase (ERK/MAPK) pathway on AR levels and activity. Hyperactivation of ERK/MAPK signaling in breast cancers has been associated with overexpression of the HER2 cell surface receptor, low oestrogen receptor levels and poor responsiveness of tumours to antioestrogen therapies.

Hyperactivation of ERK/MAPK signaling by stable transfection of the human breast cancer cell line, MCF-7 with a constitutively active ΔMEK1 (C3 cells), did not alter basal or DHT induced increases in AR protein levels. Similarly, prevention of ERK/MAPK activation by culture of MCF-7 cells in the presence of a MEK-specific inhibitor, U0126 did not inhibit DHT-induced increases in AR protein levels indicating that ERK/MAPK is not essential for ligand-induced receptor stabilisation. However, ERK activity may be required to maintain basal AR protein as AR levels were reduced overall in U0126-treated MCF-7 cultures.

Cytoplasmic localisation of the AR in the absence of DHT was not altered in C3 cells in comparison to parental MCF-7 cells and treatment of MCF-7 cells with U0126 did not impede AR translocation to the nucleus in the presence of DHT. However, DHT treatment of C3 cells resulted in a more rapid nuclear translocation of the AR (10 minutes following DHT treatment), suggesting that ERK/MAPK hyperactivation facilitated trafficking of the AR in the presence of DHT.

Importantly, DHT was growth inhibitory to both MCF-7 and C3 cells, with \(10^{-10}-10^{-7}\)M DHT resulting in ~40-60% inhibition of proliferation following 8 days of treatment. These studies suggest that MCF-7 cells with hyperactivated ERK/MAPK signaling remain susceptible to DHT and provide further support for the development of AR-targeted therapies for breast cancer treatment.
X-LINKED INHIBITOR OF APOPTOSIS PROTEIN EXPRESSION AND CHEMORESISTANCE IN OVARIAN CANCER

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Epithelial ovarian cancer is the most lethal gynaecological cancer in the Western world and ranks fourth among the most common female cancers. Cisplatin therapy has recently been linked to high levels of the inhibitor of apoptosis (IAP) family of proteins. XIAP has been shown to bind to, and prevent activation of, Caspase-3,-7 and -9, a key group of intrinsic apoptotic signalling molecules. Up-regulation of XIAP in response to Cisplatin treatment has been indicated as a potential mechanism for chemoresistance in ovarian cancer. Two cell lines were used, representing a chemoresistant (SKOV-3) and chemosensitive (OVCAR) cancer lineage. Cells were seeded at 25,000 cells/ml and grown for three days in 6-well plates. Media was removed and cells treated with media (control) or 20μM Cisplatin in media. After 24 hours treatment cells were harvested, DNA, RNA and protein was isolated. Cisplatin significantly (p<0.001) increased DNA fragmentation in both cell lines but the degree of DNA fragmentation was much greater in the OVCAR cell line. XIAP protein was found to be down-regulated between control and treatment groups in the SKOV cell line, and below detectable levels in the OVCAR. AIF protein was found similar in both cell lines. Expression of XIAP, caspase 3 and AIF mRNA was analysed in OVCAR and SKOV cells. The levels of expression of XIAP mRNA was significantly (p<0.05) decreased in both cell lines. Caspase-3 mRNA levels increased significantly (p<0.05) only in SKOV treated cells. The mRNA levels of AIF were significantly reduced in both cell lines. These results suggest that XIAP plays a significant role in the regulation of apoptosis in human ovarian cancer cells and their sensitivity to Cisplatin. Cell survival factors are an important determinant in chemoresistance and serve as potential molecular targets for the development of novel therapies for chemoresistant ovarian cancer.

DIFFERENTIAL KINETICS AND AFFINITIES OF LIGAND-INDUCED G-PROTEIN COUPLED RECEPTOR INTERACTIONS WITH BETA-ARRESTINS AS DETECTED BY EXTENDED BIOLUMINESCENCE RESONANCE ENERGY TRANSFER.

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G-protein coupled receptors (GPCRs) are seven transmembrane receptors that interact with intracellular scaffolding proteins such as beta-arrestins (Barrs) in a ligand-dependent manner. Class A GPCRs (eg. beta2AR, TRHR2) preferentially interact with Barr2 in a weak and transient manner, with dissociation occurring shortly after movement of the receptor from clathrin-coated pits into endosomal vesicles. In contrast, Class B GPCRs (eg. AT1AR, TRHR1, V2R) form stronger, more stable interactions with both Barr1 and Barr2, trafficking together into deep core endosomes. The aim of this study was to investigate the interactions between GPCRs (Class A and B) and beta-arrestins (1 and 2) using bioluminescence resonance energy transfer (BRET). The GPCRs and arrestins were tagged with either donor (Rluc), or acceptor (EGFP) and by using the strict dependence on the molecular proximity between donors and acceptors for energy transfer we were able to monitor GPCR-arrestin interactions in living cells. Using a new long-acting Rluc substrate, EnduRen™, we attempted to elucidate the extended use of beta-arrestins by a range of GPCRs. Classically it was thought that signalling ceased once a receptor was internalised. However, there is now increasing evidence for a receptor/beta-arrestin complex acting as a secondary signalling platform, potentially enabling a host of signalling pathways to be activated hours after initial GPCR activation. Therefore, it is becoming increasingly important to be able to monitor such complexes in live cells over this timeframe. Our results show that prolonged ligand-induced receptor-arrestin interactions occur with both Class A and Class B receptors, reflecting either a steady state of successive transient interactions occurring over time and/or prolonged interactions of individual proteins. The BRET ratios observed with Class A receptors are significantly lower than those observed for Class B receptors, even at maximal agonist doses, thereby corroborating previous evidence for distinct Class-dependent differences in the strength of receptor/arrestin interactions.
MAPPING OF FUNCTIONAL DOMAINS OF OESTROGEN RECEPTOR-α THAT MEDIATE REGULATION OF GROWTH HORMONE SIGNALLING BY OESTROGEN AND SERMS

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We have recently reported that oestrogen inhibits while selective oestrogen receptor modulators (SERMs) potentiate GH signalling. To understand the molecular basis by which ER mediates the opposite effects of oestrogen and SERMs, we undertook structure-function studies of ERα. ERα is a modular protein with a DNA binding domain (DBD) and two activation functions, AF1 and AF2 (also known as LBD) (Figure). AF1 and AF2 mediate the ligand-independent and dependent transcriptional action of ER, respectively. We studied the functions of three murine ERα mutants in mediating the effects of 17β-oestradiol (E2), 4-hydroxytamoxifen (4HT) and raloxifene (Ral) on GH activation of JAK2/STAT5 signalling. The mutants were designated DBD-AA (inactivated DBD), ΔAF1 (AF1-deleted) and ΔAF2 (AF2-deleted). HEK293 cells stably expressing human GH receptor were transfected with a STAT5-responsive luciferase reporter and expression plasmids with wild type (WT) or mutant ERα, and then treated with 500ng/ml GH and 100nM of E2, 4HT or Ral. In cells expressing WT ERα, E2 inhibited while SERMs augmented GH-induced reporter activity (Figure). In cells expressing DBD-AA, there were no effects. In ΔAF1-expressing cells, E2 exerted a lesser effect, whereas SERMs had no effects. In ΔAF2-expressing cells, the GH-induced reporter activity was increased constitutively. In summary, (i) DNA binding of ERα was crucial for mediating the effects of E2 and SERMs, (ii) both AF1 and AF2 were required for maximal E2 inhibition, and (iii) AF1 was sufficient for mediating the enhancing effects of SERMs. We conclude that different regions of ERα are responsible for mediating the disparate effects of oestrogen and SERMs on GH signalling. (This work was supported by NHMRC)

DIRECT COMPARISON OF THE EFFECTS OF RALOXIFENE AND OESTROGEN DURING GH THERAPY ON BODY COMPOSITION IN HYPOPITUITARY WOMEN

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Oral oestrogen (E) attenuates GH action (1). The effects of selective estrogen receptor modulators on GH action is unknown. We compared the effects of raloxifene (R, 60mg/d) with E (17β-oestradiol 2mg/d) in 16 hypopituitary women treated with GH for 24 months. They were randomised into 2 groups in a parallel open-label cross-over study. One group (n=8) received E for the first 6m before cross-over to R for the remaining 18m; the other received the reverse sequence. Lean body mass [LBM], fat mass [FM] and lumbar spine bone mineral density [LSBMD] by DEXA were measured at 0, 6, 12 and 24m with IGF-I and IGFBP-3. Treatment effects were analysed as a 6m cross-over comparison in both groups and 18m longitudinal comparison after cross-over in each group by least-square ANOVA model. Results are expressed as mean % change±SE from baseline or from cross-over.

<table>
<thead>
<tr>
<th></th>
<th>Cross-over 6 months</th>
<th>Longitudinal 18 months</th>
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<tbody>
<tr>
<td></td>
<td>E</td>
<td>R</td>
</tr>
<tr>
<td>LBM</td>
<td>3.3±2.2*#</td>
<td>0.2±2.2</td>
</tr>
<tr>
<td>FM</td>
<td>-4.2±2.3*#</td>
<td>-1.3±2.3</td>
</tr>
<tr>
<td>LSBMD</td>
<td>2.8±1.4*</td>
<td>0.6±1.4</td>
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GH treatment significantly increased IGF-I and IGFBP-3 levels. While the IGF-I increase did not differ between treatments, the rise in IGFBP-3 was greater (p<0.05) with R (36.4±8.4 vs 22.6±8.4%). During the 6m cross-over evaluation, GH significantly reduced FM and increased LBM and LSBMD with E but not R; these differences between treatments were significant for LBM and FM. Over the last 18m from cross-over, GH treatment induced a further fall in FM, increased LSBMD and a trend towards higher LBM with E but not R. These changes differed significantly between treatments. Changes in IGFBP-3 correlated positively with FM (p=0.03) and negatively with LBM (p=0.06) and LSBMD (p=0.08). In conclusion R attenuates GH action more than E. The mechanism is unknown but may involve IGFBP-3 modulation. (Supported by the Swiss National Foundation, Lilly Australia, NHMRC of Australia)

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OVARIAN HYPERSTIMULATION AND AMENORRHOEA SECONDARY TO PITUITARY ADENOMA COSECRETING FSH AND PROLACTIN

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Ovarian hyperstimulation is most commonly seen as a complication of ovulation induction therapy. Rarely, elevated endogenous gonadotrophins may result in such a presentation. A 43 year old Sudanese refugee was referred for investigation of pelvic pain and secondary amenorrhoea at a gynaecological outpatient clinic. She was noted to have marked uterine enlargement and was placed on the waiting list for a hysterectomy. She was also referred for assessment of progressive visual loss occurring over the past 3 years. At presentation, she was only able to see shadows and shapes. A CT scan of the brain revealed a pituitary macroadenoma and she was referred for neurosurgery. Notable findings on examination included visual acuity of hand movements only, no galactorrhoea and a large non-tender midline pelvic mass, equivalent to a 20 week gravid uterus. An ultrasound showed bilaterally enlarged ovaries with multiple cysts, and a large fibroid uterus. Biochemistry revealed markedly elevated oestradiol levels of 7971pmol/L, FSH 33.1U/L, LH 1.7 U/L, prolactin 5147 miU/L. Other pituitary investigations revealed an early morning cortisol 291nmol/L, TSH 1.95 m U/mL, T4 12 pmol/L and IGF-1 0.17U/mL. MRI demonstrated a giant pituitary macroadenoma deforming the optic chiasm, third ventricle and corpus callosum. Following trans-sphenoidal surgery, FSH, prolactin and oestradiol levels fell. There was a modest improvement in vision. The uterine enlargement regressed such that hysterectomy was no longer required. Immunohistochemical staining was positive for FSH and prolactin. Commencement of cabergoline resulted in prolactin suppression but no additional tumour shrinkage. Further debulking surgery was undertaken. While most clinically non-functioning pituitary adenomas arise from a gonadotroph lineage, increased FSH secretion resulting in ovarian hyperstimulation is uncommon. This case represents only the second reported case in the literature characterized by FSH and prolactin co-secretion.

ACROMEGALY SECONDARY TO INTRA-THECAL MORPHINE ADMINISTRATION FOR CHRONIC PAIN

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Acromegaly is an uncommon condition characterized by excessive growth hormone (GH) secretion. Over 99% of cases are caused by a pituitary adenoma. There has been extensive previous research regarding the effects of endogenous opioid peptides and narcotic drugs on the endocrine system. Morphine inhibits corticotrophin and gonadotrophin secretion, and elevates prolactin. While the intravenous administration of morphine has no effect on GH or TSH levels in humans, both these hormones increase in response to both intracerebroventricular (icv) and intravenous (iv) morphine in laboratory animals. There is a small body of evidence which indicates the icv administration of morphine in cancer patients may elevate GH levels, at least in the short term. Since this mode of analgesia is generally reserved for patients with a terminal disease, opportunities to study the long term effects of this form of analgesic delivery have been limited. A 48 year old woman had a history of a chronic pain syndrome which had been treated with continuous intra-thecal morphine for several years. Other symptoms included sweating and an inability to wear her rings. Examination revealed clinical features of acromegaly. Investigations demonstrated an elevated IGF-1 of 2.18U/mL (reference range 0.39-1.18). An oral glucose tolerance test showed failure to suppress GH with a nadir of 2.6miU/L at 2h (normal range <2.0miU/L). A day profile of GH showed a mean level of measured hourly between 3.1 and 4.7 miU/L. She had documented corticotrophin and gonadotrophin deficiency, with mild hyperprolactinaemia, but normal thyroid function. Her MRI pituitary showed an empty sella, with no evidence of a pituitary adenoma. Treatment with Lanreotide Autogel 90mg per month has resulted in symptomatic improvement and reduced IGF-1 to 0.6U/mL (0.39-1.18). This is likely to represent the first reported case of acromegaly and partial hypopituitarism induced by chronic intra-thecal morphine.
EFFECT OF SPORTING TYPE ON GROWTH HORMONE-RESPONSIVE MARKERS IN ELITE ATHLETES.
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Biochemical markers of the IGF system and of collagen turnover are potential indicators of growth hormone (GH) abuse in sport. The aim was to investigate any associations between sporting type and these markers in elite athletes. Serum samples were obtained from elite athletes (586 M, 350 F) aged 22±5 years. Concentrations of IGF-I, IGFBP-3 and ALS and of bone turnover markers: N-terminal propeptide of type I collagen (PINP), C-terminal telopeptide of type I collagen (ICTP), and N-terminal propeptide of type III collagen (PIIINP) were measured by radioimmunoassay. Analysis was performed of 7 sporting categories: athletics (n=80), combat (n=179, including boxing, judo), endurance (n=95, including cycling, marathon, triathlon), power (n=45, including weightlifting, hammer), power/endurance (n=171, including rowing, swimming), racket (n=67) and team ball sports (n=299). Data were adjusted for age, sex, BMI and ethnicity in a multiple linear regression model.

IGF-I was significantly higher (p<0.001) in power sports and lower (p<0.005) in team ball and combat sports (adjusted means±SE: power 180±6.6, team ball 146±2.7, combat 149±3.3 μg/L). Similar differences were observed for IGFBP-3, PINP, ICTP and PIIINP were all significantly higher (p<0.001) in combat sports and lower (p<0.02) in racket sports. Similar differences were observed using analysis of z scores derived for each individual and stratified by decile. The proportion of variance accounted for by sporting type for adjusted IGF markers was 2-6% (total explained variability 18-28%), and for adjusted collagen markers was 4-5% (total explained variability 21-34%).

In conclusion, using this sports classification, IGF axis markers in general were higher in power sports whereas bone turnover markers were significantly higher in combat sports. Sporting type however accounted for a minor proportion of variance of these GH-responsive markers compared to age and gender. (Supported by the World Anti-Doping Agency and Australian Government Anti-Doping Research Program).

ABERRANT STRESS RESPONSES IN AUTONOMIC FAILURE SYNDROMES: MECHANISMS AND POTENTIAL CLINICAL IMPLICATIONS.
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Introduction: Neuroendocrine, behavioural and autonomic cardiovascular responses to the 35% CO₂ challenge, a novel means of investigating the stress response in humans, have been studied in several forms of autonomic failure.

Methods: Responses to a single breath of 35% CO₂ in healthy controls were compared to patients with diabetic autonomic neuropathy (DAN), multiple systems atrophy (MSA) or pure autonomic failure (PAF). The normal response includes initial bradycardia due to direct vagal stimulation followed by a noradrenaline mediated pressor response. HPA axis activation (cortisol and ACTH), prolactin release and emotional arousal also occur.

Results: Compared with normal controls, in DAN, baseline and stimulated cortisol, prolactin, systolic blood pressure and emotional arousal responses were normal. However, these patients failed to demonstrate any of the expected CO₂-induced bradycardia (p<0.0001). MSA and PAF subjects showed smaller and delayed pressor responses (p<0.01 for all), whilst MSA was also associated with a smaller cortisol response and fewer somatic symptoms of emotional arousal. MSA showed a blunted reduction in skin blood flow (SBF) response compared with normal controls, whilst PAF showed an increase in SBF (p<0.0001 for all).

Conclusion: Explanations for observed differences and clinical implications are proposed based on differences in the pathophysiology of each condition. The CO₂ test clearly distinguishes subjects with various forms of autonomic neuropathy and could be an important adjunct in the investigation and diagnosis of these conditions.
THE COUPLING OF ACTH AND CORTISOL SECRETION IS GREATER IN FEMALE THAN MALE SHEEP UNDER BASAL CONDITIONS

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There are substantial differences between the hypothalamo-pituitary-adrenal (HPA) axes of males and females, and an incomplete understanding of the regulatory mechanisms underlying these differences. We have measured the concentrations of ACTH and cortisol in plasma of male and female sheep (n=5/group) over 8 hours under basal conditions. Blood samples were obtained every ten minutes. The resulting hormone profiles were analysed using entropy and cross-correlation analysis. Entropy analysis revealed that the approximate entropy (ApEn) of ACTH profiles was higher in female than male sheep (P<0.05), though no sex differences in ApEn were observed for cortisol profiles. Cross approximate entropies for ACTH-cortisol and cortisol-ACTH were also not different between the sexes. When cross correlation analyses were performed on matched profiles of ACTH and cortisol concentrations, a significant (P<0.05) positive correlation was observed in female sheep at 10 mins, with significant (P<0.05) negative correlations observed at 60 and 70 mins. In contrast, no significant correlations were observed between the hormone profiles in male sheep. The correlations seen in the female animals were also greater (P<0.05) than those seen in the male animals at 10 and 70 mins. Taken together, these observations indicate a greater control of ACTH secretion in female sheep than male sheep under basal conditions. In addition, there is evidence of a stronger, short-term (10 mins), positive drive of cortisol secretion by ACTH in female sheep than male sheep, and more tightly coupled negative feedback of ACTH secretion by cortisol in female sheep with a time lag of 60 to 70 mins. Therefore, these results indicate that under basal conditions the HPA axis of the female sheep is under tighter control than that of the male. Further investigations are targeted at determining the nature of the control of the axes of males and females under situations of stress.

REFERENCE INTERVALS FOR REPRODUCTIVE HORMONES IN HEALTHY FERTILE YOUNG MEN: EVALUATION OF AUTOMATED PLATFORM ASSAYS.

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Introduction: The management of infertility and/or androgen deficiency in men requires accurate hormonal measurement with valid reference intervals. This study aimed to develop a valid reference panel of blood samples from healthy young men to examine the performance of 7 fully automated multiplex immunoassay platforms used for the measurement of serum total testosterone (T), luteinizing hormone (LH) and follicle stimulating hormone (FSH).

Methods: A reference panel of sera from 124 healthy reproductively normal men (age 21-35yrs) with normal sperm output were used. The coherence and consistency between different commercial automated immunoassays for total T, LH and FSH were evaluated according to 7 different immunoassay methods. T concentration was also measured by gas chromatography/mass spectrometry (GC/MS).

Results: Descriptive statistics and reference intervals for serum T, LH and FSH differed widely and significantly between methods but variation between laboratories were negligible. None of the T methods corresponded well with the independent GC/MS reference methods with all showing significant deviations in regression slope and intercept, in deviance plots and reference intervals. The lower T reference limit ranged from 7.5 to 12.7 nmol/L whereas the upper limit ranged from 25.8 to 34.4 nmol/L compared with 10.4 – 29.8 for GC/MS. Although similar method differences existed between gonadotropin assays, the discrepancies were smaller with a reference interval for FSH (1.3-8.4 IU/L) and LH (1.6-8.0 IU/L); limits differed from manufacturers quoted values.

Conclusion: We conclude that substantial differences between commercial T immunoassays and their major divergences from the GC/MS standard affect their clinical diagnostic utility. These findings point to the need for substantial improvements in automated T immunoassay technologies or a switch to GC/MS. Gonadotropin assays showed less variability, potentially allowing the use of a common reference interval, but currently used intervals were suboptimal for assisting in the diagnosis of azoospermia or androgen deficiency.
INTERACTION BETWEEN TESTOSTERONE AND APOE E4 ON COGNITION IN NORMAL OLDER MEN: THE FREMANTLE ENDOCRINOLOGY OF AGEING RESEARCH STUDY.

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Reduced testosterone levels have been implicated as a potential causative factor in cognitive decline with old age. However, no studies have examined whether effects of testosterone to influence cognition in healthy older men might be modulated by genetic predisposition for dementia. We investigated the association between serum testosterone concentrations and cognitive functioning in healthy older men, taking into account apolipoprotein E (APOE) ε4 status. Healthy men (n=45) had fasting early morning blood samples for testosterone and sex hormone binding globulin (SHBG) and were assessed for mood and indices of general cognition, verbal and visual episodic memory, executive functioning, working memory and attention. There was a significant interaction between calculated free testosterone (CFT) and APOE ε4 on general cognitive function (p=0.01) and executive function, working memory and attention (p<0.01). Higher levels of CFT were associated with better general cognitive performance in non-ε4 carriers (p=0.01). By contrast, in ε4 carriers, higher CFT levels were associated with lower scores on tests of executive functioning, working memory and attention (p=0.02). Men who possess the APOE ε4 allele have an increased risk of developing Alzheimer’s disease, but higher testosterone levels in these men were associated with worse cognitive performance. Cross-sectional and prospective studies of cognition in older men should allow for an interaction between testosterone and APOE genotype by stratifying men based on APOE ε4 status.

ANDROGENS ACT DIRECTLY THROUGH THE ANDROGEN RECEPTOR IN SKELETAL MUSCLE TO MAINTAIN MUSCLE MASS

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We are studying the anabolic actions of androgens that are mediated directly through the androgen receptor (AR) in skeletal muscle, using the cre/lox system to generate muscle-specific AR knockout (mARKO) mice. To generate mARKO mice, we bred together two mouse lines: the floxed AR line, with exon 3 of the AR gene flanked by loxP sites; and the α-actin-cre line, in which cre is expressed predominantly in proliferative myoblasts and post-proliferative muscle fibres.

We examined skeletal muscle mass in 12 week old male mARKO mice (n=9), compared to three groups of control males: wild-type (n=7), floxed AR (n=5) or α-actin-cre (n=9) littermates. Muscle mass is reduced in a muscle-specific manner in the mARKO male mice. The highly androgen-responsive levator ani (LA) muscle is reduced by 51% in mARKO males compared to all control male groups (p<0.001, one-way ANOVA, Tukey’s post-hoc analysis). The fast-twitch tibialis anterior muscle mass is reduced by 15% in mARKO vs control male littermates (p<0.01). The slow-twitch soleus is reduced by 12% in mARKO vs floxed AR and cre male controls (p<0.05), whereas there is no change in the mass of the fast-twitch EDL or the mixed-fibre gastrocnemius muscles. Preliminary data from another mARKO mouse line, generated using an MCK-cre line that expresses cre only in post-proliferative muscle fibres, indicates that the mass of the LA is also reduced by 50% in MCK-cre mARKO mice (n=5, p<0.001).

These data demonstrate that the anabolic actions of androgens are mediated in part via direct actions through the AR in skeletal muscle. We are currently examining the level of AR mRNA expression in all muscles of the mARKO mice, to determine if the differences in muscle mass relate to the degree of AR deletion in the different muscles, or reflect different levels of androgen responsiveness of these muscles.
DECREASED SKELETAL MUSCLE MASS AND STRENGTH IN MALE BUT NOT FEMALE UNIVERSAL ANDROGEN RECEPTOR KNOCKOUT MICE

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We are studying mechanisms via which androgens increase muscle size and strength. To investigate actions mediated directly through the androgen receptor (AR), we generated universal AR knockout (ARKO) mice. Universal ARKO mice have exon 3 of the AR gene deleted in all tissues, leading to an in-frame deletion of the 2nd zinc finger of the DNA binding domain. Male ARKO mice have female external genitalia, confirming this deletion abolishes AR function.

We examined the mass of hind limb muscles including the fast-twitch extensor digitorum longus (EDL) and tibialis anterior (TA), the slow-twitch soleus (SOL), and mixed fibre gastrocnemius (GAST), from 9 wo. mice (n=12-16/grp). Data were analyzed by one-way ANOVA and Tukey's post-hoc analysis. Muscle mass was decreased in male universal ARKO mice. The TA mass was reduced by 23% in universal ARKO vs control males (p<0.001), and was the same as control females. The mass of other muscles in universal ARKO males, including the EDL, SOL and GAST, was reduced by 11-20% vs control males (p<0.001), but was higher than control females. The highly androgen-responsive levator ani (LA) muscle did not develop in universal ARKO males. There was no difference in muscle mass between universal ARKO female and control female mice, for all muscles examined. In vitro functional testing was performed on the EDL and SOL muscles (n=6/grp). Maximal force production of the EDL was reduced by 18% in universal ARKO males vs control males (p<0.01). There was no significant difference in the SOL, nor was there any difference in the fatigue resistance of the ARKO male vs control male muscles.

Our data demonstrate that the genomic actions of the AR are required in males to mediate the anabolic actions of androgens in fast- and slow-twitch muscles. We are currently examining potential molecular targets mediating these actions.

AROMATASE-OVEREXPRESSING MICE (AROM1) GAIN EXCESS ADIPOSE TISSUE.

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Estrogen (E2) is known to play a significant role in the regulation of energy homeostasis, specifically in the accretion and distribution of adipose tissue. Previously we have demonstrated that mice lacking aromatase expression (ArKO mice) become fat and administration of exogenous estrogens reverses the phenotype1. The aromatase over-expressing (Arom+) mouse model was recently generated, in which aromatase is ubiquitously and permanently expressed under control of the ubiquitin C promoter2.

In male Arom+ mice, serum E2 concentrations were elevated while testosterone (T) levels were markedly reduced3. Interestingly, both male and female Arom+ mice appeared to gain adipose tissue in excess of their wildtype (WT) littermates, hence we are currently characterising this observation. Six month old female Arom+ mice were significantly heavier than WT littermates (Arom+ 43.38g ± 2.11, n=10 vs WT 35.28g ± 2.90, n=5; p = 0.04; mean ± SEM), with concomitant heavier visceral fat pads (discrete pad dorsal to kidney) masses (0.63g ± 0.07, n=10 vs 0.37g ± 0.06, n=5; p = 0.03). A trend existed for gonadal fat pads to be heavier and their adipocyte complement to be greater in Arom+ female mice. Adipocyte density in the gonadal fat pads was not different between the genotypes. Older (40-52 weeks old) male Arom+ mice were significantly heavier than WT littermates (Arom+ 54.57g ± 1.47, n=4 vs WT 43.77g ± 1.26, n=3; p < 0.001; mean ± SEM), correlated with significantly heavier gonadal fat pads (Arom+ 1.82g ± 0.14, n=4 vs WT 0.65g ± 0.03, n=3; p = 0.007). Livers of both Arom+ genders were pale in colour, indicative of hepatic steatosis. These preliminary results indicate that in the presence of excess circulating estrogens, the finely-regulated balance governing fat storage and/or utilisation is compromised, and expands our current understanding of the role E2 plays in fat homeostasis. Analyses are ongoing.

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DIHYDROTESTOSTERONE INHIBITS ACTIVATION OF AMPK IN ADIPOSE TISSUE OF FEMALE MICE
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There is evidence that androgens influence adipose tissue metabolism and insulin resistance in women. However, the signalling pathways that mediate these effects of androgens remain poorly understood.

Acetyl-CoA carboxylase (ACC) plays a critical role in lipid metabolism by catalysing the ATP-dependent formation of malonyl-CoA. Malonyl-CoA inhibits carnitine palmitoyltransferase 1, the enzyme that controls the transfer of long-chain fatty acyl residues from the cytoplasm to the mitochondria where they are oxidised. The activation of 5'-AMP-activated protein kinase (AMPK) phosphorylates ACC which results in inhibition of fatty acid synthesis and stimulation of fatty acid oxidation. Here we show that phosphorylation of AMPK and ACC is impaired in adipose tissue of mice treated with the active androgen, dihydrotestosterone (DHT). Mice were ovariectomised and DHT was administered via a subcutaneous pellet at 2 or 8µg/mouse/day for 6 weeks. DHT-treated mice gained significantly more weight than control mice (control; 1.2±0.10g, 2µgDHT; 5.2±1.7g, 8µgDHT; 7±1.05g) and had significantly increased gonadal fat-pad masses (control; 0.94±0.18g, 2µgDHT; 1.998±0.29g, 8µgDHT; 2.241±0.243g). Phosphorylation of AMPK and ACC was determined by Western blot in gonadal fat. Phosphorylation of AMPK and ACC was significantly decreased in DHT-treated mice compared to control mice. AMPK: control; 1.20±0.052 AU, 2µgDHT; 1.051±0.019 AU, ACC: control; 4.377±0.335 AU, 2µgDHT; 2.459±0.642 AU, 8µgDHT; 2.549±0.503 AU. In addition mice treated with DHT had significantly lower levels of hepatic insulin receptor substrate-1 (IRS-1) (control; 4.215±0.762 AU, 2µgDHT; 1.576±0.117 AU, 8µgDHT; 1.261±0.259 AU), which plays a key role in insulin signaling. Low expression of IRS-1 in target tissues of insulin action has been considered as a marker of insulin-resistant states such as obesity and diabetes.

This is the first study to show that androgens effect the activation of AMPK and ACC and has implications for the treatment of hyperandrogenic states in women such as polycystic ovary syndrome.

ROLE OF THE HU PROTEINS, HUR AND HUD, IN THE REGULATION OF ANDROGEN RECEPTOR EXPRESSION AND ACTIVITY IN PROSTATE CANCER CELLS.
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The primary treatment for prostate cancer (PCa) involves androgen ablation, halting tumour growth through down-regulation of important proliferative genes under control of the androgen receptor (AR) (1). Frequently, PCa progresses to an androgen-independent state and untreatable disease. Recent data indicates that the AR continues to be expressed in many of these tumours, often associated with androgen-independent activation of the AR signalling pathway. Thus, understanding the mechanisms that regulate AR expression in these cells is an important goal. We have previously shown that mRNA decay usually restricted to neurons, is expressed in a range of primary PCa samples. In these studies, we aimed to determine the functional role of the Hu proteins, HuR and HuD, and αCP binding sites reduce luciferase reporter activity when inserted 3' of the luciferase gene. Significantly, HuD, which is usually restricted to neurons, is expressed in a range of primary PCa samples. In these studies, we aimed to determine the functional role of the Hu proteins and αCP1 in the regulation of AR expression and activity in PCa cells. We found that AR protein levels were decreased in cells with levels of HuR or αCP1 reduced by RNAi treatment, and preliminary results indicate that knockdown of HuR alters half-life of AR mRNA. Taken together, these data implicate the Hu proteins, HuR and HuD, and αCP1, as novel AR mRNA-binding proteins that play an important role as regulators of AR expression and signalling in PCa cells. As such, they may represent potential therapeutic targets.

4) de Silanes IL, et al. Oncogene. 22: 7146-54
HORMONAL REGULATION OF PHOSPHORYLATED PROTEINS IN SEMINIFEROUS TUBULES; POSSIBLE INVOLVEMENT IN SPERMATION.

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Spermiation is the final step of spermatogenesis, where mature spermatids are released from Sertoli cells in the testis. The suppression of testosterone (T) and FSH causes spermiation failure in rats, monkeys and men, whereby spermatids are not released but are instead retained by the Sertoli cell and phagocytosed. As spermiation is a critical determinant of sperm output from the testis, elucidation of the mechanisms involved in spermiation will aid in male contraceptive development. We have previously shown that an unknown adhesion complex containing β₁-integrin is present during spermiated release¹, and in vitro data suggests that protein phosphorylation downstream of integrins may regulate spermiation. This study aimed to identify changes in phosphorylated proteins during spermiation and spermiation failure in order to gain further insights into the mechanisms of sperm release and retention. FSH and T was suppressed in adult Sprague-Dawley rats (n=4) via a combination of steroid implants, FSH immunoneutralisation and an androgen receptor antagonist (flutamide) treatment for 4 days. This treatment induced 90% spermiation failure. Control rats (n=4) received vehicle. Western blot analyses on whole seminiferous tubules assessed: phospho-serine, -threonine and -tyrosine proteins, MAP kinase ERK 1/2, (phosphorylated (active) and total-ERK forms) and FAK (non-phosphorylated forms and a specific FAK⁴³⁹⁷ form that is present at the site of spermiation). Hormone suppression caused a significant reduction in an ~82kDa tyrosine-phosphorylated band (p=0.02), a ~148kDa threonine-phosphorylated band (p=0.02), active ERK2 (p=0.03) but not ERK1, and non-phosphorylated FAK (p=0.002), but not FAK⁴³⁹⁷. Current studies are assessing these proteins during normal spermiation (using segments taken before and after sperm release), and in spermiation-specific segments with or without spermiation failure. In summary, these data support the hypothesis that the MAP kinase-FAK-integrin pathway is involved in regulating sperm release, and that this pathway is hormonally regulated in Sertoli cells.

(1) Beardsley & O'Donnell Biol Reprod 2003;68:1299

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ESTRADIOL INDUCTION OF MOUSE SPERMATOGENESIS REQUIRES A FUNCTIONAL ANDROGEN RECEPTOR

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Using the congenitally gonadotropin and androgen deficient hypogonadal (hpg) mouse, we showed that testosterone (T) and its non-aromatisable metabolite DHT induce qualitatively full spermatogenesis in the absence of gonadotropins (Endo 136: 5311, 1995). Yet surprisingly estradiol (E₂) has also been reported to induce spermatogenesis in hpg mice (Endo 141: 2861, 2000). We therefore undertook experiments to verify this observation and clarify its mechanism. Weanling homozygous hpg mice (n=7-8 per group) had Silastic implants (2, 1 & 0.5 cm) filled with E₂ (diluted 1:1000 with cholesterol) placed under the dorsal skin for a further 6 weeks. Testis weight was increased to a similar extent by all three E₂ doses compared with untreated hpg mice (27±4 vs 2.3±0.1 mg) and serum FSH was also increased (5.8±0.3 vs 0.6±0.1 ng/mL) into the low-normal range (4-25 ng/mL) using a mouse-specific DELFIa FSH assay (Biol Reprod 72:78, 2005).

To clarify the mechanism of E₂-induction of spermatogenesis, high dose E₂ treatment (3cm implant) was administered to mice (n=8 per group) with an exon 3-deleted non-functional androgen receptor (AR) created through crossing CMV-cre mice with a mouse line which has loxP sites flanking exon 3 of the AR introduced by homologous recombination. High dose E₂ fully suppressed LH levels (0.2±0.04 vs 4.2±0.4 ng/mL) but did not increase testis weight (5.7±0.2 vs 5.5±0.4 mg) or initiate spermatogenesis, whereas serum FSH levels were partly suppressed but remained very high (24±3 vs 77±6 ng/mL) compared with untreated AR null males or E₂-treated hpg mice. These findings suggest that a functional AR is required for E₂ induction of mouse spermatogenesis. The significance of the E₂-induced rise in serum FSH remains to be clarified.


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ISOFORM SPECIFIC FOLLISTATIN MOUSE MODELS
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Follistatin (FS) is a single-chain glycolysated protein initially identified in porcine follicular fluid by its ability to inhibit FSH secretion [1]. The expression of two splice variants from the FS gene results in the two isoforms; a 288 amino acid (FS-288) and a 315 amino acid (FS-315) protein. FS-288 binds to heparin sulfate proteoglycans and is tissue bound, while FS-315 is the circulating form.

Genetically engineered transgenic mice have been created to express these specific human isoforms in the absence of endogenous mouse FS expression. FS-KO mice die within hours of birth due to pulmonary and muscular defects [2]. Mice expressing human FS-288 are partially rescued and survive until 24 hours after birth, but still show similar defects to the FS-KO mice. In contrast, the mice expressing FS-315 survive until adulthood but show micro-opthalmia with disoriented whisker and fur growth. Further, they have considerably shorter tails due to gangrene at the tip of the tail, which is possibly due to impaired angiogenesis.

The FS-KO mice show defects in pulmonary structure with thickened interalveolar septa and limited alveolar spaces. This is essentially unchanged in FS-288 mice, but the FS-315 mice show essentially normal alveolar morphology. The renal medulla of FS-288 mice is small, but restored to normal in FS-315 mice. Skin of FS-288 and FS-KO mice is taut and shiny with an absence of dermal and epidermal ridges; however skin morphology appears essentially normal in FS-315 mice. The FS-288 mice also have a skeletal defect with only 12 ribs or a partial 13th; while FS-315 mice mostly have the full complement of 13 complete ribs. Finally, FS-315 males are fertile, however the FS-315 females are infertile despite showing evidence of a normal reproductive cycle. These data suggest that the FS isoforms may have specific functions that require further study.


DIFFERENTIAL EFFECTS OF EARLY PREGNANCY TREATMENT WITH NATURAL AND SYNTHETIC GLUCOCORTICOIDS IN SHEEP
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Over the past 10 years we have been studying the effects of treating sheep, early in pregnancy (26-28d, term ~150d) with the synthetic steroid dexamethasone (DEX) or the natural steroid, cortisol (F). Both treatments, had prolonged effects, long after the steroid treatment ceased, on the offspring--a 'programmed' effect. In both sexes the blood pressure was significantly increased by both steroid treatments, from 4 months after birth. In the case of the DEX offspring the hypertension was due to increased cardiac output; in the F offspring the hypertension was due to increased peripheral resistance. There were major differences in the gene expression in the brains of the 'programmed' sheep, depending on whether the early steroid used was DEX or F. In the DEX animals there was an upregulation of angiotensinogen gene expression in the hypothalamus, and of the AT1 receptor in the medulla oblongata. This had functional consequences, as the DEX offspring had significantly increased pressor responses to intra-ventricular infusions of graded doses of angiotensin II. There were no similar effects in the F offspring, in spite of an equal or greater hypertension. In the kidneys of both groups (DEX,F) there was a reduction in nephron number, of ~ 30%: a decrease in gene expression for components of the renin-angiotensin system (RAS) during active nephrogenesis, and and upregulation of RAS gene expression in the kidney after nephrogenesis was completed. These results show that differential effects are programmed by natural and synthetic glucocorticoid treatment early in development.
PEPTIDE PROFILE CHANGES BETWEEN LABOURING AND NON LABOURING HUMAN MYOMETRIUM IDENTIFIED USING SELDI-TOF MS

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Mechanisms controlling the transition of myometrium to a contractile phenotype are poorly defined. We aim to identify changes in the myometrial proteome associated with uterine activation using samples from Caesarean section prior to labour (NL, n=10) or following spontaneous term labour (n=4). Peaks of interest were also examined in placenta, decidua and fetal membranes.

Decidua, placenta and fetal membranes were collected and separated within 30 minutes of delivery. Proteins were extracted in Urea/CHAPS buffer (7M Urea, 2M Thiourea, 30mM Tris, 4% CHAPS, pH 8.5) . Protein profiles were generated by surface enhanced laser desorption/ionisation-time of flight mass spectrometry (SELDI-TOF MS) on strong anion or weak cation exchange ProteinChips. Peaks were determined as significantly different by Mann-Whitney U Test (p<0.05) and only those with a greater than 2-fold difference in mean peak intensity were examined.

In the myometrium, a total of 220 peaks were detected. Of these, 21 peaks were significantly different between term L and term NL with 11 increasing and 10 decreasing with labour. The Swiss-Prot (TagIdent) protein database was interrogated for proteins with matching mass/charge ranges and three peaks were identified as adrenomedullin (8.6 fold decrease in labour), corticotrophin releasing hormone (9-fold increase in labour) and α-defensin (4 fold increase in labour). α-defensin was also identified in all other intra-uterine tissues with elevated levels being detected in amnion and decidua with labour onset.

Using SELDI-TOF MS, changes in the myometrial proteome were successfully identified. The increase of α-defensin in the myometrium, decidua and amnion supports a potential inflammatory aetiology for labour with decreasing levels of adrenomedullin, a potent vasodilator, possibly involved in the switch of the myometrium to a contractile phenotype. There is a well characterised link between CRH levels and the length of gestation however its role in the initiation of labour remains unknown.

PROGESTERONE RECEPTOR PROTEIN EXPRESSION IN HUMAN FETAL MEMBRANES AND MYOMETRIUM WITH THE ONSET OF LABOUR:

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In all mammals, the onset of labour is associated with the withdrawal of the pro-pregnancy action(s) of progesterone. “Functional” progesterone withdrawal, mediated by decreased uterine responsiveness to progesterone, is thought to be involved in human parturition. Previous studies have suggested that desensitization to progesterone is due to changes in progesterone receptor (PR) expression.

The human PR exists in two forms: PR-A and PR-B. Generally, PR-B mediates the genomic effects of progesterone, whereas PR-A suppresses the actions of PR-B. Increasing PR-A:PR-B ratio is expected to decrease progesterone responsiveness. We have shown previously that PRA and PRB mRNA levels are very low in the amnion, chorion and placenta and are higher in the decidua and the myometrium. The onset of labour is associated with an increase in both mRNA isoform abundance in the myometrium, but not in the other tissues, resulting in a net increase in myometrial PR-A:PR-B mRNA ratio (1). The purpose of this investigation was to determine the expression of PRA and PRB proteins in these tissues at term labour.

We used western blotting to determine the abundance of PR proteins in labouring and non-labouring human amnion, chorion, decidua, placenta (n=12 each) and myometrium (n=8). Preliminary results show that PR-A protein is present in the amnion, chorion, decidua, and placenta before and during labour whereas PR-B is not detectable in any case. In the myometria, PR-A protein, but not PR-B protein, is detectable before labour. During labour, neither PR-A nor PR-B has been detected in the myometrium, suggesting a labour-associated loss of PR-A protein. Thus, PR mRNA and protein expression levels are in agreement in the fetal membranes and the placenta. In the myometrium, however, a loss of both PR proteins, in addition to increasing PRA:PRB mRNA ratio, might block progesterone action during term labour.

MODULATION OF PROGESTERONE RECEPTOR EXPRESSION BY CORTICOTROPHIN RELEASING HORMONE IN HUMAN MYOMETRIAL CELLS

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It is now generally accepted that in humans and higher primates a 'functional' progesterone withdrawal occurs in association with labour. One possible mechanism is through increased expression of progesterone receptor-A (PR-A), a repressor of progesterone actions mediated through progesterone receptor-B (PR-B). Corticotrophin Releasing Hormone (CRH) levels have been shown to increase exponentially with gestation. We hypothesise that at low concentrations, CRH stimulates both PR-A and PR-B via PKA mechanisms while at high concentrations of CRH, PKC mechanisms dominate leading to preferential synthesis of PR-A and functional progesterone withdrawal.

To test this hypothesis, we determined whether CRH had an effect on PR isoform expression in both primary human myometrial cells and in the PHM1-31 human myometrial cell line. Cells were exposed to CRH (0.001 to 10nM) for 18h. Relative abundance of PR-A and PR-B (normalised to 18S rRNA) was determined by quantitative real-time RT-PCR. We also tested for the presence of CRH receptors in cells from the two model systems.

mRNA for CRH-R1 and CRH-R2 was detectable in the primary cells and the myometrial cell line and both models displayed a similar response to CRH stimulation. Both PRs were increased at low concentrations of CRH with no change in the PR-A/PR-B expression ratio but at higher physiological concentrations (>0.1nM) a two-fold increase in the PR-A/PR-B expression ratio was observed.

These data support our hypothesis that early in gestation when CRH levels are low, the PR-A/PR-B expression ratio is low and the uterus remains quiescent. However, in late gestation, CRH increases, leading to a relative increase in the PR-A/PR-B expression ratio and a possible functional progesterone withdrawal.

HUMAN MYOMETRIAL HISTOLOGY AT TERM

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Studies have demonstrated that inflammatory cells infiltrate the human myometrium during spontaneous labour at term1. Recent murine studies2 have suggested that Surfactant Protein A (SP-A) levels rise during gestation, and cause migration of fetal macrophages into the uterus and subsequent activation may play a part in the initiation of parturition. To determine if evidence of inflammation exists in the human myometrium prior to the onset of labour, myometrium was sampled at caesarian section prior to labour (n=12, mean gest age 38.5, range 38.0 – 39.6, 9 male, 3 female fetuses). The samples were formalin fixed and paraffin embedded. The presence of macrophages was examined for using immunoperoxidase staining for CD68 and Chromogenic In Situ Hybridisation (CISH) was used to detect the presence of Y chromosome. Y chromosomes were demonstrated in the trophoblast layer in the samples from mothers carrying a male baby, but not in the body of the myometrium. No Y chromosome was demonstrated in samples from mothers carrying a female fetus. Fetal squames were found in maternal blood in four samples and confirmed by staining with anti-AE1/3 to broad spectrum cytokeratin. Variable degrees of muscle damage was seen in all samples and dislodgement of the endothelial lining was common. In spite of evidence of myometrial damage, very few inflammatory cells were identified. This data is more consistent with the inflammatory infiltrate seen in labour being a consequence of labour rather than an antecedent.

(1) Condon, J.C. et al. Surfactant protein secreted by the maturing mouse fetal lung acts as a hormone that signals the initiation of parturition. PNAS 2004(101):14, 4978-4983

(2) Thompson, A.J. et al. Leukocytes infiltrate the myometrium during human parturition: further evidence that labour is an inflammatory process. Human Reproduction 1999 (14):229-236
CORONARY HEART DISEASE IN ELDERLY WOMEN

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Coronary heart disease (CHD) accounts for nearly 50% of female deaths. The negative effects of exogenous estrogen on cardiovascular function have recently been described. However, the effects of endogenous estrogen on CHD or coronary events have not been reported for elderly women. The association between endogenous estrogen concentration and death due to CHD were studied in a prospective 6 year study of 1500 women mean 75±3 years. Estrogen and SHBG concentrations were measured at baseline. Cause of death was ascertained from death certificates. Cox proportional hazard model was used to examine time to death due to CHD or time to a coronary heart event. Over the 72 months of observation, 91 deaths were recorded of which 37 (40.6%) were attributed to CHD disease. Compared to patients surviving to 72 months, those patients who died of CHD had a higher concentration of estradiol and a higher prevalence of hypertension and CHD at baseline. There was an association of higher estradiol concentration with risk of death due to CHD before (HR per 1 SD increase 1.18, 95% CI: 1.02-1.37) and after adjustment for age, BMI and smoking (HR per 1 SD increase 1.23, 95% CI: 1.05-1.44). Further adjustment for baseline CHD and hypertension indicated that CHD (HR 5.55, 95% CI: 2.28-13.53) and hypertension (HR 2.44, 95% CI: 1.20-4.50) were significant independent predictors of CHD death (HR per 1 SD increase in FEI 1.21, 95% CI: 1.02-1.23 after adjustment). A similar analysis for time to diagnosis of CHD or death due to CHD (coronary heart event) showed that baseline estradiol concentration was associated with a coronary heart event before (HR per 1 SD increase 1.16, 95% CI: 1.03 – 1.31) and after adjustment for age, BMI and smoking history (HR per 1 SD increase 1.18, 95% CI: 1.03-1.35). SHBG concentration was not associated with coronary heart death. These data suggest that higher endogenous estrogen may be associated with increased risk of death due to CHD in elderly women.

A NEW APPROACH OF RECOMBINANT FSH DOSES IN SUB-FERTILITY PATIENTS WITH POLYCYSTIC OVARIAN SYNDROME.

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Introduction: Polycystic ovarian syndrome (PCOS) is the endocrinological disorder in female presenting with hirsutism, menstrual disturbance, anovulatory infertility and other signs acne and crown pattern baldness. These patients were induced with clomiphene citrate (CC). Patients who are resistant to CC are subjected to Gonadotrophin stimulation. Study comprises of different Protocol of recombinant FSH for ovulation induction so as to have unifollicular development and avoiding multiple pregnancies.

Material and Method: 100 patient were selected attending OPD of the Centre with PCOS. All were given Estrogen and Progesterone for one month before starting the induction. Two groups of 50 patients were formed:

In-group I recombinant FSH was started with 37.5 units and increasing in low dose step up protocol on alternate days for 4 cycles. In-group II recombinant FSH was started with 37.5 units daily and doses were increased according to the response. Continuous ultrasound monitoring to know ovarian response. Base line LH and FSH were estimated. HCG was given to trigger ovulation when follicle reached 18-20mm.

Patient’s age, duration, tubal patency, sLH, sFSH, sTSH, sE 2, sProlactin, sTestosteron, androstenedione, fasting insulin level and glucose tolerance tests were performed and Patients with Thyroid disorder were excluded. The prime outcome was the duration of ovulation induction, total doses of recombinant FSH required, number of follicle development, pregnancy rate per cycle, incidence of OHSS, Cumulative birth rate, multiple pregnancy rate and miscarriage rate were evaluated.

Results: Ovulation rate, pregnancy rate and miscarriage rate were same in both the groups. Unifollicular development, duration of induction of ovulation, total doses of recombinant FSH required was more in group I. Multiple pregnancy were more in group II and a few patient suffered from mild OHSS in group II.

Conclusion: Various clinical trials have been done before to optimize the protocol for ovulation induction in PCOS patients. The study suggest that alternate day recombinant FSH therapy is also effective as daily dose therapy but it is cost effective and with least side effects.
PARITY AND THE RISK OF AUTOIMMUNE THYROID DISEASE: A COMMUNITY-BASED STUDY
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The pathogenesis of autoimmune thyroid disease is incompletely understood. Recent studies have suggested that fetal microchimerism (transplacental passage of fetal cells followed by engraftment into maternal tissues) may play a pathogenic role. If that is true, then parity should be risk factor for autoimmune thyroid disease. We tested this hypothesis in a cross-sectional, community-based study. Thyrotropin, thyroid peroxidase antibody and thyroglobulin antibody concentrations were measured on archived sera from 1045 female participants in a 1981 community health survey in Busselton, Western Australia. Associations between parity and positive thyroid antibodies (increased concentration of either antibody) or thyroid dysfunction were examined by logistic regression. After adjustment for age, women who had previously been pregnant did not have a significantly increased risk of positive thyroid antibodies (odds ratio [OR] 1.20, 95% confidence interval [CI] 0.74-1.97, P=0.46), raised TSH (OR 0.93, 95% CI 0.46-1.87, P=0.84) or reduced TSH (OR 0.87, 95% CI 0.33-2.30, P=0.79) compared with women who had never been pregnant. For each additional pregnancy, the OR increased by a factor of 1.02 (95% CI 0.94-1.11, P=0.57) for positive antibodies, 1.02 (95% CI 0.91-1.14, P=0.67) for raised TSH and 1.03 (95% CI 0.87-1.22, P=0.73) for reduced TSH. Analysis using number of live births rather than pregnancies gave similar results. The results were similar in younger (<50 years) and older women. We conclude that, in a community setting, parity is not a risk factor for thyroid autoimmunity or thyroid dysfunction. These data do not support the hypothesis that fetal microchimerism is a key pathogenic factor in chronic autoimmune thyroid disease.

ISOLATED ACTH DEFICIENCY PRESENTING AS SEVERE HYPERCALCAEMIA
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A 64 yo woman presented with altered conscious state and progressive weakness on the background of a two-month history of weight loss, lethargy, and low grade fever. Significant past medical history included premature menopause at age 38. Her serum calcium was found to be 3.29 mmol/L with a PTH of 0.9 pmol/L (NR 1.1-7.7). Her TSH was 0.01 mIU/L (0.3-5.0), fT4 20.1 pmol/L (9-19), and fT3 6.2 pmol/L (1.5-2.2). The calcium improved with hydration to 2.6 mmol/L, but she remained weak and confused. During the first week in hospital, she was reviewed by a Neurologist, Haematologist and Infectious Disease Physician and had multiple laboratory and imaging studies which failed to establish the aetiology of her hypercalcaemia. After Endocrine review on day 8, cortisol and ACTH levels were performed and both found to be repeatedly undetectable. Cortisone replacement led to prompt resolution of her symptoms, and the calcium fell to 2.2 mmol/L with no other medical therapy. Pituitary function was otherwise normal, and dedicated pituitary MRI showed a partial empty sella. The thyroid function tests normalised when reviewed 6 weeks later. While hypercalcaemia is well described in Addison's disease, it is not a recognized feature of hypoadrenalism due to pituitary failure. This is thought to be due to coexistence of secondary hypothyroidism: thyroid hormone appears necessary for the development of hypercalcaemia in the setting of cortisol deficiency: thyroidectomy prevents the development of severe hypercalcaemia in adrenalectomized dogs (1), and commencement of thyroxine for primary hypothyroidism can cause life-threatening hypercalcaemia in patients with untreated Addison’s disease (2). There are 5 case reports of secondary hypoadrenalism presenting with hypercalcaemia, all of which, like our patient, had isolated ACTH deficiency with concomitant transient thyrotoxicosis (3). Transient thyrotoxicosis may have precipitated our patient’s acute presentation by two mechanisms: firstly by accelerated cortisol clearance, and secondly by development of severe hypercalcaemia. Serum cortisol and thyroid hormone levels should therefore be measured in unexplained PTH-independent hypercalcaemia.

(2) Vasikaran SD et al., Clin Endocrinol 41 :261, 1994
THE EVOLUTIONARY ENIGMA OF HEREDITARY CAROTID BODY TUMOURS
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Carotid body paragangliomas and adrenal or extra-adrenal phaeochromocytomas occur sporadically and also in the familial setting. Hereditary paraganglioma is a rare condition and is inherited in an autosomal-dominant fashion. In the literature there are only a few reported cases of concurrent familial carotid body tumours and phaeochromocytomas. The implication of germline mutations in succinate dehydrogenase, which encodes subunits of mitochondrial complex II, in the tumourigenesis of familial phaeochromocytomas and paragangliomas has been previously well documented. We report the case of a 43-year-old man who presented 14 years ago with a painless right neck lump which was diagnosed as a carotid body tumour. He underwent a right carotid body excision, complicated by right glossopharyngeal, vagal and hypoglossal nerve palsies. Two years later he had a left carotid body excision for recurrent disease and developed an intra-operative hypertensive crisis to 260/130, prompting further investigation. A phaeochromocytoma was diagnosed on MIBG with elevated urinary and plasma catecholeamines and a right adrenalectomy was performed two weeks later once adequately alpha- and beta-blocked. Despite remaining normotensive, urinary and plasma noradrenaline levels began to rise again three years later. Four extra-adrenal paragangliomas, three in the neck with associated left recurrent laryngeal nerve palsy, left vocal cord paralysis and progressive difficulties with speech and swallowing, one in the posterior mediastinum and one in the left adrenal gland were identified. Avid uptake was seen on MIBG in the vicinity of three of five lesions. Serial MIBG, CT, MRI, serum and urine catecholeamines were used to monitor disease over six years and the lesions remained indolent. This case demonstrates the physiological sequelae of removal of both carotid bodies, the challenges in management of phaeochromocytoma evolution in hereditary paraganglioma disease and that careful interpretation of imaging modalities has significant effects on clinical decision making.

CHANGE IN BONE DENSITY OVER TIME IN ADULTS WITH CYSTIC FIBROSIS.
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Children with stable Cystic Fibrosis are reported as having similar bone density (BMD) to their non-CF counterparts, but in adolescence and particularly adulthood they fall behind most likely do a failure to achieve the same peak bone mass. Previous studies examining bone loss have included both children and adults over a short period of time (1 to 2 years). Because these children may reach their peak bone mass later (due to developmental delay, chronic illness and hypogonadism), their BMD measurements may be misleading. To overcome some of these difficulties, we examined sequential BMD changes in 46 adult cystic fibrosis patients over an average of 48 months. All patients were on calcium and vitamin D supplementation and no patients were on bisphosphonates. Lung transplant recipients were excluded. Fracture incidence, vitamin D and calcium status, hospital admissions, glucocorticoid use and lung function data were also obtained.

46 patients (aged 16-67 years, 46% male) with at least 2 BMD scans were studied. Scans were performed an average of 48 months apart (range 12 to 78 months). Initially, 8 (17%) patients had normal a BMD, 28 (61%) had osteopenia and 10 (22%) had osteoporosis. Final scanning revealed 11 (24%) with normal BMD, 27 (59%) with osteopenia and 8 (17%) with osteoporosis. During follow-up, a significant fall in hip and lumbar spine BMD occurred in 22 (48%) and 13 (28%) respectively. 12 (26%) and 9 (20%) respectively, improved their hip and lumbar spine BMD. Vitamin D status did not appear to alter this result.

Low bone density remains a problem in adult cystic fibrosis patients with much higher rates of osteopenia and osteoporosis than in the general population. They also appear to lose bone faster with higher rates of bone loss than would be expected in this age group.
AN ASSOCIATION BETWEEN NON ALCOHOLIC STEATOHEPATITIS AND POLYCYSTIC OVARIAN SYNDROME

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OBJECTIVES: The aim of this study was to determine if there is an association between non-alcoholic steatohepatitis (NASH) and polycystic ovarian syndrome (PCOS). NASH and PCOS are known to be associated with metabolic syndrome/insulin resistance. METHOD: The study recruited 14 consecutive female patients of reproductive age (20-45) either with liver biopsy proven NASH (50%) or positive abdominal ultrasound US (50%). Diagnosis of PCOS was defined by criteria from 2003 Rotterdam consensus meeting. Other causes of hyperandrogenism were excluded. All subjects underwent relevant questionnaire and clinical exam, had appropriate serum hormonal assays, pelvic (1) or transvaginal US (13) and were screened for evidence of the metabolic syndrome.

RESULTS:

Eleven subjects (79%) had clinical evidence of PCOS with oligo/amenorrhoea (8), hyperandrogenism (9), and infertility (6). Seven women (50%) had evidence of biochemical hyperandrogenism with low SHBG, raised free testosterone and elevation of serum LH concentration. Seven subjects (50%) fulfilled US criteria for PCOS. Overall ten out of fourteen subjects matched diagnostic criteria for PCOS (71%). CONCLUSION: Despite limitations of the study due to the sample size we found evidence of PCOS in the majority of subjects with NASH. The diabetic patients were in PCOS group (3/10). IMPLICATIONS: Women with NASH should be routinely screened for presence of PCOS, Diabetes Mellitus and metabolic risk factors for cardiovascular disease. Equally women with PCOS should be screened for NASH. The serum fasting insulin level was not helpful in discriminating between women with or without PCOS.

SRB ORALS FOR JOINT SESSIONS

EXPRESSION OF COMPONENTS OF THE HEDGEHOG SIGNALLING PATHWAY DURING MURINE SPERMATOCYGENESIS

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Hedgehog (Hh) signalling is best known for its involvement in regulating patterning, driving cell proliferation, promoting cell survival and directing differentiation during embryonic development (1). The role that Hedgehog signalling plays in the testis is not yet clearly defined, though deletion of one Hedgehog ligand, Dhh, leads to male infertility. The Gli family of zinc finger TFs, consisting of Gli1, Gli2 and Gli3, are mediators of the Hh signalling cascade in vertebrates. We have previously shown that the mRNA transcripts encoding all three Gli genes in the adult mouse testis are expressed highly in spermatogonia, spermatocytes and to a lower extent in the round spermatids. To understand the potential sites of action of Hh proteins in spermatogenesis, we have extended our analysis to other genes involved in the Hh signalling pathway in the adult mouse testis. Using in situ hybridization, Patched2, a transmembrane receptor for Hh, was detected in spermatogonia and spermatocytes, with an apparently lower expression in the round spermatids. The mRNA of Smoothened, another transmembrane protein which forms a membrane receptor complex with Patched, is highly expressed in spermatogonia and spermatocytes, again showing lower expression in round spermatids and interstitial cells. Fused, a positive regulator of Hh signalling, is highly expressed in spermatogonia and spermatocytes with slightly lower expression in round spermatids. SuFu is a negative regulator of Hh signalling, known to repress Gli1 function in part by tethering it in the cytoplasm. The mRNA encoding SuFu is absent from spermatogonia, detected in spermatocytes and persists in round spermatids where its expression appears highest, suggesting that the SuFu protein may be acting to switch off Hh signalling at that stage of spermatogenesis. Overall, the regulated expression pattern of these genes in the adult mouse testis suggests a role for Hh signalling in the regulation of spermatogenesis.

OESTROGEN RECEPTOR BETA IS INVOLVED IN THE REGULATION OF LEYDIG CELL NUMBER IN THE MOUSE.

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Recent evidence suggests that oestrogen plays a physiological role in the testis. Both oestrogen receptor alpha and oestrogen receptor beta (ERb) are present in the testis and administration of oestrogen has been shown to inhibit the development of Sertoli, Leydig and germ cells. This study investigates the effect of ERb on the testis using ERb knockout mice (bERKO).

Adult male bERKO mice (n=8) and their wild-type littermates (n=7) were killed at 11 weeks postpartum. One testis from each animal was fixed in Bouin's fluid and embedded. Each testis was fractionated and thick sections cut and stained with PAS. The optical disector method was used to count the number of Leydig cells, Sertoli cells, spermatogonia, spermatocytes and spermatids in each testis. Trunk blood was collected and plasma testosterone concentrations measured by radioimmunoassay.

No significant differences in body or testis weight were seen between the bERKO or wild-type mice. Similar numbers of Sertoli cells, spermatogonia, spermatocytes and spermatids were also observed between the two groups. The number of Leydig cells was significantly increased in bERKO mice compared with their wild-type littermates (P< 0.05). Despite the increased number of Leydig cells in the bERKO mice there was no significant difference in plasma testosterone concentrations in this group compared to the wild-type mice. Oestrogen has been reported to inhibit proliferation of adult-type Leydig cells and to inhibit steroidogenesis. This study suggests that the regulation of Leydig cell proliferation may be mediated by ERb. The presence of normal circulating testosterone concentrations in bERKO mice suggests that the effects of oestrogen on steroidogenesis are not brought about by ERbeta.

FIBROBLAST GROWTH FACTOR RECEPTOR-1 (FGFR-1) IS ESSENTIAL FOR SPERMIOGENESIS, CAPACITATION AND MALE FERTILITY

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Male infertility is often a result of irregular sperm development/function. The identification of snt-2 (Suc-1 associated Neurotrophic Factor Target 2) and Fgfr-1 to the sperm tail, lead to the hypothesis that Fgf signalling through snt-2 is involved in sperm tail development/function. To test this hypothesis, transgenic mice carrying a dominant-negative variant of Fgfr-1, driven by the protamine 1 promoter (haploid specific) were created. Breeding experiments confirmed male fertility, however one line was significantly sub-fertile and demonstrated a significantly reduced daily sperm production, (DSP, 30%↓).

Transgene expression levels were up to 70 times above native mRNA levels in wt mice; however there was a concurrent up-regulation of the native receptor in transgenic mice, resulting in only a 6x over-expression in transgenic: native mRNA. To increase transgene expression, independent lines were crossed (double heterozygous, DH). DH transgene expression levels were up to 120 times above the native mRNA in wild type mice, resulting in a 20x over-expression in transgenic : native mRNA. Breeding experiments showed males from 1 cross were significantly subfertile with DSPs further reduced, (41%↓). Collectively this data shows Fgfr-1 signalling is required for quantitatively normal spermiogenesis. Given the millions of sperm that mice produce, a 40%↓ in DSP is unlikely to be responsible for the sub-fertility observed ie. 2 versus 9 pups/litter. Therefore, a disruption of Fgfr-1 signalling may also induce a post-testicular phenotype. Western blot analysis, using tyrosine phosphorylation as a surrogate marker of sperm capacitation, showed transgenic mice had a significantly attenuated ability to initiate capacitation. As capacitation is an absolute requirement for fertilisation, the absence of capacitating capability is probably the major contributor to the sub-fertility seen in the transgenic mice. This research demonstrates for the first time that the Fgfr-1 signalling cascade is one of several pathways associated with sperm development and function.
ADULT EXPOSURE TO DIETARY PHYTOESTROGENS REDUCES FERTILITY OF MALE RATS

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Phytoestrogens are plant-derived compounds that are particularly abundant in soy-based foods. Exposure to exogenous oestrogenic chemicals has been implicated in declining male fertility. The aim of this study is to deduce whether adult phytoestrogen exposure affects the reproductive function of male rats, and by what mechanisms phytoestrogens may be acting.

Six male rats were transferred from a low soy diet (control) to an experimental high soy diet, while 9 males remained on the control diet. On days 3, 6 and 12 all males were mated and litter sizes recorded. A second group of male rats kept on the same dietary regimen were killed after 3, 6 or 12 days on the diets. The epididymides were collected from the rats. Real-time PCR was performed to measure mRNA quantities of oestrogen receptors alpha (ERα) and beta (ERβ), and androgen receptor (AR). The TBARS assay for lipid peroxidation was performed on epididymal sperm samples from rats fed the high or low phytoestrogen diet for 3 days.

The average litter size following 3 days on the high soy diet was significantly lower than that for rats maintained on the control diet. Litter sizes returned to control levels by day 12. ERα and AR expression decreased in the cauda region of the epididymis following 3 days on the high soy diet, but returned to control levels by day 6. Lipid peroxidation of epididymal sperm was increased in rats fed the high phytoestrogen diet for 3 days.

Short-term exposure to high phytoestrogen levels transiently reduces male fertility, and alters hormone receptor expression. Endocrine disruption may impair fertility by reducing antioxidant protection of sperm stored in the epididymis.

GONADOTROPHIC HORMONES AFFECT THE UTERUS, IMPLANTATION AND FETAL DEVELOPMENT IN MICE


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Although gonadotrophin stimulation using equine chorionic gonadotrophin (eCG) adversely influences pregnancy and fetal development, the effects of stimulation using recombinant human follicle stimulating hormone (rhFSH) are largely unknown. Evidence from human studies indicates that rhFSH may also be detrimental to the endometrium and implantation. We investigated the effect of gonadotrophin stimulation on ovarian hormones and uterine characteristics in the peri-implantation period, and pregnancy outcomes in mice. Adult female mice were stimulated with 2.5IU or 10IU rhFSH or 5IU eCG, followed by 5IU human chorionic gonadotrophin (hCG). Control mice received saline injections. On day 4 of pseudopregnancy mice either had embryos transferred to the uterus or were sacrificed and blood and uterine samples collected. Plasma progesterone and estradiol concentrations were determined by radioimmunoassay. Uterine mRNA levels of the estrogen and progesterone receptors (ERα and PR), leukaemia inhibitory factor (LIF), homeobox gene Hoxa10 and vascular endothelial growth factor (VEGF) were determined by real-time RT-PCR. Uterine protein distribution of PR was determined by immunohistochemistry. Embryo transfer recipients were sacrificed on day 15 to assess pregnancy outcomes. Gonadotrophin stimulation increased plasma progesterone concentration compared to controls, while estradiol concentrations were not affected. Stimulation also reduced total LIF mRNA and altered the spatial distribution of PR protein in the uterus on day 4. Embryo transfer recipients administered eCG or 10IU rhFSH had lower pregnancy rates compared to controls (11, 35 and 75% respectively) and fetuses from the rhFSH group had reduced mean weight (94 ± 6 vs 176 ± 8 mg), length (11 ± 0.2 vs 12 ± 0.1 mm) and maturity (14.1 ± 0.09 vs 14.6 ± 0.05 days) compared to controls. These results demonstrate that gonadotrophin stimulation induces changes to the maternal reproductive tract prior to implantation that have consequences for the establishment of pregnancy and fetal development in the mouse.
SUPPRESSOR OF CYTOKINE SIGNALLING 3 REGULATES IL-11 INDUCED HUMAN ENDOMETRIAL STROMAL CELL DECIDUALIZATION.

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Decidualization of endometrial stromal cells is critical for embryo implantation and establishment of pregnancy. Locally produced cytokines such as interleukin (IL)-11 enhance decidualization of human endometrial stromal cells (HESC). IL-11 signaling is negatively regulated by suppressor of cytokine signaling (SOCS) proteins. IL-11 stimulates SOCS3 in human pituitary cells. The aim of this study was to examine the role of SOCS3 on IL-11 induced HESC decidualization. Decidualization of HESC was assessed using an *in vitro* model in which estrogen (E)+progesterone (P) or cAMP was administered for 8 days to cells. Medium was collected for prolactin (PRL) assay (a decidual marker). Cellular protein was extracted for Western analysis and cellular RNA for real-time RT-PCR analysis. SOCS3 was overexpressed in HESC cells and the effect on decidualization assessed. HESC treated with E+P or cAMP secreted PRL from day 6. Treatment of HESC with E+P or cAMP increased the abundance of SOCS3 protein, coinciding with an increase in PRL secretion. cAMP maximally stimulated SOCS3 protein and mRNA during decidualization. Antiprogestin (onapristone) added to E+P or cAMP treated cells at day 6 reduced PRL secretion but had no influence on SOCS3 abundance suggesting that SOCS3 protein was not regulated via the P-receptor pathway. Addition of IL-11 to HESC increased SOCS3 abundance from 1 hour. SOCS3 abundance returned to control levels following treatment of cells with IL-11 and IL-11 neutralising antibody. SOCS3 overexpression in HESC treated with cAMP reduced PRL secretion compared to mock- or non-transfected HESC. Furthermore, IL-11 mediated decidualization was diminished by SOCS3 overexpression. We have demonstrated for the first time that SOCS3 regulates IL-11 induced decidualization and that SOCS3 overexpression in HESC disrupts decidualization. This knowledge is important in understanding the mechanisms by which IL-11 promotes decidualization of HESC and thus the formation of decidua, an essential component of a functional placenta.

INTERLEUKIN-10 INHIBITS TNFA SYNTHESIS AND PROTECTS AGAINST LPS-INDUCED MISCARRIAGE AND PRETERM LABOUR

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The immune-deviating and anti-inflammatory cytokine interleukin-10 (IL-10) is expressed throughout pregnancy in the decidual and placental tissues. Mice with a null mutation in the IL-10 gene are fertile with no reduction in litter size, although fetal growth trajectories and placental structure are altered. IL-10 is known to terminate inflammatory responses and to limit inflammation-induced tissue pathology by inhibiting macrophage synthesis of tumor necrosis factor-α (TNFα). To investigate the anti-inflammatory role of IL-10 in pregnancy, the susceptibility of null mutant mice to low dose LPS-induced miscarriage and preterm labour has been evaluated. When IL-10 null mutant C57Bl/6 (IL-10-/-) and control (IL-10+/+) mice were given low dose E.coli LPS on d10 of pregnancy, IL-10 deficiency was associated with greater fetal loss with fewer mated IL-10-/- mice carrying viable fetuses at day 18 and increased rate of fetal resorption. In mice treated with LPS on day 17, preterm delivery within 24 h occurred in a higher proportion of IL-10-/- mice than IL-10+/+ mice. LPS induced very high and sustained TNFα and IL-6 content in serum, uterine and placental tissue in IL-10-/- mice, associated with upregulated mRNA expression of both cytokines in gestational tissues. These data show that IL-10 modulates placental resistance to inflammatory stimuli by down-regulating expression of the pro-inflammatory cytokines TNFα and IL-6. We conclude that IL-10 has a dual role in pregnancy, acting to regulate placental morphogenesis and fetal growth trajectory, and to protect against inflammation-induced miscarriage and preterm labour.
EXPERIMENTALLY INDUCED HYPOGLYCEMIA: A MODEL TO EXAMINE THE EFFECTS OF LACTATION ON REPRODUCTIVE FUNCTION IN DAIRY COWS?

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The metabolic changes subsequent to lactogenesis have been associated with poor reproduction in high-producing dairy cows [1,2]. Periods of hypoglycaemia reflect severe energy deficit and are associated with changes in plasma levels of growth hormone (GH), insulin-like growth hormone-I (IGF-I) and insulin-like growth hormone-II (IGF-II). Somatotropic activity has been shown to influence reproductive functions [3-5].

This study evaluated the effects of experimentally induced hypoglycaemia in seven non-lactating cows, over a 7-day period. Phloridizin treatment (8 g/d) resulted in urinary glucose loss (control: 3.5 ± 1.0 g/d and phloridizin: 468 ± 46 g/d) and decline in plasma glucose (control: 60.6 ± 0.6 mg/dL and phloridizin: 71.8 ± 0.4 mg/dL; P<0.001). Treatment increased plasma beta hydroxybutyrate (BOH), non-esterified fatty acids (NEFA) and IGF-I concentrations (P<0.001). Plasma insulin and GH concentrations did not differ. During treatment, expression of mRNA for total growth hormone receptor (GHR(tot); P=0.012) and GHR(1A) (P<0.001) in liver tissue declined. Luteal and follicle diameters in ovaries recovered after treatment did not differ. Expressions of mRNA for IGF-I (P=0.052) and interleukin-1β (IL-1β) in corpus luteum and for 3β-hydroxysteroid dehydrogenase (3β–HSD) within dominant follicles were significantly elevated, while mRNA for GHR(tot), cytochrome P450 cholesterol side chain cleavage enzyme (P450-SCC), and steroidogenic acute regulatory protein (StAR) tended to increase (P<0.1) with treatment.

The treatment resulted in changes similar to those of nutritional stress or the initiation of lactogenesis. Phloridizin-induced hypoglycaemia may be a model to investigate mechanisms linking glucose metabolism, and the somatotropic axis to reproductive function. The advantages of such a model, is that it allows for strict control of the level of hypoglycaemia. The use of non-lactating cows also removes the feedback mechanisms that modulate mammary gland requirements, and therefore will minimize the between cow variance when using lactating cows.

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9-HODE-INDUCED APOPTOSIS IN U937 MONOCYTES IS NOT INHIBITED BY BLOCKADE OF PPARγ, AND IS ENHANCED BY ACTIVATION OF PPAR δ.

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The oxidation product of linoleic acid, (±)-9-hydroxy-10E,12Z-octadecadienoic acid (9-HODE) is found abundantly in oxidised LDL and in atherosclerotic lesions. 9-HODE has previously been shown by this laboratory to be a mild peroxisome proliferator activated receptor gamma (PPARγ) agonist, and to selectively induce apoptosis in U937 monocytes. Furthermore a related compound, 13-HODE, has been shown to induce apoptosis in colorectal cancer cells by down-regulating PPAR δ. The mechanism by which 9-HODE induces apoptosis in U937 monocytes is currently unknown.

Aims: To determine whether 9-HODE-induced apoptosis in U937 monocytes occurs via a PPAR γ- or PPAR δ-dependent mechanism.

Methods: U937 monocytes were treated for 48h with either 9-HODE (10μM), PPARγ antagonist GW9662 (10μM) or PPAR δ agonist GW501516 (10μM), and combinations of either 10μM GW9662 or 10μM GW501516 with 10μM 9-HODE. Monocytes were gently harvested and briefly incubated with a mixture of Annexin-V-FITC and propidium iodide, then analysed by flow cytometry.

Results: Compared to vehicle treated cells (apoptotic RR=1), GW9662 (RR=1.46 ± 0.24, p=0.083 v vehicle (mean ± SD)) or GW501516 (RR=1.29 ± 0.01, p=0.0004 v vehicle) had modest effects on apoptosis. 9-HODE alone caused a 3-fold induction of apoptosis compared to vehicle treated cells (RR=3.16 ± 0.47, p=0.015 v vehicle), but this increased when 9-HODE was combined with either GW9662 (RR=4.16 ± 0.19, p=0.086 v 9HODE alone) or GW501516 (RR=4.82 ± 0.48, p=0.016 v 9HODE alone).

Conclusions: Both GW9662 and GW501516 alone resulted in small increases in apoptosis. The presence of PPAR γ antagonist GW9662 does not prevent 9-HODE related apoptosis. The PPAR δ agonist GW501516 increased apoptosis in conjunction with 9-HODE. While 9-HODE is a PPAR γ agonist, its apoptotic action is mediated independently of PPAR γ, and is enhanced by the PPAR δ agonist GW501516.


HEATED PALM OIL IS NOT DETRIMENTAL TO BONE METABOLISM IN ESTROGEN DEFICIENT RATS

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Objectives: The effects of heated palm oil and soya oil on bone metabolism in ovariectomised Sprague-Dawley rats were studied. Methods: Sixty-four 3-month old female rats, weighing between 180-250 g were equally divided into 8 groups: normal control (NC), ovariectomised control (OVX C), fresh palm oil (POF), fresh soybean oil (SOF), once-heated palm oil (PO1), once-heated soya oil (SO1), 5x-heated palm oil (PO5) and 5x-heated soya oil (SO5). All the groups were ovariectomised except for the NC group. Rat chow fortified with the respective oils (15% w/w) was given to the tested groups. Blood and urine samples were obtained before and after four weeks of treatment. Parameters measured were serum levels of the bone resorbing cytokines, interleukin-1 and interleukin-6; as well as the bone biomarkers, serum osteocalcin (bone formation) and urine deoxypyridinolines (bone resorption). Results: Serum levels of IL-1α for all groups were undetectable. Rats in the POF and SO5 showed higher levels of IL-6 after treatment compared to the NC and OVX C (p<0.01). IL-6 was lower in the SOF group compared to the POF group (p<0.01), but higher in the SO5 group compared to the PO5 group (p<0.01). Osteocalcin levels in the NC, SOF and SO5 were decreased after treatment. The POF and PO1 group of rats had higher osteocalcin levels compared to NC. Only the POF group had lower urinary Dpd/creatinine levels after treatment. Bone remodeling index (BRI) for POF and PO1 after treatment was increased. Percentage change for BRI was higher in the POF and PO5 groups compared to the NC and SO5 groups. Conclusion: Heated palm oil has no detrimental effects on bone metabolism in estrogen deficient ovariectomised rats. On the other hand, heated soya oil induced negative changes in serum IL-6 and osteocalcin levels which may lead to osteoporosis in the long term.
AORTIC HISTOMORPHOMETRIC FINDING IN OVARIECTOMIZED RATS FED WITH HEATED VEGETABLE OILS
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This study examined the effects of heated vegetable oils in estrogen deficiency rats. Eighty female Sprague–Dawley rats were divided equally into eight groups (all the rats were ovariectomized except for rats in group I), each treated with the following prescribed course of food: (I) basal diet as the control (without oil) non ovariectomized, or (II) basal diet as the control (without oil), or (III) basal diet fortified with 15% weight/weight (w/w) fresh Soya bean oil (FSO), or (IV) heated once Soya bean oil (1H-SO), or (V) heated five times Soya bean oil (5H-SO), or (VI) fresh Palm oil (FPO), or (VII) heated once Palm oil (1H-PO), or (VIII) heated five times Palm oil (5H-PO) for 24 weeks. At the end of the study, aortic tissue was taken from consistent segment of the ascending aorta for histopathological examination. The specimens were stained with haematoxylin eosin and Verhoeff van Gieson for light microscopy. Intimal thickness was calculated using computerised image analyser. Both fresh and heated Soya or Palm oil diet did not alter the tunica intima over tunica media ratio. There was no obvious focal or diffuse atherosclerotic plaque formation seen in all groups. The intact and continuous internal elastic lamina did not support the smooth muscle cells migration as seen in atherosclerosis. There is no obvious thickening or swelling of tunica media indicates that no lipid-laden foam cells formation.
In conclusion, heated Soya and Palm oil appeared comparable in their effect on aorta. Heating did not render it more atherogenic in ovariectomized female rats. Electron microscopic study is required to see ultra structural changes.

EFFECT OF HEATED SOYA AND PALM OIL ON SERUM HOMOCYSTEINE, INTERLEUKIN AND MDA IN OVARIECTOMIZED RATS
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Objective: The aim of this study is to determine the changes in serum MDA, homocysteine and interleukin 6 levels of estrogen deficient rats fed with heated vegetable oils (soya and palm oil diet). Methods: Eighty female Sprague–Dawley rats were divided equally into eight groups (all the rats were ovariectomized except for rats in group I), each treated with the following prescribed course of food: (I) basal diet as the control (without oil) non ovariectomized, or (II) basal diet as the control (without oil), or (III) basal diet fortified with 15% weight/weight (w/w) fresh Soya bean oil (FSO), or (IV) heated once Soya bean oil (1H-SO), or (V) heated five times Soya bean oil (5H-SO), or (VI) fresh Palm oil (FPO), or (VII) heated once Palm oil (1H-PO), or (VIII) heated five times Palm oil (5H-PO) for 24 weeks. Serum samples for interleukin and MDA were taken prior to ovariectomy and later repeated every 4 weeks post ovariectomy for 24 weeks. Serum samples for homocysteine assay was carried out prior to ovariectomy and once 24 weeks post ovariectomy. Results: Fresh and heated Palm oil did not increase serum MDA, Homocystein and Interleukin 6.
Conclusion: Consumption of fresh and heated palm oil did not render it more atherogenic in ovariectomized rat.
CHANGES IN SERUM LIPID PROFILES IN ESTROGEN DEFICIENT RATS FED WITH SOYA AND PALM OIL DIET

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Objective: The aim of this study is to determine the changes in lipid profiles of estrogen deficient rats fed with heated vegetable oils (soya and palm oil diet). Methods: Eighty female Sprague–Dawley rats were divided equally into eight groups (all the rats were ovariectomized except for rats in the first group; each fed with basal diet as the control (without oil), the second group fed with basal diet as the control (without oil), third group fed with basal diet fortified with 15% weight/weight (w/w) fresh Soya bean oil, fourth group with heated once Soya bean oil, fifth group with heated five times Soya bean oil, sixth group with fresh Palm oil, seventh group heated once Palm oil, and the last group with heated five times Palm oil for 24 weeks. Serum sample for lipid profile was taken prior to ovariectomy and every four weeks post ovariectomy for 24 weeks. Results: Ovariectomy increased serum level of triglyceride and total cholesterol. There is a tendency for fresh and heated Palm oil to cause transient increase in serum triglyceride, LDL-cholesterol and HDL-cholesterol. Conclusion: Consumption of fresh and heated palm oil did not render it more atherogenic in ovariectomized rat.

EFFECTS OF ANDROGENS ON MYOBLAST PROLIFERATION

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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 androgens on the proliferation of myoblasts in vitro, using C2C12 and Sol8 cells, two well established myoblast cell lines from mouse skeletal muscle. Firstly, AR expression was identified in these two cell lines by reverse transcript PCR (RT-PCR). The conditions for proliferation of cells were optimised. Cells were cultured in charcoal strip fetal calf serum (CS-FCS) with the addition of androgen every 24 hours. The MTT assay was used to quantitate proliferation of cells. No significant effect of androgens on proliferation of C2C12 and Sol8 was observed using either testosterone or dihydrotestosterone, at concentrations ranging from 10-6 to 10-9 M for up to 6 days of treatment. However, C2C12 cells treated with 10 ng/ml IGF-I showed a higher proliferation rate compared with controls, indicating the cells are capable of responding to a mitogenic stimulus. Levels of AR protein in the cell lines were investigated. No AR protein was detectable in either cell line by Western analysis, indicating that protein levels of the AR were below the limit of detection. This low level of AR protein might explain the lack of response of the myoblast cell lines to androgen treatment. In order to further study androgen actions in myoblasts in vitro, we have stably transfected 13 C2C12 cell lines to overexpress the mouse AR. We have confirmed AR protein levels are detectable in 7 cell lines, and are currently examining the effects of androgens on proliferation and differentiation in myoblasts in these cell lines.
GONADOTROPHIN-INHIBITORY HORMONE (GnIH) SUPPRESSES LH SECRETION IN THE RAT
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The secretion of the gonadotrophins, LH and FSH, and hence the control of fertility is driven by gonadotrophin-releasing hormone (GnRH). The concept of direct inhibition of pituitary gonadotrophin secretion by the brain was unknown until very recently, when a ‘new’ hypothalamic hormone, which suppressed gonadotrophin release from cultured quail pituitary glands, was discovered and named GnIH. Since we have observed GnIH-immunoreactive cells in the rodent brain as well as fibres projecting to the median eminence, we hypothesized that GnIH acts at the pituitary gland to suppress gonadotrophin secretion in mammals as well as birds. In Experiment 1, sparrow GnIH-related peptide 2 (GnIH-RP2; 10 μg) or vehicle was injected iv into ovariectomized rats. Blood was collected at 0, 1.5, 3 and 10 minutes for LH analysis. After 3 minutes, plasma LH was suppressed to 50% of basal values by GnIH-RP2 (P < 0.05). To test if GnIH inhibited GnRH-induced LH secretion (Experiment 2), GnRH (0.1 μg) was injected iv either alone or with GnIH-RP2. Rats were blood sampled as before. GnRH caused a robust release of LH, but in the presence of GnIH the peak LH response was suppressed to 60% of control values (3 minutes after injection; P < 0.05). We also tested whether GnIH can affect pulsatile GnRH release (Experiment 3), since we and others have observed GnIH fibres projecting to GnRH cell bodies. Rats were blood sampled every 10 minutes for 4 hours. GnIH (1 μg) or vehicle was injected iv after 2 hours. LH pulse frequency (an index of GnRH pulse frequency) was unaffected by GnIH. These results support a neuroendocrine role for GnIH and are the first to suggest that a direct inhibitory system governing pituitary gonadotrophin release exists in mammals. However, they do not support a role for GnIH in central regulation of GnRH.

OREXIN RECEPTOR SUBTYPES-1 AND -2 EXHIBIT DISTINCT BETA-ARRESTIN PROFILES DETERMINED USING BIOLUMINESCENCE RESONANCE ENERGY TRANSFER (BRET) AND CONFOCAL MICROSCOPY
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Since their discovery in 1998, the role of orexin neuropeptides has been the subject of considerable interest. While familial canine narcolepsy is clearly correlated with functionally null type-2 orexin receptor (OxR2), the human condition is less clearly defined. By examining the two orexin receptor subtypes and their interactions with beta-arrestins 1 (Barr1) and 2 (Barr2), we aim to gain a greater understanding of the desensitization/internalization mechanisms regulating this system. A comprehensive series of BRET dose-response assays were performed, using both OxR1 and OxR2 in combination with Barr1, Barr2 and the respective phosphorylation-independent mutants. These data show no significant differences between Barr1 and Barr2 for these receptors, in keeping with their classification as Class B for beta-arrestin usage. Orexin B (OxB) is selective for OxR2 over OxR1 and our data showing much greater affinity of beta-arrestins for OxB-activated OxR2 reflects this selectivity. Interestingly, despite Orexin A (OxA) demonstrating similar efficacy for both OxR1 and OxR2, our data shows a significantly greater affinity of beta-arrestins for OxA-activated OxR2 compared with OxR1. Furthermore, phosphorylation-independent beta-arrestins had a significantly greater affinity for OxR1 than wild-type beta-arrestins, whereas this was not the case for OxR2. This suggests that OxR2 possesses additional components compared to OxR1 that enable stronger interactions with beta-arrestins and that phosphorylation-independence at least partially overcomes the need for these additional components. This receptor-specific difference correlates with our extended BRET kinetic studies showing a more robust beta-arrestin interaction with OxA-activated OxR2 compared with OxR1 over time. Confocal microscopy studies correlate with the BRET data, with receptor and/or beta-arrestin translocation evident for both OxA-activated receptors and OxB-activated OxR2, but little if any translocation evident with OxB-activated OxR1. Our results indicate receptor subtype-specific differences in beta-arrestin regulation of orexin receptor desensitization/internalization, providing a mechanism for distinct roles in the intricate functioning of the orexigenic system.
PRENATAL BETAMETHASONE EXPOSURE SIGNIFICANTLY ALTERS FETAL ADRENAL STEROIDOGENIC ENZYME P450C17 GENE EXPRESSION IN SHEEP

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Fetal exposure to synthetic glucocorticoids increases fetal and postnatal hypothalamic-pituitary-adrenal (HPA) axis activity. Peri-conceptional undernutrition alters fetal adrenal receptor and steroidogenic enzyme expression, but little is known about the effects of fetal exposure to synthetic glucocorticoids on adrenal cortisol synthesis. Our aim was to determine the levels of fetal adrenal steroidogenic enzyme P450c17 mRNA after one, two or three doses of betamethasone administered to the mother. Pregnant ewes were injected with saline or 1 (104 days of gestation; dG), 2 (104, 111 dG) or 3 (104, 111, 118 dG) doses of betamethasone. Fetal blood samples and fetal and postnatal pituitary and adrenal tissue was collected at 84 (control only), 109, 116, 133 dG and 6 weeks postpartum. Plasma was collected for ACTH and cortisol (F) analyses. Fetal pituitaries and adrenals were collected and adrenals processed for mRNA determination of steroidogenic enzyme P450c17 (real time RT-PCR). One dose of maternal betamethasone reduced fetal pituitary weight at 109 dG (P=0.027) and three doses decreased pituitary and adrenal weights at 133 dG (P=0.052; P=0.01). Three doses of betamethasone resulted in a modest increase in adrenal weight at 6 weeks of age (P=0.08). Plasma ACTH and F levels increased with increasing pre- and postnatal age in both groups (P=0.001). ACTH levels were reduced at 6 weeks of age after 3 doses of betamethasone (P=0.038). Adrenal P450c17 mRNA levels increased with increasing pre- and postnatal age in both groups (P<0.001). P450c17 mRNA levels were reduced at 109 dG after one dose of betamethasone (P=0.004), and modestly reduced after two (at 116 dG; P=0.1) or three doses (at 133 dG; P=0.057). The ratio of F to P450c17 mRNA increased with increasing pre- and postnatal age in both groups but was higher after one dose (at 109 dG; P=0.004), two doses (at 116 dG; P=0.03) or three doses (at 133 dG; P=0.029) of betamethasone. Fetal exposure to betamethasone alters fetal adrenal P450c17 enzyme expression. An increase in F: P450c17 may be indicative of an increase in adrenal efficiency in producing cortisol or alternatively a reduction in cortisol clearance.


USE OF A DIURNAL CORTISOL BLOOD SPOT PROFILE FOR ADJUSTMENT OF HYDROCORTISONE DOSE.

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Adjustment of glucocorticoid replacement therapy for patients with pituitary or adrenal disease based on clinical evaluation can be imprecise. Obtaining a diurnal profile of cortisol levels requires multiple venepunctures. We have developed a method for obtaining a diurnal profile of cortisol concentrations from dried capillary bloodspots collected at home by the patient. This study evaluates the use of this method to adjust hydrocortisone replacement regimens. We further assess the effect of dose adjustment on bone turnover and peripheral white blood cell counts; markers of end-organ response to glucocorticoid action. This method utilises methanol extraction and reconstitution in an assay buffer to allow measurement of cortisol from small volume samples in a standard commercial assay (DPC Immulite 2000). Results from the bloodspot assay were validated against simultaneously collected serum cortisol measurements collected by venepuncture, yielding a highly significant linear correlation (r=0.93, n=80) across a range of serum cortisol concentrations of 83 – 659 nmol/l. The relationship y=0.138x was subsequently used to transform bloodspot cortisol concentrations to equivalent serum cortisol estimations. In a single day, 10 patients with either primary or secondary hypoadrenalism who were clinically glucocorticoid sufficient, collected 5-6 capillary blood samples by fingerprint, absorbed onto blotting paper. The samples were obtained before and 2 hours after each hydrocortisone dose. The dried blotting paper was mailed to our laboratory. Patients were taking either twice or thrice daily hydrocortisone replacement (total daily dose range 12mg-30mg). Dose adjustment was made according to predetermined target ranges based on expected physiological serum cortisol concentrations. Doses adjustments were required in 7 patients. 2 patients were changed to thrice daily dosing. Data will be presented on changes in urinary deoxypyrindinoline and white cell counts following hydrocortisone dose adjustment. Bloodspot cortisol measurement enables convenient and effective optimisation of hydrocortisone replacement therapy, detecting subclinical under or over-replacement.
EFFECTS OF TOCOTRIENOL AND TOCOPHEROL ON CORTICOSTERONE LEVEL AND GASTROINTESTINAL CHANGES IN RATS EXPOSED TO STRESS

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This study investigates the effects of supplementation with either tocotrienol (TT) or tocopherol (TF) on corticosterone level and gastrointestinal changes in rats exposed to restraint stress. Twenty-four male Sprague-Dawley rats were randomly assigned into 4 equal sized groups, two control groups were given a commercially prepared normal rat diet, while the treated group was fed with an identical diet with supplementation of either tocotrienol or tocopherol orally at the dose of 60mg/kg body weight. After 28 days of treatment, one control group, TT group and TF group were subjected to restraint stress, 2 hours daily for 4 consecutive days. After the last exposure to stress, plasma samples were taken to determine the corticosterone level and after which the rats were killed. The stomach was excised for the evaluation of gastric lesion, malondialdehyde (MDA) and prostaglandin E\(_2\) levels. The results showed that TT and TF were able to maintained the corticosterone level to pre-stress values in rats exposed stress. Tocotrienol was found to be better in preventing formation of gastric lesion compared to rats supplemented with TF while both TT and TF were proved to significantly reduce the gastric MDA content in stress condition compared to control group. We also found that both TT and TF have the ability to reduce prostaglandin E\(_2\) loss which was apparent with stress exposure. As a conclusion, tocotrienol and tocopherol are capable in reducing stress-induced damages in the gastric tissue probably through inhibiting stress induced elevation of corticosterone levels as well as through free radical scavenging activities.

A COMPARISON OF BODY COMPOSITION IN CUSHING’S SYNDROME AND GROWTH HORMONE DEFICIENCY

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Glucocorticoid excess and growth hormone deficiency (GHD) both perturb body composition by increasing fat mass (FM) and reducing lean body mass (LBM). Because these occur via different metabolic mechanisms, it is likely that distinct differences in the severity and distribution of body composition abnormalities exist. The aim was to compare body composition in subjects with Cushing’s syndrome (CS) and GHD. Eighteen subjects with CS (12F, age=41.5±3.0yrs, 24h UFC=1471±329nmol/d, normal<300nmol/d), 22 subjects with GHD (14F, age=42.9±2.9yrs) and 18 normal subjects (11F, age=46.8±2.8yrs) were studied. LBM, FM, bone mineral content (BMC) and regional body composition analysis were assessed by DXA. In both CS and GHD, FM was significantly greater and LBM significantly lower than normal subjects (Table). There were no significant differences in FM and LBM between subjects with CS and GHD. BMC was significantly lower in CS, but not GHD, than normal subjects. In CS, limb lean tissue was significantly less than both groups. Truncal fat was significantly greater than normal subjects in CS and tended to be greater than in subjects with GHD. In summary FM is increased and LBM reduced in both CS and GHD. However CS results in greater perturbation of regional body composition, with a greater reduction in limb lean mass and a greater increase in truncal fat. This may result in subjects with CS having greater functional impairment and a less favourable metabolic profile.

<table>
<thead>
<tr>
<th></th>
<th>Weight (kg)</th>
<th>FM (%)*</th>
<th>LBM (%)*</th>
<th>BMC (%)*</th>
<th>Limb lean mass (%)*</th>
<th>Truncal fat (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>72.4±4.2</td>
<td>33.8±2.4</td>
<td>62.1±2.3</td>
<td>4.1±0.1</td>
<td>29.7±1.1</td>
<td>15.8±1.2</td>
</tr>
<tr>
<td>CS</td>
<td>75.1±2.6</td>
<td>43.9±1.6</td>
<td>52.7±1.6</td>
<td>3.5±0.1</td>
<td>32.4±0.7</td>
<td>23.2±1.3</td>
</tr>
<tr>
<td>GHD</td>
<td>76.9±3.9</td>
<td>41.1±1.2</td>
<td>55.1±1.9</td>
<td>3.8±0.1</td>
<td>26.5±1.0</td>
<td>20.2±1.0</td>
</tr>
</tbody>
</table>

* Expressed as % of body weight
a = p<0.05 vs normal subjects
b = p<0.05 vs GHD
c = p<0.01 vs GHD
ACTIVIN AS A NOVEL MARKER IN CLINICAL INFLAMMATORY PROCESSES: ELEVATIONS IN BURNS AND TRAUMATIC BRAIN INJURY PATIENTS.

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Activin A is a member of the Transforming Growth Factor-β superfamily with diverse roles, but it has only been recently identified as a cytokine elevated in inflammatory processes. In animal models of acute inflammation, activin A is released into the circulation within 40 minutes of challenge with agents such as lipopolysaccharide. The relevance of this response to clinical inflammatory syndromes was confirmed where circulating activin levels were high in patients with septicemia. However, whether this applies to other clinical settings of acute inflammation is not currently known. Here, the response of activin was assessed in two major inflammatory conditions, in patients who have suffered a major burns episode and those that have undergone a traumatic brain injury (TBI), typically following a vehicle accident. In burns patients, activin was elevated both systemically in the circulation and locally in burns blister fluid as assessed by an immunoassay specific for activin A. Tissue immunohistochemistry using an antibody specific for the activin βA subunit (activin A is a dimer of two βA subunits) showed that fibroblasts in the dermis of the burns wound were immunopositive for activin, along with infiltrating leukocytes and neutrophils. In TBI, activin A was elevated in a subset of patients in the cerebrospinal fluid (CSF), but there were only minor or no elevations in the systemic circulation. There were no apparent relationships between the degree of activin response and coma/injury score, but CSF activin was correlated with markers of ischaemia and/or neural injury. Furthermore, the length of stay in intensive care was associated with the degree of activin response in the CSF. These findings add further evidence that activin is upregulated in a number of clinical inflammatory settings and offer new perspectives in terms of potential diagnostic and/or therapeutic opportunities involving this protein.

TESTOSTERONE ADMINISTRATION PREVENTS SKELETAL MUSCLE ATROPHY AND ENHANCES RESISTANCE TO FATIGUE IN ORCHIDECTOMISED MALE MICE

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The mechanism of androgen action in muscle growth and function remains poorly understood. Our aim is to investigate the effect of androgens on muscle mass and function using an in vivo androgen withdrawal/replacement mouse model. Eight week-old C57BL6 male mice were orchidectomised (Orx) or sham-operated (Sham) and treated for 10-11 weeks with 3 intraperitoneal testosterone (T) or vehicle (V) implants (n=6/gp). Maximum force and fatigue of fast-twitch (extensor digitorum longus; EDL) and slow-twitch (soleus; SOL) hind limb muscles were determined by in vitro muscle function testing. Histology was performed to assess fibre cross-sectional area (CSA) and fibre type distribution. Orx+V mice had decreased mass of the androgen-responsive seminal vesicles (87%, p<0.005) and levator ani (LA) muscle (85%, p<0.001) compared to Sham+V, and this was prevented with testosterone treatment. Sham+T mice had greater mass of both fast (EDL; 25%, p<0.001) and slow-twitch (SOL; 30%, p=0.001) muscles compared to Orx+V mice, and this was accompanied by greater maximum force production (EDL, 25%; SOL, 25%) (p<0.05). Orx+T mice had a 2-fold increase in fatigue resistance of slow-twitch (p<0.05) but not fast-twitch muscles compared to Orx+V controls. In the LA, orchidectomy-induced muscle atrophy correlated with a decrease in fibre CSA (p<0.001), and this was prevented with testosterone treatment. Proportion of fast-twitch fibres in the SOL muscle was significantly decreased in Orx+V mice compared to Sham+V controls (p<0.05). Assays for metabolic enzyme activity are underway to further investigate the role of androgens in regulation of muscle fibre type characteristics. We have demonstrated that muscle mass and force in male mice is androgen-dependent, and that orchidectomy-induced muscle atrophy is prevented with testosterone treatment. Our results indicate that androgen-induced changes in muscle mass lead to a proportional change in muscle force. In slow-twitch muscle, androgens alter fatigue resistance and fibre type proportion.
EFFECTS OF LOW DOSE ESTRADIOL SILASTIC IMPLANTS IN AROMATASE DEFICIENT (ARKO) MICE.

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The vital role of estrogen in male as well as female physiology, notably in gonads, bone, brain and fat, has been studied using estrogen deficient mice created by global knock-out of the unique aromatase enzyme which mediates irreversible conversion of testosterone (T) to estradiol (E₂). Studies of physiological E₂ replacement in males are limited by the ability to deliver very low E₂ doses. We developed silastic implants that deliver low dose E₂ to maintain physiological blood E₂ in castrate mice for prolonged periods(1). To verify E₂ effects in male mice lacking non-gonadal sources of E₂ production, we examined these E₂ implants in global aromatase knockout (ArKO) mice.

Male ArKO mice at 8-10 weeks had subdermal insertion of a 1cm silastic implant filled with E₂ recrystallized and diluted with cholesterol for 2 or 4 weeks, a dose maintaining physiological blood E₂ levels for >6 weeks in castrate normal male mice (1). Untreated wild type (WT) and ArKO male mice were controls. Increased serum T in ArKO mice (49±9 nM) was normalized at 2 (12±3nM) and 4 (14±3 nM) weeks compared with WT (16±4 nM). Similarly, E₂ treatment reduced the increased seminal vesicles in ArKO towards that of WT mice while testis weight was unaffected by E₂ deficiency or treatment. Prostate lobes were hypertrophied in control ArKO mice but displayed lobe-specific response to low dose E₂ treatment, with ventral, anterior and dorsal lobe weights suppressed by E₂ treatment towards WT levels. By contrast, the lateral prostate lobe was unaffected by aromatase deficiency or E₂ treatment. This study using the congenitally estrogen deficient ArKO male mice demonstrates prostate lobe-specific responses to E₂ deficiency and treatment while further validating the modified silastic implants for the sustained delivery of low dose, physiological E₂ levels.

(1) Spaliviero et al Proceedings ESA 2004, Sydney

INHIBIN-ALPHA IN PROSTATE CANCER: TUMOR SUPPRESSOR AND PRO-METASTATIC FACTOR


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Inhibin-alpha (INHA) has been shown to act as a tumor suppressor in mice yet is elevated in women with ovarian cancer. Similarly, studies investigating the role of INHA in prostate cancer (PCa) yield conflicting results. We have previously reported the down-regulation of INHA in PCa yet prostate tumors from patients with high risk of recurrence have been reported to show increased INHA expression.

We have suggested a unifying hypothesis that addresses this apparent contradiction and propose that INHA may function as a tumor suppressor in the early stages of tumorigenesis but switches to become a pro-metastatic factor during later stage disease. This study examines the effect of expressing INHA in two INHA negative prostate cancer cell lines, which represent androgen dependent, well differentiated, poorly tumorigenic LNCaP cell line and androgen independent, poorly differentiated highly tumorigenic PC3 cell line.

In vitro and in vivo functional studies using LNCaP cells demonstrated over-expression of INHA significantly reduces rate of cell growth, proliferation and tumor size. This supports INHAs role as a tumor suppressor in the early stages of tumorigenesis. Similar studies involving PC3 cells over-expressing INHA demonstrated significantly increased rate of cell growth and proliferation in vitro consistent with pro-metastatic or loss of tumor suppressive role of INHA, but invasive capacity, tumor size and the incidence of bone lesions were not increased, rather they were decreased (consistent with tumor suppressive effects); orthotopic inoculation showed increased tumor size and increased incidence of lymph node metastasis. This supports the role of INHA as a pro-metastatic factor but indicates that this role maybe tissue specific. The increase in lymph node metastasis was further supported by increased in lymphangiogenesis factor (VEGF-C) in the cells.

Overall, this study demonstrates that the biological function of INHA is dependent on the stage of carcinogenesis thereby supporting the hypothesis and indicates that there is indeed a correlation between INHA levels and end stage PCa but the precise role of INHA during this stage is dependent on the environment.
TESTICULAR HYPERTROPHY AFTER HEMICAstration IN THE NEONATAL BOAR REQUIRES GONADOTROPHIN BUT NOT TESTOSTERONE SUPPORT

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Circulating concentrations of testosterone were unchanged after hematicastration in boars (1) implying that the synthesis and secretion of testosterone by the remaining testis was increased. To determine the requirement for gonadotrophin and testosterone support for testicular hypertrophy after hematicastration in neonatal boars animals were assigned at birth to one of six treatments (n = 5 per treatment): Group 1, control, no treatment; Group 2, implanted with a gonadotrophin releasing hormone (GnRH) agonist (Deslorelin) within 24h of birth (Day 0) to suppress gonadotrophins; Group 3, hematicastrated on Day 10; Group 4, implanted with agonist (Day 0) and hematicastrated (Day 10); Group 5, implanted with agonist (Day 10) and hematicastrated (Day 10); Group 6, implanted with agonist (Day 0), hematicastrated (Day 10) and treated with testosterone from Day 10. The GnRH agonist Deslorelin (Peptech Animal Health Pty Ltd) was administered as a controlled-release implant (10-20 mg/24h) and testosterone (VR Testoprop, Jurox Pty Ltd) was injected i.m. (100mg) on alternate days from Day 10-24. The right testis was removed on Day 25. Treatment with agonist from Day 0 (Group 2, 4) or Day 10 (Group 5) suppressed (P<0.01) testicular growth, irrespective of whether boars were also hematicastrated (Group 4, 5). Hematicastration alone (Group 3) resulted in an enhanced rate of testicular growth and on Day 25 the right testis (6.7 ± 0.8g; mean ± SEM) was heavier (P<0.01) than in controls (4.3 ± 0.4g). Boars implanted with agonist, hematicastrated, and treated with testosterone (Group 6) did not show testicular hypertrophy and on Day 25 had a smaller (P<0.01) testis (0.8 ± 0.1g) than controls. The epididymis (an androgen dependent organ) for boars in Group 6 was similar (1.4 ± 0.2g) to controls (1.2 ± 0.1g). It was concluded that testosterone is not essential for testicular hypertrophy after hematicastration in boars but gonadotrophin support is necessary.

(1) Theriogenology 1981: 16; 249-257

OVARIAN STATUS AND EMBRYO YIELDS IN SUPEROVULATED EWES: THE EQUILIBRIUM BETWEEN ABSENCE OF DOMINANT FOLLICLES AND PRESENCE OF SIZE-ADEQUATE FOLLICLES

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In sheep, ideal ovarian conditions for higher embryo yields are related to the presence of a high number of gonadotrophin-responsive follicles at start the superovulatory treatment (1), in absence of a dominant follicle (2). However, there are no studies regarding how size of subordinate follicles may affect the embryo outputs. Evaluation of such effect was carried out in forty-three Manchega ewes treated with an intravaginal progestagen for 14 days and superovulated with eight decreasing doses of oFSH (OVAGEN™), starting on Day 12 after sponge insertion. The diameter of the largest follicle (LF1) and the second largest follicle (LF2) were determined by ultrasonography at the first two FSH doses (0 and 12 hours, respectively). Embryos were recovered and evaluated on Day 21. Neither size of LF1 nor size of LF2 were found to affect ovulation rate; however, a higher size of both largest follicles decreased recovery rates (r = 0.608, P < 0.05 for LF1 and: r = 0.884, P < 0.05 for LF2). Thereafter, embryo viability was also negatively affected by a larger LF1 due to a lower fertilization rate and a higher number of degenerated embryos (r = 0.464, P < 0.05 and r = 0.509, P < 0.05, respectively). Conversely, a lower LF2 at both first FSH doses was found to be related to a decreased embryo viability (r = 0.877, P < 0.05). Current results confirmed previous reports of effects of dominant follicles on rates of embryo recovery, fertilization and degeneration. However, is the first report that highlights the role of the size limit of accompanying follicles; the second large follicle may exert co-dominance whilst too small subordinate follicles ovulate, but may be too immature to give rise a viable embryo.

(1) Gonzalez-Bulnes et al. Theriogenology; 2003; 60:281–8
(2) Brebion and Cognie 1989, 5th AETE Proc
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EXPRESSION PROFILES OF STEROID RECEPTOR-ASSOCIATED IMMUNOPHILINS IN HUMAN ENDOMETRIUM DURING THE MENSTRUAL CYCLE

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Hormone-free steroid receptors assemble into heterocomplexes with several molecular chaperone components, including the major heat shock protein Hsp90 and Hsp90-associated co-chaperones such as the immunophilins cyclophilin 40 (CyP40), FKBP51, FKBP52 and PP5. There is emerging evidence that these immunophilins have a role in modulating receptor responsiveness to hormone. This may be determined by the relative expression of these proteins within target cells with certain immunophilins (e.g. FKBP52) acting to potentiate receptor activity and others (e.g. FKBP51) to attenuate receptor function. In addition to their pivotal role in hormonal signalling, the steroid receptor-associated immunophilins are themselves hormonally regulated. Human endometrium undergoes profound changes through the course of the menstrual cycle, largely attributable to the effects of estrogen and progesterone. The aim of the present study was to determine the expression profiles of the immunophilin co-chaperones in human endometrium throughout the menstrual cycle.

Using immunohistochemical techniques we have preliminary data that shows expression of the immunophilins FKBP52 and CyP40 in glandular epithelium follows a parallel expression pattern, revealing elevated expression during the secretory phase of the menstrual cycle. With FKBP52, increased expression was evident from the early secretory phase and remained elevated through to the late secretory phase. CyP40 expression was more variable during the secretory phase, with maximal expression occurring in mid-secretory phase and then decreasing slightly in the late secretory phase. In the stroma there was a trend to a more gradual increase in expression levels of both FKBP52 and CyP40 from the proliferative phase through to the late secretory phase. For both immunophilins staining was more intense in the glandular epithelium relative to stromal tissue. Our data suggest that CyP40 and FKBP52 might be required to support further growth and development of secretory phase endometrium. Expression profiling of FKBP51 and PP5 is in progress.

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A PHARMACOGENOMIC APPROACH TO THE TREATMENT OF INFERTILITY IN POLYCYSTIC OVARIAN SYNDROME

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Polycystic ovarian syndrome (PCOS) is a very prevalent and heterogeneous disease, affecting five to ten percent of women. The aetiology of PCOS remains unknown; however, genetic involvement is recognised. Patients suffer significant reproductive and metabolic morbidity. Obesity is strongly associated with the disease, and in New Zealand, obese individuals are ineligible for the standard infertility treatment (clomiphene citrate). As these patients have very few treatment options and poor fertility prognosis, there has been a significant drive to treat these patients by other means.

Recently, metformin, an insulin sensitising agent, has been used to treat the metabolic and endocrine abnormalities of PCOS. Small scale observational studies indicate that only 60% of women ovulate in response to metformin. Currently, no parameters exist to predict which patients will be sensitive to metformin therapy. The aim of this research was to investigate novel genetic and phenotypic markers of therapeutic outcome to metformin treatment.

In collaboration with the first large scale controlled trial of metformin at National Women’s Hospital, Auckland, New Zealand, pharmacogenomics was investigated as a predictor of fertility response in an obese PCOS patient cohort. Genetic analysis of polymorphisms in candidate genes revealed significant predictive value of polymorphisms in two genes, namely the steroidogenetic enzyme, CYP17, and insulin receptor substrate-2. Phenotypic markers of insulin resistance also appeared to predict drug response, where a higher degree of insulin resistance was associated with a positive therapeutic response to metformin.

Preliminary results of this novel study warrant further investigation. Pharmacogenomic and phenotypic predictors may identify patients sensitive to therapy prior to treatment. Clinical application of this approach is likely to have a substantial impact on the treatment of PCOS, and hence help alleviate the clinical and social morbidity that is caused by this prevalent reproductive disorder.
THE ENDOCRINE RESPONSE OF MAIDEN EWES TO THE RAM EFFECT IS NOT DEPENDENT ON PRIOR EXPERIENCE WITH RAMS

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Murtagh et al., (1984) found that pre-exposure of maiden ewes to rams during anoestrus increased the proportion of ewes ovulating in response to the ram effect. This study tested whether pre-exposure of maiden mule ewes to rams during mid-anoestrus would enhance their endocrine response when subsequently introduced to rams during late anoestrus. During mid-anoestrus (June) ewes were kept with rams for 7 days (RE, ram-experienced; n=6) or isolated from ram contact (RN, ram-naïve; n=6). All ewes were subsequently isolated from ram contact. During late anoestrus (September), the ewes were introduced to rams midway through a frequent blood-sampling regime (every 12 minutes for 12 hours) Both RE and RN ewes had a significant increase in LH pulse frequency and basal LH concentrations in response to ram introduction. RE ewes had a significant increase in mean LH concentrations and this response tended towards significance in RN ewes. Overall there was no significant effect of prior ram experience on the LH response or on the proportion of ewes having an LH surge.

In conclusion, the endocrine response to the ram effect is not dependent on and does not appear to be greatly enhanced by prior ram experience.


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Table 1. LH characteristics of RE and RN ewes before and after ram introduction within treatment. S P<0.01, *P<0.05; **P<0.01; ***P<0.001.

SECRETION PATTERNS OF LH AND FSH AROUND ESTRUS IN MITHUN (BOS FRONTALIS)

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Mithun (Bos frontalis) a semi-domesticated bovine, reared primarily for beef, is mainly distributed in parts of India, Myanmar, Bhutan, China and Bangladesh. At present no information is available on the secretion pattern of gonadotropins around estrus in mithun. The study was conducted on 10 adult mithuns to establish the secretion patterns of LH and FSH around estrus. From estrous onset jugular vein blood samples were collected every 2 hr for 72 and 96 hr, respectively from animals without and with standing heat to determine the plasma concentrations of LH and FSH.

Frequent short-duration low-amplitude LH and FSH surges were observed throughout the estrous period. Average LH concentration (ng/ml) on the day of estrous onset was found higher in animals with (7.1± 0.9) or without (7.1± 0.8) standing heat but was insignificant. The amplitude of peak LH concentration (ng/ml) and interval (h) between estrous onset to peak LH did not differ significantly in animals with (15.8 ± 0.4, 37.0 ± 9.9) or without (16.4 ± 1.2, 42.3 ± 11.6) standing heat. Average FSH concentration (ng/ml) did not vary significantly among different days of estrus in animals without standing heat. But it was found significantly (p<0.01) higher (6.8 ± 0.4) on the second day of estrus in animals with standing heat. The amplitude of peak FSH concentration (ng/ml) and interval (h) between estrous onset to peak FSH did not differ significantly in animals with (14.8 ± 1.2, 51.0 ± 16.5) or without (15.8 ± 0.6, 33.6 ± 8.7) standing heat. A definite temporal coupling between LH and FSH secretions was absent.

The results suggest 1) frequent short-duration low-amplitude LH and FSH surges may be important for the final maturation of ovulatory follicle and subsequent ovulation; 2) differential regulatory mechanism probably exists to control the LH and FSH secretions.
RETINOIC ACID (RA) REGULATION OF TYPE II RECEPTORS FOR THE TGF-β SUPERFAMILY IN A MOUSE ADRENOCORTICAL CELL LINE

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Inhibin A binds its co-receptor, betaglycan, and the type II receptors for activin or bone morphogenetic protein (BMP) in high affinity complexes that block activin and BMP actions. We previously correlated changes in the levels of betaglycan mRNA induced by physiological factors in several mouse cell lines with changes in inhibin binding to betaglycan on the cell surfaces. However, RA increased the binding of inhibin A to mouse adrenocortical AC cells with little stimulation of betaglycan expression. The present studies were undertaken to examine possible explanations for this anomalous finding.

Treatment of AC cells with RA (30 μM) for 18 h increased total $[^{125}\text{I}]$inhibin A binding to 124 ± 5% of the DMSO-treated control (mean ± SEM, n=12), whereas it suppressed the betaglycan mRNA level to 59 ± 7% of control (n=6), measured using real-time RT-PCR. RA treatment increased the $[^{125}\text{I}]$inhibin A affinity labelling of a protein species of deduced size 75 kDa, consistent in size with several type II receptors for the TGF-β superfamily. AC cells were found to express mRNA encoding type II receptors for activin (ActRII, ActRIIB), BMP (BMPRII) and Mullerian Inhibiting Substance (MISRII), but expression of the type II receptor for transforming growth factor-β (TβRII) was minor. RA dose-dependently suppressed the levels of mRNA for ActRIIB and MISRII by around 50% in each case, and further decreased the low level of TβRII expression, while the level of mRNA for ActRII was little changed. However, the level of BMPRII mRNA in AC cells was increased more than 2.5-fold in response to RA (30 μM).

We conclude from these studies that the AC cells may respond to many TGF-β superfamily members, but not TGF-β, and that the BMP type II receptor is a candidate for the protein that mediates the increase in inhibin binding following stimulation of AC cells with RA.

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EFFECTS OF CORTICOTROPHIN RELEASING HORMONE AND SALBUTAMOL ON TERM PREGNANT HUMAN MYOMETRIAL CONTRACTILE ACTIVITY IN VITRO

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The function of CRH in pregnancy is unknown. One potential role is modulation of myometrial contractility. CRH stimulation of myometrial cells/tissue in vitro increases cAMP suggesting it should cause uterine relaxation. These findings contrast with earlier studies which indicate that CRH augments the contractile effects of oxytocin [1,2]. Furthermore recent studies suggest that high levels of CRH, as found at term, can down-regulate CRH receptors [3].

We hypothesized that high concentrations of CRH may lead not only to homologous desensitization of CRH receptors but also to heterologous desensitization of other receptors linked to adenylate cyclase/cAMP production such as the β2-adrenergic receptor.

To test this hypothesis, isometric tension recordings were performed on term, spontaneously contracting myometrial strips (n=8) obtained at Caesarean section. Strips (7 x 2 mm) were mounted in organ baths containing Krebs’ (37°C, pH 7.4, 95% O2 /5% CO2) . The effects of CRH 10^{-7} M alone and as pretreatment (for 30 mins) prior to salbutamol (10^{-10}–10^{-4} M, log intervals) were examined. Responses were measured by integrating the area under the tension curve. Responses to CRH and salbutamol were compared as a percentage of spontaneous activity prior to treatment.

Salbutamol caused a 42±13% (mean±SEM) reduction in contractility, ranging between 13-100% relaxation. Pretreatment with CRH under the same conditions resulted in a 26±7% reduction in contractility, ranging from 4-60%. There was no significant difference in relaxation between salbutamol alone or with CRH. Treatment with CRH 10^{-7} M alone exerted no significant effects.

Conclusions: We did not show a significant difference between salbutamol alone or in the presence of CRH. However, there was significant interpatient variability in the responses to salbutamol, increasing the possibility of a type 2 error.

(1) Quartero et al. Placenta 10(5),1989,pp439
(3) Grammatopoulos et al. Molec Endocrinol 19(2),2005,pp474
A PREGNANCY WITH HYDATIDIFORM MOLE, CO-EXISTING TWIN AND SEVERE THYROTOXICOSIS: A CASE REPORT

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Hyperthyroidism in pregnancy occurs with a prevalence of 0.1-0.2%(1). Clinical hyperthyroidism is rarely described nowadays in patients with a molar pregnancy (2). The association of a live fetus and a co-existing mole within a twin pregnancy is an unusual event, occurring with a frequency of 1 per 22,000-100,000 pregnancies (3). We present the case of a 28-year-old woman with hydatidiform mole and co-existing twin. She presented to her general practitioner at 15 weeks gestation with a history of palpitations, tremor and weight loss of 5kg over a period of 3 weeks. Thyroid function tests showed a free T4 at 42.0pmol/L (8.0-22.0), Free T3 at 21.6pmol/L (2.5-6.0) and TSH at 0.009mU/L (0.3-4.0). Treatment was initiated with propylthiouracil (PTU). The serum β-hCG was noted to be grossly elevated at 3503219 IU/L at 17 weeks gestation (6000 – 50,000). Ultrasound evaluation demonstrated a normal fetus co-existing with a mass consistent with a hydatidiform mole at which point she was referred to the local base hospital. An endocrinology opinion was sought on admission and her PTU dose was reduced as she had marginally low thyroid function on testing. The pregnancy ended prematurely a few days later when pre-eclampsia developed and fetal death in-utero occurred at 23 weeks gestation. Following uterine evacuation clinical and biochemical resolution of the hyperthyroidism resulted with decline in serum β-hCG levels, enabling cessation of anti-thyroid treatment. She was later found to be thyroid receptor antibody negative. The fetus appeared morphologically normal. Histopathology of the mass confirmed a complete hydatidiform mole. We provide details of this distinctive case in which thyrotoxicosis was the initial presenting feature of the patient's condition. A review of the literature indicates that despite numerous case reports of a complete mole with co-existing twin only a few report complicating hyperthyroidism.


SEX DIFFERENCES IN PLACENTAL CYTOKINE EXPRESSION AND THEIR RELATIONSHIP TO FETAL CORTISOL


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In human pregnancy, there are sexually dimorphic differences in morbidity and mortality with the male fetus being more at risk of a poor outcome than the female fetus in association with complications such as placental insufficiency, pre-eclampsia, infection, intra-uterine growth restriction and pre-term delivery. The physiological mechanisms that confer differences in mortality in human male and female fetuses are unknown. The hypothalamic-pituitary-adrenal (HPA) axis and immune system are closely linked as adrenal glucocorticoid production modulates the inflammatory response by inhibiting pro-inflammatory cytokine mRNA expression. Sex specific differences in HPA and immune function have previously been observed in neonatal animal models and adult humans due to differences in cortisol bioavailability. In the human placenta, we have identified that sexually dimorphic cortisol bioavailability is controlled by 11beta-hydroxysteroid dehydrogenase type 2 (11b-HSD 2) activity and this may confer differences in fetal-placental immune function. We hypothesise that there are sex specific differences in placental cytokine expression due to differences in cortisol bioavailability. In this study we aim to determine if there are sex specific differences in human placental cytokine mRNA expression and whether this is related to cord blood cortisol concentrations. Fetal cord blood was collected at the time of delivery and cortisol levels were measured using a commercial radioimmunoassay. Quantitative Real Time RT-PCR was used to examine the expression of cytokines in the placenta. The expression of cytokine mRNA in males (n=10) was positively correlated to the concentration of cortisol in cord blood. This occurred for TNFa (R²=0.444, P=0.0255), IL-6 (R²=0.4984, P=0.0289), IL-1 (R²=0.5117, P=0.0310), IL-8 (R²=0.6745, P=0.0038), and IL-5 (R²=0.4524, P=0.0331). There was no correlation between cortisol and female (n=11) placental cytokine mRNA expression. This data suggests there is a sexually dimorphic difference in the relationship between cortisol and placental cytokine expression.
MATERNAL AND CORD PLASMA CYTOKINE / CHEMOKINE PROFILES IN PREGNANCIES COMPLICATED BY ASTHMA

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The influence of pregnancy on maternal asthma is not unimodal but studies have indicated that in about a third of patients, asthma worsens as gestation progresses particularly in the last trimester. The reason for this effect is unknown. Asthma is an inflammatory disease characterised by the activation of T helper (Th) -2 response and expression of chemokines promoting the recruitment and survival of activated immune cells such as T-lymphocytes, eosinophils and neutrophils. In addition pregnancy is also associated with a Th-2 response. The aim of this study was to determine whether inflammatory mediators interleukin (IL) -6, IL-8, eotaxin and regulated upon activation normal T cell expressed and secreted (RANTES) are increased in pregnancies complicated by asthma. Peripheral blood was collected from asthmatic (n = 35) and nonasthmatic patients (n = 13) in the third trimester (30-32 weeks) of pregnancy. Cord blood (n = 24) was also collected after normal vaginal delivery. Blood samples were centrifuged at 1000g for 15 minutes and plasma samples were collected and assayed for IL-6, IL-8, eotaxin and RANTES using an enzyme linked immunosorbent assay (ELISA) kit. The levels of all the cytokines measured were significantly lower in the maternal circulation compared with the cord plasma (Kruskal Wallis, P < 0.01) in both asthmatic and nonasthmatic patients. There were no significant differences in the levels of maternal IL-6, IL-8, eotaxin and RANTES between asthmatics and nonasthmatics, though there was a trend towards increased level in asthmatic individuals. Interestingly, eotaxin was the only asthma specific chemokine that was significantly higher in cord plasma of asthmatic patients (Mann-Whitney, P = 0.004) compared with cord plasma from normal pregnancies. The results of this study suggest that eotaxin production is increased in the feto-placental unit of asthmatic pregnancies. The presence of asthma does not enhance Th-2 cytokine production during pregnancy.

IDENTIFICATION AND CHARACTERISATION OF MACROPHAGES IN PLACENTAE FROM PREGNANCIES COMPLICATED BY ASTHMA

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Maternal asthma during pregnancy has been associated with poor foetal outcome. Further studies have demonstrated that the outcome of pregnancy in optimally treated asthmatic women with inhaled glucocorticoids does not significantly differ from that of healthy women. Prospective studies reveal gender-specific outcomes in pregnancies complicated by asthma. In the presence of a female foetus, studies have observed the exacerbation of maternal asthma and an increased requirement for treatment of symptoms. It has also been identified that when asthma, regardless of its severity, is not treated during pregnancy with inhaled steroids female foetal growth is significantly reduced. The male foetus appears unaffected by asthma or its treatment. The increased inflammation evident with asthma is thought to alter placental function. Studies have noted an increase during gestation of monocytes in the maternal circulation in asthmatic women who did not use inhaled steroids and were pregnant with a female foetus.

Since monocytes are the precursors to macrophages and increased recruitment of placental macrophages has been associated with poor pregnancy outcome, this study aimed to identify and quantify the population densities of placental macrophages. Immunohistochemistry was utilised with specific staining of macrophages using CD68, to assess the effect of asthma on these inflammatory cells. Populations were also assessed in relation to glucocorticoid treatment and the presence of a female or male foetus, with these assessments compared to a non-asthmatic control group. No significant difference was observed between the placental macrophage number in pregnancies complicated by asthma, with (n=50) or without glucocorticoid treatment (n=50), and non-asthmatic pregnancies (n=39). In addition, there was no evidence of a gender-specific difference in the placental macrophage population. This study demonstrates that the poor foetal outcome associated with pregnancies complicated by asthma may not be due to increased infiltration of placental macrophages.
PLACENTAL GLUCOCORTICOID RECEPTOR EXPRESSION IN PREGNANCIES COMPLICATED BY ASTHMA


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Foetal growth and neonatal birth weight are significant contributing factors to the development of adult disease states in later life. Most animal models indicate that glucocorticoids are central in the regulation of organ maturation and foetal growth with increased bioactive cortisol being associated with reduced foetal growth. In human pregnancy, we have identified sexually dimorphic differences in foetal growth with the female foetus reducing growth in response to maternal asthma and the male foetus continuing to grow at a normal rate but being at an increased risk of *in utero* death. Interestingly, both the male and female foetuses were exposed to the same concentration of cortisol and yet there was a differential growth response. These findings lead to the question of whether there were sexually dimorphic differences in glucocorticoid receptor expression in placentae of male and female foetuses in pregnancies complicated by asthma that may confer differences in the response to cortisol. Both total GR and the alpha isoform mRNA expression were measured using quantitative RT-PCR in asthmatic and non-asthmatic pregnancies. GR total and GRα mRNA expression was significantly greater in placentae of female foetuses (n=6) from non-asthmatic pregnancies when compared to placentae of males (n=10). In the presence of asthma, GR mRNA was decreased in placentae of female foetuses (n=16) and increased in the placentae of male foetuses (n=21). These findings indicate that the sexually dimorphic response to a change in cortisol concentration may be mediated by differences in GR expression and regulation.

DIABETIC PREGNANCY & CONGENITAL MALFORMATIONS: WHEN SHOULD WE STOP WORRYING?

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Pregnancy outcomes for women with pregestational diabetes in the last decade have been disappointing despite the targets set by the St Vincent’s Declaration in 1989. It has been well established that congenital malformations are strongly associated with poor metabolic control prior to and during the pregnancy. A recent Australian audit in 10 teaching hospitals with an interest in high risk obstetric medicine demonstrated a four times increase in the stillbirth and congenital malformation rate, similar to the findings in reported studies from Europe, UK and New Zealand. Of the major malformations, congenital heart disease is one of the commonest. Fetal echocardiogram is currently recommended in pregestational diabetes in the early second trimester. There is a persistent lack of awareness about the need for pre-conception planning, tight maternal diabetes control and antenatal screening. We report 2 cases that highlight these important issues.

Case 1: A 38 year old woman with type 1 diabetes who had suboptimal glycaemic control prior to and during her pregnancy. Tight control was limited by frequent and unexpected episodes of hypoglycaemia. Antenatal fetal structural and growth ultrasound scans were normal although a fetal echocardiogram was not performed. A subsequent neonatal echocardiogram revealed a ventricular septal defect with pulmonary regurgitation requiring surgical correction.

Case 2: A 34 year old woman with type 1 diabetes had an unplanned pregnancy and poor metabolic control in the first trimester. Fetal echocardiogram at 24 weeks gestation was normal. Postnatal paediatric echocardiogram demonstrated moderate pulmonary stenosis and atrial septal defect with left to right shunting. This was managed conservatively with close monitoring.

A literature review and a local audit currently in process will be discussed.
ONCOGENIC OSTEOMALACIA: A CASE OF DIAGNOSTIC DILEMMA AND MANAGEMENT CHALLENGE
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A 75 year old previously independent woman presents with a significant decline in mobility preceded by progressive proximal myalgia and weakness. Investigations revealed multiple minimal trauma fractures, marked renal phosphate wasting and apparent impaired vitamin D metabolism consistent with oncogenic osteomalacia. Diagnosis was further confirmed by a bone biopsy with tetracycline label and an elevated fibroblast growth factor 23 (FGF-23) level. Imaging studies demonstrated a left adrenal mass, left superior parathyroid adenoma and left thyroid nodule, all of which were octreotide avid. The patient responded well to vitamin D and phosphate supplements. A clinical decision was made to have all 3 lesions surgically excised. Pathology revealed benign adenoma of both the adrenal and parathyroid mass. However, papillary carcinoma with capsular invasion was demonstrated in the hemithyroid specimen. FGF-23 levels fell postoperatively but rose again in 3 months, coinciding with a clinical recurrence upon cessation of the supplements. Review of the MRI scans raised suspicion of multiple hemangiomas in a number of thoracic vertebrae and an unusual lesion anterior to the mandible. Selective venous sampling and positron emission tomography are currently considered. Oncogenic osteomalacia is an acquired paraneoplastic syndrome. Traditionally, there is a long lag time to diagnosis and location of culprit lesions. Phosphatonin is produced by the responsible tumour. Fibroblast growth factor 23 (FGF-23) has been demonstrated to be a major phosphatonin and other candidates have also been discussed in literature. Thyroid carcinoma has not been reported in literature to be associated with oncogenic osteomalacia but the findings in this patient may be coincidental. Whilst oncogenic osteomalacia is a rare disease, it is important to consider it in the assessment of patients presenting with weakness and pain. A literature review of novel therapies will be discussed.

CASE PRESENTATION: WIDESPREAD OSTEITIS FIBROSA CYSTICA AND PRIMARY HYPERPARATHYROIDISM IN A 41 YEAR OLD WOMAN.
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Osteitis fibrosa cystica (OFC) is a rare complication of primary hyperparathyroidism. We present the case of a 41 year old Sri Lankan lady who presented with atraumatic fracture of her right ulnar associated with hypercalcaemia up to 4.22mmol/l (ref range 2.15-2.55). X-rays revealed multiple lytic lesions throughout her long bone, pelvis, ribs and skull consistent with OFC. Subsequent investigations confirmed a diagnosis of primary hyperparathyroidism with an elevated PTH of 140 pmol/l (ref range 1.6-7), a low 25-OH vitamin D 21nmol/l (ref range 31-107) and a bone specific ALP of 95.9ug/l (ref range 2.9-14.5). Bone scan revealed multiple "hot" lesions throughout her skeleton and a sesamibi scan revealed uptake in the mediastinum, due to an intrathymic parathyroid tumour measuring 4.5 x 3.5 x 1.5cm. Representative radiology, bone scans and parathyroid imaging will be shown.
Therapy instituted to try to prevent the post operative occurrence of the “hungry bone syndrome” (including pre-operative calcitriol) will be described. Her post operative course, with respect to calcium, phosphate, Vitamin D, bone mineral density and radiology will be described in detail. Theories as to why OFC is rare will be discussed.
In summary she had a dramatic improvement in her plasma calcium, 24hour urinary calcium excretion initially, and with follow up until the time of the meeting we hope to show improvement in her radiology and BMD.
2. Rao et al, JBMR 2002
3. Harinarayan, Clin Endo 1995
THE MONITORING OF VITAMIN D IN OSTELIN-TREATED PATIENTS
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The measurement of serum 25-hydroxyvitamin D (25-OHD) has become routine in our unit as part of the investigation and management of patients with low bone density. Our usual practice is to titrate the dose of replacement vitamin D2 (Ostelin 1000U) to achieve blood levels of 25-OHD in the middle of the normal range. Recently we observed that despite increasing oral doses of Ostelin, serum 25-OHD levels failed to rise as expected. Literature reports have called attention to inconsistencies in specificity and standardisation of assays for 25-OHD (1) and accordingly we questioned whether our current automated method (the competitive protein binding method (CPB) run on the AdvantageR analyser (Nichols Institute Diagnostics)), was appropriate for patients supplemented with vitamin D2. To assess this, we compared the results using the Nichols AdvantageR Autoanalyser to those obtained using a manual RIA method (Diasorin) in a cross-sectional study of patients receiving Ostelin (Vit D2) supplements. Baseline values from a group of untreated patients (who were not vitamin D deficient) were also included. Mean 25-OHD levels are shown in the table.

<table>
<thead>
<tr>
<th>Ostelin Dose</th>
<th>Nichols CPB (nmol/L)</th>
<th>Diasorin RIA</th>
<th>Significance (RIA vs CPB)</th>
<th>RIA:CPB Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (n=72)</td>
<td>68.3</td>
<td>73.4</td>
<td>ns</td>
<td>1.12</td>
</tr>
<tr>
<td>1000U/d (n=62)</td>
<td>53.0</td>
<td>72.2</td>
<td>p &lt; 0.0001</td>
<td>1.44**</td>
</tr>
<tr>
<td>2000U/d (n=40)</td>
<td>48.0</td>
<td>78.8</td>
<td>p &lt;0.0001</td>
<td>1.68**</td>
</tr>
<tr>
<td>3000U/d (n=6)</td>
<td>44.2</td>
<td>62.2</td>
<td>ns</td>
<td>1.53</td>
</tr>
<tr>
<td>4000U/d (n=2)</td>
<td>36.9</td>
<td>74.0</td>
<td>ns</td>
<td>2.01</td>
</tr>
</tbody>
</table>

** Highly significant (p<0.0001) for mean ratio (vs. untreated). At higher doses the significance was lost possibly due to small sample sizes.

For patients naive to supplementation, the RIA and CPB assays produced similar measures of 25-OHD. However, their ratio was increased for each of the dosage groups, possibly reflecting the relative underestimation by the Nichols AdvantageR of an increased proportion of 25-OHD2 present in the serum.

Conclusion: The Nichols CPB method appears to be underestimating 25-OHD2 in patients receiving vitamin D2 replacement. In its present form, it may not be suitable for monitoring vitamin D sufficiency when vitamin D2 is prescribed.


IMPACT OF A HOSPITAL-BASED INTERVENTION ON THE OUTCOME OF MINIMAL TRAUMA FRACTURES
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Women who sustain a low trauma fracture are at significantly increased risk of subsequent fracture, and suffer increased morbidity and mortality. A number of medical therapies have now been proven in randomized controlled trials to reduce the incidence of minimal trauma fracture in selected high-risk women. Despite this, most minimal trauma fracture patients are discharged from hospital without the initiation of effective medical therapy to prevent recurrent fractures. Public healthcare institutions have an obligation to act to close this “care gap”. This study is a prospective randomised evaluation of the efficacy of a hospital-based intervention delivered to patients admitted with a minimal-trauma fracture at our institution. Patients with a diagnosis of a fragility fracture were randomised to two groups: an intervention group and a conventional treatment group. The intervention consisted of: clinical review, dual energy X-ray absorptiometry (DEXA) scan, basic biochemistry, a letter of recommendation to the LMO, provision of educational materials and if appropriate initiation of Calcium and Vitamin D, a Bisphosphonate or Raloxifene. The conventional group had no specific intervention and were managed by orthopaedic and rehabilitation specialists. Both groups were followed up (and will continue to be followed up at 1 and 2 years) to determine the proportion of patients who are initiated and remain on effective anti-fracture therapy (primary outcome). At this stage, baseline data for this study is available: 112 patients have been enrolled: 56 in intervention group, 56 in standard-care group. 12 patients have completed the biochemistry, DEXA and clinic review. Of these results are as follows: Biochemistry – one male with mild testosterone deficiency; one case of subclinical hyperthyroidism. All had 25(OH) Vitamin D3 < 100. Average level was 30-40. Skeletal risk score – three patients with a score between 2 and 5 with the remainder <1. DEXA scan: One patient with osteoporotic range T scores. 2 patients with T < -1.5 (at Femoral Neck T score (-1.66 and -2.02) Lumbar spine (-1.7 and -1.83) All except 2 patients had a T score < -1 at both femoral neck and lumbar spine. Treatment initiated: Risedronate on one patient, Vitamin D initiated on 2 patients. The data will be updated prior to presentation to present a more complete overview of our baseline population.
ASSESSMENT OF THE CLINICAL UTILITY OF URINARY NTX IN OSTEOPOROSIS—AN AUDIT

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Background: Urinary levels of cross-linked N-terminal telopeptide of type I collagen (NTX) are used as a marker of bone resorption and are useful for the diagnosis of metabolic bone diseases and monitoring response of patients treated with anti-resorptive agents. We aimed to determine how urinary NTX results alter clinical decision making by physicians treating patients with osteoporosis in a tertiary hospital setting.

Methods: We reviewed patient notes of all new NTX requests in 2002 and 2003 with at least one subsequent repeat measurement. Patients with a diagnosis of osteoporosis and both pre- and post-treatment measurements of bone mineral density (BMD) and NTX were included. Urinary NTX was measured with the Osteomark enzyme-linked immunosorbent assay (Wampole Laboratories, Princeton NJ, USA). BMD of the hip and lumbar spine was measured using dual energy x-ray (DEXA).

Results: A total of 357 patients had serial NTX requests during the time period. Sixty five of these patients had a diagnosis of osteoporosis. Out of 37 patients treated for osteoporosis who had complete data available, 29 patients had concordant results between BMD and NTX and 8 patients had discordant results. Of these only one patient had treatment changed as a result of a lack of reduction in NTX following treatment. Thirteen patients had therapy altered. Common reasons for altering therapy were patient non-compliance, side effects and failure of BMD to increase.

Conclusions: Alteration to therapy in this patient population is mainly dictated by issues such as patient compliance, medication side effects and bone mineral density results rather than urinary NTX values.

CARDIOVASCULAR EFFECTS OF MEDICAL THERAPIES IN POLYCYSTIC OVARY SYNDROME

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Aim: To determine the impact of commonly used treatments for PCOS on CV risk factors including IR and on non invasive surrogate markers of CV disease.

Methods: 100 overweight women with PCOS were randomly assigned to either 35mcg ethinyl estradiol (EE)/2mg cyproterone acetate (CPA), Metformin (1g twice daily) or a low dose OCP (20mcg EE/100mcg levonorgestrel) combined with an Aldactone 50mg bd for 6 months. Arterial structure and function and metabolic parameters were assessed before and 6 months after treatment. Arterial structure and function was assessed using carotid intimal media thickness [IMT], pulse wave velocity [PWV] and flow mediated vasodilation [FMD]. Metabolic parameters assessed included insulin and glucose during an OGTT, lipid parameters and serum androgens.

Results: All 3 treatments significantly improved hirsutism and menstrual cycle length however only the OCP groups showed improvements in androgens. IR improved in the Metformin group and deteriorated in the higher dose OCP group. Arterial stiffness worsened in the higher dose OCP group (PWV 7.46 vs. 8.03 m/s P<0.05). The increase in IR was a significant predictor of the increased arterial stiffness.

Conclusions: In overweight women with PCOS Metformin, the low dose OCP/Aldactone combination and a higher dose OCP had similar clinical efficacy. Metformin decreased IR, the low dose OCP had a neutral effect but the high dose OCP was associated with worsening IR and arterial stiffness. If an OCP is required by women with PCOS a low dose estrogen preparation should be used. Insulin sensitizing agents should be considered as the primary therapy in the symptomatic management of women with PCOS particularly in those with additional CV risk factors.
CLINICAL EXPERIENCE WITH 2 YEARS OF Zoledronic Acid in Osteoporosis

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²Endocrinology, Concord Hospital, NSW, Australia
³Endocrinology, RPAH, NSW, Australia

Intravenous (IV) zoledronic acid (ZA:Zometa, Novartis Pharmaceutical) therapy is used in the management of osteoporosis (Reid et al. 2002). In this study we report our experience with ZA in routine clinical practice in regard to effect on bone mineral density (BMD) in osteoporosis. We reviewed the records of patients who had been given IV ZA (4mg) as a single annual dose. BMD was routinely measured on the day of the first, second and third annual infusion. Results were available at 0 and 1 years (Group 1) in 55 patients (36 women and 14 men), with a mean age of 68 years (range, 19-92 years) and 0, 1 and 2 years in a subset of 39 patients (all postmenopausal women) (Group 2). The analyses used paired t tests. Results are mean + SEM. In group 1, lumbar spine BMD was increased significantly (3.8 + 0.7%) at 12 months (P<0.0001), femoral neck also increased significantly (2.0 + 0.5%) at 12 months (P=0.0001). In group 2, who were followed for a further 12 months, the change in BMD compared to baseline were at the lumbar spine +3.1 + 0.7% and +4.3 + 0.9% respectively at 1 and 2 years, and at the femoral neck 2.5 + 0.6% and 2.5 + 0.8%. No new incident fractures were observed.

(1) Reid, IR et al. NEJM 2002; 346:653-661

THYROTOXIC HYPOKALAEMIC PERIODIC PARALYSIS IN A YOUNG AUSTRALIAN CAUCASIAN MAN: A CASE REPORT

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²Orange Campus, University of Sydney, Orange, NSW, Australia
³Cairns Base Hospital, Cairns, QLD, Australia

A 23-year-old Australian Caucasian male presented to a country hospital with rapid onset quadriperisis with a several weeks' history of palpitations increased anxiety, tremors and tiredness suggestive of thyrotoxicosis. Examination revealed tachycardia, hypotonia, and quadriperisis. He was haemodynamically stable. Neck examination showed a smooth goiter with a loud Thyroid bruit and he was positive for thyrotoxic eye signs.

On admission his investigation results were as follows. Serum Potassium = 1.7 mmol/L (3.5-5.6), TSH = 0.0 uU/ml, FT4 = 69.9 pmol/L, FT3 > 35 pmol/L, ESR = 15, Cr = 44 umol/L, Urea = 4.1 mmol/L, Thyroid receptor Antibodies = 14.9 IU/L, TC99 thyroid uptake scan showed a homogeneous diffuse uptake of 17%. His family history was unremarkable for thyroid disease or periodic paralysis.

He was treated with intravenous and oral potassium supplementation, Carbimazole and Propranolol. Following correction of his hypokalaemia his quadriperisis improved rapidly and he walked out of hospital upon discharge two days later on Carbimazole and Propranolol therapy. For the last twelve months he has maintained euthyroidism on a small dose of Carbimazole therapy with out any further episodes of thyrotoxicosis or periodic paralysis.

Thyrotoxic Hypokalaemic Periodic Paralysis is relatively common amongst Asians and Latin Americans but remain very rare amongst the Caucasians. A total of about twenty cases are reported in the literature and only a single case was previously published of a white Australian with this condition(1).

(1) Sivagangabalan G et al., Internal Medicine Journal 2003 Sep-Oct; 33(9-10):475
INSULIN LIKE GROWTH FACTOR-2 OCCUPANCY OF THE INSULIN RECEPTOR ISOFORM A: SIGNIFICANCE IN NORMAL AND MALIGNANT TISSUES.
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Insulin and insulin-like growth factor-2 (IGF-2) play disparate roles in adult tissue. While insulin is predominantly a metabolic hormone, IGF-2 plays a significant role in promoting cell growth. Despite this, recent results have suggested that an alternatively spliced isoform of the insulin receptor missing exon 11 (IR-A) binds both insulin and IGF-2 with high affinity. Although both hormones can activate IR-A to produce their specific effects, the full-length insulin receptor, IR-B, is apparently specific only for insulin. Expression of these isoforms is highly tissue-specific. However, it has never been determined if the IGF-2 preference for IR-A correlates with increased IGF-2 occupancy of the insulin receptor, and thus increased signaling. In this study, we investigated the affinity and occupancy of insulin and IGF-2 for insulin receptors purified from rat liver and brain, which express predominantly IR-B and IR-A, respectively. These calculations revealed that, in tissues expressing predominantly IR-B, insulin receptors are 41% and 3% occupied by insulin and IGF-2, respectively. However, in tissues expressing predominantly IR-A, insulin occupancy of receptors increased marginally to 49%, while IGF-II occupancy increased significantly to 16%. However, in the brain where IR-A is also predominant, insulin receptors were 93% and 1% occupied by insulin and IGF-II, respectively. These results suggest that IGF-2 signaling by IR-B, or by IR-A in the brain, play only a minor role. However, IGF-2 signaling in other tissues predominantly expressing IR-A, likely plays a significant role, especially in some malignant tissues that secrete IGF-2 and over-express IR-A.

THE MULTIFUNCTIONAL PROTEIN CREAP IS A NUCLEAR PROTEIN
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The cAMP regulatory element (CRE) is one of the most important regulatory elements determining up regulation of corticotropin releasing hormone (CRH) in the placenta and hypothalamus. A placental cDNA library has previously been screened using the yeast one-hybrid system to identify proteins capable of functionally binding the CRE. A human cDNA encoding a protein with a distinctive combination of modular domains was discovered. This new protein has been named CRE Associated Protein 1 (CREAP1). CREAP contains two leucine-zipper-like domains typical of bZIP transcription factors, a zinc finger-like domain with potential DNA binding properties, a zinc finger-like domain typical of RNA binding proteins, two coiled-coil domains typically found in transcription factors and an SR-rich domain characteristic of proteins involved in RNA splicing.

CREAP has been shown to specifically bind to the CRE of the CRH promoter using an electrophoretic mobility shift assay. A multiple tissue expression array has shown that CREAP is present in a wide variety of human adult and fetal tissues. The CREAP peptide sequence was compared to the protein databases and two highly related proteins of unknown function were found. They are 95% similar to each other over the N-terminal two-thirds and are all very similar (60%) to CREAP1. All three proteins share the coiled-coil, zinc finger, leucine zipper and SR domains. These protein sequence and domain similarities suggest that a new family of human proteins uniquely capable of binding to the CRE and to function in RNA splicing has been identified.

Western blotting has shown that CREAP is only detected in nuclear or total protein extracts and not in cytosolic protein extracts. This nuclear localization further supports a role for CREAP as a transcription factor and/or splicing protein.
IDENTIFICATION OF RENIN AND REGULATORY ROLE OF THE RENIN mRNA-BINDING PROTEINS HUR, HADHB AND CPI IN HUMAN BREAST CANCER CELLS.
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Renin is the enzyme responsible for the cleavage of angiotensinogen to produce angiotensin I and forms a key part of the renin-angiotensin system (RAS). This system is not only crucial for the regulation of blood pressure, fluid and electrolyte balance, but can also affect both cell proliferation and apoptosis. Although renin is typically expressed in the juxtaglomerular cells of the kidney, it has now also been detected in extra-renal sites such as the pancreas, and in the renin-expressing pulmonary carcinoma cell line Calu-6, suggesting renin may be more widely expressed than was once perceived. Of interest, preliminary results from our laboratory have identified (via RT-PCR) expression of renin mRNA in both MDA-MB-468 and MCF-7 breast cancer cells. To our knowledge, this is the first evidence of renin expression in breast cancer cells. We have previously demonstrated (¹) that the RNA-binding proteins (RBPs) HuR, HADHB and CPI bind to the renin 3'UTR and that both renin mRNA and protein levels can be regulated by these RBPs. As such, we wished to determine whether the renin mRNA and protein detected in two breast cancer cell lines was regulated by these RBPs. Immunoprecipitation-RT-PCR and GST pulldown assays are being utilised to assess whether these RBPs immunoprecipitate and interact with renin mRNA in MDA-MB-468 and MCF-7 breast cancer cell lines. In addition, we have utilised siRNA and Western blotting to determine the effect of reducing endogenous RBP levels on renin mRNA and protein expression, while also examining these cells for any resultant effects on proliferation. Our results to date indicate that reducing the levels of HuR significantly reduces renin expression, suggesting a key role for this protein in the renin expression and signaling pathway. In sum, our data demonstrate renin mRNA for the first time in breast cancer cells, and that renin expression is likely to be governed, at least in part, by renin RBPs, such as HuR. Further studies are underway to explore the effect of these RNA-protein interactions on breast cancer cell growth and proliferation.


EXPRESSION OF SFRP-4 AND β-CATENIN IN SEROUS OVARIAN CARCINOMA
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²School of Surgery and Pathology, The University of Western Australia, Crawley, WA, Australia

Ovarian cancers may arise from mutations that activate the Wnt signalling pathway, as elevated Wnt expression has previously been demonstrated in ovarian cancer. Secreted frizzled-related proteins (sFRPs) comprise a family of five secreted glycoproteins that antagonize Wnt signaling. Thus, a role for sFRPs as negative regulators of Wnt signalling may have important implications in ovarian tumorigenesis. In the present study, we investigated the expression of sFRP-4, together with the downstream marker of Wnt activation, phosphorylated β-catenin, in 163 serous ovarian adenocarcinoma samples by using tissue microarrays (TMA) and immunohistochemistry (IHC).

Ovarian cancer samples expressed higher levels of sFRP-4 compared to adjacent normal stroma. However, sFRP-4 expression was not correlated with any clinico-pathological features such as age, International Federation of Gynecological Oncologists (FIGO) stage or histological grade. Therefore, sFRP-4 expression was not an independent prognostic marker for serous carcinoma of the ovary. In addition, FIGO stage was significantly correlated with patient survival (p<0.01) but there was no significant relationship between histological grade and survival.

A trend towards improved survival was observed in patients whose tumours exhibited high levels of sFRP4 staining. Therefore, this research may help to explain previous contradicting results from studies which examined sFRP4 as a prognostic marker in tumours of the prostate and colon. The proposal that sFRP4 may be used as a potential survival marker for prostate but not colon tumours could relate to the hormone-dependent nature of the tissue from which the cancer has derived. Expression of sFRP4 in these hormone dependent tumours suggests that sFRP4 could be over-expressed in order to reverse the uncontrolled cell proliferation.
TWO STRUCTURALLY-RELATED COUMARIN ANTIBIOTICS EXERT DIFFERENT EFFECTS ON DISRUPTION OF HSP90 DIMERIZATION

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Heat shock protein 90 (Hsp90) is a molecular chaperone that is found in steroid receptor heterocomplexes and functions as a dimer, in association with p23 and one of four immunophilins, CyP40, FKBP51, FKBP52 or PP5, all of which are essential for promoting steroid hormone signalling. The C-terminal region of Hsp90 contains a site for chaperone function, a dimerization domain and a recognition site for the immunophilins. Also within this region is an overlapping binding site for coumarin antibiotics, such as novobiocin, which has been shown in our laboratory to interfere with binding to immunophilins. The binding site for novobiocin has been shown to be located within the dimerization domain and the aim of this project was to determine if novobiocin and another coumarin drug, coumermycin A₁, could disrupt Hsp90 dimerization.

Treatment with 0-20 mM novobiocin on a his-tagged Hsp90 C-terminal fragment (527-724) was assessed by native PAGE to determine the effect of the coumarin on dimerization. A dimer band was observed at all concentrations of novobiocin tested and under different temperature conditions, suggesting that novobiocin is unable to disrupt pre-formed Hsp90 dimers.

Treatment with 0-1 mM coumermycin A₁, which is almost twice the size of novobiocin, on his-tagged Hsp90 527-724, was analysed by a chemical cross-linking assay to determine its effect on dimerization. Analysis by Western blotting revealed that the level of Hsp90 dimer decreased in the presence of coumermycin A₁ in a concentration-dependent manner. Native gel experiments using coumermycin A₁ are now underway to confirm these results. These data indicate that coumermycin A₁ is more potent at disrupting Hsp90 dimerization than novobiocin. In conclusion, our results suggest that the coumarin antibiotics antagonize Hsp90 function through a unique mode of action by destabilising Hsp90 dimerization.

THE HEAT SHOCK PROTEIN 90-BINDING COUMARIN NOVOBIOCIN INHIBITS STEROID RECEPTOR ACTIVITY WITHOUT THE STRESS

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Heat shock protein 90 (Hsp90) is a molecular chaperone that regulates the stability of many key cell-signalling proteins such as steroid hormone receptors and protein kinases. Many of these signalling molecules are associated with cancer development and progression and thus Hsp90 presents as a unique anticancer target that influences many signalling pathways in tumour formation. C-terminal dimerisation of Hsp90 followed by the chaperone's ATPase activity are required for client protein activation in a multistep process. Hsp90 forms distinct heterocomplexes with steroid receptors and protein kinases by associating with tetratricopeptide repeat (TPR)-containing immunophilins or p50cdc37, respectively. The antitumour agent cisplatin binds to the Hsp90 C-terminal domain and inhibits steroid receptor transcriptional activity through depletion of receptor protein. The coumarin antibiotic novobiocin has been shown to also bind to the Hsp90 C-terminal domain and cause depletion of the signalling kinases erbB2 and Raf-1, unlike cisplatin, which did not deplete kinases. Geldanamycin is a well-documented Hsp90 inhibitor that also causes signalling protein depletion but also induces a cellular stress response, which in the case of its derivative 17-AAG, causes osteoclast formation and subsequent bone destruction. Although cisplatin also impacted on steroid receptor activity, it was reported to not induce a stress response through the heat shock factor 1 transcription factor.

Previous findings in our laboratory have revealed that novobiocin is able to inhibit immunophilin and p50cdc37 association with Hsp90, but not disrupt Hsp90-immunophilin interaction. We have also observed decreased glucocorticoid receptor transcriptional activity in HeLa cells and now show that this reduced activity may be due to depleted receptor protein levels in the cells. Furthermore, like cisplatin, novobiocin was found to not induce a cellular stress response, as seen by monitoring Hsp70 protein production.