42nd Annual Meeting

Fetal and Neonatal Physiological Society

August 9 – 12, 2015, Vancouver, Canada

42nd Annual Meeting

Fetal and Neonatal

Physiological Society

August 9 12, 2015

University of British Columbia

Vancouver, BC, Canada

Organizing Committee

Dan Rurak
Ken Lim
Rajavel Elango
John Smyth
KS Joseph
Alex Beristain

Index

5

Welcome

42 years ago, the first meeting of our society was held in Oxford. It was focused on fetal breathing movements, which had been first directly measured only a few years earlier. Since that time, there has been an explosive growth in our knowledge and understanding of fetal and neonatal development and physiologic functions from both animal studies and from the application of largely non-invasive methods to obtain data from the human fetus and neonate_ These methodologies have greatly increased the information that we have on fetal and neonatal functions under both normal and pathophysiological conditions. And now of course there is the abundant basic science and clinical evidence that perturbations in the intrauterine and postnatal environments can have long term impact of health and disease over the lifespan — the DOHAD hypothesis. Overall it has been a very exciting time to be involved in perinatal research

The FNPS has been involved in all of these developments through the oral and poster presentations from researchers and research trainees that have been given at the successive meetings. We hope that the 2015 meeting will continue this tradition and we are grateful for all of you that have registered for the meeting and submitted abstracts. We invite you to experience UBC, Vancouver and Beautiful British Columbia and participate in the exchange of results and ideas.

Sincerely,

Dan Rurak

KS Joseph

Rajavel Elango

Ken Lim

John Smyth

Alex Beristain

Welcome from Professor Martin Dawes, Head, Department of Family Practice and Midwifery, University of British Columbia

Professor Rurak asked if I could share a few words with the meeting. I had met with Dan over a pint at the pub in the early part of this summer reminiscing and chatting about research. Some things never change!

I work at UBC and my research is on genetics in primary care. It would have been a pleasure and honor to be at the meeting in person. Unfortunately I am getting away from the challenges of running a large University Department by tracking grizzly bears in Northern British Columbia.

Geoffrey would have been delighted to be at this meeting in Vancouver, to enjoy the science and the sparking of ideas. He would have joined us at the pub and after the meeting, on the Fraser River fly-fishing for salmon.

He relentlessly pursued both the fish as well as the scientific answers to help understand the amazing changes that happen in the fetus. Of course life was somewhat simpler back at the beginning of FNPS. Geoffrey wrote his PhD thesis in the late nineteen forties on the boat across from Southampton to New York. I asked him how he dealt with getting all the published literature and he told me that he took all seven references with him!

He relished the increase in complexity over the following years. Whether it was electronic calculators or the increasing efficiency of intra-arterial probes his excitement was palpable and always shared with the family. As children we enjoyed being made aware of the discoveries whether they were his, or his colleagues in the FNP crowd. We became part of the FNP experience and as such are delighted to be able to share a few thoughts at this meeting and bring a personal welcome from the Dawes family.

FNPS Mission Statement

The FNPS stimulates discussion and exchange of ideas between physiologists, obstetricians and neonatologists on basic science, clinical and population health issues in the perinatal area. The FNPS considers an informal gathering and presentations of new and preliminary data, especially by students and in-training investigators, essential to achieve goals.

The Society was founded in 1974 during an informal meeting in Oxford. Professor Geoffrey Dawes (1918-1996) and Dr. Gerhard (Bo) Gennser (1929-2010) took the initiative and were made honorary members of the society in 1995. At every meeting since Professor Dawes death, a Geoffrey Dawes Lecture has been held in his honor.

The name of the annual conference (and Society) has changed several times, reflecting the widening scope of the society:

1974-80 Conference on Fetal Breathing
1981-83 International Conference on Fetal Breathing and other Movements
1984-95 Society for the Study of Fetal Physiology
1996-present Fetal and Neonatal Physiological Society

Over the years the Society has maintained its informal character and a lack of rigid structures. Those who have attended at least one of the previous three meetings are members of the Society and will be informed about the next meeting. Abstracts for the Annual Meeting are requested two months before the meeting and are compiled in the Book of Abstracts to encourage recent and preliminary data to be presented.

The Organizational Coordinator will be selected by the Organizational Committee and shall serve the three years. The Organizational Committee shall consist of representatives from Africa, Asia, Australia, Canada, continental Europe, South America, the United Kingdom and the United States of America and shall be selected by the committee. The Annual Meeting will be held in Europe, North America and the Southern Hemisphere, in June-September, as determined by the Organizational Committee. Approximately half of the meetings will be held in Europe.

Any residual funds from the prior meeting shall be passed on to the coordinator for the next meeting. Audit will not be required if the residual funds are less than 10,000 USD. The (local) Organizing Committee shall have the right to solicit funds in the name of the Society from organizations for the purpose of providing financial support for students and fellow-in-training to attend the meeting of the Society.

FNPS Previous Meetings

1974 Oxford, United Kingdom
1975, Oxford, United Kingdom
1976, Malmo, Sweden
1977, Oxford, United Kingdom
1978, Nijmegen, The Netherlands
1979, Paris, France
1980, Oxford, United Kingdom
1981, Maastricht, The Netherlands
1982 London, Canada
1983 Malmo, Sweden
1984 Oxford, United Kingdom
1985 Haifa, Italy
1986, Banff, Canada
1987, Groningen, The Netherlands
1988, Cairn, Australia
1989, Reading, United Kingdom
1990, Pacific Grove, United Kingdom
1991, De Eernhof, The Netherlands
1992, Niagara-on-the Lake, Canada
1993, Plymouth, United Kongdom
1994 Palm Cove, Australia
1995, Malmo, Sweden
1996, Arica, Chile
1997, S. Margherita, Italy
1998, Lake Arrowhead, USA
1999, Vineland, The Netherlands
2000, Southampton, United Kingdom
2001 Auckland, New Zealand
2002 Prague, Czech Republic
2003 Banff, Canada
2004 Tuscany, Italy
2005 Glenelg, South Africa
2006, Cambridge, United Kingdom
2007, Senday, Japan
2008, Maastricht, The Netherlands
2009, Lake Arrowhead, USA
2010, Winchester, United Kingdom
2011, Palm Cove, Australia
2012, Utrecht, The Netherlands
2013, Puerto Varas, Chile
2014, Saint Vincent, Italy

FNPS Board Members 2015

Laura Bennet, New Zealand
Dino Giussani (President Elect), UK
Emilio A. Herrera (Scribe Elect), Chile
Charles Ducsay, USA
Jan Derks, The Netherlands
Tomoaki Ikeda, Japan
Carina Mallard, Sweden
Suzie Miller, Australia
Tim Moss, Australia
Jan G. Nijhuis, The Netherlands
Julian T Parer, USA
Donald Peebles, UK
Dan Rurak, , Canada
Charles Wood, USA
Luc Zimmermann, The Netherlands
Robert Galinsky, In-training member, New Zealand

FNPS Annual Board Meeting

41st FNPS Meeting, 31 August – 3 September 2014

St Vincent, Italy

Present: Laura Bennett, Dino Giussani (President), Emilio Herrera (Scribe), Tomoaki Ikeda, Tim Moss (minute taker), Jan Nijhuis, Bill Parer, Donald Peebles, Dan Rurak, Luc Zimmerman.

Apologies: Carina Mallard

MINUTES

Agenda Items:

1. Minutes of the last meeting
   - The minutes were accepted.
   - It was restated that the conference program should be structured in such a way that nominees for prizes do not present on the final day of the meeting (to allow sufficient time for collating judges' scores and preparation of certificates).
2. Matters arising not included in the agenda
   - None
3. Vote of thanks to the 2015 conference organisers
   - Prof Giussani expressed his appreciation for the efforts of the conference organisers. All board members agreed that the meeting was successful and enjoyable.
4. Xenosite web hosting
   - Prof Giussani reported that he had arranged for Cambridge University to host the FNPS website whilst during his Presidential term. He and Dr Herrera will manage content of the site.
5. FNPS board members update (including in-training member)
   - It was restated that members of the board must attend the annual meeting at least once every 3 consecutive years to retain their position on the board.
   - It was restated that it is expected that board members would bring at least 1 other members of their research group to the annual meeting.
   - Dr Lucy Green has not attended the annual meeting for 4 years. The board agreed that she should step down. Prof Giussani will write to Lucy.

- The board agreed that Dr Suzie Miller (Australia), Dr Jan Derks (Netherlands) and Dr Charles Ducsay (USA) should be invited to join the board. Prof Giussani will ask each of these nominees to join the board.
- The board agreed that it should invite a member of the society who is in training onto the board. The board agreed that Dr Robert Galinsky (New Zealand) should be invited onto the board. Prof Giussani will ask Dr Galinsky to join the board.

6. FNPS bank account

After some discussion about the difficulties establishing an official account for The Society, the board agreed that Prof Laura Bennett should open an account in her name to be used solely for FNPS business. Prof Bennet will report all transactions for the account to the board.

Awards

YOUNG INVESTIGATOR AWARDS

There will be two prizes, one for the Best Oral Presentation, and one for the best poster to support young investigators in the field of Fetal and Neonatal Medicine. Young investigators are defined as young investigator ore researchers in full-time training under the age of 40.

Awards Committee
President: Tim Moss
Members, Dan Rurak, Dino Giussani

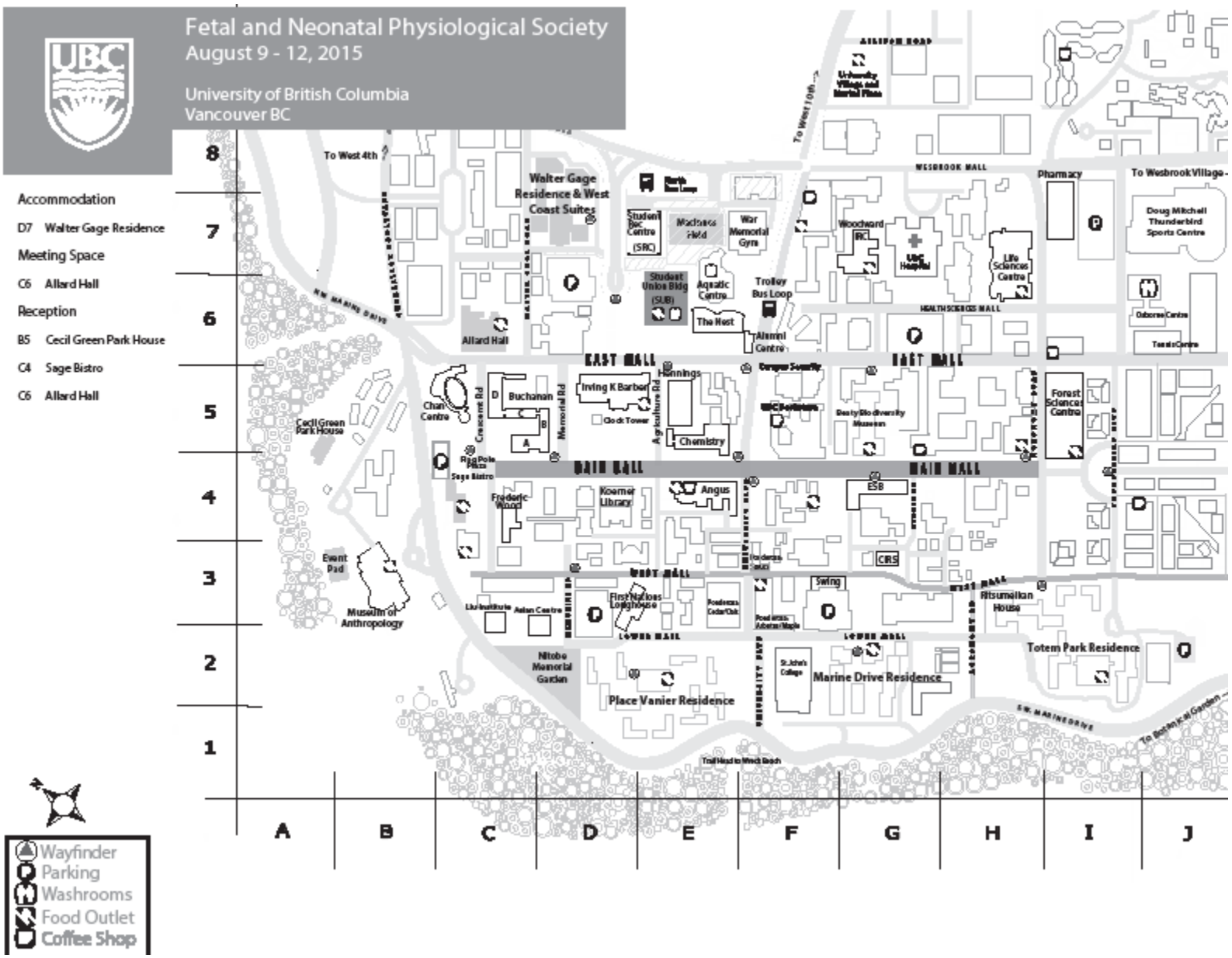
Tania Gunn Memorial Prize

The prize was introduced to the FNPS in 2011 in memory ot Tania Gunn (1931-1999), Professor of Neonatology at Auckland, New Zealand. She is remembered for her important studies of the control of thermoregulation at birth and the safety of hypothermia for babies with acute encephalopathy.

Bo Gennser Memorial Prize (Poster)

This prize was introduced in 2013 in memory of Bo Gennser (1929-2010), Professor in Obstetrics and Gynecology at Lund University, Sweden. He is recognized for developing new methods in recording fetal breathing and the evaluation of fetal real-time ultrasound in 1974. In 1974, he and Geoffrey Dawes initiated the Fetal Breathing Conference, that later ended up as the Fetal and Neonatal Physiological Society.

UBC Campus Map



We thank the following for their generous financial support:

Child & Family Research Institute
Reproduction & Healthy Pregnancy Cluster

Department of Obstetrics & Gynecology
Division of Maternal-Fetal Medicine

Department of Pediatrics
Division of Neonatalogy

Canadian Institutes of Health Research

Program at a glance

Sunday August 9, 2015

17:30- 18:00 Registration Desk Open Allard Hall Atrium

18:30 – 21:30 Geoffrey Dawes Lecture: Professor Stephen Lye

19:30 - 21:30 Reception: Allard Hall Terrace Lounge

Monday August 10, 2015

7:00 – 11:00 Registration Desk Open Allard Hall Atrium

7:00 – 8:00 Breakfast Pacific Spirit Place

08:00 – 1030 Oral Session 1: Fetal Brain - Chairs – Ken Lim (Vancouver) and John Smyth (Vancouver)

10:30 – 11:00 Refreshment Break and Poster viewing – Allard Hall Lounge Area

11:00 – 11:45 Moderated Poster Discussion - Chair – Dan Rurak (Vancouver)

11:45 – 12:30 Invited Lecture - Dr. Angela Devlin, *Department of Pediatrics, University of British Columbia*

12:30 – 14:00 Lunch – Allard Hall Lounge Area

14:00 – 15:30 Oral Session 2: DOHAD - Chair – Alex Beristain (Vancouver)

15:30 – 16:00 Refreshment Break – Allard Hall Lounge Area

16:00 – 17:15 Oral Session 3: Fetal & Neonatal Cardiorespiratory Function = Chair – Laura Bennet (New Zealand)

18:00 – 23:00 Dinner Cecil Green Park

Tuesday August 11, 2015

7:30 – 11:00 Registration Desk Open Allard Hall Atrium

7:00 – 8:30 Breakfast Pacific Spirit Place

8:30 – 9:15 Invited Lecture Dr. Samantha Benton, *Department of Cellular and Molecular Medicine, University of Ottawa*

9:15 – 10:15 Oral Session 4: Fetal Monitoring - Chair – KS Joseph (Vancouver)

10:15 – 10:45 Refreshment Break – Allard Hall Lounge Area

10:45 – 11:30 Panel Discussion: Fetal Monitoring - Jan Nijhuis,(Chair) Bill Parer, Ken Lim, Samantha Benton

11:30 – 12:30 Oral Session 5: Uterus and Placenta - Chair – David Walker (Australia)

12:30 – 14:00 Lunch – Allard Hall Lounge Area

13:30 – 17:00 Sporting Event

18:00 – 23:00 Dinner Sage Bistro

Wednesday August 12, 2015

7:30 – 8:30	Registration Desk Open Allard Hall Atrium
7:00 – 8:00	Breakfast Pacific Spirit Place
8:00 – 8:45	Invited Lecture: Dr. Rajavel Elango, Department of Pediatrics, University of British Columbia
8:45 – 10:30	Oral Session 6: Fetal Brain, DOHAD, parturition - Chair – Rajavel Elango (Vancouver)
10:30 – 11:00	Refreshment Break – Allard Hall Lounge Area
11:00 – 12:00	Oral Session 7: Translational Studies - Chair – Dino Giussani
12:00 – 12:30	Trainee Awards
12:30 – 1300	Business Meeting and Closing Remarks
13:00 – 14:00	Lunch – Allard Hall Lounge Area

Program

Fetal and Neonatal Physiological Society 2015 Meeting Program

Sunday August 9, 2015

17:30- 18:00 Registration Desk Open Allard Hall Atrium

18:15 – 18:30 Welcome and Introduction: Geoffrey Dawes Lecture – Dan Rurak

18:30 – 21:30 Geoffrey Dawes Lecture: Allard Hall Lecture Forum- **The Initiation of Labour – New Understanding from an Old Hormone**
Professor Stephen Lye, Executive Director, Fraser Mustard Institute for Human Development, University of Toronto, Toronto

19:30 - 21:30 Reception: Allard Hall Terrace Lounge

Monday August 10, 2015

7:00 – 11:00 Registration Desk Open Allard Hall Atrium

7:00 – 8:00 Breakfast Pacific Spirit Place

8:00 Sessions start – Allard Hall

Oral Session 1 Fetal Brain

Chair – Ken Lim (Vancouver) and John Smyth (Vancouver)

8:00 – 8:15 **O1 White Matter and Cortical Brain Injury in the Very Immature Rat Following Lipopolysaccharide Induced Mild Systemic Inflammation**
S Ranchhod, K Gunn, T Fowke, J Chan, J Prasad, J Bai, J Dean, *Department of Physiology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand*

8:15 – 8:30 **O2 Hyaluronan controls formation of neuronal lamellipodia and filopodia**
Tania M Fowke[1,2], Ji-Zhong Bai[1,2], Jaya Prasad[1,2], Katherine Gunn[1,2], Justin M Dean[1,2] *1. Department of Physiology, University of Auckland, New Zealand.2. Centre for Brain Research, University of Auckland, New Zealand.*

8:30 – 8:45 **O3 The effect of MgSO4 on asphyxia-induced brain injury in preterm fetal sheep**
Robert Galinsky, Joanna Tse, Joanne O Davidson, Christopher A Lear, Paul P Drury, Guido Wassink, Lotte Van den Heuij, Alistair Jan Gunn and Laura Bennet, The Department of Physiology, The University of Auckland, Auckland, New Zealand

8:45 – 9:00 **O4 Intranasal human amniotic epithelial cell therapy and long-term recovery after asphyxial brain injury in preterm fetal sheep**
Lotte van den Heuij[1], Suzie L. Miller[2], Mhoyra Fraser[1], Graham Jenkin[2], Alistair J. Gunn[1], Laura Bennet[1].
[1] Fetal Physiology and Neuroscience Group, Dept. Physiology, The University of Auckland, New Zealand, [2] Ritchie Centre, Hudson Institute of Medical Research, Monash University, Australia

9:00 – 9:15 **O5 Antenatal Melatonin Administration Protects Neurovascular Development In Growth Restricted Lambs**
Margie Castillo-Melendez, Tamara Yawno, Euan Wallace, Graham Jenkin, Suzie Miller.
The Ritchie Centre, The Hudson Institute, Monash University, Clayton, Vic, Australia

9:15 – 9:30 **O6 Dexamethasone induced hyperglycaemia during asphyxia is associated with severe cystic white and grey matter brain injury in preterm fetal sheep**
Christopher A. Lear[1], Joanne O. Davidson[1], Robert Galinsky[1], Alistair J. Gunn[1], Laura Bennet[1], [1]*Fetal Physiology and Neuroscience Group, Department of Physiology, The University of Auckland, Auckland, New Zealand*

9:30 – 9:45 **O7 Non-additive effects of delayed connexin hemichannel blockade and hypothermia after cerebral ischemia in near-term fetal sheep**
Joanne O Davidson, Alexandra Rout, Guido Wassink, Caroline A Yuill, Frank G Zhang, Colin R Green, Laura Bennet, Alistair J Gunn, *Department of Physiology, The University of Auckland, New Zealand*

9:45 – 10:00 **O8 Early infusion of recombinant erythropoietin is partially neuroprotective after profound asphyxia in preterm fetal sheep**
Guido Wassink; Joanne O. Davidson; Laura Bennet; Mhoyra Fraser; Alistair J. Gunn
Fetal Physiology and Neuroscience Group, Department of Physiology,The University of Auckland, Auckland, New Zealand

10:00 – 10:15 **O9 Umbilical cord blood cell administration is neuroprotective following preterm hypoxic-ischemic insult**
Jingang Li, Tamara Yawno, Amy Sutherland, Jan Loose, Ilias Nitsos, Flora Wong, Graham Jenkin, Suzie Miller, *The Ritchie Centre, Hudson Institute of Medical Research, Monash University, The Departments of Obstetrics and Gynaecology, and Paediatrics, Monash Health, Monash University.*

10:15 – 10:30 **O10 Understanding the therapeutic potential of umbilical cord blood cells for cerebral palsy.**
Courtney McDonald[1], Nicole Jones[2], Margie Castillo-Melendez[1], Graham Jenkin[1] and Suzie Miller[1], [1]*The Ritchie Centre, Hudson Institute of Medical Research, Clayton, Australia,* [2]*Department of Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, Australia*

10:30 – 11:00 Refreshment Break and Poster viewing – Allard Hall Lounge Area

11:00 – 11:45 **Moderated Poster Discussion**

Chair – Dan Rurak (Vancouver)

P1 Adenosine Contributes to the Fetal Coronary Vasodilatory Response in Acute Hypoxia
Sonnet S Jonker1, Eileen I Chang1, Samantha Louey1 and George D Giraud1,2.
1Knight Cardiovascular Institute, Oregon Health & Science University, Portland, OR, United States and 2Cardiology, VA Portland Health Care System, Portland, OR, United States.

P2 Thalamic Locus Mediates Hypoxic Respiratory depression In Young Lambs
Arezoo Rajaee[1], Basil Ibe[3], Catalina Guerra[3], Lawrence Kruger[2] and Brian J. Koos[1]
Departments of [1]Obstetrics & Gynecology and [2]Neurobiology, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095; and [3]Dept of Pediatrics, David Geffen School of Medicine at UCLA, Torrance, CA 90502

P3 Inflammatory and profibrotic markers in the lung of growth restricted offspring before and after birth

Jia Yin Soo[1], Sandra Orgeig[2], Erin V. McGillick[1], Song Zhang[1], I. Caroline McMillen[1], Janna L. Morrison[1], *[1]Early Origins of Adult Health Research Group and [2]Molecular & Evolutionary Physiology of the Lung Laboratory, School of Pharmacy & Medical Sciences, Sansom Institute for Health Research, University of South Australia, Adelaide, SA, Australia, 5001*

P4 Reduced cortisol response to AVP+CRH challenge in offspring of ewes undernourished around conception improves with age.

Mark H Oliver, Anne L Jaquiery, Jane E Harding, Hui Hui Phua, Eric B Thorstensen and Frank H Bloomfield, *Liggins Institute, University of Auckland, Auckland, New Zealand*

P5 Maternal Hyperglycemia Affects Rat Neonatal Cardiovascular Development

Lara LEHTORANTA[1], Olli VUOLTEENAHO[2], Jukka LAINE[3], Mervi HAAPSAMO[2], Juha RÄSÄNEN[4], *[1]University of Turku, Obstetrics and Gynecology, Turku, Finland ; [2]University of Oulu, Physiology, Oulu, Finland; [3]Turku University Hospital, Dep. of Pathology, Turku, Finland; [4]University of Eastern Finland, Obstetrics and Gynecology, Kuopio, Finland*

P6 Structural and molecular regulation of lung maturation by intratracheal vascular endothelial growth factor (VEGF) administration in the normally grown and placentally restricted fetus

Erin V McGillick[1,2], Sandra Orgeig[1] and Janna L Morrison[2], *[1]Early Origins of Adult Health Research Group and [2]Molecular & Evolutionary Physiology of the Lung Laboratory, Sansom Institute for Health Research, School of Pharmacy & Medical Sciences, University of South Australia, Adelaide, Australia*

P7 Creatine synthesis and CrT transporter expression in the placenta: an interspecies comparison

Syed Baharom [1,3,4] , Paul Della Gatta[2], Stacey Ellery[1],Hayley Dickinson[1], Richard Harding[3], Rod J Snow[2], David W Walker[1], [1]The Ritchie Centre, Hudson Institute of Medical Research, Monash Medical Centre, Clayton, VIC. [2]Centre for Physical Activity & Nutrition, Deakin University, Burwood, VIC. [3]Department of Anatomy & Developmental Biology, Monash University, Clayton VIC, Australia. [4]Faculty of Medicine, Universiti Teknologi MARA, Sg. Buloh, Malaysia.

P8 Late gestation overnutrition decreases the numerical density of type II alveolar epithelial cells and surfactant protein gene expression in the fetus but this is not maintained after birth

Mitchell C. Lock[1], Erin V. McGillick[1,2], Sandra Orgeig[2], I. Caroline McMillen[1], Beverly S. Mühlhausler[1], Song Zhang[1] and Janna L. Morrison[1]
[1] Early Origins of Adult Health Research Group and [2] Molecular & Evolutionary Physiology of the Lung Laboratory, Sansom Institute for Health Research, University of South Australia, Adelaide, SA, Australia

P9 Late gestation overnutrition alters the cardiac metabolic profile

Jack R Darby, Song Zhang, I Caroline McMillen, Beverly S. Mühlhausler and Janna L Morrison, *Early Origins of Adult Health Research Group, Sansom Institute, University of South Australia, SA 5000, Australia*

P10 Mitochondrial function in ovine skeletal muscle during late gestation

O.R. Vaughan, K.L. Davies. A.J. Murray and A.L. Fowden, *Department of Physiology, Development and Neuroscience, University of Cambridge, UK*

P11 Effect of Altered Systolic Load on Fetal Heart Growth

Divya Soman[1,2], Debra Anderson[1,2], Fred Tibiyan[1,2], Jobe Faber[1,2], Samantha Louey[1,2], Sonnet Jonker, Kent Thornburg[1,2] and George Giraud[1,2,3,4]. *[1]Center for Developmental Health, [2]Knight Cardiovascular Institute, [3]Department of Physiology and Pharmacology, Oregon Health & Science University and [4]Portland VA Medical Center, Portland.*

11:45 – 12:30 Invited Lecture - **Maternal Folate and Vitamin B12 Status during Pregnancy and Programming of Offspring Adiposity**
Dr. Angela Devlin, *Department of Pediatrics, Child & Family Research Institute, University of British Columbia*

12:30 – 14:00 Lunch – Allard Hall Lounge Area

Oral Session 2 DOHAD

Chair – Alex Beristain (Vancouver)

14:00 – 14:15 **O11 Isolating the long-term cardiovascular effects on adult offspring of human clinically relevant doses of antenatal glucocorticoids**
Kimberley J Botting, Youguo Niu, Katie L Skeffington, Beth J Allison, Kirsty L Brain, Nozomi Itani, Christian Beck, Ana-Mishel Spiroski & Dino A Giussani.
Physiology, Development & Neuroscience, University of Cambridge, United Kingdom

14:15 – 14:30 **O12 A sheep model to examine the effects of maternal asthma on fetal outcomes**
Robert J Bischof[1,2], Vicki L Clifton[5,6], Amy L Wooldridge[5], Kathryn L Gatford[5], Bahar Liravi[2], Dasom Kim[2], Beverly S Muhlhausler[7], Janna L Morrison[8], Andrew Davies[2,9], Robert De Matteo[4], Megan J Wallace[1,3] & Timothy JM Moss[1,3] , *[1]The Ritchie Centre, Hudson Institute of Medical Research, Clayton VIC 3168, Australia; [2]Department of Physiology, [3]Department of Obstetrics and Gynaecology and [4]Department of Anatomy and Developmental Biology, Monash University, Clayton VIC 3800, Australia; [5]Robinson Research Institute and School of Paediatrics & Reproductive Health, University of Adelaide, Adelaide SA 5005, Australia; [6]Mater Medical Research Institute, University of Queensland, Brisbane, Qld 4101, Australia; [7]FOODplus Research Centre, School of Agriculture, Food and Wine, The University of Adelaide, Adelaide SA 5005, Australia; [8]Early Origins of Adult Health Research Group, School of Pharmacy and Medical Sciences, Sansom Institute for Health Research, University of South Australia, Adelaide SA 5001, Australia; [9]School of Biomedical Sciences, Peninsula Campus, Monash University, Frankston VIC 3199, Australia.*

14:30 – 14:45 **O13 Isolated direct adverse effects of glucocorticoids on the fetal heart and circulation**
Noor Teulings[2], Youguo Niu[1], Tessa Garrud[1], Katie L Skeffington[1], Christian Beck[1], Nozomi Itani[1], Jan B Derks[2] & Dino A Giussani[1]., *[1]Physiology Development & Neuroscience, University of Cambridge, UK; [2]University Medical Center Utrecht, The Netherlands.*

14:45 – 15:00 **O14 Maternal administration of cyclic-glycine-proline alters the apoptosis of mammary cells during lactation and involution and enhances cognition of offspring in rats**
Gagandeep Singh Mallah[1, 2], Kuljeet Singh[2], Chris McMahon[2], Jian Guan[1, 2]
[1]Liggins Institute, [2]Gravida, University of Auckland, Auckland, New Zealand

15:00– 15:15 **O15 Developmental and Cortisol Regulation of Mitochondrial Function in Fetal Skeletal Muscle during Late Gestation**
Katie L. Davies[1], Owen R. Vaughan[1], Alison J. Forhead[1], Miles J. De Blasio[1], Andrew J. Murray[1] & Abigail L. Fowden[1], *[1]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, CB2 3EG, UK.*

15:30 – 16:00 Refreshment Break – Allard Hall Lounge Area

Oral Session 3 Fetal & Neonatal Cardiorespiratory Function

Chair – Laura Bennet (New Zealand)

16:00 – 16:15 **O16 Meis1 drives metabolic maturation of cardiomyocytes**
Isa M Lindgren and Kent L Thornburg, *Center for Developmental Health, Knight Cardiovascular Institute, Oregon Health and Science University, Portland OR*

16:15 – 16:30 **O17 Aortic displacement as a surrogate for intertwin pulse pressure differences in monochorionic pregnancies with and without twin-twin transfusion syndrome**
Christoph Wohlmuth[1,2], Frank A. Osei1, Kenneth J. Moise[1], Anthony Johnson1, Ramesha Papanna[1], Michael Bebbington[1], Helena M. Gardiner[1], *[1]The Fetal Center, Children's Memorial Hermann Hospital and the Department of Obstetrics and Gynecology, The University of Texas Health Science Center at Houston, TX, USA, [2]Department of Obstetrics and Gynecology, Paracelsus Medical University Salzburg, Salzburg, Austria.*

16:30 – 16:45 **O18 Cardiac dysfunction in the hypoxic chick embryo is rescued by treatment with the mitochondrial targeted antioxidant MitoQ**
Katie L Skeffington[1], Nozomi Itani[1], Christian Beck[1], Youguo Niu[1], Angela Logan[2], Michael P Murphy[2] and Dino A Giussani[1], *[1]Department of Physiology Development & Neuroscience, [2]Mitochondrial Biology Unit, Addenbrookes; University of Cambridge, UK.*

16:45 – 17:00 **O19 Store operated channel blockade partially reverses pulmonary artery hyper reactivity and remodeling in newborn sheep exposed to hypoxia in the last part of gestation**
Sebastián Quezada[1], Sebastián Castillo-Galán[1], Renato Ebensperger[1], Felipe Beñaldo[1], Fernando Moraga[3], Ismael Hernández[1], German Ebensperger[1], Emilio A. Herrera[1,2], Aníbal J. Llanos[1,2], Roberto V. Reyes[1], *[1]Programa de Fisiopatología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile. [2]International Center for Andean Studies (INCAS), Universidad de Chile, Santiago, Chile. [3]Facultad de Medicina, Universidad Católica del Norte, Coquimbo, Chile*

18:00 – 19:00 Social Hour Cecil Green Park

19:00 – 23:00 Dinner Cecil Green Park

Tuesday August 11, 2015

7:30 – 11:00 Registration Desk Open Allard Hall Atrium

7:00 – 8:30 Breakfast Pacific Spirit Place

8:30 – 9:15 Invited Lecture **The Pregnancy Diaries: Chronicling placenta health in obstetrical complications and postpartum health**
Dr. Samantha Benton, *Department of Cellular and Molecular Medicine, University of Ottawa*

Oral Session 4 Fetal Monitoring

Chair – KS Joseph (Vancouver)

9:15 – 9:30 **O20 Effect of maternal position on fetal heart rate variability and behavioural state in healthy term pregnancy**

Jordan PR McIntyre[1,4,5], Wendy Burgess[1,6], Christopher A Lear[3], JMD Thompson[2], Alistair J Gunn[2,3], Laura Bennet[3], and PR Stone[1,6] on behalf of the Maternal Sleep in Pregnancy Research Group.
Departments of Obstetrics & Gynaecology[1], Paediatrics: Child and Youth Health[2], and Physiology[3], Faculty of Medical and Health Sciences, University of Auckland, New Zealand. New Zealand Respiratory and Sleep Institute[4], Auckland, NZ.Starship Children's Hospital Respiratory Physiology Laboratory[5] and National Women's Health[6], Auckland District Health Board, New Zealand.

9:30 – 9:45 **O21 Diurnal changes in fetal middle cerebral artery blood flow parameters in a normal population**

Tehila Avitan[1], Kenneth Lim[1], Dan Rurak[1], Meisan Brownlum[2], Ursula Brain[2], Tim Oberlander[2],
Department of Obstetrics and Gynecology, 2 Department of Pediatrics, University of British Columbia, Canada

9: 45 – 10:00 **O22 Sinusoidal (-like) fetal heart rate: recognition of the pattern is crucial!**

Jan Nijhuis, *Dept Ob Maastricht University Medical Center (MUMC), GROW-school for oncology and developmental biology /Gyn, POBox 5800, 6202AZ NL-Maastricht.*

10:00 – 10:15 **O23 Fetal Urine Production Rate in Preterm Premature Rupture of Membranes And Adverse Neonatal Outcomes: a pilot study**

Tehila Avitan, Hadas Pri-Chen, Ron Rabinovitz, Nurit Algor, Michal Kovo, Letizia Screiber, Michael S. Schimmel ,Arnon Samueloff, Sorina Grisaru-Granovsky
Departments of Obstetrics & Gynecology and Neonatology and Biochemical Analyses Laboratory, Shaare Zedek Medical Center, affiliated with the Hebrew University Medical School, Jerusalem; and Department of Pathology, Edith Wolfson Medical Center, Holon, affiliated with the Tel Aviv University; Israel

10:15 – 10:45 Refreshment Break – Allard Hall Lounge Area

10:45 – 11:30 **Panel Discussion Fetal Monitoring**
Jan Nijhuis,(Chair) Bill Parer, Ken Lim, Samantha Benton

Oral Session 5 Uterus and Placenta

Chair – David Walker (Australia)

11:30 – 11:45 **O24 The placental phenotype adapts to chronic hypoxia and mitochondrial-targeted antioxidant (MitoQ) therapy in rat pregnancy**

Emily J Camm [1], Anna M Nuzzo [1,2], Amanda N Sferruzzi-Perri [1], Alessandro Rolfo [2], Tullia Todros [2], Michael P Murphy[3] & Dino A Giussani [1], *[1] Department of Physiology, Development and Neuroscience, University of Cambridge, United Kingdom, [2] Department of Surgical Sciences, University of Turin, Italy; [3] MRC Mitochondrial Biology Unit, Cambridge, United Kingdom.*

11:45 – 12:00 **O25 Maternal obesity drives decidual natural killer cell imbalances in early pregnancy**

S Perdu[1], Y Kim[1], K Chan[1], AG Beristain[1], *[1]Department of Obstetrics & Gynecology, The University of British Columbia, Vancouver, Canada*

12:00 – 12:15 **O26 Effects of chronic hypoxia and the mitochondria-targeted antioxidant MitoQ on uterine artery reactivity in rodent pregnancy**

Zhongchao Wang[1,3], Emily J Camm[1], Ana-Mishel Spiroski[1], Katie L Skeffington[1], Thomas J Ashmore[1], Youguo Niu[1], Michael P Murphy[2], Jin Ma[3] and Dino A Giussani[1], *[1]Department of Physiology Development & Neuroscience, University of Cambridge, UK; [2]Mitochondrial Biology Unit, Addenbrookes; University of Cambridge, UK; [3]Department of Aerospace Physiology, Fourth Military Medical University, China*

12:15 – 12:30 **O27 A parallelized, pumpless artificial placenta system significantly prolonged survival time in preterm ovine fetus.**
Takushi Hanita[1], Tadashi Matsuda[1], Yuichiro Miura[1, 2], Haruo Usuda[1], Shinpei Watanabe[1], Ryuta Kitanishi[1], Masatoshi Saito[1], Yoshiyasu Kobayashi[3], *1 Centre for Perinatal and Neonatal Medicine, Tohoku University Hospital, Sendai, Miyagi, Japan*
2 School of Women's and Infants' Health, The University of Western Australia, Perth, WA, Australia, 3 Department of Veterinary Pathology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan

12:30 – 14:00 Lunch – Allard Hall Lounge Area

13:30 – 17:00 Sporting Event

18:00 – 19:00 Social Hour Cecil Green Park

19:00 – 23:00 Dinner Sage Bistro

Wednesday August 12, 2015

7:30 – 8:30 Registration Desk Open Allard Hall Atrium

7:00 – 8:00 Breakfast Pacific Spirit Place

8:00 – 8:45 Invited Lecture **Protein and amino acid needs during pregnancy – Application of novel stable isotope techniques in humans**
Dr. Rajavel Elango, Department of Pediatrics, Child & Family Research Institute, University of British Columbia

Oral Session 6 Fetal Brain, DOHAD, parturition

Chair – Rajavel Elango (Vancouver)

8:45 – 9:00 **O28 The effect of magnesium sulphate on post-asphyxial preterm fetal seizures**
Vittoria Draghi, Robert Galinsky, Chris Lear, Alistair Jan Gunn, Laura Bennet.
Fetal Physiology and Neuroscience group, Department of Physiology, The University of Auckland, Auckland, New Zealand

9:00 – 9:15 **O29 Magnesium sulphate for neuroprotection reduces punctate white matter laesions at 30 weeks MRI in the human neonate**
Sol C, Benders M, Van Leeuwen J, Keunen K, Mulder E, De Vries L, Groenendaal F, Lemmers P, Van Bel F, Derks JB, *Dept. of Perinatal Medicine, Wilhelmina Children's Hospital, University Medical Centre Utrecht, The Netherlands*

9:15 – 9:30 **O30 The impact of an adverse in utero environment followed by a postnatal western diet, upon muscle and liver development and function and evidence of a pre-diabetic state in young adulthood**
Ousseynou Sarr[1,2,4]*, Kristyn P Dunlop[2]*, Lin Zhao[1], and Timothy RH Regnault[1,2,3,4]
*Departments of [1]Obstetrics and Gynecology, [2]Physiology and Pharmacology, [3]Lawson Health Research Institute, [4]Children's Health Research Institute, Western University, London, ON, Canada. * = Equal contribution*

9:30 – 9:45 **O31 Reactive oxygen species (ROS) as an early gestation mediator of cardiac programming in the growth restricted sheep fetus**

Song Zhang[1], Michelle Tie[1], Kimberley J. Botting[1], I. Caroline McMillen[1], Sheridan Gentili[1], Severance M. MacLaughlin[1], Doug A Brooks[2] and Janna L. Morrison[1, 1] Early Origins of Adult Health Research Group, [2] Mechanisms in Cell Biology and Disease Research Group, Sansom Institute for Health Research, University of South Australia, Adelaide

9:45 – 10:00 **O32 Markers of allostatic load associate with adverse pregnancy and offspring outcomes in rats**
David Olson[1], Ashlee Matkin[2], J. Keiko McCreary[2], Erin Falkenberg[2], Barbara Verstraeten[1,3], Gerlinde A.S. Metz[2], *[1]University of Alberta, [2]Canadian Centre for Behavioural Neurosciences, University of Lethbridge,[3]University of Ghent.*

10:00 – 10:15 **O33 Complex interacting mechanisms regulating contraction and inter-contraction relaxation in term human labour myometrium**
[1]Helena C Parkington, [1]Marius Jigau, [1]Crystal Goundar, [1]Mary Tonta, [2]Janet Stevenson, [2]Penny Sheehan, [2]Shaun Brennecke, [1]Harold Coleman., *[1]Department of Physiology, Monash University, Clayton, Victoria, Australia and [2]Royal Women's Hospital, Parkville Victoria, Australia.*

10:15 – 10:30 **O34 Interleukin (IL) 1-breceptors are regulated in human myometrial smooth muscle cells (HMSMC) and in rats at delivery by IL-1b, prostaglandin (PG) F2a and progesterone (P4).**
David Olson1, Tomohito Ishiguro1,2, Barbara Verstraeten1,3, Kelycia Leimert1
1Dept of Obstetrics and Gynecology, University of Alberta, 2Juntendo Medical University, 3University of Ghent

10:30 – 11:00 Refreshment Break – Allard Hall Lounge Area

Oral Session 7 Translational Studies

Chair – Dino Giussani

11:00 – 11:15 **O35 Heterogeneity of respiratory distress syndrome: risk factors and morbidity associated with early and late gestation disease**
Azar Mehrabadi[1], Sarka Lisonkova[1], K.S. Joseph[1 2]
[1]Department of Obstetrics and Gynaecology, University of British Columbia and the Children's and Women's Hospital and Health Centre of British Columbia; [2]School of Population and Public Health, University of British Columbia, Vancouver, British Columbia, Canada

11:15 – 11:30 **O36 Human amnion epithelial cells modulate the initial pulmonary inflammatory response to injurious ventilation in preterm lambs**
Jacqueline Melville, Courtney McDonald, Robert Bischof, Graeme Polglase, Rebecca Lim, Graham Jenkin and Tim Moss.
The Ritchie Centre, Hudson Institute of Medical Research; Department of Obstetrics and Gynaecology, Monash University.

11:30 – 11:45 **O37 5-minute Apgar score as a marker for developmental vulnerability at 5 years of age**
Neda Razaz[1], Thomas Boyce[2], Marni Brownell[3], Douglas Jutte[2], Helen Tremlett[1], Ruth Marrie[3], K.S. Joseph[1], *University of British Columbia, Vancouver, Canada, [2] University of California, San Francisco, United States, [3] University of Manitoba, Winnipeg, Canada*

11:45 – 12:00 **O38 Maternal creatine levels throughout human pregnancy and the association of these with maternal demographics and indices of fetal growth – a retrospective cohort study.**

Hayley Dickinson[1,2], Miranda Davies-Tuck[1,2], Stacey Ellery[1,2], Jessica Grieger[3], Rod Snow4, Euan M Wallace[1,2], David W Walker[1,2], Vicki Clifton[5], *[1]The Ritchie Centre, Hudson Institute of Medical Research, Australia; [2]Department of Obstetrics and Gynaecology, Monash University, Australia; [3]Robinson Research Institute, University of Adelaide, Australia; [4]Centre for Physical Activity and Nutrition Research, School of Exercise and Nutrition Sciences, Deakin University, Australia; [5]Mater Research, Queensland, Australia*.

12:00 – 12:30 Trainee Awards

12:30 – 1300 Business Meeting and Closing Remarks

13:00 – 14:00 Lunch – Allard Hall Lounge Area

Geoffrey Dawes Lecture

The Initiation of Labour – New Understanding from an Old Hormone
Professor Stephen Lye, Executive Director, Fraser Mustard Institute for Human Development, University of Toronto, Toronto

The timely onset of labour is critical to newborn survival. However, the mechanisms that maintain pregnancy and initiate labour are poorly understood and this likely accounts for our limited ability to prevent preterm birth. Our research has demonstrated that there is an intricate interaction between maternal endocrine and immune systems that supports healthy fetal development during pregnancy and at term, results in the onset of labour. During pregnancy interactions between the maternal immune system and fetal/uterine tissues support placentation and the increase in placental blood flow required for fetal growth and development. Prior to the onset of labour signals from the uterine tissues activate uterine endothelial cells as well as maternal peripheral immune cells. This leads to an influx of maternal monocytes into the uterine tissues where they differentiate into macrophage and induce a physiologic inflammation that contributes to the onset of labour.

The maternal endocrine environment balances the pregnancy maintenance and labour inducing properties of the maternal immune system through the effects of the pregnancy hormone, progesterone. During pregnancy, progesterone supports myometrial quiescence and suppression of immune cell activation. These actions of progesterone must be withdrawn in order for labour to occur. In most species this is achieved through reduced levels of circulating progesterone at term. In human pregnancy, however, progesterone levels do not fall, raising questions as to the mechanisms by which labour is initiated in women. We have recently discovered that the progesterone receptor isoforms (PRA and PRB) act within the myometrium to mediate the switch from pregnancy to labour. Liganded PRB maintains myometrial quiescence throughout pregnancy through suppression of labour gene expression. At term there is an increase in intracellular progesterone metabolism (through increased expression of the enzyme 20□HSD) and the reduced intracellular levels of progesterone results in un-liganded PRB exiting the nucleus and being replaced by un-liganded PRA. Remarkably, un-liganded PRA, rather than suppressing labour gene (connexin43) expression, activates expression of connexin43. This new understanding is aiding the development of new diagnostic and therapeutic approaches that will enhance our ability to prevent preterm birth and its consequent newborn mortality and morbidity.

Invited Lecture

Maternal Folate and Vitamin B12 Status during Pregnancy and Programming of Offspring Adiposity

Dr. Angela Devlin, *Department of Pediatrics, Child & Family Research Institute, University of British Columbia*

Background: The concept of developmental programming suggests that prenatal and early postnatal exposure to shifts in environmental factors, such as maternal diet and nutritional status, may play a role in the development of cardiometabolic disease later in life. Folate and vitamin B12 are micronutrients required for DNA synthesis, amino acid metabolism, and the generation of methyl groups. Fortification of grain products with folic acid was mandated in North America in the late 1990s for the prevention of neural tube birth defects. Given this fortification policy, the folic acid intake of Canadians and Americans has increased and folate deficiency is rare. However, poor vitamin B12 status in women during pregnancy is common, and estimated to be present in 1 in 20 Canadians. This is concerning because population studies from South Asia have reported greater adiposity and insulin resistance in children from mothers with adequate folate but marginal vitamin B12 status during pregnancy. This suggests that maternal folate and vitamin B12 status during pregnancy may program offspring cardiometabolic health.

Objective: The goal of this study is to investigate the mechanisms by which maternal folate and vitamin B12 imbalance during pregnancy can program adiposity and glucose homeostasis in adult offspring.

Methods: Female C57BL/6J mice were fed one of the following diets and their adult offspring were studied: supplemental folic acid/no vitamin B12 (SFA-B12 offspring), supplemental folic acid/adequate B12 (SFA+B12 offspring) or a control diet adequate in folic acid and vitamin B12 (control offspring) for six weeks prior to breeding, and during pregnancy and lactation. Offspring male and female mice were weaned onto the control diet or a western diet [45% fat, 35% carbohydrate] and maintained on the diet for 20-40 weeks. At the end of the feeding period, intraperitoneal glucose tolerance tests were performed followed by collection of tissues.

Results: No effect of maternal diet was observed on body weight during pregnancy and lactation, litter size, and serum folate concentrations in dams. Dams fed the SFA-B12 diet had lower serum vitamin B12 concentrations than dams fed the control diet and SFA+B12 diet. Sex-specific differences in adiposity and glucose homeostasis were observed in the offspring mice.

In male offspring, control and western diet-fed SFA-B12 and SFA+B12 mice had smaller visceral and subcutaneous adipose tissue depots than control mice. No effect of maternal diet on offspring glucose tolerance was observed. Control-fed SFA-B12 and SFA+B12 male offspring had lower serum total adiponectin concentrations, lower NADPH Oxidase 2 subunit (Nox2) expression in aorta, and lower serum vitamin B12 concentrations compared to control offspring; this was not observed in western diet-fed offspring. No effect of maternal diet on offspring endothelial-dependent relaxation of aorta and mesenteric arteries, indicators of vascular function, was observed.

In female offspring, control-diet fed SFA-B12 mice had larger visceral adipose tissue fat pads and greater glucose intolerance compared to control offspring. This was accompanied by alterations in methyl nutrient metabolism in the liver. In female offspring fed the post weaning western diet we found no effect of maternal diet on adiposity but found lower blood glucose and insulin concentrations in SFA-B12 female offspring compared to control female offspring.

Conclusion: Our findings suggest that maternal folate and vitamin B12 status during pregnancy and lactation programs adiposity, glucose homeostasis, and liver metabolism in adult mice; the effects are different in male and female adult offspring.

Supported by funding from NSERC and CIHR.

Invited Lecture

The Pregnancy Diaries: Chronicling placenta health in obstetrical complications and postpartum health
Dr. Samantha Benton, *Department of Cellular and Molecular Medicine, University of Ottawa*

A healthy placenta is vital for healthy pregnancy. When the placenta is not functioning properly or function is compromised, the health and well-being of mother and baby can be adversely affected both during gestation and in later life. Preeclampsia, fetal growth restriction and some cases of stillbirth are serious complications that arise from placental compromise and are leading causes of maternal and fetal/neonatal mortality and morbidity. Reliable markers of placental health would allow for appropriate surveillance and early intervention to optimise outcomes for these mothers and babies. Additionally, while the exact mechanisms leading to placental compromise have yet to be fully elucidated, markers that specifically reflect the underlying mechanisms leading to placental compromise could allow for targeted therapies and refined risk assessment. This talk will review our current understanding of placental-mediated pregnancy complications and markers of placental health being investigated in the prediction and/or diagnosis of these complications. The importance of including the placenta in outcome definitions, disease subtyping and marker discovery will be emphasized.

Invited Lecture

Protein and amino acid needs during pregnancy – Application of novel stable isotope techniques in humans

Dr. Rajavel Elango,
Department of Pediatrics, Child & Family Research Institute, University of British Columbia

Maternal-child health issues are fairly complex and multifactorial. But, there is no denying the critical role and impact of maternal nutrition on the growth of the fetus. Infant birth weight, a key indicator of infant health, as well as growth during the first few years of life is modulated by diet and nutrition. Furthermore, there is accumulating evidence that the prevalence of chronic diseases in adult life is related to the availability of adequate nutrition during pregnancy. Several key vitamins and minerals are well known to influence maternal-child health, such as folic acid in the prevention of neural tube defects. Recently, the role of dietary protein in promoting health has become the focus of several studies. There is also evidence that current national and international protein intake recommendations, which are based on factorial (mathematical) calculations may not be adequate to meet the body needs during pregnancy. These studies were performed using state-of-the-art stable isotope based techniques directly in pregnant women. This talk will outline the methodology, principles and major results from studies conducted using this new technique. The talk will also highlight protein and amino acid nutrition as a key factor in positive maternal-child health outcomes.

ABSTRACTS

ORAL SESSION 1 FETAL BRAIN

Chairs: Ken Lim and John Smyth

O1 White Matter and Cortical Brain Injury in the Very Immature Rat Following Lipopolysaccharide Induced Mild Systemic Inflammation

S Ranchhod, K Gunn, T Fowke, J Chan, J Prasad, J Bai, J Dean
Department of Physiology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

Background: Low level systemic infection in preterm infants is associated with brain injury and disability, including cerebral palsy and impaired cognition. The underlying mechanisms of infection-mediated injury remain unclear. The aim of this study was to examine the effect of exposure to mild systemic inflammation on white matter and cortical injury in newborn rats.

Methods: Postnatal day (PND) 1–3 Sprague-Dawley rats received daily injections of lipopolysaccharide (LPS; 0.3mg/kg/day i.p.). Animals were recovered until PND2, 4, 7, 14, or 21. Brains were processed for immunohistochemical analysis of cell death, oligodendrocyte survival/maturation, white matter and cortical volumes, and cortical neuronal survival/maturation.

Results: LPS was associated with acute cytokine upregulation in the blood and brain, and long-term reductions in white matter (Sham, 24.6±0.9 *vs.* LPS, 17.0±0.3mm^3; P<0.01) and cortical (Sham, 321.3±9.6 *vs.* LPS, 287.0±8.6mm^3; P<0.05) volumes at PND21. LPS was associated with an acute increase in degenerating nuclei in the white matter (PND4: Sham, 720±60 *vs.* LPS, 1164±126cells/mm^3; P<0.01) and a decrease in total olig2$^+$-oligodendrocytes (PND4: Sham, 93,334±5570 *vs.* LPS, 73,3044±4358 cells/mm^3; P<0.05). However, total olig2$^+$-oligodendrocytes in the LPS group returned to control levels by PND14 and PND21. LPS was associated with an acute decrease in PDGFRα+ oligodendrocytre progenitor cells in the white matter (PND4: Sham, 52,488±3,226 *vs.* LPS, 34,133±2,930 cells/mm^3; P<0.01), followed by a marked proliferative response at PND7–PND21 (peak PND14: Sham, 34,070±1,988 *vs.* LPS, 58,526±9,287 cells/mm^3; P<0.05). At PND14 and PND21 there was a decrease in the ratio of CC1$^+$ mature oligodendrocytes/total olig2$^+$ oligodendrocytes (PND21: Sham, 80±4% *vs.* LPS, 62±7%; P<0.01). LPS had no effect on numbers of cortical neurons (PND21: Sham, 2.22x10^7 *vs.* LPS, 2.15 x10^7 cells).

Conclusions: Mild systemic inflammation in the very immature rat is associated with persisting alterations in white matter and cortical development. Studies examining mechanisms of oligodendrocyte arrest and regenerative treatment are undergoing.

O2 Hyaluronan controls formation of neuronal lamellipodia and filopodia

Tania M Fowke[1,2], Ji-Zhong Bai[1,2], Jaya Prasad[1,2], Katherine Gunn[1,2], Justin M Dean[1,2]
1. Department of Physiology, University of Auckland, New Zealand.
2. Centre for Brain Research, University of Auckland, New Zealand.

Background: Hyaluronan is a linear, unbranched glycosaminoglycan that forms an integral part of the extracellular matrix throughout the body. Hyaluronan is synthesised by endogenous hyaluronan synthases (HAS1-3) and degraded by hyaluronidases (Hyals). Hyaluronan is abundant in the CNS, particularly during brain development, where it is produced by astrocytes. However, the function of hyaluronan in developing cortical neurons is unknown. The aim of this study was to characterise the production and function of hyaluronan during cortical neuron development.

Methods: Primary cortical neuronal cultures were established from E16 rats. For expression studies, cells were collected at days *in vitro* (DIV)0 (4h), 1, 3, 7 and 14 and processed for immunocytochemistry (hyaluronan binding protein; MAP2; actin) or qPCR (HAS1 -3). For hyaluronan function experiments, DIV0 neurons were treated with the broad HAS inhibitor 4-methylumbelliferone (4-MU; 0.1 -0.3 mM), and recovered until DIV1 or 3. Neurons were stained with actin/MAP2/Hoechst and morphological analysis performed using fluorescence microscopy and Neurolucida software.

Results: Neurons at all ages expressed HAS1 -3 mRNA. Neuronal progenitors exhibited limited hyaluronan expression, which progressively increased with neuronal maturation. By DIV1, punctate hyaluronan expression was observed on the cell body, processes and lamellipodia of neurons (MAP2+), which developed with time in culture. 4-MU markedly reduced neuronal hyaluronan expression and significantly reduced: (i) lamellipodia number (DIV1: 2.0±0.1 *vs.* 1.5±0.1, DIV3: 2.1±0.3 *vs.* 1.2±0.1; $p<0.01$) and mean area (DIV1: 44.3±3.4 *vs.* 33.0±2.5µm^2; $p<0.05$); (ii) number (DIV1: 4.2±0.3 *vs.* 2.6±0.2, DIV3: 2.5±0.3 *vs.* 1.7±0.2; $p<0.05$) and length (DIV1: 3.3±0.1 *vs.* 2.5±0.2µm; $p<0.0001$) of filopodia on lamellipodia; and (iii) filopodia per dendrite (DIV3: 6.0±0.5 *vs.* 4.6±0.4; $p<0.05$).

Conclusion: These data suggest that neurons themselves synthesise hyaluronan that is important for formation/stability of lamellipodia and filopodia on developing cortical neurons. Neuronal hyaluronan production may therefore represent a novel mechanism for regulation of neuronal growth.

O3 The effect of MgSO4 on asphyxia-induced brain injury in preterm fetal sheep.

Robert Galinsky, Joanna Tse, Joanne O Davidson, Christopher A Lear, Paul P Drury, Guido Wassink, Lotte Van den Heuij, Alistair Jan Gunn and Laura Bennet
The Department of Physiology, The University of Auckland, Auckland, New Zealand

Background: There is an important, unmet need to improve the outcome of neonatal encephalopathy in preterm infants. It remains controversial whether MgSO4 is clinically neuroprotective, and thus, it is unclear whether it would be appropriate to use MgSO4 for treatment of encephalopathy in preterm infants. We aimed to test whether MgSO4 infusion can reduce white or grey matter damage after profound asphyxia in preterm fetal sheep.

Methods: At 104 ± 1 days (0.7) of gestation, fetal sheep were randomly assigned to receive either a continuous infusion of i.v. MgSO4 (n=7) or vehicle (control; n=10) starting 24 hours before 25 minutes of complete umbilical cord occlusion, and continued for 24 hours after occlusion. Mean arterial pressure (MAP), fetal heart rate (FHR), and carotid blood flow (CaBF), fetal electroencephalography (EEG) and movement (nuchal electromyography (EMG)) were measured continuously. 72 h after occlusion, fetal brains were processed for neuropathological assessment of the subcortical grey matter, including the mid-striatum, hippocampus, dentate gyrus and thalamus, and intragyral and periventricular white matter.

Results: MgSO4 infusion increased fetal plasma magnesium levels from 0.78 to 1.89 mmol/L ($p<0.05$). The MgSO4 infusion before asphyxia was associated with reduced EEG power ($p<0.05$; MgSO4 vs. control). MgSO4 did not affect neuronal survival in subcortical grey matter. MgSO4 treated fetuses had reduced numbers of oligodendrocytes in the intragyral white matter ($p<0.05$), but no difference in the numbers of activated microglia or reactive astrocytes.

Conclusions: A clinically comparable dose of MgSO4 was associated with reduced EEG power before asphyxia, but did not reduce asphyxia-induced preterm brain injury and may increase oligodendrocyte loss.

O4 Intranasal human amniotic epithelial cell therapy and long-term recovery after asphyxial brain injury in preterm fetal sheep.

Lotte van den Heuij[1], Suzie L. Miller[2], Mhoyra Fraser[1], Graham Jenkin[2], Alistair J. Gunn[1], Laura Bennet[1].
[1] Fetal Physiology and Neuroscience Group, Dept. Physiology, The University of Auckland, New Zealand, [2] Ritchie Centre, Hudson Institute of Medical Research, Monash University, Australia

Introduction: Preterm infants have very high risks of perinatal asphyxia and brain injury. At present treatment is only symptomatic. Stem cell therapy is a promising option for improving neural outcomes after brain ischemia. Human amnion epithelial cells (hAECs) may be safer than other stem cell approaches and can attenuate cerebral inflammation. In this study we investigated whether intranasal administration of hAECs could improve recovery from asphyxial brain injury in preterm fetal sheep.

Methods: Preterm fetal sheep (103-4 days gestation; term is 147 days) were continuously monitored before, during and for 21 days after 25 min umbilical cord occlusion. Intranasal (IN) boluses of either hAECs ($30*10^6$ cells/2ml) or vehicle were given at 1 d, 3 d and 10 d post-insult. Brains were histologically assessed after post-mortem at 21 days.

Results: hAEC treatment did not affect any physiological parameters, although there was an apparent trend to better EEG recovery. Histologically, surviving hAECs were identified in the white matter tracts. There were no differences in total, olig-2+ve oligodendrocyte numbers between sham, asphyxia only and treatment groups. In contrast, hAEC treatment was associated with greater numbers of more mature, CNPase and MBP+ve oligodendrocytes than asphyxia only ($p<0.05$), and with reduced microglial infiltration and astrogliosis.

Conclusion: These findings strongly suggest that delayed intranasal administration of repeat doses of hAECs are associated with intracerebral migration of the hAECs, which act to reduce white matter inflammation and associated oligodendrocyte maturational arrest after severe asphyxial brain injury.

O5 Antenatal Melatonin Administration Protects Neurovascular Development In Growth Restricted Lambs.

Margie Castillo-Melendez, Tamara Yawno, Euan Wallace, Graham Jenkin, Suzie Miller.
The Ritchie Centre, The Hudson Institute, Monash University, Clayton, Vic, Australia

Introduction: Chronic hypoxia, such as occurs in intrauterine growth restriction (IUGR), results in oxidative stress, white matter (WM) injury and cerebrovascular abnormalities in newborn lambs. We previously demonstrated the neuroprotective and antioxidant effects of maternal administration of melatonin in growth-restricted lambs. In this study we examined whether antenatal melatonin treatment reduced vascular abnormalities in the IUGR brain.

Methods: Single umbilical artery ligation was performed at 110d gestation to induce placental insufficiency and IUGR. Melatonin was administered (0.25 mg/hr, i.v.) to a subset of IUGR pregnancies from surgery until term. Ewes delivered spontaneously and lambs were sacrificed 24h after birth. Single-label immunohistochemistry was performed with anti-laminin, and albumin. Doble-label immunofluorescence was performed with anti-desmin and anti-alpha smooth muscle actin (pericytes) and end feet blood vessel coverage using GFAP (astrocytes) and laminin.

Results: IUGR brains (n=7) demonstrated a reduction in the number laminin positive blood vessels versus controls (n=8): from 1650.0±283.9 to 416.2±46.61 cells/mm2 in subcortical WM (SCWM) 1793.1±297.5 to 384.5±20.47 cells/mm2 in periventricular WM (PVWM), and 1717.0±160.8 to 405.3±84.21 cells/mm2 in the subventricular zone (SVZ). Maternal melatonin had no effect on blood vessel numbers (481.3±24.6 cell/mm2; SCWM; 211.75±21.8 cell/mm2, PVWM; 300.4±17.8 cell/mm2, SVZ) in IUGR. A 71% reduction in vascular pericytes was seen in PVWM, 66% in CWM and 73% in SVZ of IUGR lambs, compared to controls. Maternal melatonin restored pericyte coverage to levels seen in control animals in all brain regions examined (69-75% reduction in controls vs IUGR+melatonin). Reduced astrocytes end-feet coverage and albumin extravasation into the brain parenchyma (increased BBB permeability) was observed in the WM and SVZ of IUGR lambs only.

Conclusions: Antenatal maternal melatonin has no effect on restoring the reduced vascular density seen in the IUGR brain, but prevents disruption of the BBB by protecting perivascular cells essential for maintenance of vascular homeostasis and stability.

O6 Dexamethasone induced hyperglycaemia during asphyxia is associated with severe cystic white and grey matter brain injury in preterm fetal sheep

Christopher A. Lear[1], Joanne O. Davidson[1], Robert Galinsky[1], Alistair J. Gunn[1], Laura Bennet[1]
[1]Fetal Physiology and Neuroscience Group, Department of Physiology, The University of Auckland, Auckland, New Zealand

Introduction: Antenatal glucocorticoids improve outcomes among preterm babies but are also associated with hyperglycaemia. In human pregnancy hyperglycaemia is associated with adverse neonatal outcomes; however, it is unclear whether this link is causal. In newborn rodents, both glucocorticoids and hyperglycaemia before hypoxia-ischaemia are highly neuroprotective, but there is little evidence from large animal studies. In this study we investigated whether glucocorticoids modulate asphyxial brain injury through induction of hyperglycaemia.

Methods: At 0.7 of gestation, chronically instrumented singleton sheep fetuses were exposed to maternal injection of 12 mg DEX i.m. (n=7), saline (n=8), or a fetal i.v. infusion of glucose dissolved in saline (2 mmol/ml, Glucose group, n=7), titrated to increase fetal plasma glucose levels to those observed in the DEX group. After 4 hours fetuses received 25 minutes of complete umbilical cord occlusion. Post-mortems were performed after 7 days recovery for cerebral histology.

Results: DEX and glucose treatment increased fetal blood glucose levels before asphyxia (DEX: 2.0±0.2 and Glucose: 2.4±0.3 *vs.* saline: 1.0±0.1 mmol/l, $p<0.05$). Occlusion with saline was associated with subcortical neuronal loss ($p<0.05$) and loss of mature oligodendrocytes ($p<0.05$) without cystic lesions. DEX and Glucose were associated with increased grey and white matter injury compared to saline treatment, including severe cystic infarcts in the thalamus and periventricular white matter in all DEX and Glucose fetuses ($p<0.001$, Fisher exact test). Within the DEX and Glucose groups, higher peak plasma glucose values were correlated with more extensive injury, including cystic damage of the striatum and sagittal gyrus.

Conclusions: Maternal DEX injection and fetal hyperglycaemia were associated with dramatic, dose-related exacerbation of neural injury after subsequent fetal asphyxia, including severe cystic infarction. These data strongly suggest that the clinical association of perinatal hyperglycaemia with adverse outcomes is causal.

O7 Non-additive effects of delayed connexin hemichannel blockade and hypothermia after cerebral ischemia in near-term fetal sheep

Joanne O Davidson, Alexandra Rout, Guido Wassink, Caroline A Yuill, Frank G Zhang, Colin R Green, Laura Bennet, Alistair J Gunn
Department of Physiology, The University of Auckland, New Zealand

Hypothermia is partially neuroprotective after neonatal hypoxic-ischemic encephalopathy. Blockade of connexin hemichannels can improve recovery of brain activity and cell survival after ischemia in near-term fetal sheep.

In this study we investigated whether combining delayed hypothermia with connexin hemichannel blockade with intracerebroventricular (i.c.v.) infusion of a mimetic peptide can further improve outcomes after cerebral ischemia. Fetal sheep (0.85 gestation) received 30 min cerebral ischemia followed by a three hour recovery period before treatment was started. Fetuses were randomized to one of the following treatment groups: normothermia (n=8), hypothermia for three days (n=8), connexin hemichannel blockade (50μM i.c.v. over one hour followed by 50μM over 24 h, n=8) or hypothermia plus hemichannel blockade (n=7).

After 7 days recovery, hypothermia was associated with reduced seizure burden, improved EEG power, and a significant increase in neuronal and oligodendrocyte survival and reduced induction of Iba1-positive microglia. In contrast, although hemichannel blockade reduced seizure burden, there was no effect on EEG power or histology ($p<0.05$). There was no further improvement in outcomes with combined hypothermia plus hemichannel blockade.

In conclusion these data show that there is no additive neuroprotection with combined hypothermia and hemichannel blockade after cerebral ischemia in near-term fetal sheep.

O8 Early infusion of recombinant erythropoietin is partially neuroprotective after profound asphyxia in preterm fetal sheep

Guido Wassink; Joanne O. Davidson; Laura Bennet; Mhoyra Fraser; Alistair J. Gunn
Fetal Physiology and Neuroscience Group, Department of Physiology,The University of Auckland, Auckland, New Zealand

Background. Perinatal asphyxia in preterm infants remains a significant contributor to neonatal death and abnormal long-term neurodevelopmental outcomes. The pleiotropic growth factor recombinant erythropoietin has potent non-hematopoietic neuroprotective properties, but there is limited evidence for whether it can reduce white or grey matter injury after asphyxia in the preterm brain.

Methods. Preterm (0.7 gestation) fetal sheep received sham asphyxia (sham) or asphyxia induced by complete umbilical cord occlusion for 25 min, followed by an intravenous infusion of vehicle (occl-veh) or recombinant human erythropoietin (occl-epo) starting from 30 min and continued until 72 hours after asphyxia. Post-mortems were performed at 72 hours.

Results. Recombinant human erythropoietin was associated with reduced neuronal loss in the striatal caudate nucleus ($p<0.01$; vs. occl-veh) but not the putamen, with greater expression of Ki67-positive proliferative cells in the periventricular and intragyral white matter ($p<0.01$; vs. sham and occl-veh) after 3 days recovery. This was associated with more rapid recovery of EEG power ($p<0.05$; 30-42 h) and spectral edge ($p<0.05$; 54-72 h) after asphyxia, with markedly greater carotid blood flow ($P<0.05$; 48-72 h) mediated by a proportionate increase in carotid vascular conductance.

Conclusions. These preliminary findings suggest that early treatment with recombinant human erythropoietin is associated with partial striatal neuroprotection and greater proliferation in subcortical white matter regions. This was associated with faster recovery of fetal EEG power and higher frequency EEG activities and a striking increase in cephalic blood flow that may help support cerebral metabolism during recovery from asphyxia.

O9 Umbilical cord blood cell administration is neuroprotective following preterm hypoxic-ischemic insult.

Jingang Li, Tamara Yawno, Amy Sutherland, Jan Loose, Ilias Nitsos, Flora Wong, Graham Jenkin, Suzie Miller
The Ritchie Centre, Hudson Institute of Medical Research, Monash University, The Departments of Obstetrics and Gynaecology, and Paediatrics, Monash Health, Monash University.

Introduction: Preterm infants, particularly infants born <32 weeks gestational age, are at high risk for cerebral palsy and other neurological deficits. There is increasing evidence that umbilical cord blood (UCB) cells are neuroprotective if administered following term perinatal asphyxia. However, there is limited information on the potential benefits of UCB treatment for the preterm brain, or optimal timing of administration in high-risk preterm infants. This study examined the efficacy and mechanism/s of action of allogeneic UCB cell administration to preterm fetal sheep following umbilical cord occlusion (UCO).

Methods: UCO, or sham occlusion, was performed for 25 minutes in fetal sheep at 100d gestational age; equivalent to very preterm human brain development. 50 million CFSE-labelled UCB mononuclear cells, derived from term lambs, or saline, were administered iv to the fetus at 12h or 5d after UCO. Fetal brains were collected at 10d after UCO.

Results: Histology revealed that CFSE-labelled cells were detected in the fetal brain at post mortem. UCO induced cell death (TUNEL+), reduced the number of oligodendrocytes (olig2+) and myelinated axon density (CNPase+), increased the density of inflammatory cells (Iba+), and increased proliferation (Ki67+), in white matter compared to that in control fetal brains. We found a strong correlation between increased inflammatory cells and oligodendrocyte cell loss within the white matter. UCB cell administration at 12h after UCO prevented cell death, loss of oligodendrocytes, neuroinflammation and hypomyelination. Delaying administration of UCB cells (5d) did not reduce inflammatory cells within the brain, and showed an intermediate improvement in oligodendrocyte number, when compared to UCO alone and UCO+UCB at 12h.

Conclusion: Administration of UCB cells should occur as early as possible in preterm infants at high-risk of brain injury. Administration at 12h after hypoxia-ischemia reduced white matter injury through anti-inflammatory and anti-apoptotic effects.

O10 Understanding the therapeutic potential of umbilical cord blood cells for cerebral palsy.

Courtney McDonald [1], Nicole Jones **2**, Margie Castillo-Melendez [1], Graham Jenkin [1] and Suzie Miller[1].
[1] The Ritchie Centre, Hudson Institute of Medical Research, Clayton, Australia, [2] Department of Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, Australia

Background:
Cerebral Palsy (CP) is the most prevalent cause of chronic disability in children. Recently, human clinical trials have reported safety data and shown some efficacy following treatment of established CP with umbilical cord blood (UCB) cells. However, it remains unknown how UCB cells can reverse or prevent brain injury. Furthermore, UCB is made up of many different cell types including endothelial progenitor cells (EPCs), T regulatory cells (Tregs) and monocyte derived suppressor cells (MDSCs). It is not known how each cell type within UCB contributes to repairing brain injury. In this study we examined whether UCB cells or the individual cell types could reduce inflammation and promote brain repair when given early after neonatal brain injury.

Methods:
Human UCB was collected from uncomplicated pregnancies undergoing elective cesarean section at term. UCB mononuclear cells were isolated and EPCs, T regs and MDSCs were separated using magnetic activated cell sorting. To assess the therapeutic potential of the cells, hypoxic-ischemic brain injury was induced in postnatal day 7 Sprague-Dawley rat pups, by single left carotid artery ligation followed by exposure to 8% oxygen for 3 hours. UCB cells were administered 24 hours post HI injury. MRI scans were performed using a 9.4T MRI at 24h and 7 days post HI. Immediately following the day 7 MRI, brains and spleens were collected. Spleens were assessed for immune cell phenotype. Brains were analysed for cell death (Caspase-3), inflammation (IBA-1), immune cell phenotype (Th1, Th17, Th2), neuronal injury (NeuN) and white matter injury (MBP).

Results:
We will present results for immune cell phenotype within the spleen and brain and demonstrate how UCB, EPCs and Tregs influence cell death, inflammation and markers of neuronal loss following neonatal brain injury.

Conclusions:
We propose that each cell type has a specific effect on inflammation, neuroprotection and neurorepair that we will be able to tease out with this model. This study will inform us to the cells types that are present in UCB that mediate neuroprotective actions, and future work could therefore utilise specific cell types for tailored therapies.

ORAL SESSION 2

DOHAD

Chair – Alex Beristain

O11 Isolating the long-term cardiovascular effects on adult offspring of human clinically relevant doses of antenatal glucocorticoids

Kimberley J Botting, Youguo Niu, Katie L Skeffington, Beth J Allison, Kirsty L Brain, Nozomi Itani, Christian Beck, Ana-Mishel Spiroski & Dino A Giussani, *Physiology, Development & Neuroscience, University of Cambridge, United Kingdom*

Introduction: Antenatal glucocorticoid therapy (AGT) in threatened preterm labour is globally implemented as it reduces the incidence of respiratory distress and death in preterm infants (Roberts & Dalziel. *Cochrane Dat Syst Rev.* **3**:CD004454, 2006). Both synthetic glucocorticoids as well as preterm birth may have adverse long-term effects on cardiovascular function (Kelly et al. *Pediatrics* **129(5):**e1282, 2012; Parkinson et al. *Pediatrics* **131(4)**:e1240, 2013). However, this is difficult to prove in humans as ex-preterm adults have likely been exposed to synthetic glucocorticoids during the perinatal period. Here, we isolated the long-term effects of AGT used in humans on cardiovascular function at adulthood in sheep not born preterm.

Methods: At 0.8 of gestation, pregnant ewes carrying singletons were treated with steroids (2 x 12mg dexamethasone i.m. 24h apart, D; n=7) while the other received saline vehicle i.m. (C; n=10). After natural delivery, offspring were maintained until 9 months, and then chronically instrumented with vascular catheters and a femoral flow probe to assess *in vivo* cardiovascular physiology followed by *ex vivo* cardiac (Langendorff) and vascular (wire myography) function analysis.

Results: Both C and D ewes delivered at term (148+3 *vs*. 148+2 d). At adulthood, C and D offspring had similar basal arterial blood pressure (89.8+1.8 *vs*. 92.8+2.6 mmHg) and heart rate (97.5+3.2 *vs*. 97.3+8.5 bpm). However, baroreflex function and pressure and femoral blood flow responses to nitroprusside were altered in D offspring (Fig. 1 a-c). Isolated hearts in D relative to C offspring showed markedly elevated left ventricular end diastolic pressure (LVEDP), an index of diastolic dysfunction, and impaired dP/dtmax, a measure of myocardial contractility (Fig. 1 d & e). Constrictor and dilator reactivity of isolated femoral vessels was comparable between C and D offspring.

Conclusions: Human AGT adversely affects cardiovascular function in adult offspring independent of preterm birth.
Support: British Heart Foundation

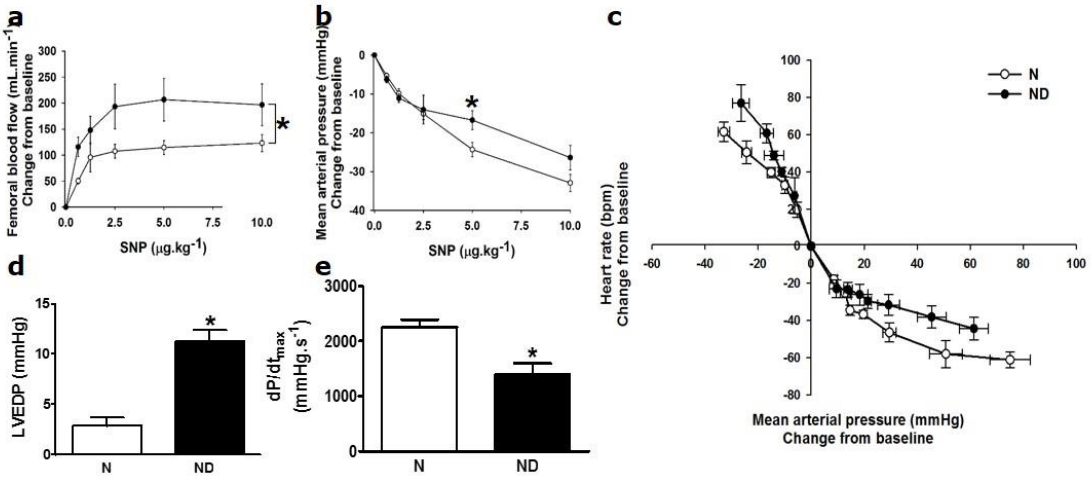


Figure 1. Data are mean±SEM for change from baseline in femoral blood flow (a) and in mean arterial pressure (b) to increasing doses of sodium nitroprusside (SNP, 0.6 - 10mg/kg i.v.), cardiac baroreflex function induced by increasing i.v. doses of SNP or of phenylephrine 0.5 - 64mg/kg i.v.(c), *ex vivo* left ventricular end diastolic pressure (LVEDP; d) and dP/dtmax (e). Significant differences are: *P<0.05; C, Control (white) *vs.* D, dexamethasone (black), Student's *t* test for unpaired data or two-way RM ANOVA plus Tukey Test.

O12 A sheep model to examine the effects of maternal asthma on fetal outcomes

Robert J Bischof[1,2], Vicki L Clifton[5,6], Amy L Wooldridge[5], Kathryn L Gatford[5], Bahar Liravi[2], Dasom Kim[2], Beverly S Muhlhausler[7], Janna L Morrison[8], Andrew Davies[2,9], Robert De Matteo[4], Megan J Wallace[1,3] & Timothy JM Moss[1,3]

[1]The Ritchie Centre, Hudson Institute of Medical Research, Clayton VIC 3168, Australia; [2]Department of Physiology, [3]Department of Obstetrics and Gynaecology and [4]Department of Anatomy and Developmental Biology, Monash University, Clayton VIC 3800, Australia; [5]Robinson Research Institute and School of Paediatrics & Reproductive Health, University of Adelaide, Adelaide SA 5005, Australia; [6]Mater Medical Research Institute, University of Queensland, Brisbane, Qld 4101, Australia; [7]FOODplus Research Centre, School of Agriculture, Food and Wine, The University of Adelaide, Adelaide SA 5005, Australia; [8]Early Origins of Adult Health Research Group, School of Pharmacy and Medical Sciences, Sansom Institute for Health Research, University of South Australia, Adelaide SA 5001, Australia; [9]School of Biomedical Sciences, Peninsula Campus, Monash University, Frankston VIC 3199, Australia.

Introduction:

Maternal asthma during pregnancy has an adverse effect on pregnancy outcomes, but identifying the cause(s) and the ability to evaluate interventions, is limited by the lack of an appropriate animal model. In this study we developed a sheep model of maternal allergic asthma to examine maternal responses and effects on fetal development.

Methods:

Allergic asthma was induced in sheep using an established protocol that included parenteral immunisation prior to mating, followed by chronic weekly airway challenges with house dust mite allergen (HDM) that continued throughout pregnancy. Maternal and fetal lung and placental phenotypes were characterised in singleton-bearing ewes (allergic and non-allergic controls) at 138–142 d gestational age (term ~147 d).

Results:

The eosinophil influx into lungs was greater after HDM challenge in allergic ewes than after saline challenge in control ewes before mating and in late gestation. Airway resistance increased throughout pregnancy in allergic but not control ewes, consistent with the accumulation of airway smooth muscle seen in allergic airways. Maternal allergic asthma decreased relative fetal weight (-12%) and altered placental phenotype to a more mature form. In fetal lungs, expression of surfactant protein (SP) -B mRNA was 48% lower in fetuses from allergic ewes than controls, with a similar trend for SP-D. Thus, allergic asthma in pregnant sheep modifies placental phenotype and inhibits fetal growth and lung development consistent with observations from human pregnancies.

Conclusion:

This study for the first time establishes a large animal model of maternal asthma, based on allergen sensitization prior to conception followed by repeated airway challenges in pregnant sheep, that can be used to investigate mechanisms of altered fetal development and adverse pregnancy outcomes caused by maternal asthma in pregnancy.

O13 Isolated direct adverse effects of glucocorticoids on the fetal heart and circulation

Noor Teulings[2], Youguo Niu[1], Tessa Garrud[1], Katie L Skeffington[1], Christian Beck[1], Nozomi Itani[1], Jan B Derks[2] & Dino A Giussani[1].

[1]Physiology Development & Neuroscience, University of Cambridge, UK; [2]University Medical Center Utrecht, The Netherlands.

Background: Antenatal glucocorticoid therapy (AGT) in pregnancy threatened with preterm labour is one of the best examples of the successful translation of basic science into effective clinical therapy as it significantly reduces respiratory distress syndrome and neonatal mortality. However, accumulating evidence suggest adverse side-effects on the developing cardiovascular system. Whether adverse effects of AGT are direct or secondary to effects on the placental physiology is unclear. Here, we isolated the direct effects of AGT in the chick embryo, the only model in experimental medicine that permits investigation of the effects of therapy on the fetus independent of effects on the maternal and/or placental physiology.

Methods: Fertilized chicken eggs were randomly divided into two groups. Dexamethasone was administered by injection into the egg air cell using a human clinically relevant dose (0.1 mg/kg) on day 14 of the 21 day incubation period. At day 19, embryos were euthanized, cardiac function assessed under a Langendorff preparation and second order femoral artery reactivity studied with *in vitro* wire myography. A separate cohort of embryos was perfusion fixed (4% paraformaldehyde; 2.66kPa) and the cardiac morphology established via unbiased stereology.

Results: Treatment with dexamethasone restricted fetal growth (C: 38.3±0.6, D: 31.9±0.8 %, P<0.05), increased left ventricular end diastolic pressure (LVEDP, Fig.1A), impaired LV inotropic responses to carbachol (muscarinic agonist) and to isoprenaline (β1 adrenergic agonist, Fig.1B), altered femoral constrictor responses to serotonin and to endothelin (Fig. 1C & D) and lead to a heavy dilated heart (Fig. 1E & F).

Conclusion: Human clinically-relevant doses of AGT directly impair fetal growth and directly induce significant fetal cardiac and vascular dysfunction.

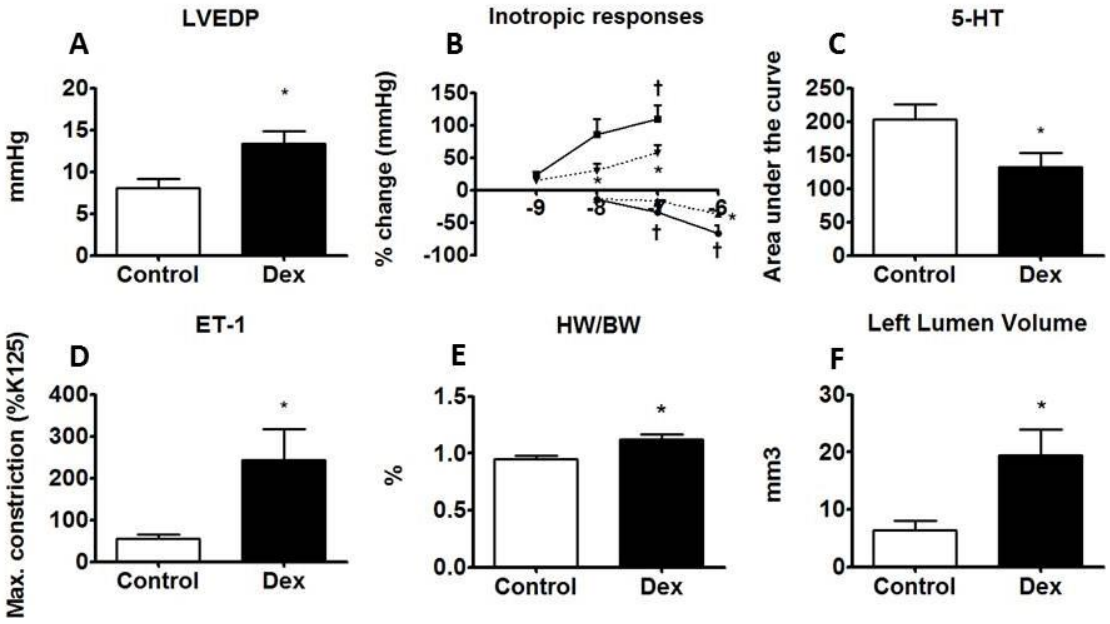


Figure1. Cardiovascular function in the dexamethasone-treated chick embryo. Data are mean+SEM for LVEDP (n=12, A), LV inotropic responses to carbachol and to isoprenaline (B, n= 6-8), dotted line: dexamethasone treated, solid line: control, X-axis: [Log] doses. Femoral artery constrictor responses to 5-HT and ET-1 (C and D, n=10), the relative heart weight (E, n=50) and the absolute LV lumen volume (F, n=10). Significant differences (P<0.05) are * *vs.* control , † *vs.* lowest dose (For B: Two-way ANOVA with Tukey test, A, C-F. Student's *t* test for unpaired data).

O14 Maternal administration of cyclic-glycine-proline alters the apoptosis of mammary cells during lactation and involution and enhances cognition of offspring in rats

Gagandeep Singh Mallah[1, 2], Kuljeet Singh[2], Chris McMahon[2], Jian Guan[1, 2]
[1]Liggins Institute, [2]Gravida, University of Auckland, Auckland, New Zealand

Background: Cyclic-glycine-proline (cGP), an Insulin-like growth factor-1 metabolite, provides neuro-protection in rodents following brain injury. Maternal administration of cGP in rats may increase mammary cell proliferation during lactation. We investigated the effects of maternal administration of cGP on mammary cell proliferation and apoptosis during lactation and involution in rats. We also tested the cognitive function of their offspring at adulthood.

Methods: cGP or saline (Control) was gavaged in lactating Sprague-Dawley rats during postnatal (PN) d8-22. Involution was enforced by sealing a pair of glands for either 24h or 72h before collecting tissues at PNd23. Concentrations of cGP were measured by High Performance Liquid Chromatography - Mass Spectrometry in milk samples collected from dams from PNd7-23, and in plasma samples collected from dams (PNd23) and their pups (PNd23 and 77). Mammary cell proliferation and apoptosis was quantified by Immunohistochemistry. Litter body weight was measured at PNd7, 14, 21 and 23. Behavioral tests were carried out (PNd35-70) to evaluate learning and memory (Novel Object Recognition test (NORT) and Morris Water Maze (MWM)), anxiety-like behavior (Light/Dark Box test) and general locomotory function (Open Field test) of pups.

Results: Milk and plasma cGP concentrations were higher ($P<0.01$) in cGP treated dams and their pups than in Controls except at PNd7 and 77 ($P>0.1$). Maternal cGP administration decreased the extent of apoptosis in lactating glands ($P<0.05$) but increased it in glands involuted for 72h ($P<0.05$), with no effect on mammary cell proliferation. Maternal cGP administration increased the litter growth rate between PNd7-14 ($P=0.06$), and enhanced the learning and memory abilities of pups ($P<0.05$ both NORT and MWM) without affecting their anxiety-like behaviour or general locomotory function.

Conclusions: Administering cGP to lactating dams may improve the persistency of lactation by regulating the apoptosis of mammary cells, leading to improved growth and cognitive function of the offspring.

015 Developmental and Cortisol Regulation of Mitochondrial Function in Fetal Skeletal Muscle during Late Gestation

Katie L. Davies[1], Owen R. Vaughan[1], Alison J. Forhead[1], Miles J. De Blasio[1], Andrew J. Murray[1] & Abigail L. Fowden[1]
[1]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, CB2 3EG, UK.

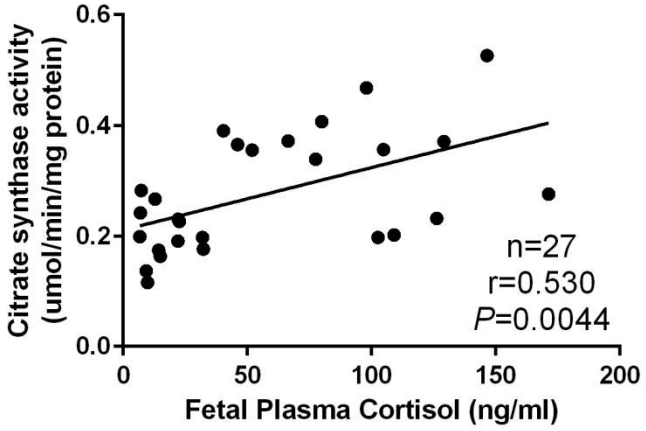
Background: At birth, skeletal muscle requires increased energy for its new locomotive and thermoregulatory functions. Mitochondria are the primary energy source and also regulate oxidative stress when oxygen tension rises at birth. Fetal cortisol concentrations increase towards term and cause tissue maturation essential for neonatal survival. However, little is known about prepartum mitochondrial maturation. This study examined the ontogeny and cortisol regulation of mitochondrial function in skeletal muscle of fetal sheep during late gestation.

Methods: Fetuses were catheterised under anaesthesia at 120 days (d) of gestation and infused with cortisol (1-2mg/kg/d; n=9) or saline (n=7) between d125-d130 (term ~d145). After maternal and fetal euthanasia, *biceps femoris* muscle was collected from d130 infused fetuses and from additional untreated fetuses at d130 (n=6) and d143 (n=5). Citrate synthase (CS) activity, a marker of mitochondrial density, was measured spectrophotometrically and electron transport system (ETS) complexes I-IV and ATP-synthase, used for oxidative phosphorylation, quantified using western blotting. Fetal plasma cortisol was measured by radio-immunoassay. Treatment and age comparisons were made using *t-test*.

Results: Muscle CS activity, but not ETS complex nor ATP-synthase abundance, was higher in cortisol- than saline-infused fetuses (d130, $P<0.05$). CS activity increased with gestational age in untreated fetuses ($P<0.01$), and correlated positively with fetal plasma cortisol concentrations when combining all data (Figure). Additionally, expression of all ETS complexes was higher at d143 than d130, although the increment was significant only for complex I ($P<0.05$). When normalised to CS activity, muscle abundance of all ETS complexes was unaffected by fetal cortisol infusion, whereas complexes II, IV and ATP-synthase were lower in untreated fetuses at d143 than d130 ($P<0.05$).

Conclusions: Maturational changes in mitochondrial biogenesis and oxidative capacity occur in skeletal muscle towards term and may depend partly on the fetal prepartum cortisol surge.

Figure: *Citrate synthase activity with respect to fetal cortisol.*



ORAL SESSION 3

FETAL & NEONATAL CARDIORESPIRATORY FUNCTION

Chair – Laura Bennet

O16 Meis1 drives metabolic maturation of cardiomyocytes

Isa M Lindgren and Kent L Thornburg
Center for Developmental Health, Knight Cardiovascular Institute, Oregon Health and Science University, Portland OR.

Introduction
The metabolic strategy of the heart shifts at birth from glycolytic to oxidative. The mechanisms that regulate this shift are little understood and there is an urgent need for new knowledge that will improve the care of premature babies. The transcription factor Meis1 maintains glycolysis in hematopoietic stem cells (HTCs) through its regulation of hypoxia-inducible factor 1α (HIF1α). By suppressing Meis1 expression, the metabolic strategy of the HTCs shift from glycolysis to oxygen consuming mitochondrial respiration, similar to what happens in the fetal heart at birth. We hypothesize that fetal cardiac Meis1/HIF1α expression decreases in fetal sheep cardiomyocytes with gestational age as the myocardium prepare to augment its workload at birth in a high oxygen environment.

Methods
RNA expression and protein levels of Meis1 and HIF1α was assayed in left ventricular cardiac tissue from fetal (90-95 and 135 days of gestation), neonatal (7-12 days) and adult sheep by qRT-PCR and Western blot, respectively. Metabolic measurements were performed on primary cultured cardiomyocytes (100 and 135 days of gestation) with the Seahorse cell metabolism analyzer.

Results
Meis1 expression decreased with fetal age ($P<0.01$, 90 vs. 135d, n=4-5) and decreased further postnatally ($P<0.05$, neonate vs. adult n=4-5) [Figure] (One-way ANOVA with Newman-Keuls post hoc test). HIF1α mirrored Meis1 levels and Western blot showed a decrease in protein and a change in Meis1 splice variants with ontogeny. Cardiomyocyte metabolic analysis showed an increase in oxygen consumption between 100 and 135 days of gestation.

Conclusions
The decrease in expression of Meis1 and HIF-1α during late gestational development and the increase of cardiomyocyte oxygen consumption are consistent with the hypothesis that Meis1 drives glycolysis in fetal cardiomyocytes and that a decrease in expression of Meis1 leads to metabolic changes that favor oxidative phosphorylation. Meis1 could be a target for improving oxygen utilization in premature myocardium.

O17 Aortic displacement as a surrogate for intertwin pulse pressure differences in monochorionic pregnancies with and without twin-twin transfusion syndrome.

Christoph Wohlmuth[1,2], Frank A. Osei1, Kenneth J. Moise[1], Anthony Johnson1, Ramesha Papanna[1], Michael Bebbington[1], Helena M. Gardiner[1]
[1]The Fetal Center, Children's Memorial Hermann Hospital and the Department of Obstetrics and Gynecology, The University of Texas Health Science Center at Houston, TX, USA, [2]Department of Obstetrics and Gynecology, Paracelsus Medical University Salzburg, Salzburg, Austria.

Background: Twin-twin transfusion syndrome (TTTS) complicates 10-15% of monochorionic diamniotic (MCDA) twin pregnancies. Donor responses to resultant hypovolemia include net transfer of vasoactive mediators to the recipient causing increased recipient afterload and hypertension. We hypothesize that Aortic Fractional Area Change (AFAC) reflects fetal pulse pressure thus providing an estimate of fetal blood pressure.

Methods: High frame rate four chamber views, including the mid-thoracic aorta, were collected prospectively in 56 MCDA twin pairs using GE Voluson E8 with RAB6-D probe (GE Health Care, Austria). Four groups were studied: uncomplicated MCDA (n=14); selective growth restriction (sIUGR, n=5, weight discordance > 25%); TTTS stages 1+2 (n=21) and TTTS stages 3+4 (n=16). AFAC, defined as (max(area)-min(area))/min(area), was calculated off-line using dedicated speckle-tracking software (TomTec, Germany). The endovascular border was traced manually during systole with timing guided by anatomical M-mode and AFAC averaged over 3 cardiac cycles.
Intertwin-pair differences were calculated. Inter- and intraobserver reproducibility was tested using intercorrelation coefficient (ICC).

Results: Mean gestational age was 20.9±3.1 weeks. Intertwin-pair heart rates were similar at 142±7 bpm. Mean frame rate was 82±29 Hz with no significant difference between twin pairs in MCDA, sIUGR and TTTS stage 1+2, but higher donor frame rates in TTTS stage 3+4 (88+/-42 vs. 76+/-30, p=0.012). Inter- and intra- ICC were 0.894 and 0.888. Significant intertwin-pair differences were seen in both TTTS groups (Table 1) consistent with higher pulse pressure in the recipient twin compared to its donor co-twin.

Conclusions: AFAC provides a quantifiable and reproducible method to assess aortic area change. This represents aortic distensibility and may demonstrate, differences in intertwin-pair pulse pressure. Thus it is a promising tool to monitor therapeutic interventions.

Category	AFAC Twin 1 (%)	AFAC Twin 2 (%)	p
MCDA (n=14)	64.1 ± 20.4	69.0 ± 19.3	0.335
sIUGR (n=5)	60.8 ± 30.6	60.5 ± 13.0	0.974
TTTS Stage 1/2 (n=21)	43.7 ± 19.3	72.3 ± 29.9	0.001
TTTS Stage 3/4 (n=16)	42.5 ± 18.4	75.2 ± 29.2	<0.001

Table 1: Intertwin AFAC in uncomplicated MCDA pregnancies and those complicated by sIUGR and TTTS. Twin 1 was defined as the smaller twin in MCDA, sIUGR and the Donor in TTTS cases.

O18 Cardiac dysfunction in the hypoxic chick embryo is rescued by treatment with the mitochondrial targeted antioxidant MitoQ

Katie L Skeffington1, Nozomi Itani1, Christian Beck1, Youguo Niu1, Angela Logan2, Michael P Murphy2 and Dino A Giussani, *1Department of Physiology Development & Neuroscience, 2Mitochondrial Biology Unit, Addenbrookes; University of Cambridge, UK.*

Introduction: Chronic fetal hypoxia triggers an early origin of cardiovascular dysfunction secondary to increased oxidative stress. Therefore, treatment with the antioxidant vitamin C is protective (Giussani et al. *PLoS ONE* **7(2)**:e31017, 2012). However, vitamin C is only effective in high doses incompatible with human treatment. Hence, the search for alternative translational antioxidant therapies. Mitochondria are a major source of cellular oxidative stress. Therefore, here, we established the efficacy of MitoQ in a chick embryo model of hypoxic development.

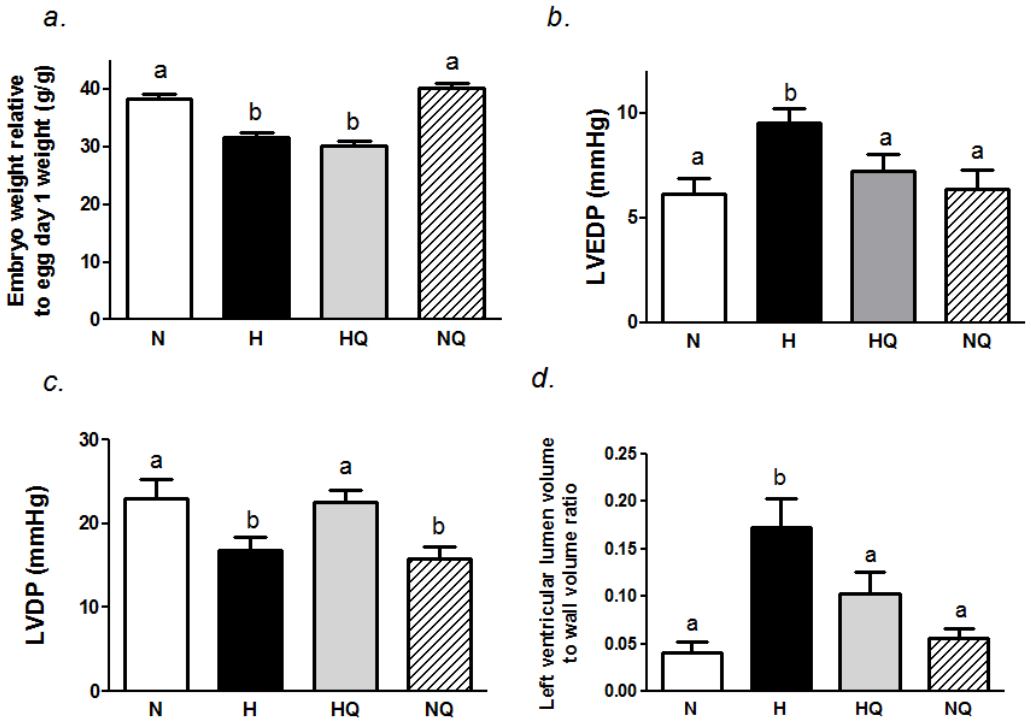
Methods: Fertilised chicken eggs were incubated under normoxic or hypoxic (14% O_2) conditions from day (d) one (term is 21d). Between d13-18 eggs were dosed daily with either vehicle (water) or MitoQ (0.2mg/kg/d) by injection onto the chorioallantoic membrane. On d19 cardiac function was determined using the Langendorff preparation. A separate cohort of animals was perfusion fixed (4% paraformaldehyde, 30mmHg) and stereological analysis performed on paraffin-embedded sections of the heart.

Results: Hypoxic embryos were growth restricted (Fig.1a), showed increased left ventricular end diastolic pressure (LVEDP, Fig.1b) and decreased left ventricular developed pressure (LVDP, Fig.1c), indicative of both systolic and diastolic dysfunction. Left ventricular lumen volume was increased whilst left ventricular wall volume was reduced, increasing the lumen-to-wall volume ratio (Fig.1d). Treatment with MitoQ had no effect on embryo growth but reduced the cardiac dysfunction and dilatation.

Conclusions: Development under hypoxia promoted significant cardiac dysfunction in the chick embryo. Treatment with MitoQ had beneficial effects, suggesting that mitochondrial derived oxidative stress contributes to the dysfunction. MitoQ is already used at these doses in human clinical practice. Therefore, MitoQ may be an alternative, clinically-viable antioxidant therapy for pregnancy complicated by chronic fetal hypoxia.

Supported by The British Heart Foundation.

Figure 1



O19 Store operated channel blockade partially reverses pulmonary artery hyper reactivity and remodeling in newborn sheep exposed to hypoxia in the last part of gestation.

Sebastián Quezada[1], Sebastián Castillo-Galán[1], Renato Ebensperger[1], Felipe Beñaldo[1], Fernando Moraga[3], Ismael Hernández[1], German Ebensperger[1], Emilio A. Herrera[1,2], Aníbal J. Llanos[1,2], Roberto V. Reyes[1].
[1]Programa de Fisiopatología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile. [2]International Center for Andean Studies (INCAS), Universidad de Chile, Santiago, Chile. [3]Facultad de Medicina, Universidad Católica del Norte, Coquimbo, Chile.

Introduction. Exposure to chronic hypoxia during the last 100 days of gestation results in pulmonary artery hypertension (PAH) that persists at sea level in sheep (1). Store-operated channels (SOC) are involved in PAH since they promote both pulmonary artery contraction and remodeling (2). We addressed the effect of a ten-day treatment of SOC blocker 2-aminoethyl diphenylborinate (2-APB) in newborn lambs exposed to hypoxia in the last 100 days of gestation over the cardiovascular function *in vivo*, isolated pulmonary arteries function *ex vivo* to vasodilator and vasoconstrictor stimuli and pulmonary artery remodeling *in vitro*.

Materials and Methods. Ten four-days old newborn lambs gestated the last 2/3 at highlands and returned to lowlands immediately after delivery (LHL) were catheterized and monitored over a ten-day treatment with either 2-APB (10 $mg \cdot kg^{-1} \cdot day^{-1}$, n=5) or its vehicle (DMSO:saline 1:10, n=5). At the end of the treatment, newborns were euthanized and lung samples were collected for *ex vivo* and *in vitro* experiments.

Results. 2-APB treatment lowered mean pulmonary artery pressure (mPAP) and cardiac output (CO) from the fourth day of treatment relative to vehicle group. However, no changes in pulmonary vascular resistance were observed along treatment. Further, 2-APB did not change the *ex vivo* contractile responses to potassium or serotonin, but it decreased the maximal contraction to ET-1 and U46619. In addition, 2-APB treatment increased maximal relaxation to 8Br-cGMP, sildenafil and fasudil, but with similar dilatation responses to sodium nitroprusside, relative con controls. Finally, 2-APB reduced small pulmonary artery wall thickness.

Conclusion. 2-APB treatment lowers pulmonary hypertension in LHL lambs, and this effect may be the combined result of CO reduction, associated with thinning of pulmonary artery wall and establishment of a new balance between vasoconstrictor and vasodilator mechanisms involving ET1, thromboxane, PDE5, PKG and RhoK signaling.

Supported by FONDECYT 1120605, 1151119, 1140647, 1130424.

References.

1. Herrera et al. *Am J Physiol*. **299(**6):R1676-84, 2010.
2. Kuhr et al. *Am J Physiol.* **15**;302(8):H1546-62, 2012.

ORAL SESSION 4

FETAL MONITORING

Chair – KS Joseph

O20 Effect of maternal position on fetal heart rate variability and behavioural state in healthy term pregnancy

Jordan PR McIntyre[1,4,5], Wendy Burgess[1,6], Christopher A Lear[3], JMD Thompson[2], Alistair J Gunn[2,3], Laura Bennet[3], and PR Stone[1,6] on behalf of the Maternal Sleep in Pregnancy Research Group.
Departments of Obstetrics & Gynaecology[1], Paediatrics: Child and Youth Health[2], and Physiology[3], Faculty of Medical and Health Sciences, University of Auckland, New Zealand.
New Zealand Respiratory and Sleep Institute[4], Auckland, NZ.
Starship Children's Hospital Respiratory Physiology Laboratory[5] and National Women's Health[6], Auckland District Health Board, New Zealand.

Background/Introduction: Reduced risk of stillbirth has been associated with mothers sleeping in the left lateral position [1]. Although such catastrophic events are rare, we hypothesised that maternal position would also have more subtle effects on healthy fetuses. In this study we examined whether fetal behavioural state (FBS) or fetal heart rate variability (FHRV) were affected by maternal position.

Methods: Healthy pregnant women (35-38 weeks gestation, n=29) were monitored lying supine, left-lateral, right-lateral or semi-recumbent for 30 minutes each, in randomized order. Trans-abdominal fetal-ECG (Monica AN24™) was recorded on three channels at 300 Hz, with an interpolated sample rate of 2.2 kHz. FHRV was measured using the Dawes algorithm for short term variability (STV). Mean Minute Range (MMR) and one minute means of SD-RR and RMSSD. Two independent observers blinded to maternal position assessed FBS by cardiotocography (inter-observer Kappa = 0.8).

Results: Fetal 4F (active awake) state occurred significantly more in left-lateral (45% of time) than supine (13%); $p<0.0001$. Conversely, when mothers were supine, the fetus was more likely to be in 1F (quiet sleep) and to change state more frequently. There was no effect of the sequence of maternal positions.

Consistent with these state changes, SD-RR fell by 20% [-3.96; (95% CI = -5.08, -2.84)] and RMSSD by 10% [-0.47; (-0.85, -0.09)] in the supine position compared with left-lateral. Similarly, SD-RR fell by 10% [-2.18; (-3.3, -1.06)] and RMSSD by 5% [-0.46; (-0.84, -0.08)] when mothers were semi-recumbent. There were no changes in fetal STV or MMR.

Conclusions: This study demonstrates that when mothers lie supine, and to a lesser extent semi-recumbent, their fetuses show an overall shift to quiet sleep, as well as more frequent state changes. FHRV was reduced, consistent with previous reports in quiet sleep [2]. Thus, in healthy pregnancies, maternal sleep position affects fetal behaviour.

1. Stacey, T., et al., *Association between maternal sleep practices and risk of late stillbirth: a case-control study*. BMJ, 2011. **342**: p. d3403.
2. Brändle, J., et al., *Heart rate variability parameters and fetal movement complement fetal behavioral states detection via magnetography to monitor vegetative development.* Frontiers in Human Neuroscience, 2015. **9**: p. 147.

O21 Diurnal changes in fetal middle cerebral artery blood flow parameters in a normal population

Tehila Avitan[1], Kenneth Lim[1], Dan Rurak[1], Meisan Brownlum[2], Ursula Brain[2], Tim Oberlander[2]
Department of Obstetrics and Gynecology, 2 Department of Pediatrics, University of British Columbia, Canada

INTRODUCTION: The aim of this study is to examine whether there are diurnal changes in fetal MCA Doppler blood flow parameters in a normal population.

METHODS: Prospective observational study. Singleton, uncomplicated pregnancies between 35 and 36 wks were recruited. Patients with pre-existing medical conditions, and/or taking medications were excluded. MCA and Umbilical Artery Doppler parameters were measured at 08:00 and then repeated 4.5 hours later. All parameters were measured 5 times during fetal quiescence and in absence of fetal breathing. The mean value was taken for analysis. Standard descriptive statistics and student T test (2 tailed) used as appropriate. $P < 0.01$ was considered significant for this analysis.

RESULTS: Data for 55 subjects were obtained. Table 1 outlines the results. Statistically significant increases in MCA Mean flow ($p = 0.0045$) and MCA PSV ($P=0.00007$) were observed between AM and PM. Magnitude of change was + 35% and + 10.7% respectively. There was a significantly decreasing resistance to flow as measured by RI ($p = 0.00037$) and PI ($p = 0.00037$). There were no statistical differences in HR or MCA diameter between AM and PM. There were no statistical differences in UA resistance to flow between AM and PM.

CONCLUSION: In this study, we observed significant diurnal changes in MCA Doppler flow parameters. Increased PSV and flow were not associated with changes in vessel diameter or heart rate. The clinical implications of these findings are that when serial MCA Doppler parameters are used to guide clinical management, consideration should be given to performing these measures at the same time of day.

Table 1- Comparison of MCA flow characteristics AM vs PM

	AM	PM	P value
MCA Peak systolic velocity (PSV) (cm/sec)	57.4	63.5	0.000075
MCA RI	0.81	0.78	0.000376
MCA PI	1.77	1.634	0.000376
MCA S/D	5.52	5.0363	0.005651
FHR	134.7	136.9	0.032382
MCA diameter (cm)	0.336	0.359	0.035640
MCA flow (ml/min)	138.9	187.5	0.004512
UA PI	0.94	0.891	0.031289
UA RI	0.61	0.60	0.041
UA S/D	2.67	2.55	0.049002

O22 Sinusoidal (-like) fetal heart rate: recognition of the pattern is crucial!

Jan Nijhuis, *Dept Ob Maastricht University Medical Center (MUMC), GROW-school for oncology and developmental biology /Gyn, POBox 5800, 6202AZ NL-Maastricht.*

At the SGI in San Francisco in 2015, it was postulated that sinusoidal fetal heart rate patterns (SHR) `have most commonly been associated with anemic fetuses'. For the correct assessment of the fetal condition a broader knowledge is important.
First, the definition and recognition of a SHR may be difficult if one puts too much emphasis on the difference between a `pure' SHR and a fetal heart rate (FHR) pattern `resembling' a SHR. Second, a SHR can be caused by many more clinical situations than fetal anemia only, and may also occur under physiological conditions.
Third, a SHR can be intermittently present.Therefore, when a SHR is considered, the workup of this clinical problem should be based on the knowledge of the differential diagnosis.
Crucial is the context: `what is the gestational age, and why was a FHR pattern (CTG) recorded'. Fetal anemia is the most ominous diagnosis. Ultrasonography with Doppler flow measurements are the quickest actions, a Kleihauer-Betke test in maternal blood for the presence of fetal cells takes more time.
There are at least two physiologic situations in which rhythmic fetal movements produce a SHR. An oscillating pattern with a small bandwidth during non-REM sleep (behavioral state 1F) can be caused by `regular mouthing'. A SHR with an oscillating pattern with a greater amplitude can be caused by `fetal sucking' movements during fetal wakefulness (state 3F), similar to the `nutritive' sucking in the neonate.
During labor, a SHR may be entrained by pain-relieving narcotic substances such as Meperidine. We have also recorded a SHR caused by Remifentanil, Also, cases are reported a SHR in other conditions (e.g.cardiac anomaly). These considerations are needed to decide whether the fetus needs immediate help or whether an expectant management is justified.
In conclusion, we suggest that is of great importance to recognize a `sinusoidal-like' FHR pattern. Context is crucial for the workup (e.g. `less movements') of the differential diagnosis. Finally, this pattern can also be seen with the use of modern substances such as Remifentanil.

O23 Fetal Urine Production Rate in Preterm Premature Rupture of Membranes And Adverse Neonatal Outcomes: a pilot study

Tehila Avitan, Hadas Pri-Chen, Ron Rabinovitz, Nurit Algor, Michal Kovo, Letizia Screiber, Michael S. Schimmel ,Arnon Samueloff, Sorina Grisaru-Granovsky
Departments of Obstetrics & Gynecology and Neonatology and Biochemical Analyses Laboratory, Shaare Zedek Medical Center, affiliated with the Hebrew University Medical School, Jerusalem; and Department of Pathology, Edith Wolfson Medical Center, Holon, affiliated with the Tel Aviv University; Israel

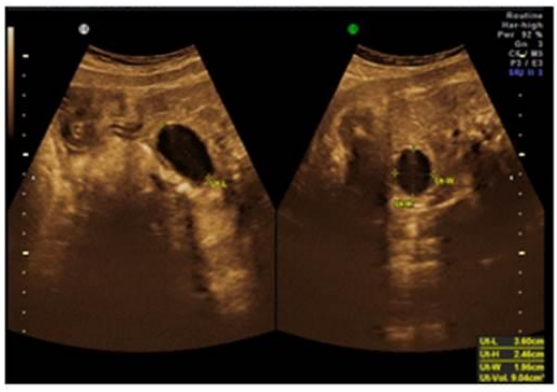
Background: Preterm premature rupture of membranes (PPROM) continues to be a leading cause of neonatal mortality and remains a major source of short and long term neonatal morbidity. The aim of this study is to evaluate associations between Fetal Urinary Production Rate (FUPR) measured by ultrasound in women with Preterm Premature Rupture of Membranes (PPROM), and adverse neonatal outcome (ANO).

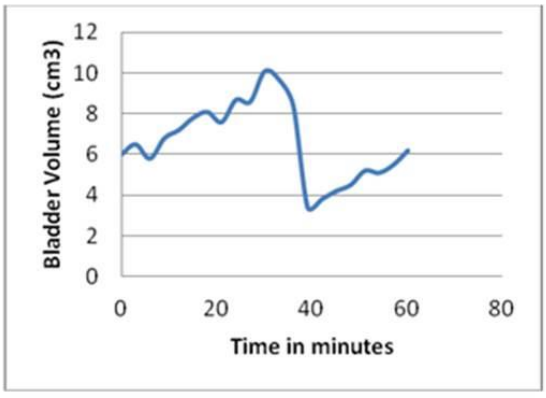
Methods: Prospective cohort of PPROM in women with singleton pregnancy. All women were hospitalized for conservative management until spontaneous labor, chorioamnionitis, or induction at 35 weeks. Fetal Urine Production Rate (FUPR) was determined after 24-48 hours of PPROM, adjusted for GA. Assessment of FUPR made by ultrasound measurement of the change in fetal bladder volume over time (Figure 1). Maternal and umbilical venous samples were analyzed for IL-6 level. Maternal and neonatal outcomes were recorded.

Results: The study included 38 women diagnosed with PPROM. FUPR at admission (mean±SD) was 20.8±12.9 ml/hour. We found that the lower the admission FUPR, the greater the risk of spontaneous labor <35 weeks, the longer NICU admission, higher risk for neonatal blood transfusion, Neonatal Enterocollitis (NEC) and Intra Ventricular Hemorrhage (IVH). However, we found no significant association between FUPR and admission oligohydramnios, placental histologic inflammation grading, and maternal or neonatal positive cultures. Higher IL-6 values were measured in women who developed chorioamnionitis and in neonates with early sepsis.

Conclusion: FUPR evaluation at admission for PPROM was found to be indicative of increased risk of prematurity and adverse neonatal outcome. This non-invasive fetal assessment may provide further insight into early subtle intrauterine inflammatory processes that impact on the neonatal outcome.

Figure 1a and 1b: Changes in fetal urine volumes (expressed in cm³) over time (in minutes) determined by ultrasound in a single fetus and illustrative ultrasound, respectively





ORAL SESSION 5

UTERUS AND PLACENTA

Chair – David Walker

O24 The placental phenotype adapts to chronic hypoxia and mitochondrial-targeted antioxidant (MitoQ) therapy in rat pregnancy

Emily J Camm 1, Anna M Nuzzo [1,2], Amanda N Sferruzzi-Perri [1], Alessandro Rolfo [2], Tullia Todros [2], Michael P Murphy[3] & Dino A Giussani [1]

[1] Department of Physiology, Development and Neuroscience, University of Cambridge, United Kingdom, [2] Department of Surgical Sciences, University of Turin, Italy; [3] MRC Mitochondrial Biology Unit, Cambridge, United Kingdom.

Introduction: The placenta responds to adverse environmental conditions, such as maternal glucorticoid over-exposure and dietary manipulation, by adapting its capacity for substrate transfer to maintain appropriate fetal growth and development (Fowden et al. *J Neuroendocrinol* **20(4)**:439, 2008). Comparatively little is known about placental adaptations to hypoxia and/or oxidative stress. Here, we determined the effect of maternal chronic hypoxia on placental morphology and established whether maternal treatment with MitoQ protected against hypoxia-induced alterations in placental structure.

Methods: Wistar dams were exposed to normoxia (N, 21% O_2) or hypoxia (H, 13% O_2) from days 6-20 of pregnancy +/- MitoQ (500μM in drinking water). On day 20, animals were sacrificed and weighed and the placentae were processed for stereology. One placenta per litter per group was used. This model does not affect maternal food intake.

Results: Neither hypoxic pregnancy or MitoQ treatment affected fetal growth (N: 3.83±0.07 g; H: 3.44±0.03 g; HM: 3.59±0.04 g; NM: 3.41±0.03 g). Relative to normoxia, the placental absolute volume and the labyrinthine fetal capillary surface area were significantly increased in hypoxic pregnancy (Fig. 1 A and B, P<0.05). Maternal MitoQ treatment in hypoxic pregnancy additionally increased the maternal blood space surface area (Fig. 1 C, P<0.05).

Conclusions: The data show that the placenta adapts to chronic hypoxic pregnancy by increasing the fetal capillary surface within the labyrinthine transport zone to maintain fetal growth. Maternal mitochondrial targeted antioxidant therapy during hypoxic pregnancy improves perfusion on the maternal side of the placenta.

Supported by the British Heart Foundation

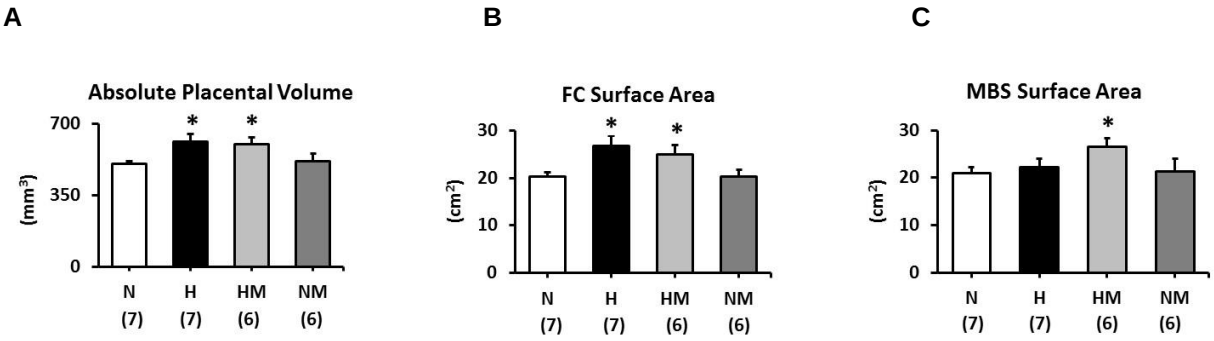


Figure 1. Placental morphology following chronic hypoxic pregnancy. Mean + S.E.M. for (A) absolute placental volume, (B) labyrinthine zone surface area of fetal capillaries (FC) and (C) maternal blood spaces (MBS). N, normoxia, H, hypoxia, HM, hypoxia+MitoQ, NM, normoxia+MitoQ. * P<0.05 *vs*. N, General Linear Model. *n* numbers in parenthesis.

O25 Maternal obesity drives decidual natural killer cell imbalances in early pregnancy

S Perdu[1], Y Kim[1], K Chan[1], AG Beristain[1]

[1]*Department of Obstetrics & Gynecology, The University of British Columbia, Vancouver, Canada*

Problem: Maternal obesity associates with obstetrical and perinatal morbidity, where in North America, over 20% of women of childbearing age are obese. Obese women are three times more likely to develop major complications during pregnancy, and excess adiposity increases the risk of preterm birth. Obesity strongly associates with low-grade inflammation characterized in part by immunological alterations in peripheral blood and adipose tissue. Natural killer cells (NKs), the dominant uterine immune cell population in early pregnancy, play key roles in vascular remodeling, trophoblast function, and fetal tolerance. The effect of obesity-linked inflammation on decidual NK function is unknown. Our study aims to examine the effects maternal obesity on decidual NK biology.

Methods of Study: Decidual NK populations were examined from consenting women undergoing elective terminations of pregnancy between 8 and 10 weeks gestation (grouped as control BMI, 20-24.9 kg/m^2 and obese BMI, 30 kg/m^2). Flow cytometry and fluorescence microscopy strategies quantified decidual NK (CD56^{bright}) proportions and absolute numbers in decidua. Global gene expression comparisons between control and obese decidual NKs were examined using the Illumina BeadChip platform. Functional assays examining NK cytotoxicity and cytokine expression were performed examining CD107a degranulation and K562 target cell cytotoxicity.

Results: Maternal obesity results in reduced numbers of decidual CD56^{bright} NKs. Phenotypically, decidual NKs from obese pregnancies expressed TNFa and IFNg at higher levels than NKs from control BMI pregnancies, and baseline cytotoxic markers also were elevated. Global gene expression differences were observed between control and obese decidual NKs. Briefly, alterations in multiple inflammatory pathways and cell adhesion networks were identified.

Conclusions: This work demonstrates that maternal obesity affects decidual NK proportions in early pregnancy. Further, maternal obesity associates with alterations in cytotoxic receptor expression, increased cytotoxicity, and changes in gene pathways linked to inflammation and immune cell function.

O26 Effects of chronic hypoxia and the mitochondria-targeted antioxidant MitoQ on uterine artery reactivity in rodent pregnancy

Zhongchao Wang[1,3], Emily J Camm[1], Ana-Mishel Spiroski[1], Katie L Skeffington[1], Thomas J Ashmore[1], Youguo Niu[1], Michael P Murphy[2], Jin Ma[3] and Dino A Giussani[1].

[1]Department of Physiology Development & Neuroscience, University of Cambridge, UK; [2]Mitochondrial Biology Unit, Addenbrookes; University of Cambridge, UK; [3]Department of Aerospace Physiology, Fourth Military Medical University, China

Background: Chronic hypoxic pregnancy promotes oxidative stress and adversely alters uterine artery reactivity, increasing the risk of complications, such as preeclampsia (Keyes et al. *Pediatr Res.* **54**:20, 2003; Giussani & Davidge. *Dev Orig Health Dis*.**4**:328, 2013). However, effective therapy remains unidentified. Here, we investigated whether MitoQ protects against adverse changes in uterine artery reactivity during hypoxic pregnancy in rats.

Methods: From days 6-20 of gestation (term ~22 days), female Wistar rats were randomly divided into normoxic or hypoxic (13% O_2) pregnancy with or without daily maternal treatment with MitoQ (500 μmol MitoQ in drinking water). This model of hypoxia does not affect maternal food intake. At 20 days of gestation, following euthanasia, uterine arteries were harvested and constrictor and dilator reactivity determined by *in vitro* wire myography.

Results: Chronic hypoxia increased uterine artery constriction to norepinephrine (NE), angiotensin II and the protein kinase C activator PDBu (Fig.1 a-c). Chronic hypoxia also enhanced dilator reactivity to nitroprusside (SNP), acetylcholine (ACh) via increased NO-dependent mechanisms and the large conductance Ca^{2+}-activated K^+-channel (BK_{Ca}) activator NS1619 (Fig.1 d-f). MitoQ in hypoxic pregnancy prevented all effects. MitoQ in normoxic pregnancy increased endothelial NO-dependent uterine artery dilator reactivity (Fig. 1f).

Conclusions: In the uterine vascular bed, chronic hypoxic pregnancy increases constrictor reactivity via mechanisms involving mitochondria-derived oxidative stress, leading to up-regulation of compensatory dilator mechanisms. Maternal MitoQ treatment prevents the hypoxia-induced increased uterine artery constrictor reactivity, this vessels no longer require dilator compensation. Maternal MitoQ treatment may protect against preeclampsia in pregnancy complicated by hypoxia.

Supported by The British Heart Foundation

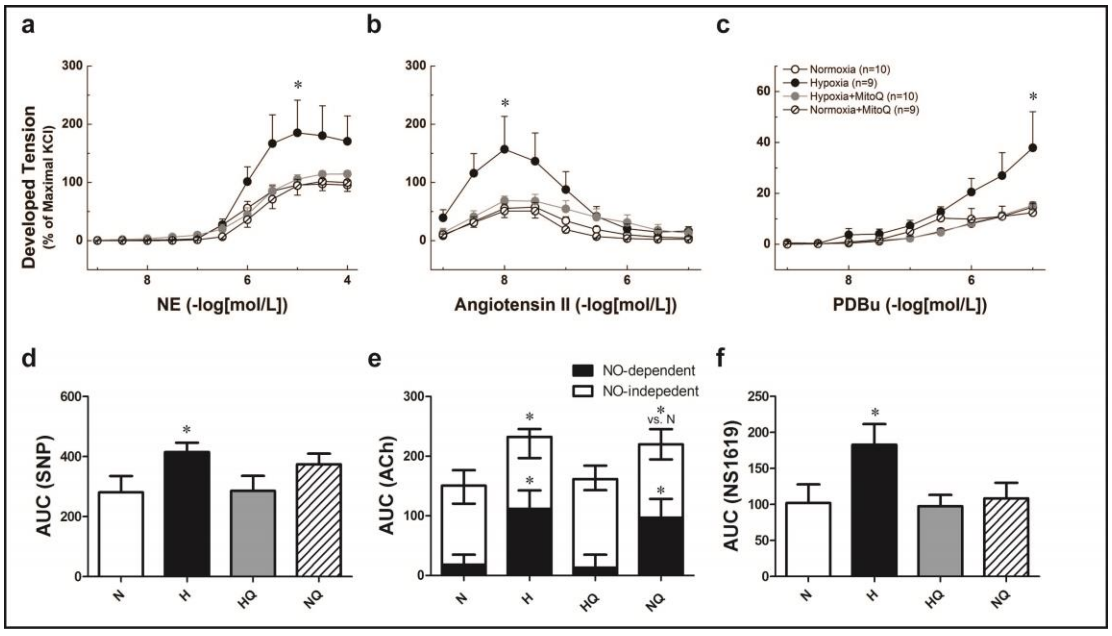


Figure. 1 Values (mean±S.E.M) for constrictor reactivity to norepinephrine (NE), Angiotensin II and the protein kinase C activator PDBu (a-c) and area under the curve (AUC) representing dilator reactivity to nitroprusside (SNP), acetylcholine (ACh) and the Ca^{2+} activate K^+ channel BK_{Ca} activator NS1619 (d-f) in uterine arteries isolated from normoxic (N, open symbols), hypoxic (H, closed symbols), hypoxic+MitoQ (HQ, grey symbols) and normoxic+MitoQ (NQ, hatched symbols) rodent pregnancy. *P<0.05 *vs.* all (a-c, f) or *vs.* N and HQ (d and e) (ANOVA+Tukey Test).

O27 A parallelized, pumpless artificial placenta system significantly prolonged survival time in preterm ovine fetus.

Takushi Hanita[1], Tadashi Matsuda[1], Yuichiro Miura[1, 2], Haruo Usuda[1], Shinpei Watanabe[1], Ryuta Kitanishi[1], Masatoshi Saito[1], Yoshiyasu Kobayashi[3]

1 Centre for Perinatal and Neonatal Medicine, Tohoku University Hospital, Sendai, Miyagi, Japan
2 School of Women's and Infants' Health, The University of Western Australia, Perth, WA, Australia
3 Department of Veterinary Pathology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan

Background/Introduction: An artificial placenta (AP) is an extracorporeal life support system that allows very premature babies to be treated as fetuses. We previously developed a pumpless AP system with a single circuit in preterm lambs, which suggested that reducing circuit resistance would be a key to elongate survival time. Subsequently, we developed a parallelized AP system aiming to reduce circuit resistance. The objective of this study was to compare the efficacy of the parallelized AP system with the single circuit AP system.

Methods: Ovine fetuses were surgically delivered between 109-135 days of gestational age and umbilical vessels were cannulated. In a parallel circuit group (n=6), each arterial catheter was connected to one oxygenator separately. In a single circuit group (n=5), both arterial catheters were connected to one single oxygenator. The fetus was submerged in a warm saline bath. Fetal physiological parameters and blood chemical and gas data were serially obtained and statistically compared between groups.

Results: The fetal survival period was significantly prolonged (60.4 ± 8.5 *vs* 18.2 ± 7.1 hours, mean ± SD; $p<0.01$); and gestational age of the fetuses (112.2 ± 2.4 *vs* 130 ± 3.5 days, $p<0.01$), birth weight (1,996 ± 377 *vs* 2,924 ± 793 g, $p<0.05$), minimal blood lactate levels (24.9 ± 21.3 *vs* 120.5 ± 75.0 mg/dl, $p<0.05$), and circuit resistance (0.41 ± 0.02 *vs* 0.85 ± 0.05 mm Hg·min·kg·ml^{-1}, $p<0.01$) were significantly lower in parallel circuit group compared to single circuit group. Circuit blood flow (118.5 ± 18.3 *vs* 61.1 ± 30.5 ml·kg^{-1}·min^{-1}, $p<0.01$) was significantly larger in parallel circuit group while there was no significant difference in mean arterial pressure between two groups.

Conclusions: Parallelization of AP system decreased the circuit resistance and enabled lamb fetuses to survive for a significantly longer period accompanied by significantly lower blood lactate level.

ORAL SESSION 6

FETAL BRAIN, DOHAD, PARTURITION

Chair – Rajavel Elango

O28 The effect of magnesium sulphate on post-asphyxial preterm fetal seizures

Vittoria Draghi, Robert Galinsky, Chris Lear, Alistair Jan Gunn, Laura Bennet.
Fetal Physiology and Neuroscience group, Department of Physiology, The University of Auckland, Auckland, New Zealand

Introduction: Magnesium sulphate ($MgSO_4$) is increasingly being given to pregnant mothers to delay preterm labour as it may provide perinatal neuroprotection. $MgSO_4$ can modify maternal seizures in pre-eclampsia and other types of seizures. Seizures are common after asphyxia in utero, but we do not know the effect of $MgSO_4$ on perinatal seizures.

Methods: 0.7 gestation fetal sheep received $MgSO_4$ (n=7; 160 mg bolus+ 48 mg/h constant infusion), or saline (n=8) for 48h starting 24 hours before asphyxia induced by 25 min of complete umbilical cord occlusion (UCO, starting at 10.00h). Fetuses were studied for 3 days post-UCO. The lab daylight cycle was 06.30-18.30h. Seizures were visually identified from the EEG as high amplitude, repetitive, evolving wave forms of >10sec. Seizure onset, duration, number and amplitude were assessed.

Results: Seizures markedly increased in controls ~16h post-UCO, peaking at 21h post-UCO (06-07.00h). Seizures increased again ~38h, peaking around 42h (05-06.00h). Seizures were of higher amplitude in this phase. $MgSO_4$ did not stop seizures onset time (14.4±4.1 vs. 13.5±3.3h, $MgSO_4$ vs control), but reduced the total duration (25.0±7.0 vs. 28.2±6.2h, $P<0.05$) and seizure number (33.3± 6.4 vs 65.0±20.0, $P<0.05$). There was a temporal pattern to the reduction in number, but not amplitude, between 18-34h post-UCO, and significantly reduced amplitude but not number in phase two ($p<0.05$).

Conclusion: These data suggest that preterm fetal seizures are circadian in nature, with seizures most active during the early morning. Glutamate levels increase at night and $MgSO_4$, in its capacity as a glutamate antagonist, may reduce this circadian release. Seizure amplitude increased in phase two, potentially due to greater glutamate release or synchronisation of neuronal clusters. Fewer first phase seizures may reduce cluster synchronisation or $MgSO_4$ levels may remain sufficiently high enough to affect glutamate. A longer infusion may have prevented a second phase of seizures.

O29 Magnesium sulphate for neuroprotection reduces punctate white matter laesions at 30 weeks MRI in the human neonate

Sol C, Benders M, Van Leeuwen J, Keunen K, Mulder E, De Vries L, Groenendaal F, Lemmers P, Van Bel F, Derks JB
Dept. of Perinatal Medicine, Wilhelmina Children's Hospital, University Medical Centre Utrecht, The Netherlands

Introduction: In preterm infants, antenatal magnesium sulphate (MgSO4) improves neurological outcome when administered during premature labor before 32 weeks of gestation (Rouse et al.). Antenatal MgSO4 especially reduces the incidence of cerebral palsy (CP). However, controversy remains about the benefits of MgSO4 in the Dutch population, since the incidence of CP is lower than in the USA and because of its potential side effects, including cardiorespiratory depression.

Aim of the study: To assess the effect of MgSO4 on brain injury (and neonatal morbidity) in a Dutch cohort of extremely premature infants born before 28 weeks of gestation.

Study Design: Retrospective cohort study performed at the UMCU evaluating the effect of MgSO4 on brain injury assessed on MRI at 30 and 40 weeks corrected age and, as a secondary outcome, neonatal complications. The infants born in the two years after implementation of the protocol were compared with a historical cohort of infants born in the two years before implementation of MgSO4. Patients were included if they were born at the UMCU between 24 and 28 weeks of gestation between January 2011 - 2015.

Outcome: In 4 years time, a total of 207 children were included for analysis by MRI at 30 and 40 weeks (108 before implementation of the protocol and 99 after). Punctate white matter lesions (PWML) were reduced at 30 weeks MRI (19,4% vs 45,2%, p value 0.002), but not at 40 weeks MRI. There was no increase in neonatal complications, including early intubation for respiratory insufficiency or hypotension.

Discussion: Magnesium sulphate does not increase neonatal side effects and is associated with less PWML at 30 weeks MRI. The clinical consequences need to be elucidated in long term followup.The data have to be interpreted with caution due to the retrospective character of the study.

O30 The impact of an adverse in utero environment followed by a postnatal western diet, upon muscle and liver development and function and evidence of a pre-diabetic state in young adulthood.

Ousseynou Sarr[1,2,4]*, Kristyn P Dunlop[2]*, Lin Zhao[1], and Timothy RH Regnault[1,2,3,4]
*Departments of [1]Obstetrics and Gynecology, [2]Physiology and Pharmacology, [3]Lawson Health Research Institute, [4]Children's Health Research Institute, Western University, London, ON, Canada. * = Equal contribution*

Background: The pre-diabetic state is characterized by development of insulin resistance (IR), mitochondrial dysfunction and recently hepatic steatosis. Due to previous associations between Low Birth Weight (LBW – signalling an adverse in utero environment) and later life IR, and independent effects of a Western diet (WD) in promoting pre-diabetic markers, we investigated the degree to which LBW and a postnatal WD interact to promote a pre-diabetic state.

Methods: LBW was induced in guinea pigs via uterine artery ablation. Male pups with a significantly reduced body length/body weight ratio below the 25th percentile were classified LBW. Pups were weaned onto control (CD) or Western (WD) diet. At young adulthood (postnatal day 150), Gastrocnemius muscle and liver were harvested.

Results: In LBW/CD muscle, altered phosphorylation of insulin signaling intermediates was observed similar to normal BW (NBW) and LBW WD offspring and in liver Akt activation was inhibited in WD offspring only. Muscle TGs and DAG were not impacted by birth weight or diet, however liver TGs and metabolite-based lipogenic parameters were significantly elevated in WD offspring independent of birth weight. Genes involved in mitochondrial biogenesis and oxidative function, were reduced in LBW/CD muscle and LBW/WD liver. In muscle, accumulation of acylcarnitines, indicating impaired β-oxidation substrate utilization, occurred in LBW and following WD. In liver, long-chain acylcarnitines accumulated in WD offspring with a lesser increase in LBW/WD versus NBW/WD. NBW/WD animals displayed a macrovesicular steatosis and in LBW/WD offspring microvesicular steatosis was predominant.

Conclusions: These studies highlight that an adverse *in utero* environment resulting in LBW, is associated with pre-diabetic markers, independent of postnatal diet. These potentially programmed metabolic alterations, in concert with WD, further promote muscle and liver dysfunctional lipid metabolism, inhibition of key mitochondrial genes and hepatic microvesicular steatosis. The early interactive development of these pre-diabetic markers highlights a potential premature development of diabetes.

O31 Reactive oxygen species (ROS) as an early gestation mediator of cardiac programming in the growth restricted sheep fetus

Song Zhang[1], Michelle Tie[1], Kimberley J. Botting[1], I. Caroline McMillen[1], Sheridan Gentili[1], Severance M. MacLaughlin[1], Doug A Brooks[2] and Janna L. Morrison[1]

[1] Early Origins of Adult Health Research Group, [2] Mechanisms in Cell Biology and Disease Research Group, Sansom Institute for Health Research, University of South Australia, Adelaide, SA, 5001

Uterine carunclectomy (CX) in sheep results in fetal growth restriction with fewer cardiomyocytes in the fetal heart in the absence of changes in cardiomyocyte proliferation or apoptosis in late gestation. The cardiomyocytes are also larger with increased cardiac expression of insulin-like growth factor (IGF)-2 and its receptor IGF2R. There are, however, no studies investigating the impact of CX on cardiac growth and development in early gestation.

CX was induced by removing endometrial caruncles in ewes prior to conception. At 55d gestation, Control and CX ewes were humanely killed and fetuses and hearts were weighed. Changes in gene and protein expression were determined by real-time PCR and Western blotting, followed by Students' t-test.

The heart weight was lower in CX than Control fetuses at 55d gestation. CX resulted in an increase in cardiac mRNA expression of molecules involved in the regulation of ROS including SOD1 and cytochrome B. CX increased mRNA and protein expression of the acetyltransferase GCN5 and decreased protein abundance of the deacetylase SIRT1. Cardiac mRNA expression of IGF2 and IGF2R was higher in CX than Control fetuses. There was an increase in mRNA expression of the cell cycle inhibitor p27, a decrease in protein abundance of the anti-apoptotic BCLXL, and an increase in mRNA and protein expression of the autophagy marker BECLIN1 in CX fetuses.

These data suggest that cardiac oxidative stress induced in early gestation may alter cardiac GCN5 and SIRT1 expression, which in turn may further increase acetylation and subsequent expression of key genes involved in cardiac growth and development. Changes in apoptosis and autophagy of cardiomyocytes may also play roles in reducing cardiomyocyte endowment in the heart of the growth restricted fetus in late gestation.

032 Markers of allostatic load associate with adverse pregnancy and offspring outcomes in rats

David Olson[1], Ashlee Matkin[2], J. Keiko McCreary[2], Erin Falkenberg[2], Barbara Verstraeten[1,3], Gerlinde A.S. Metz[2]
[1]University of Alberta, [2]Canadian Centre for Behavioural Neurosciences, University of Lethbridge,[3]University of Ghent.

Research question: Allostatic load is an index of multisystem physiologic risk that can be used as a measure of the cumulative toll (the 'wear and tear') of physiologic and psychological stress. It assumes multisystemic interactions including transgenerational inheritance and incorporates subclinical biomarkers of neuroendocrine, inflammatory, and metabolic function into a single index score. We developed a new model of allostatic load in pregnant Long-Evans rats and hypothesized that markers of allostatic load are risk factors for preterm birth and are passed on to subsequent generations.

Methods: Pregnant dams were untreated controls (saline injected) or treated to stress (swimming and restraint) from gestational days 12-18 alone, interleukin (IL)-1b alone or both stress + IL1b. The timing of gestation, pregnancy outcomes, behaviour and neurological assessments were monitored.

Results: Stress alone or IL1b alone had no effect upon gestational length or adverse outcomes whereas stress + IL1b dams had more preterm deliveries and more adverse outcomes. Offspring from stressed, IL1b and stress + IL1b-treated dams displayed behavioural, neurological and neurodevelopmental changes compared to offspring from control dams. Frequently stress + IL1b produced the worst results.

Conclusion: Allostatic load markers associate with adverse pregnancy and offspring outcomes and may be used to predict risk.

O33 Complex interacting mechanisms regulating contraction and inter-contraction relaxation in term human labour myometrium

[1]Helena C Parkington, [1]Marius Jigau, [1]Crystal Goundar, [1]Mary Tonta, [2]Janet Stevenson, [2]Penny Sheehan, [2]Shaun Brennecke, [1]Harold Coleman.

[1]Department of Physiology, Monash University, Clayton, Victoria, Australia and [2]Royal Women's Hospital, Parkville Victoria, Australia.

Obesity is associated with prolonged labour and failure to progress in labour, necessitating caesarean delivery (CD). Successful vaginal delivery requires strong forceful uterine contractions. Poor contractions that result in failure to progress in labour necessitate CD. Full uterine relaxation must occur between contractions since this permits restoration of placental blood flow, mandatory in the long hours of human labour. Failure of adequate relaxation results in non-reassuring fetal welfare necessitating CD. The aim of our study was to investigate the mechanisms underpinning contraction and relaxation before and during established labour.

Myometrium was obtained during CD at term before labour and during established labour. Contraction was recorded simultaneously with membrane potential in strips, using sharp intracellular microelectrodes. Following collagenase treatment, ionic currents were recorded from isolated myocytes using patch-clamp electrophysiology. Tissue was frozen at -80 C for later western blotting.

Resting membrane potential was similar in lean and obese myometrium before labour and in lean tissues in labour. However, in obese tissue in labour the myometrial cells were some 17mV more hyperpolarized rendering the tissue difficult to excite and contract. Contraction was underpinned by an action potential (AP) that included a plateau of depolarization to27mV for about 1min. The AP determined the amplitude and duration of contraction. We found that extending plateau duration had a marked influence on the level of membrane potential between action potentials and hence on the interval between contractions. Using ion substitution and blockade of ion channels we showed that Na influx during the plateau stimulates the Na/K ATPase pump and Na/Ca exchanger, both electrogenic in nature. Ca influx during the plateau activated small-conductance Ca-activated K channels.

In conclusion, the AP sets up mechanism for repolarization, and this is exaggerated as the duration of the plateau increases. This sets up a strong relaxation between strong contractions, ensuring fetal wellbeing.

O34 Interleukin (IL) 1-b receptors are regulated in human myometrial smooth muscle cells (HMSMC) and in rats at delivery by IL-1b, prostaglandin (PG) F2a and progesterone (P4).

David Olson[1], Tomohito Ishiguro[1,2], Barbara Verstraeten[1,3], Kelycia Leimert[1]
[1]Dept of Obstetrics and Gynecology, University of Alberta, [2]Juntendo Medical University, [3]University of Ghent

Research question: The mechanism of parturition involves a number of inflammatory mediators, especially IL-1b and the regulation of its receptors. IL-1b signals through the IL1R1 when complexed with its accessory protein, AcP. We recently discovered the mRNA expression of its brain isoform, AcPb, in uterus. We hypothesized that IL-1b, PGF2a or P4, known to either maintain uterine quiescence or transform it for delivery, regulate the expression of ILR's.

Methods: We incubated 7th passage HMSMC with IL-1b (1ng/mL), PGF2a (10^{-7}-10^{-5}M) or the two in combination. mRNA abundance was assessed by qRT-PCR. Long Evans rats were studied from gestational day (GD)17 to delivery on GD22.5. P4 or its receptor antagonist, RU486, were administered to rats to extend or shorten gestation length, respectively. Data were analyzed by Student's t test or ANOVA. Significance was achieved at $p<0.05$.

Results: In HMSMC PGF2α stimulated IL-1R1, IL-1R2 and AcPb mRNA expression in a concentration-dependent manner ($p<0.001$, $p<0.05$). IL-1β stimulated a rise in expression of IL-1R genes ($p<0.001$). PGF2α plus IL-1β increased ILRs in an additive fashion ($p<0.05$). In rats, IL-1R1 mRNA abundance increased 3-fold in upper and lower uterine segments over the last 5d of pregnancy ($p<0.05$, 0.001), whereas there was a significant decrease in the mRNA abundance of IL-1R2 ($p<0.05$). AcP mRNA abundance increased 2-fold in both uterine segments ($p<0.01$, 0.05) during late gestation, but surprisingly, AcPb increased 4-fold in lower uterine segment ($p<0.01$). P4 delayed delivery and prevented the term increase in IL-1R1 mRNA expression whereas RU486 administration increased significantly ($p<0.05$) IL-1R1 expression and caused preterm delivery.

Conclusion: Late gestation increases in IL-1R1 and AcP's may be due to decreasing P4 and increasing IL-1b and PGF2a in rats and humans. Further studies examining the regulation of IL-1R2 and the specific role of AcPb are warranted.

Funding: CIHR, GAPPS, MOD, FWO, Juntendo Medical University

ORAL SESSION 7

TRANSLATIONAL STUDIES

Chair – Dino Giussani

O35 Heterogeneity of respiratory distress syndrome: risk factors and morbidity associated with early and late gestation disease

Azar Mehrabadi[1], Sarka Lisonkova[1], K.S. Joseph[1 2]

[1]Department of Obstetrics and Gynaecology, University of British Columbia and the Children's and Women's Hospital and Health Centre of British Columbia; [2]School of Population and Public Health, University of British Columbia, Vancouver, British Columbia, Canada

Background: Although respiratory distress syndrome (RDS) is considered a disease of prematurity, there is evidence to suggest heterogeneity between early and late gestation RDS. We examined the epidemiologic features of RDS occurring at early and late gestation.

Methods: We studied live births in the United States in 2005-06 with information obtained from the National Center for Health Statistics. Early (<32 weeks) and late gestation RDS (≥39 weeks) were contrasted in terms of risk factors and associations with pregnancy complications, obstetric intervention and co-morbidity. Logistic regression was used to quantify the effects of risk factors, while other associations were quantified descriptively.

Results: There were 27,971 RDS cases, yielding an incidence of 6.4 per 1,000 live births. Early and late gestation RDS differed in terms of risk factors, with factors such as multi-fetal gestation more strongly associated with early (adjusted odds ratio [aOR] 11.6, 95% confidence interval 11.0-12.2) compared with late gestation RDS (aOR 3.66, 95% confidence interval 2.68-4.98). The morbidity correlates of early and late gestation RDS also differed substantially; neonatal seizures were less strongly associated with early (OR 5.90, 95% confidence interval 3.67-9.47) compared with late gestation RDS (OR 33.1, 95% confidence interval 27.2-40.2), while meconium aspiration syndrome was not associated with early gestation RDS (OR 1.87, 95% confidence interval 0.94-3.72) and very strongly associated with late gestation RDS (OR 39.8, 95% confidence interval 34.7-45.6).

Conclusions: Differences in risk factors and morbidity correlates of early and late gestation RDS suggest that these entities represent two distinct diseases.

O36 Human amnion epithelial cells modulate the initial pulmonary inflammatory response to injurious ventilation in preterm lambs

Jacqueline Melville, Courtney McDonald, Robert Bischof, Graeme Polglase, Rebecca Lim, Graham Jenkin and Tim Moss.
The Ritchie Centre, Hudson Institute of Medical Research; Depertment of Obstetrics and Gynaecology, Monash University.

Background/Introduction
Initiation of mechanical ventilation in preterm neonates begins an inflammatory cascade that can lead to ventilation-induced lung injury (VILI), a systemic inflammatory response, and long-term lung disease. Human amnion epithelial cells (hAECs) reduce lung pathology resulting from pro-inflammatory or injurious stimuli, but the underlying mechanisms are unknown. We hypothesised that hAECs would modulate the initial pulmonary inflammatory response to VILI in preterm lambs.

Methods
Preterm lambs received intratracheal hAECs (90 million) or vehicle, immediately prior to 2h mechanical ventilation using a regimen known to be injurious to the lungs. Within 5 min of ventilation, lambs received intravenous hAECs (90 million) or vehicle. Indices of lung injury and inflammation (histology, cytokine mRNA and protein levels), pulmonary immune cell phenotypes (FACS), and systemic inflammation were examined in unventilated (control; n=10), vehicle-treated ventilated (vehicle; n=10) and hAEC-treated ventilated (hAEC; n=10) lambs.

Results
Histological indices of lung injury were elevated, relative to control, in vehicle but not hAEC lambs. Ventilation-induced pulmonary leucocyte recruitment was higher in hAEC than vehicle lambs, despite lower pulmonary IL-8 mRNA levels in the hAEC group. Ventilation-induced pulmonary recruitment of CD44+ cells tended higher, and CD8+ and CD21+ macrophage recruitment was lower, in hAEC than vehicle lambs. Indices of ventilation-induced systemic inflammation were not different between hAEC and vehicle groups, although plasma IL-10 levels tended higher in hAEC lambs.

Conclusion
Human amnion epithelial cells modulate the pulmonary inflammatory response to the initiation of ventilation in preterm neonates. These acute beneficial effects may be a mechanism by which hAECs reduce lung pathology in response to inflammatory and injurious stimuli, and suggest that early administration of hAECs to ventilated newborns may moderate the adverse long-term outcomes.

O37 5-minute Apgar score as a marker for developmental vulnerability at 5 years of age

Neda Razaz[1], Thomas Boyce[2], Marni Brownell[3], Douglas Jutte[2], Helen Tremlett[1], Ruth Ann Marrie[3], K.S. Joseph[1]
University of British Columbia, Vancouver, Canada, [2] University of California, San Francisco, United States, [3] University of Manitoba, Winnipeg, Canada

Background: Previous studies have not examined the developmental correlates of the Apgar score across the entire spectrum of recorded scores. We attempted to assess the relationship between the 5-minute Apgar score and developmental vulnerability at 5 years of age.

Methods: We carried out a population-based retrospective cohort study using information on children in Manitoba, Canada, born between 1999 and 2006 at term gestation. Childhood development at 5 years of age, expressed as vulnerability on 5 domains of the Early Development Instrument (EDI) was examined by 5-minute Apgar score. Logistic regression was used to develop a prognostic model based on 5-minute Apgar score, infant sex, birth weight-for-gestational age, age at EDI assessment, gestational age at birth, breastfeeding initiation and socioeconomic status.

Results: Of the 33,883 children in the study, most (82%) had a 5-minute Apgar score of 9; 1% of children had a score <7, and 5.6% had a score of 10. Children with 5-minute Apgar scores <10 had higher odds of vulnerability on the physical domain compared with children with a score of 10 (e.g., adjusted odds ratio [aOR] for 5-minute Apgar 9=1.23, 95% confidence interval [CI] 1.05-1.44). Similarly, children with 5-minute Apgar scores of <10 were more vulnerable on the emotional domain (e.g., aOR for 5-minute Apgar 9=1.20, 95% CI 1.03-1.41). Nevertheless, the Apgar-based prognostic model had a poor sensitivity for physical vulnerability (19%, 95% CI 18-20%). Although, the Apgar score-based prognostic model performed reasonably in terms of calibration and risk-stratification for identifying developmentally vulnerable children, classification accuracy was poor.

Conclusion: The risk of developmental vulnerability at 5 years of age is inversely associated with the 5-minute Apgar score across its entire range. The score can serve as a population-level indicator of developmental risk but cannot be used to create a program to mitigate developmental vulnerability.

O38 Maternal creatine levels throughout human pregnancy and the association of these with maternal demographics and indices of fetal growth – a retrospective cohort study.

Hayley Dickinson[1,2], Miranda Davies-Tuck[1,2], Stacey Ellery[1,2], Jessica Grieger[3], Rod Snow[4], Euan M Wallace[1,2], David W Walker[1,2], Vicki Clifton[5]
[1]The Ritchie Centre, Hudson Institute of Medical Research, Australia; [2]Department of Obstetrics and Gynaecology, Monash University, Australia; [3]Robinson Research Institute, University of Adelaide, Australia; [4]Centre for Physical Activity and Nutrition Research, School of Exercise and Nutrition Sciences, Deakin University, Australia; [5]Mater Research, Queensland, Australia.

Background: The spiny mouse fetus relies upon placental transfer of creatine until late in pregnancy, which is supported by significant changes in maternal creatine synthesis and excretion as pregnancy progresses. Maternal creatine supplementation improves neonatal survival and organ function following birth asphyxia. Before undertaking a randomised controlled trial of creatine supplementation in pregnancy, we need to determine how creatine biosynthesis is regulated during pregnancy. The aim of this study was to retrospectively determine creatine levels across gestation in pregnant women and identify populations of women for whom creatine supplementation may be beneficial.

Methods: Two hundred and eighty-four women were recruited from an existing larger prospective cohort study from the Lyell McEwin Hospital, South Australia, assessing the effects of asthma during pregnancy on the mother, placenta and baby. Comprehensive maternal demographic and pregnancy outcome data was recorded. Creatine levels were measured in maternal plasma and urine samples collected at 12, 18, 30 and 36 weeks of gestation.

Results: After adjustment, there was a 17% reduced odds of the baby being small for gestational age for every 50µmol/L increase in urinary creatine. For every 50 µmol/L increase in urinary creatine, there was a 4.49g increase in placental weight. Maternal smoking and asthma were associated with higher plasma creatine levels than the rest of the population. Parity of ≥1 was associated with lower plasma creatine levels than those who were nulliparous. For each unit increase in maternal BMI there was an associated increase in urine creatine levels.

Discussion: Here we report for the first time, maternal plasma and urine creatine levels across gestation and we report associations between maternal creatine levels and maternal smoking and asthma, and show relationships between low maternal urine creatine levels and reduced birth and placenta weight. These observations necessitate further work and exploration of creatine in human pregnancy.

POSTERS

P1 Adenosine Contributes to the Fetal Coronary Vasodilatory Response in Acute Hypoxia

Sonnet S Jonker[1], Eileen I Chang[1], Samantha Louey[1] and George D Giraud[1,2].
1Knight Cardiovascular Institute, Oregon Health & Science University, Portland, OR, United States and 2Cardiology, VA Portland Health Care System, Portland, OR, United States.

Background/Introduction
Although administered adenosine is known to relax fetal coronary arteries, its importance as an endogenously generated mediator is unknown. We tested the role of endogenous adenosine and nitric oxide in fetal coronary vasodilation during acute hypoxia.

Methods
Late-term fetal sheep were implanted with vascular catheters and a circumflex flow probe. Maternal tracheal catheters were placed for nitrogen infusion to produce acute hypoxia. Inhibition of hypoxia-mediated fetal coronary flow by theophylline administration (pKb in fetal sheep coronaries 23.9uM) in the right atrium was tested, followed (n=4) or preceded (n=1) by L-NG-Nitroarginine methyl ester (LNAME) infusion to inhibit nitric oxide synthesis.

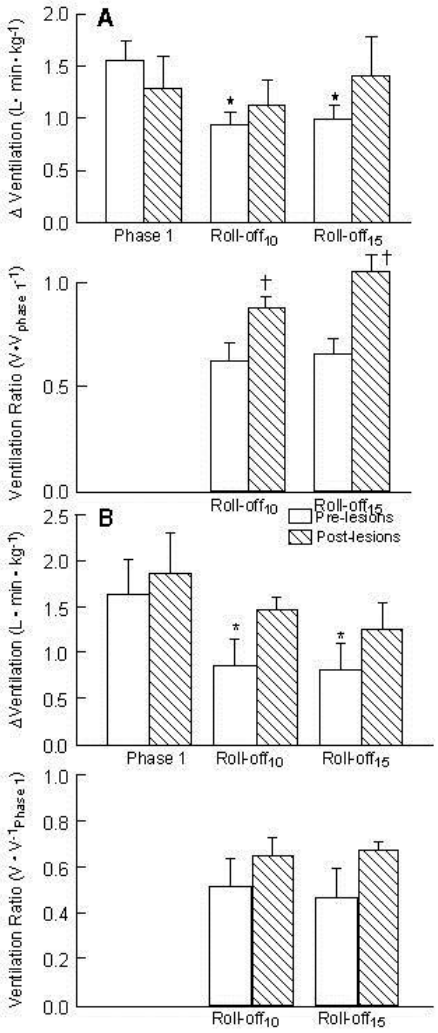
Results
Nitrogen infusion into the maternal tracheal catheter reduced maternal arterial oxygen saturation to 53±13% (SEM; $P<0.002$), and fetal arterial O2 saturation from 47±7% to 19±3% ($P<0.0004$). During hypoxia, fetal coronary flow increased from 1.2±0.1ml/min/g to 3.9±1.6ml/min/g ($P<0.02$). A graded series of theophylline infusions (to ~50uM, 60uM and 140uM), and LNAME (30mg bolus + 6mg/min), progressively reduced the increased coronary flow resulting from hypoxia ($P<0.009$). Although a maximally antagonizing dose of theophylline was not tested, at the maximum dose used, hypoxia-induced flow was reduced by 35%. LNAME further reduced the hypoxia-induced flow by 12%.

Conclusions
These data demonstrate that endogenously produced adenosine and nitric oxide contribute to coronary regulation in the fetal sheep. This developing vascular bed may be affected by maternal consumption of products containing methylxanthines (coffee, tea) during pregnancy.

P2 Thalamic Locus Mediates Hypoxic Respiratory depression In Young Lambs

Arezoo Rajaee[1], Basil Ibe[3], Catalina Guerra[3], Lawrence Kruger[2] and Brian J. Koos[1]
Departments of [1]Obstetrics & Gynecology and [2]Neurobiology, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095; and [3]Dept of Pediatrics, David Geffen School of Medicine at UCLA, Torrance, CA 90502

The postnatal hypoxic ventilatory response is biphasic: initial stimulation via the carotid chemoreflex (phase 1) followed by a decline or roll-off (phase 2). The roll-off is putatively triggered by an O_2 sensor involving neural circuitry in the pons and/or mesencephalon. We have shown that the anatomical substrate that mediates hypoxic arrest of fetal breathing movements includes a thalamic locus involving the parafascicular nuclear complex (Pf). These studies were designed to determine whether Pf is also involved in the ventilatory roll-off in lambs.



Respiratory responses to hypoxia (fraction of inspired O_2 = 0.08) were determined in 11 awake, chronically catheterized lambs (1-2 weeks of age). Ibotenic acid (IBO), a neurotoxin, was microinjected into the thalamus of 9 lambs.

In 5 lambs with lesions involving Pf, the ventilatory ratio ($\Delta V_{roll\text{-}off}/\Delta V_{phase\ 1}$) of 0.9-1.0 after 10 and 15 min of hypoxia was significantly greater than the respective ratios for experiments before IBO (Fig.1A). In 4 lambs with lesions sparing the Pf sector, the ventilatory ratios after 10 and 15 min of O_2 deficiency were similar to the pre-IBO values (Fig.1B). Control injections of vehicle into Pf did not dampen the hypoxic roll-off. In conclusion, a thalamic sector encompassing Pf is a novel component of the neuronal substrate that mediates the ventilatory roll-off in lambs.

Thus, Pf is a crucial locus involved in hypoxic depression of breathing in both prenatal and postnatal life.

P3 Inflammatory and profibrotic markers in the lung of growth restricted offspring before and after birth

Jia Yin Soo[1], Sandra Orgeig[2], Erin V. McGillick[1], Song Zhang[1], I. Caroline McMillen[1], Janna L. Morrison[1]
[1]Early Origins of Adult Health Research Group and [2]Molecular & Evolutionary Physiology of the Lung Laboratory, School of Pharmacy & Medical Sciences, Sansom Institute for Health Research, University of South Australia, Adelaide, SA, Australia, 5001

Background
Although there is epidemiological and experimental evidence that intrauterine growth restriction (IUGR) reduces lung function in later life, little is known about the molecular mechanisms involved in predisposing IUGR infants to poor lung function in childhood. Since many chronic lung diseases, including asthma and COPD, involve an inflammatory aspect and remodelling of the lung architecture, we aimed to evaluate the effect of IUGR on expression of inflammatory cytokines and regulators of extracellular matrix (ECM) in the lungs before and after birth.

Methods
Placental restriction (PR), leading to IUGR was induced by carunclectomy in ewes (n=11). Lungs were collected from fetal sheep at 130 days gestation (term =150±3d; Control, n=9; PR, n=5) and lambs at 21 days after birth (Control, n=9; PR, n=6). Lung mRNA expression of inflammatory cytokines (Interleukin 6 (IL-6) and macrophage inflammatory protein-1β (MIP-1β)) and regulators of the ECM (matrix metallopeptidase-9 (MMP-9) and tissue inhibitors of metalloproteinases -1 and -2 (TIMP-1 and -2)) were quantified. Data were analysed by two-way analysis of variance (ANOVA) for treatment and age ($P<0.05$).

Results
PR resulted in a significant increase in lung mRNA expression of the pro-inflammatory marker IL-6 in fetuses but not in lambs. Furthermore, PR decreased mRNA expression of the chemokine MIP-1β in lambs, but not fetuses. We also found that PR decreased mRNA expression of the ECM structural regulator MMP-9 in the lungs of both fetuses and lambs.

Conclusion
This study suggests that PR may lead to a dysregulation of inflammatory mediators, such as MIP-1β and may contribute to an increased risk of chronic lung disease in later life. The decrease in lung function due to PR may be caused by a dysregulation in the extracellular matrix in the lungs of the fetus and this dysregulation may be sustained up to 21 days after birth.

P4 Reduced cortisol response to AVP+CRH challenge in offspring of ewes undernourished around conception improves with age.

Mark H Oliver, Anne L Jaquiery, Jane E Harding, Hui Hui Phua, Eric B Thorstensen and Frank H Bloomfield
Liggins Institute, University of Auckland, Auckland, New Zealand

Background.
Periconceptional undernutrition (PCUN) from 60d before to 30d after mating accelerates hypothalamo-pituitary-adrenal axis (HPAA) maturation in fetal sheep but results in reduced cortisol response to AVP-CRH stimulation at 18 months of postnatal age. Whether this altered HPAA function deteriorates or improves with increasing age is not known.

Methods.
Three to four year old male (5N & 10PCUN) and female offspring (10N &10PCUN) were challenged with CRH (0.5 µg/kg) and AVP (0.1 µg/kg). Blood samples were collected over 60 min. Adrenal mRNA samples were analysed for ACTH receptor message, expressed relative to 3 housekeeping genes. Data are expressed as mean±SEM and mRNA fold difference between groups with 95% confidence intervals (CI).

Results.
Live weight was not affected by treatment but adrenals were lighter in PCUN than in N rams (5.5±0.4 vs.7.0±0.5g, $p<0.01$). Baseline plasma ACTH and cortisol concentrations and areas under the AVP+CRH challenge response curve (AUC) were not different between treatment groups although tended to be lower in PCUN than in N ewes (2976±171 vs. 3383±168 $ng.ml^{-1}.min^{-1}$, $p=0.1$). The ratio of cortisol to ACTH AUC in response to AVP+CRH challenge was slightly lower in PCUN, than in N offspring (270±34 vs. 361±39, $p=0.05$); however, adrenal ACTH receptor mRNA expression was increased in PCUN compared with N offspring (1.12, CI:1.16,1.09, $p<0.05$).

Conclusions.
Effects of PCUN on cortisol response to AVP+CRH challenge had diminished in both sexes at 3-4 years of age, although the cortisol to ACTH AUC ratio suggests some adrenal resistance may remain. Elevated adrenal ACTH receptor mRNA expression suggests ACTH sensitivity should have been increased in PCUN offspring. However, the difference was small and may reflect a compensatory increase in ACTH receptor message due to possible post-receptor defects in function.

P5 Maternal Hyperglycemia Affects Rat Neonatal Cardiovascular Development

Lara Lehoranta[1], Olli Vuolteenaho[2], Jukka Laine[3], Mervi Haapsamo[2], Juha Räsänen[4]
[1]*University of Turku, Obstetrics and Gynecology, Turku, Finland ;* [2]*University of Oulu, Physiology, Oulu, Finland;* [3]*Turku University Hospital, Dep. of Pathology, Turku, Finland;* [4]*University of Eastern Finland, Obstetrics and Gynecology, Kuopio, Finland*

BACKROUND To investigate neonatal cardiac responses to maternal pregestational hyperglycemia (HG) in an experimental rat model.

METHODS 9 HG rat dams with 82 pups (control dams = 9, pups = 74) were preconceptually injected with STZ (35 mg/kg). Pups were weaned and their hearts were gathered at perinatal days (PND) 0, 7, and 14. Evenly divided samples were used either for morphometric or mRNA detection experiments. TUNEL was performed to detect apoptosis. HE- and Gömöri silver stained samples were used to examine cardiomyocyte apoptosis, mitosis, dimensions, and erythroblasts present. Fetal heart RNA was isolated (Qiagen Rneasey reagents, Netherlands) and hybridized with cDNA probes. Expression was measured with qRT-PCR using TaqMan chemistry on a 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA).

RESULTS Newborn hearts were heavier and presented decreased cell turnover (apoptosis in Gömöri stained samples) in the HG group compared to controls. Gene expression was decreased for myosin isoforms (MYH2, MYH6), and genes related to contracitility (ATP2A2, KCNIP2), growth (TNFRSF12A), and metabolism (UCP3, GLUT3, GLUT4). BNP mRNA expression was increased. By PND 7, body weight was decreased, TUNEL positive cells in the myocardium increased, and MYH3 and GLUT 4 expression were decreased in the HG group compared to controls. At PND 14 body weight remained decreased but cell turnover was increased (mitosis in HE and Gömöri stained samples) in the HG pups. mRNA expression in the HG pups was increased for contractile (ATP2A2, KCNIP2), growth involved(TNFRSF12A), metabolic (UCP2, UCP3, GLUT3, GLUT4), myosin isoform (MYH2, MYH6), hypoxia related (EGLN3), and cardioprotective (BNP) genes.

CONCLUSIONS Maternal hyperglycemia leads to reversible newborn cardiomegaly and later to neonatal decreased growth. Newborn HG pup hearts underwent a period of downregulation in growth and gene expression. At PND 14 the HG pup hearts were regulated to increase cell turnover and gene expression.

P6 Structural and molecular regulation of lung maturation by intratracheal vascular endothelial growth factor (VEGF) administration in the normally grown and placentally restricted fetus

Erin V McGillick[1,2], Sandra Orgeig[1] and Janna L Morrison[2]

[1]Early Origins of Adult Health Research Group and [2]Molecular & Evolutionary Physiology of the Lung Laboratory, Sansom Institute for Health Research, School of Pharmacy & Medical Sciences, University of South Australia, Adelaide, Australia

Background: Inhibition of hypoxia signalling leads to respiratory distress syndrome (RDS). Administering vascular endothelial growth factor (VEGF), a key hypoxia responsive factor, protects from RDS. In the chronically hypoxemic placentally restricted (PR) fetus, there is altered regulation of hypoxia signalling[1] and reduced surfactant maturation[2]. This provides evidence for increased risk of RDS in growth restricted neonates. We evaluated the effect of VEGF administration to bypass endogenous alterations to hypoxia signalling in the lung of the normally grown and PR sheep fetus.

Methods: PR leading to fetal growth restriction was induced by carunclectomy (n=13). On two consecutive days (131-132d gestation, term=150±3d) fetuses received saline (Control=11; PR=8) or VEGF (Control+VEGF=9; PR+VEGF=5) via a non-obstructive tracheal catheter, followed by post mortem on day three. Fetal lung expression of genes regulating airway remodelling (*MMP2*, *MMP9*, *TIMP1*, *COL1A1, ELN*) and surfactant maturation (*SFTP-A*, *-B*, *-C*, *-D, PCYT1A*, *LAMP3*, *LPCAT*, *ABCA3*) were quantified by qRT-PCR. Percent air space and number of SFTP-B positive cells in fixed lung tissue were determined by point counting. Data were analysed by two-way ANOVA for treatment and drug (P<0.05).

Results: PR reduced P_aO_2, oxygen saturation, fetal weight, *SFTP-A*, *-B* and *-C* expression compared with Controls. VEGF increased *MMP9* expression (key regulator of extracellular matrix remodelling) but had no effect on genes regulating surfactant maturation. VEGF increased the percent air space in the PR, but not Control fetal lung. VEGF increased the number of SFTP-B positive cells in lungs of Control+VEGF and PR+VEGF fetuses.

Conclusion: Despite few effects of VEGF on molecular markers, there were positive effects on structural regulation in the Control and PR fetal lung. This provides evidence that VEGF promotes structural lung maturation and may result in synergistic effects if combined with current therapeutic treatments (e.g. glucocorticoids) to induce surfactant maturation and reduce the risk of RDS at birth.

References:

1. Orgeig et al., *Am J Physiol - Lung C*, 2015, ajplung-00275

2. Orgeig et al., *Am J Physiol - Lung C*, 2010, *298*(4), L575-L583

P7 Creatine synthesis and CrT transporter expression in the placenta: an interspecies comparison.

Syed Baharom [1,3,4] , Paul Della Gatta[2], Stacey Ellery[1],Hayley Dickinson[1], Richard Harding[3], Rod J Snow[2], David W Walker[1].
[1]The Ritchie Centre, Hudson Institute of Medical Research, Monash Medical Centre, Clayton, VIC. [2]Centre for Physical Activity & Nutrition, Deakin University, Burwood, VIC. [3]Department of Anatomy & Developmental Biology, Monash University, Clayton VIC, Australia. [4]Faculty of Medicine, Universiti Teknologi MARA, Sg. Buloh, Malaysia.

Introduction: Creatine-phosphocreatine (PCr) is involved with replenishing ATP via the creatine kinase reaction. Creatine is a arginine and glycine-derived metabolite necessary for fetal development, with studies in omnivorous mammals (humans, rats, spiny mouse) identifying creatine transfer across the placenta. We have reported expression of the creatine transporter (CrT) and capacity for creatine synthesis in the human placenta, and also that creatine is not transferred across the sheep placenta, leading to the hypothesis that in sheep (a herbivore) CrT is not expressed in the placenta. We have therefore examined expression of the enzymes of creatine synthesis (AGAT, GAMT) and CrT expression in carnivore, omnivore, and herbivore species.

Methods: Placentas collected from sheep, rabbit, dog, ferret, and human (n=3), were divided for formalin fixation and paraffin-embedding, or freezing in liquid nitrogen and used to determine creatine content, AGAT, GAMT and CrT mRNA by qRT-PCR, AGAT and GAMT protein abundance by western blot, and CrT by immunohistochemistry.

Results: The creatine content of sheep, human, ferret, dog and rabbit placentas (7.6±0.71, 6.7±2.3, 8.9±1.67. 9.6±2.3, 9.7±2.74 mmol/kg dry mass, respectively) was remarkably similar. AGAT was detected in human, rabbit and ferret placentas, and GAMT protein in human and rabbit placenta. CrT was present in the human syncytiotrophoblast, but not in sheep placenta.

Conclusion: Human and rabbit placentas may be able to synthesize creatine. The human placenta is the only one with unequivocal CrT expression. As the sheep is a herbivore and also does not absorb creatine from the gut, this raises the possibility that the placental transfer of creatine is restricted to some carnivore and omnivore species where creatine is also obtained from the diet. Absence of placental CrT raises questions about the source of fetal creatine before maturation of in situ fetal creatine synthesis.

P8 Late gestation overnutrition decreases the numerical density of type II alveolar epithelial cells and surfactant protein gene expression in the fetus but this is not maintained after birth

Mitchell C. Lock[1], Erin V. McGillick[1,2], Sandra Orgeig[2], I. Caroline McMillen[1], Beverly S. Mühlhausler[1], Song Zhang[1] and Janna L. Morrison[1]

[1] Early Origins of Adult Health Research Group and [2] Molecular & Evolutionary Physiology of the Lung Laboratory, Sansom Institute for Health Research, University of South Australia, Adelaide, SA, Australia

Background: Obese women are significantly more insulin resistant before pregnancy and remain so throughout gestation. Consequently they are at increased risk of developing gestational diabetes. Clinical outcomes indicate that infants of diabetic mothers have up to a 6-fold increased risk of developing respiratory distress syndrome (RDS); however the molecular mechanisms regulating these outcomes are unclear (Lock et al., Clin Exp Pharmacol Physiol, 2013).

Methods: We explored factors that are associated with the intrauterine environment of an overnourished mother and that may impact on fetal lung development, leading to an increased risk of RDS at birth. We used a sheep model in which the pregnant ewe was overnourished from 115 days gestation (term=150+/-3 days gestation). Fetuses (141 days gestation) and lambs (30 days after birth) were humanely killed, and lung samples were collected for immunohistochemistry and qRT-PCR analysis.

Results: Maternal overnutrition during late gestation increased fetal plasma glucose and insulin concentrations and decreased the numerical density of surfactant protein-B positive cells present in the alveolar epithelium of lung tissue in the fetus at 141 days gestation. Maternal overnutrition was also associated with a reduction in mRNA expression of surfactant proteins-A, -B & -C, glucose transporter (GLUT) 1 & 4 and phosphate cytidylyltransferase 1, choline, alpha (PCYT1A), a key rate limiting enzyme in the production of surfactant phospholipids. In contrast, in the lung of 30 day lambs, there were increases in the mRNA expression of peroxisome proliferator activated receptor-γ (PPARγ), PCYT1A, fatty acid synthase (FAS) & fatty acid transport protein (FATP).

Conclusions: These results indicate that maternal overnutrition during late gestation is associated with a reduced capacity for surfactant production in fetuses and altered glucose and fatty acid metabolism in the lung, as well as an upregulation of genes involved in lung maturation and surfactant lipid synthesis shortly after birth. Taken together these results suggest that maternal overnutrition may cause an increased risk of developing RDS at birth.

P9 Late gestation overnutrition alters the cardiac metabolic profile

Jack R Darby, Song Zhang, I Caroline McMillen, Beverly S. Mühlhausler and Janna L Morrison
Early Origins of Adult Health Research Group, Sansom Institute, University of South Australia, SA 5000, Australia

Background: Maternal obesity predisposes offspring to a greater risk of developing cardiovascular disease in adult life. The mechanism by which this occurs is not well elucidated. We aimed to determine the effects of maternal late gestation overnutrition (LGON) on the regulation of cardiac growth and metabolism**.**

Methods: Ewes were fed a control diet (100% metabolisable energy (ME)) or were overnourished (155% ME; LGON) from 115d to 150d gestation. In the fetal cohort, ewes were humanely killed at 145d gestation. In the lamb cohort, ewes gave birth spontaneously and lambs were humanely killed at 30d. Left ventricles were collected and the mRNA and protein expression of molecules involved in the regulation of cardiac development, growth and metabolism were measured.

Results: LGON increased the mRNA expression of cardiac GSK-3β in the fetus as well as decreased expression of its downstream targets *GATA-4* and *c-myc* in the fetus and both the fetus and lamb, respectively. LGON decreased *FABP-5* and adiponectin R2 mRNA in both cohorts. There was no effect of LGON on mRNA expression of adiponectin R1 or the fatty acid transporters FATP-1, FATP-4 and CD36. There was an effect of age such that the mRNA of these molecules was higher in the lamb than the fetus. *PDK-4* mRNA was higher in the lamb than the fetus and decreased in response to LGON in the lamb.

Conclusions: The expression of factors involved in fatty acid transport increases after the transition to postnatal life. This is consistent with the transition from a dependence on cardiac glucose metabolism in fetal life to fatty acids in postnatal life. After exposure to LGON, cardiac mRNA expression of specific factors that regulate fatty acid transport and metabolism were decreased. This suggests that maternal LGON may inhibit or delay the normal cardiac glucose to fatty acid metabolic transition.

P10 Mitochondrial function in ovine skeletal muscle during late gestation

O.R. Vaughan, K.L. Davies. A.J. Murray and A.L. Fowden
Department of Physiology, Development and Neuroscience, University of Cambridge, UK

Introduction
Developmental changes occur in metabolic rate and mitochondrial protein expression in fetal sheep during late gestation [1-3]. However, few studies have examined development of mitochondrial function in fetal skeletal muscle, a significant O_2 consumer *in utero* [4]. Therefore, this study measured *in situ* mitochondrial respiration, substrate use and coupling of fetal ovine skeletal muscle during late gestation.

Methods
Under the Animals (Scientific Procedures) Act, *biceps femoris* was collected from sheep fetuses on day (d) 119 (n=13, term ≈d145) or d137 (n=6) of gestation, under terminal anaesthesia (Na pentobarbitone, 200mg/kg). Muscle fibres were dissected, saponin-permeabilised and washed [5]. Carbohydrate-supported (pyruvate, 20µM), fat-supported (palmitoyl-carnitine, 0.1µM) and total (glutamate, 20µM; succinate 10µM) mitochondrial O_2 consumption rates were measured ±ADP (2µM). *UCP2* and *UCP3* expression was quantified by qPCR. Comparisons with age were compared by *t*-test.

Results
Mean±SEM uncoupled and ADP-coupled O_2 consumption rates did not differ significantly with increasing age using either pyruvate or palmitoyl carnitine as substrates (Fig.A&B). However, when total mitochondrial respiratory capacity was measured, the uncoupled O_2 consumption rate was higher at d137 than d119 (Fig.A). Total ADP-coupled O_2 consumption rate was similar at the two ages (Fig.B). Thus, the respiratory control ratio was lower at d137 than d119 when electron transport was maximally stimulated but not when pyruvate or palmitoyl carnitine were used alone (Fig.C). There was no difference in skeletal muscle *UCP2* or *UCP3* expression with increasing age (P>0.05).

Conclusions
In fetal sheep, total mitochondrial respiratory capacity of skeletal muscle remains stable during late gestation. However, mitochondrial uncoupling increases towards term, which may be a protective mechanism to limit production of reactive oxygen species in preparation for delivery.

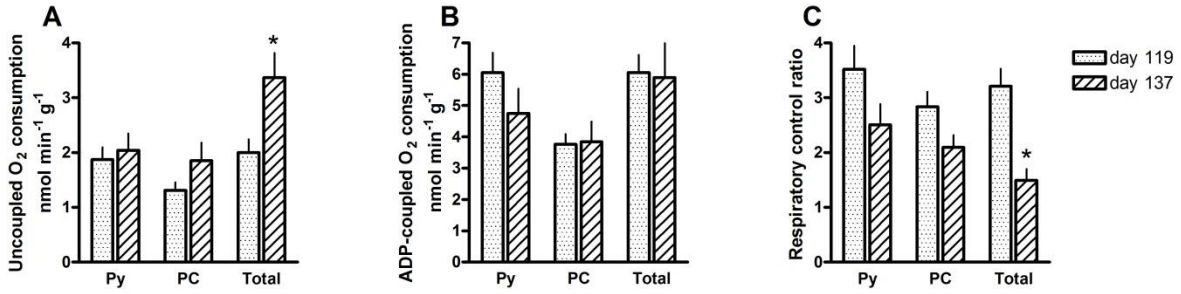


Figure
Mean±SEM mitochondrial oxygen consumption: (A) uncoupled, (B) ADP-coupled (C) respiratory control ratio (ADP-coupled O_2 consumption/uncoupled O_2 consumption) in fetal sheep skeletal muscle during late gestation *, P<0.05 t-test

P11 Effect of Altered Systolic Load on Fetal Heart Growth

Divya Soman[1,2], Debra Anderson[1,2], Fred Tibiyan[1,2], Jobe Faber[1,2], Samantha Louey[1,2], Sonnet Jonker, Kent Thornburg[1,2] and George Giraud[1,2,3,4].

[1]Center for Developmental Health, [2]Knight Cardiovascular Institute, [3]Department of Physiology and Pharmacology, Oregon Health & Science University and [4]Portland VA Medical Center, Portland.

Background/Introduction: The developing fetal heart is exquisitely sensitive to changes in hemodynamic load. We have previously shown that under conditions of increased cardiac load, the heart grows initially by hyperplasia and then by hypertrophy. Under conditions of decreased load there is marked decrease in hyperplastic growth. The effect of altered load on overall cardiac function remains poorly understood.

Methods: Eighteen fetal sheep at 120 days gestational age were instrumented with arterial and venous catheters. Flow sensors were placed around the brachiocephalic artery and the post ductal aorta to measure biventricular cardiac output. After recovery from surgery the fetuses were given 8 days of continuous IV infusions of either enalaprilat (low angiotensin II model of decreased cardiac load), plasma (low angiotensin II model of increased cardiac load) or lactated Ringer's solution (control).

Results: Fetal arterial pressure in the enalaprilat group decreased from 41.2+/- 2.5 mmHg on day 0 to 24.2+/- 3.8 mmHg on day 8, increased in the plasma group from 42.1 +/- 2.1 mmHg on day 0 to 61.7+/-2.8 mmHg on day 8 while arterial pressure in the control group was unchanged. Biventricular cardiac output and stroke volume remained unchanged although the total vascular resistance decreased in the enalaprilat infused group and increased in the plasma infused group. Compared to control, the enalaprilat group had lower wall stress but maximal isovolumetric aortic pressure was lower on day 8 than day 0. Compared to control, the plasma group had higher wall stress but the maximal isovolumetric aortic pressure was higher at Day 8 compared to Day 0.

Conclusions: Fetal hearts developing in a low cardiac load environment are undergrown and mechanically disadvantaged. Hearts growing in high cardiac load environment remodel by hypertrophy but not enough to normalize wall stress yet do not appear to be substantially mechanically disadvantaged.

Author Index

List of Delegates

Name	Location	email address
Tehila Avitan	University of British Columbia, Vancouver, Canada	tehila.avitan@cw.bc.ca
Laura Bennet	University of Auckland, Auckland, NZ	l.bennet@auckland.ac.nz
Samantha Benton	University of Ottawa, Ottawa, Canada	sbenton@uottawa.ca
Alex Beristain	University of British Columbia, Vancouver, Canada	aberista@mail.ubc.ca
Max Berry	University of Otago, Wellington, NZ	max.berry@otago.ac.nz
Rob Bischcof	Monash University, Clayton, Australia	rob.bischof@monash.edu
Kimberly Botting	University of South Australia, Adelaide, Australia	kb555@cam.ac.uk
Emily Camm	University of Cambridge, UK	ejc68@cam.ac.uk
Margie Castillo-Melendez	Monash University, Clayton, Australia	margie.castillo-melendez@hudson.org.au
Harry Coleman	Monash University, Clayton, Australia	harry.coleman@monash.edu
Joanne Davidson	University of Auckland, Auckland, NZ	joanne.davidson@auckland.ac.nz
Katie Davies	University of Cambridge, Cambridge, UK	kld47@cam.ac.uk
Lowell Davis	Oregon Health & Science University, Portland, OR	tuizing@ohsu.edu
Justin Dean	University of Auckland, Auckland, NZ	J.DEAN@AUCKLAND.AC.NZ
Jan Derks	University Medical Centre, Utrecht, The Netherlands	j.b.derks@umcutrecht.nl
Hayley Dickinson	Monash University, Clayton, Australia	hayley.dickinson@hudson.org.au
Rajavel Elango	University of British Columbia, Vancouver, Canada	relango@cfri.ubc.ca
Tania Fowke	University of Auckland, Auckland, NZ	t.fowke@auckland.ac.nz
Robert Galinsky	University of Auckland, Auckland, NZ	r.galinsky@auckland.ac.nz
Dino Giussani	University of Cambridge, Cambridge, UK	dag26@cam.ac.uk
Alistair Gunn	University of Auckland, Auckland, NZ	aj.gunn@auckland.ac.nz
Takushi Hanita	Tohoku University Hospital, Sendai, Japan	takushi.hanita@monash.edu
Tomoaki Ikeda	Mei University Graduate School of Medicine, Japan	t-ikeda@clin.medic.mie-u.ac.jp
Graham Jenkin	Monash University, Clayton, Australia	graham.jenkin@monash.edu
Sonnet Jonker	Oregon Health & Science University, Portland, OR	jonkers@ohsu.edu
K.S. Joseph	University of British Columbia, Vancouver, Canada	ksjoseph@cfri.ca
Yuki Kamimoto	Mei University Graduate School of Medicine, Japan	kozu510@clin.medic.mie-u.ac.jp
Hikari Kato	Hamamatsu University School of Medicine, Japan	ikato@saitama-med.ac.jp
Ineko Kato	Mei University Graduate School of Medicine, Japan	ikato@clin.medic.mie-u.ac.jp
Dwight Kimberly	George Fox University, Newberg, OR, USA	dkimberl@georgefox.edu

Michik Kubo	Mei University Graduate School of Medicine, Japan	michi_k_1019@yahoo.co.jp
Christopher Lear	University of Auckland, Auckland, NZ	christopher.lear@auckland.ac.nz
Lara Lehtoranta	University of Turku, Turku, Finland	lara.lehtoranta@utu.fi
Ken Lim	BC Women's Hospital, Vancouver, Canada	klim@cw.bc.ca
Isa Lindgren	Oregon Health & Science University, Portland, OR	lindgrei@ohsu.edu
Stephen Lye	University of Toronto, Toronto, Canada	lye@lunenfeld.ca
Gagandeep S Mallah	University of Auckland, Auckland, NZ	g.mallah@auckland.ac.nz
Courtney Mcdonald	Monash University, Clayton, Australia	courtney.mcdonald@hudson.org.au
Jordan McIntyre	University of Auckland, Auckland, NZ	j.mcintyre@auckland.ac.nz
Azar Mehrabadi	University of British Columbia, Vancouver, Canada	azar@alumni.ubc.ca
Suzie `Miller	Monash University, Clayton, Australia	suzie.miller@monash.edu
Janna Morrison	University of South Australia, Adelaide, Australia	janna.morrison@unisa.edu.au
Tim Moss	Monash University, Clayton, Australia	tim.moss@monash.edu
Masafumi Nii	Mei University Graduate School of Medicine, Japan	doaldosleeping@yahoo.co.jp
Jan G. Nijhuis	Maastricht University Medical Center, Maastricht, The Netherlands	jg.nijhuis@mumc.nl
Mark Oliver	University of Auckland, Auckland, NZ	m.oliver@auckland.ac.nz
David Olson	University of Alberta, Edmonton, Alberta, Canada	david.olson@ualberta.ca
Julian (Bill) Parer	University of California, San Francisco, USA	parerb@obgyn.ucsf.edu
Helena Parkington	Monash University, Clayton, Australia	helena.parkington@monash.edu
Sebastian Quezada	Universidad de Chile, Santiago, Chile	seba.quezada.r@gmail.com
Arezoo Rajaee	UCLA, Los Angeles, USA	arajaee@mednet.ucla.edu
Timothy Regnault	Western University, London, Canada	tim.regnault@uwo.ca
Dan Rurak	University of British Columbia, Vancouver, Canada	drurak@cw.bc.ca
Katie Skeffington	University of Cambridge, Cambridge, UK	kls71@cam.ac.uk
John Smyth	BC Women's Hospital, Vancouver, Canada	jsmyth@cw.bc.ca
Divya Soman	Oregon Health & Science University, Portland, OR	soman@ohsu.edu
Noor Teulings	University of Cambridge, Cambridge, UK	noorteulings@gmail.com
Kent Thornburg	Oregon Health & Science University, Portland, OR	thornbur@ohsu.edu
Lotte van den Heuij	University of Auckland, Auckland, NZ	l.vandenheuij@auckland.ac.nz
Owen Vaughan	University of Cambridge, Cambridge, UK	orv20@cantab.net
David Walker	Monash University, Clayton, Australia	david.walker@hudson.org.au
Zhongchao Wang	University of Cambridge, Cambridge, UK	5139005wzc@163.com
Guido Wassink	University of Auckland, Auckland, NZ	g.wassink@auckland.ac.nz

Christoph Wohlmuth	University of Texas Health Science Center, Houston, TX, USA	christoph.wohlmuth@outlook.com
Luc Zimmermann	Maastricht University Medical Center, Maastricht, The Netherlands	luc.zimmermann@mumc.nl

NOTES