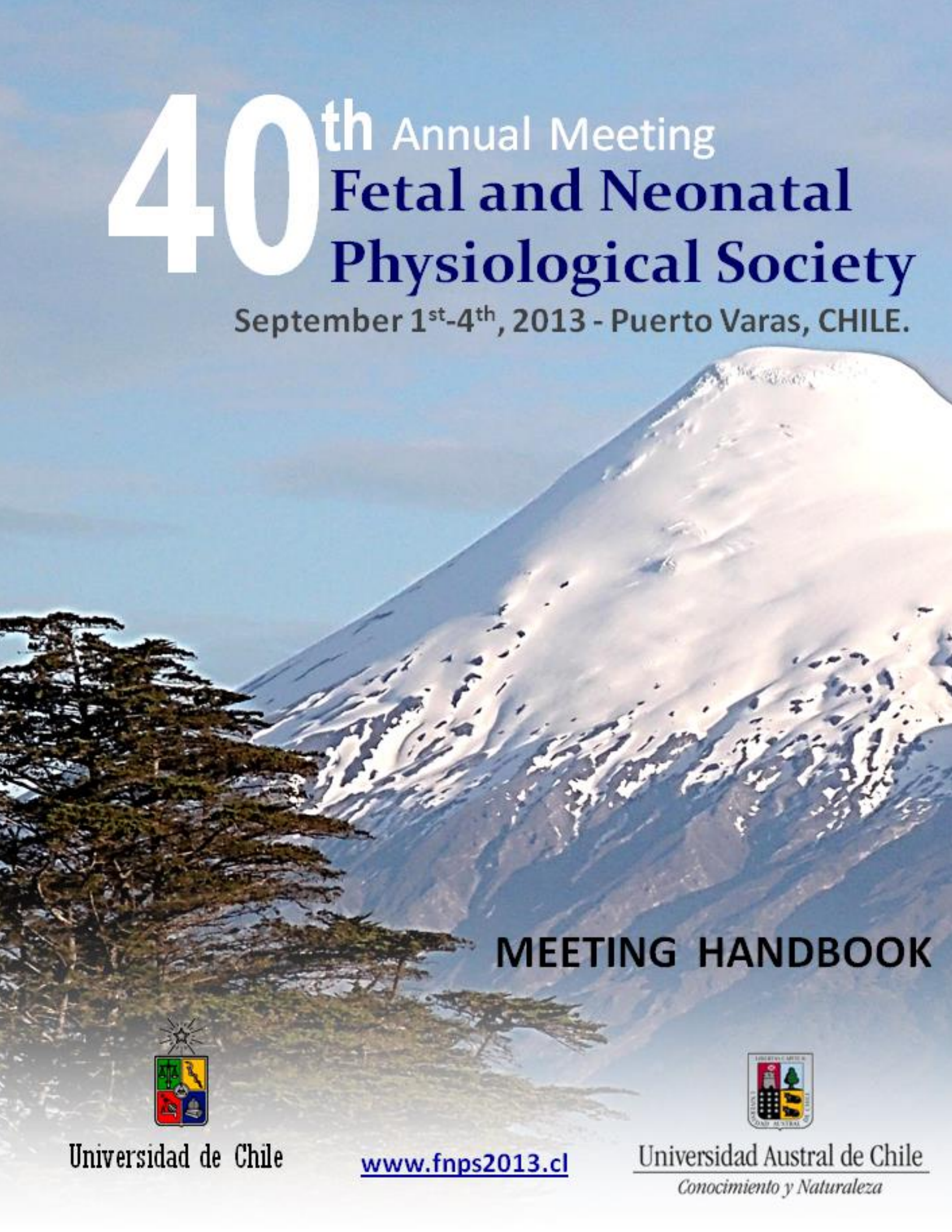
40th Annual Meeting
Fetal and Neonatal Physiological Society

September 1st-4th, 2013 - Puerto Varas, CHILE.



MEETING HANDBOOK

Universidad de Chile

www.fnps2013.cl

Universidad Austral de Chile
Conocimiento y Naturaleza

40th Annual Meeting
Fetal and Neonatal Physiological Society

September 1st-4th, 2013 - Puerto Varas, CHILE.

Meeting Organizers

Emilio A. Herrera (Chair)
Claudia Torres-Farfán (Chair)
Germán Ebensperger
Marcela Díaz
Pamela Alonso-Vazquez

Venue

Gran Hotel Colonos del Sur
Puerto Varas, CHILE

INDEX

WELCOME

Dear attendants,

The time has finally come to welcome you all to the 40th Annual Meeting of the Fetal and Neonatal Physiological Society, to be held in the North tip of the Patagonia, home of some of the most beautiful landscapes in the World. Other than the activities related to the Meeting, we strongly advise you to explore as much as you can. The Chilean Lake District is indeed unique, with attractions ranging from delicious food/drinks to many outdoor activities (trekking, fly-fishing, lake and river tours, and so on and so forth). Landscape photography is a must.

Once again, we are delighted to get the chance to share and discuss our recent findings with distinguished investigators from all continents. Thus, an updated fetal and maternal physiology and pathophysiology, and the early determinants of health and disease will be analyzed in detail. Further, an integrative developmental physiology view will conduct the discussions in the different sessions. Testimony of these are the large amount of high quality abstracts submitted to the Meeting, much of them presented by young researchers from all over the world. We are extremely grateful to all of you, as these shaped an exciting and varied scientific program.

This year, the social activities are planned to enjoy the landscapes, the local food and drinks, and the Chilean traditions. The famous *sporting event* will surely coronate these activities with fun and joy!

All members of the Local Organizing Committee truly hope that you leave Puerto Varas with vivid memories. Finally, we wish to thank the authorities from the Universidad Austral de Chile and Universidad de Chile, our main supporters. As well, we are grateful to the institutional and commercial sponsors. Undoubtedly, their support and generosity have contributed to ensure the quality of this Meeting.

Please enjoy the Meeting and Chile!

Organizing Committee - FNPS 2013

FNPS MISSION STATEMENT

The FNPS stimulates discussion and exchange of ideas between physiologists, obstetricians and neonatologists. The FNPS considers an informal gathering and presentations of new and preliminary data, especially by investigators in training, 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 took the initiative and were made honorary members of the Society in 1995.

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-date 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 JuneSeptember, 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 US$. 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 Malmö, Sweden
1977 Oxford, United Kingdom
1978 Nijmegen, The Netherlands
1979 Paris, France
1980 Oxford, United Kingdom
1981 Maastricht, The Netherlands
1982 London, Canada
1983 Malmö, Sweden
1984 Oxford, United Kingdom
1985 Haifa, Israel
1986 Banff, Canada
1987 Groningen, The Netherlands
1988 Cairns, Australia
1989 Reading, United Kingdom
1990 Pacific Grove, USA
1991 De Eemhof, The Netherlands
1992 Niagara-the-Lake, Canada
1993 Plymouth, United Kingdom
1994 Palm Cove, Australia
1995 Malmö, Sweden
1996 Arica, Chile
1997 S.Margherita Ligure, Italy
1998 Lake Arrowhead, USA
1999 Vlieland, The Netherlands
2000 Southampton, United Kingdom
2001 Auckland, New Zealand
2002 Prague, Czech Republic
2003 Banff, Canada
2004 Tuscany, Italy
2005 Glenelg, South Australia
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

FNPS BOARD MEMBERS 2013

Jan Nijhuis (Chair, The Netherlands)

Dino A. Giussani (Scribe, UK)

Luc Zimmermann (The Netherlands)

Donald Peebles (UK)

Laura Benett (New Zealand)

Tim Moss (Australia)

Tomoaki Ikeda (Japan)

Carina Mallard (Sweden)

Emilio A. Herrera (Chile)

Brian Koos (USA)

Bill Parer (USA)

Charles Wood (USA)

Dan Rurak (Canada)

Lucy Green (UK)

Minutes of the FNPS Annual General Meeting
Utrecht, The Netherlands, Wednesday 11 July 2012

Present: Laura Bennet, Jan B. Derks (guest), Dino A. Giussani (Scribe), Emilio A. Herrera, Tomoaki Ikeda, Graham Jenkin (guest), Jan Nijhuis (Chair), Bill Parer, Donald Peebles, Charles Wood, Luc Zimmermann.

1. **Meeting minutes**. The minutes of the 38th Annual FNPS meeting at The Grand Mercure Rockford Resort, Palm Cove, Australia 2011 were accepted with no amendments.

2. Jan Nijhuis expressed a vote of thanks to the organisers and sponsors.

3. The meeting welcomed Emilio A. Herrera as a new member of the FNPS board.

4. **FNPS board membership**. Some members of the society expressed an interest in becoming members of the FNPS board. Their expression of interest was discussed at length and it was decided to invite applications only when positions became vacant. The meeting also agreed that if any board member did not attend the FNPS meeting during three consecutive years, then they would be asked to step down.

5. **Joint meeting with IFPA**. This suggestion, raised by Colin Sibley and Stacy Zamudio, was entertained. The board felt in general support of a dove-tailed meeting in future years. Dino A. Giussani will communicate with IFPA organisers and determine feasibility.

6. **Sporting event**. The FNPS board felt that mixing up the teams with individuals from different groups and countries would be a positive move to encourage social integration. Future organisers should take this into consideration.

7. **Last session**. In order to facilitate the award of student prizes, the FNPS board recommended that no student should be placed in the last scientific session of the meeting. The feasibility of this proposal will be investigated by future organisers.

8. **Future meetings**:
   (i) 2013. Chile. Emilio A. Herrera and Claudia Torres-Farfan are the organizers.
   (ii) 2014. Italy. Tullia Todros will be asked to entertain organisation. This FNPS meeting may be preceded by a Satellite Workshop at Prato, organised by Graham Jenkin. Graham Jenkin to investigate possibilities with Tullia Todros.
   (iii) 2015. Possibility of Japan or Canada suggested.
   (iv) 2016. Possibility of Cambridge, UK suggested.

9. **Prizes**:

<u>The Bo Gennser Memorial Prize</u>:
Laura Thei (University College London).
'C-Jun plays a regulatory role in the neonatal brains response to hypoxia-ischemia induced injury'

The Tania Gunn Memorial Prize:
1. Joanna O Davidson (University of Auckland)
'Connexin 43 hemichannel blockade: mimetic peptide dose response after ischaemia'.
2. Miram Koome (University of Auckland)
'Exposure to maternal glucocorticoids exacerbates post-asphyxial brain injury in the preterm fetus'.

Respectfully submitted,

Dino A. Giussani, PhD

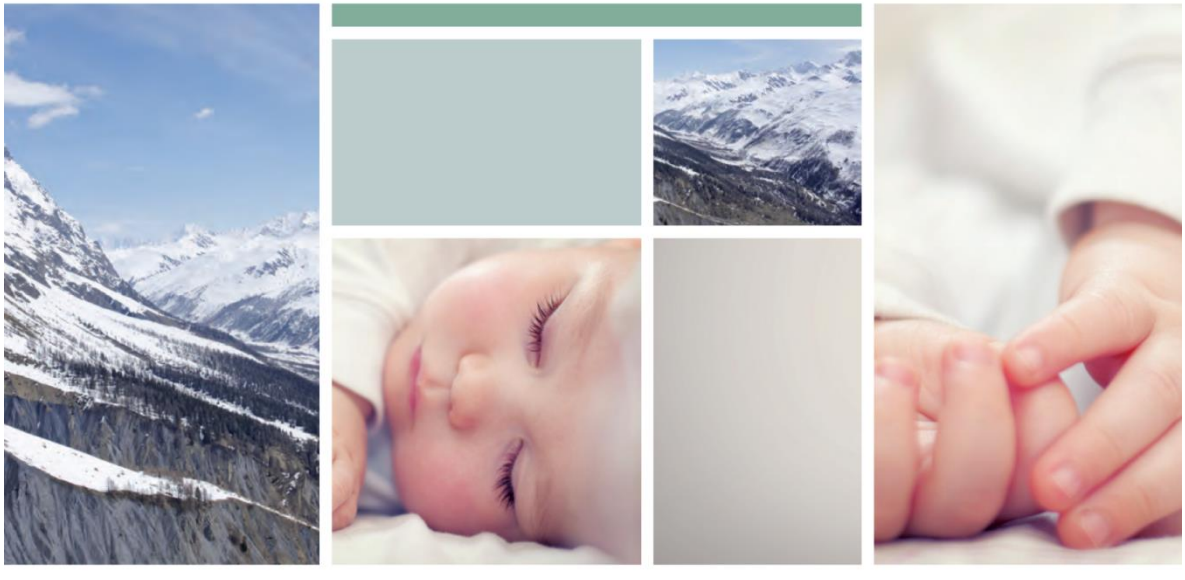
FNPS Scribe

FNPS 2014 ANNOUNCEMENT

POOMDESIGN.IT

41st ANNUAL MEETING
FETAL AND NEONATAL PHYSIOLOGICAL SOCIETY

AUGUST 31 - SEPTEMBER 3, 2014



SAINT VINCENT (ITALY)

Dear friends,

we are glad to invite you to Saint Vincent, Italy, in the charming and exciting landscape of Northern Italian Alps, for the 41st FNPS-meeting.

Enjoy with us the fascinating atmosphere of our mountains, the Italian tastes and the international knowledge of FNPS.

Prof. Tullia Todros - University of Turin
on behalf of the Organizing Committee

ORGANIZING SECRETARIAT

cci centro congressi internazionale

CENTRO CONGRESSI INTERNAZIONALE s.r.l.
Torino - Via San Francesco da Paola, 37
Ph. +39 011 2446911 - Fax +39 011 2446950
info@congressiefiere.com

WWW.FNPS2014.IT

ANNOUNCEMENT - FNPS 2014 Satellite meeting

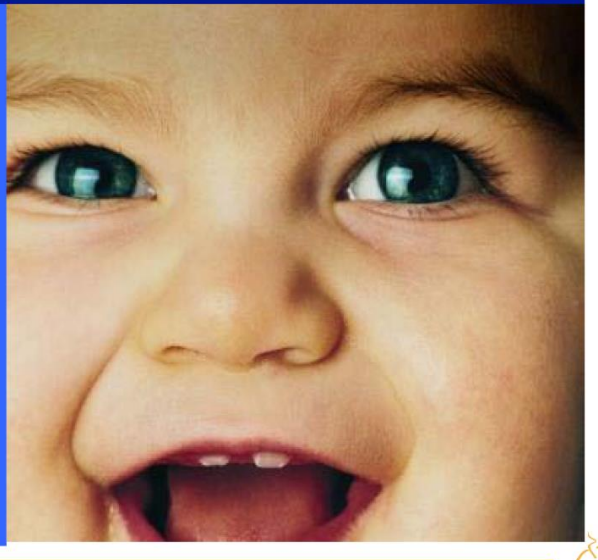
MONASH INSTITUTE OF MEDICAL RESEARCH

MONASH University

The Ritchie Centre 2014 Annual Colloquium

Premature birth

How can we make it safer for premature infants?



28-29th August, 2014
Monash University Centre,
Prato, Tuscany, Italy

Numbers are limited.
For further information please visit, www.ritchieforum.org
or contact Lisieux Jones +61 3 9594 5145
Lisieux.jones@monash.edu

MonashHealth

Monash Partners
Academic Health Science Centre

the RITCHIE CENTRE

Local Organizing Committee

Emilio A. Herrera; Universidad de Chile (Chairman)

Claudia Torres-Farfan; Universidad Austral de Chile (Chairwoman)

Germán Ebensperger; Universidad de Chile

Marcela Díaz; Universidad de Chile

Pamela Alonso Vazquez; Universidad Austral de Chile

SUPPORTERS

Universidad de Chile

Universidad Austral de Chile
Conocimiento y Naturaleza

Laboratorio de Función y Reactividad Vascular

INCAS

SOCIEDAD CHILENA DE CIENCIAS FISIOLOGICAS

SOCIEDAD CHILENA DE OBSTETRICIA Y GINECOLOGIA

SOCIEDAD CHILENA DE PEDIATRIA
RAMA NEONATOLOGIA

SPONSORS

ADINSTRUMENTS
making science easier

CONICYT
Ministerio de Educación
Gobierno de Chile
Comisión Nacional de Investigación Científica y Tecnológica

FERMELO Biotec

INSTITUTO DE CIENCIAS BIOMÉDICAS
FACULTAD DE MEDICINA
UNIVERSIDAD DE CHILE

UNIVERSIDAD DEL BÍO-BÍO

FACULTAD DE MEDICINA
UNIVERSIDAD DE CHILE

Cronobiología del Desarrollo
Anillo ACT 1116

Universidad Austral de Chile
Facultad de Medicina

ICBM
INSTITUTO DE CIENCIAS BIOMÉDICAS

FACULTAD DE MEDICINA
ESCUELA DE OBSTETRICIA

ABSTRACT AWARDS (In-trainning)

FNPS 2013 Student Awards (Funded by Sponsors)

The FNPS 2013 Organizing Committee is pleased to announce the opening of three full registration awardsto attend the meeting to be held in Chile, September 2013. The awards will recognize the best oral presentations, and will cover the full registration of undergraduate, graduate students or in-trainee who have accepted abstracts as first authors.

These awards are funded by the Sponsors: ADInstruments, Fermelo and GeneX-press.

ANILLO Awards on Materno-fetal chronobiology perinatal actions of melatonin (Funded by Research Grant ANILLO ACT 1116-Chile)

The FNPS 2013 Organizing Committee is pleased to announce the opening of three funding awards to attend the meeting to be held in Chile, September 2013. The awards will recognize novel studies regarding materno-fetal chronobiology and/or perinatal actions of melatonin, and will cover the full registration of undergraduate, graduate students or in-trainee who have accepted abstracts as first authors. These awards are funded by the Research Grant ANILLO (ACT 1116) entitled ”Pathophysiological Mechanisms of Adult Chronic Disease Imposed by Developmental Chronodisruption: therapeutic Value of Melatonin Replacement in Shift Work Pregnancy” (PIs: Dr. Claudia Torres-Farfan and Dr. Hans Richter; Faculty of Medicine, Universidad Austral de Chile).

TANIA GUNN Memorial Prize

This prize was introduced in FNPS 2011 in memory of Tania Gunn (1932-1999), professor of Neonatology at Auckland, New Zealand. She is remembered for her important studies of the control of thermoregulation at birth and safety of therapeutic hypothermia for babies with acute encephalopathy. This year there are two Tania Gunn Memorial prizes in New Zealand pounds

1. 500$ (USD 430) prize for best oral presentation by a student
2. 500$ (USD 430) prize for best oral presentation by a postdoctoral fellow, either 36 months from graduation as PhD or MD.

Bo Gerhard Gennser Memorial Prize

This prize was introduced in last year FNPS meeting in memory of Bo Gennser (1929-2010), professor in Obstetrics & Gynecology at Lund University, Sweden. He is recognized for developing new methods in recording fetal breathing movements and the evaluation of fetal real-time ultrasound. In 1974, he and Geoffrey Dawes initiated the Fetal Breathing Conferences, that later end up in the Fetal & Neonatal Physiological Society. This year the Bo Gerhard Gennser Memorial Prize is for the best scientific poster of a student or early post doc, equivalent to 5000 SEK (USD 800).

PROGRAMME *at a glance*

Fetal and Neonatal Physiological Society meeting – FNPS 2013, PUERTO VARAS, CHILE

Sunday 1 September

10.00-14.30 Arrival at location + registration + accommodation check-in
14.30-16.30 Welcome and poster session (Posters session ORAL – Chair: EA Herrera, C Torres-Farfan)
16.30-17.00 Coffee break
17.00-18.45 Session II (Preterm Birth, steroids, Miscellaneous – Chair: L Bennet, D Walker)
18.45-19.30 Dawes Lecture (Dr. Aníbal J. Llanos & Dr. María Serón-Ferré, University of Chile, Chile)
20.00-22.30 Welcome reception-dinner (Concert)

Monday 2 September

07.30-08.30 Registration + breakfast
08.30-10.15 Session III (Fetal & Neonatal Brain I – Chair: A Gunn, M Herrera-Marschitz)
10.15-11.15 Coffee break + poster viewing
11.15-13.00 Session IV (Fetal & Neonatal Brain II – Chair: EJ Camm, C Wood)
13.00-14.30 Lunch
14.30-16.15 Session V (Fetal & Neonatal Cardiovascular I – Chair: AJ Llanos, JT Parer)
16.15-16.45 Coffee break
16.45-18.30 Session VI (Fetal & Neonatal Cardiovascular II - Chair: DA Giussani, N Weissmann)
19.00-23.00 Conference dinner (+drinks & dancing)

Tuesday 3 September

07.30-08.30 Registration + breakfast
08.30-10.00 Session VII (Placenta – Chair: P Casanello, C Escudero)
10.00-10.45 Coffee break + poster viewing
10.45-12.30 Session VIII (DOHaD - Chair: M Hanson, H Richter)
12.30-13.15 Invited lecture (Prof. Dr. Ricardo Uauy, National Prize for Applied Sciences & Technology 2012, INTA, University of Chile, Chile)
13.15-14.30 Lunch
15.00-23.00 Sporting event + dinner

Wednesday 4 September

07.30-08.30 Registration
08.30-9.45 Session IX (Translational & Other - Chair: R Reyes, T Todros)
9.45-10.15 Coffee break
10.15-11.00 Invited Lecture (Prof. Dr. José Cipolla-Neto, Instituto de Ciências Biomédicas, Universidade de São Paulo, Brazil).
11.00-12.30 Session X (Fetal-maternal circadian rhythms - Chair: J Cipolla-Neto, M Serón-Ferre)
12.30-13.0 Meeting adjourns – Awards ceremony
13.00-14.30 Farewell Lunch – Closing remarks

PROGRAMME *in extenso*

Fetal and Neonatal Physiological Society meeting

FNPS 2013, PUERTO VARAS, CHILE

Sunday 1 September

10.00-14.30 Arrival at location + registration + accommodation check-in

14.30-16.30 Welcome and poster Session I - Posters session ORAL
Chairs: EA Herrera, C Torres-Farfan

Fetal & Neonatal Cardiovascular

P1. Pentose phosphate pathway and NADPH oxidase inhibition impairs the response of chicken ductus arteriosus to oxygen.
Evangelia Tserga, Marina M. Goorts, Eduardo Villamor

P2. Maternal dietary creatine supplementation attenuates changes in isolated heart function 1 month after asphyxia at birth.
Domenic A LaRosa, Stacey J. Ellery, Victor Suturin, Rod J. Snow, Helena C Parkington, David W. Walker & Hayley Dickinson

P3. Pulmonary vascular reactivity responses to pre and postnatal treatment with melatonin in chronic hypoxic newborn sheep.
Marcelino Véliz, Alejandro González-Candia, Santiago Ramírez, Claudio Araya, Sebastián Quezada, Germán Ebensperger, Emilio A. Herrera

P4. Pulmonary vascular responses to hypoxemia and cyclooxygenase blockade in highland and lowland neonatal llamas.
Marcela Díaz, Germán Ebensperger, Emilio A. Herrera, Roberto V. Reyes, Aníbal J. Llanos

P5. Role of the RhoA/ROCK pathway in neonatal pulmonary hypertension induced by chronic hypoxia during gestation.
Nandy López, Germán Ebensperger, Emilio A. Herrera, Roberto V. Reyes, Gloria Calaf[3], Aníbal J. Llanos

P6. Hemin treatment reverses right ventricle hypertrophy, decreases pulmonary arterial pressure and improves postnatal growth in higland newborn lambs.
Santiago Ramírez, Marcelino Véliz, Sebastián Quezada, Alejandro González-Candia, Claudio Araya, Emilio A. Herrera, Aníbal J. Llanos, Marcela Díaz, Patricia V. Díaz, Germán Ebensperger

P7. Hemin decreases the remodeling on the pulmonary artery trunk and small pulmonary arteries in high altitude neonatal lambs.

Alexander Riquelme, Germán Ebensperger, Gertrudis Cabello, Emilio A. Herrera, Roberto V. Reyes, Aníbal J. Llanos

P8. Hemin treatment attenuates the small pulmonary arteries vasoconstrictor function in hypertensive high altitude neonatal lambs.
Araya CE, Valenzuela JM, Uribe M, Valenzuela A, Quezada S, González A, Leiva M, Herrera EA, Moraga F Reyes RV, Díaz M, Díaz PV, Silva P , Llanos AJ, Ebensperger G

P9. Prenatal melatonin improves systemic and cerebrovascular function in neonatal lambs gestated under chronic hypoxia.
Alejandro González-Candia, Marcelino Véliz, Siri-Marie Solbakken, Sebastián Quezada, Santiago Ramirez, Claudio Araya, Germán Ebensperger, Emilio A. Herrera

P10. Administration of 2-aminoethyldiphenylborinate modifies pulmonary vascular remodeling and reactivity in chronic hypoxic newborn lambs.
Daniela Parrau, Germán Ebensperger, Emilio A. Herrera, Ismael Hernandez, Débora Santos, Sebastián Quezada Aníbal J. Llanos , Roberto V. Reyes

P11. Post-natal oxygenation at sea level reduces pulmonary hypertension in high altitude newborn lambs.
Caroll Aburto, Claudio Araya, Ismael Hernandez, Roberto V. Reyes, Germán Ebensperger, Emilio A. Herrera, Aníbal J. Llanos

Fetal & Neonatal Brain

P12. Repeated cerebral hypoxia-ischemia in the very preterm ovine fetus appears to alter brain development.
Robert De Matteo, Mary Tolcos, Nadia Hale, Amy Shields, Richard Harding, Takushi Hanita

P13. Role of membrane trafficking and adherens junctions during fetal and neonatal brain development: insights from in vivo and in vitro models.
Zahady Velásquez, Gabriela Toro, Guillermo Márquez, Camilo Muñoz, Cristian Parga, Loreto Ojeda, María Paz Miró, Cristian Oliver, César Gonzalez, Rosa Iris Muñoz, Luis Federico Bátiz

P14. Effect of dopamine or dobutamine treatment on preterm cardiovascular function and brain inflammation following hypoxia-ischaemia.
Nadine Brew, Aminath Azhan, Irene DenHeijer, Ilias Nitsos, Suzie Miller, Adrian Walker, David W. Walkerand Flora Wong

P15. Potential of autologous umbilical cord blood for the early treatment of hypoxic brain injury.
James Aridas, Courtney McDonald, Tamara Yawno, Michael Fahey, Flora Wong, Atul Malhotra, Michael Ditchfield, Madison Paton, Margie Castillo Melendez, Suzie Miller, Graham Jenkin

P16. *Pattern of brain injury after prolonged umbilical cord occlusion in near-term fetal sheep.*
Paul P. Drury, Laura Bennet, Joanne O. Davidson, Alistair Jan Gunn

DOHaD

P17. Effects of perinatal asphyxia on prepulse inhibition in rats.
Cristian Cerfogli, Mariana Garín,Diego Bustamante, Peter Gebicke-Haerter, Paola Morales, Jose Valdes, Mario Herrera-Marschitz

P18. The adult innate immune system is programmed by gestational chronodisruption through diminished C3/C4/C9 complement factors.
Jose Sarmiento, Pamela Carmona, Hugo Folch, Carlos Spichiger, Hugo Galdames, Natalia Mendez, Maria Seron-Ferre, Claudia Torres-Farfan, Hans Richter

P19. Persistent down-regulation of KChiP2 may contribute to the adult cardiac hypertrophy enforced by gestational chronodisruption.
Pamela Alonso Vazquez, Hugo Galdames, Claudia Torres-Farfan, Carlos Spichiger, Natalia Mendez, Hector Vera, Hans Richter.

P20. Maternal care and adult cognitive flexibility in a rat model of intrauterine growth restriction.
Márcio Bonesso Alves, Roberta Dalle Molle, Mina Desai, Michael G. Ross and Patricia Pelufo Silveira

P21. Maternal obesity and endoplasmic reticulum stress induces insulin resistance in human umbilical vein endothelial cells.
Villalobos-Labra R, Westermeier F, Sáez PJ, Mardones F, Kusanovic JP, Poblete JA, Sobrevia L, Casanello P, Farias M

P22. Xanthine Oxidase and Programming of Cardiac Dysfunction in Hypoxic Pregnancy: Mechanism and Intervention.
Youguo Niu, Andrew Kane, Ciara Lusby, Beth Allison, Joepe Kaandorp, Jan B. Derks , and Dino A. Giussani

Placenta

P23. The increase in oxidative stress in the umbilical vein endothelium from IUGR pregnancies involves changes in GTPCH and DHFR expression.
Ernesto Muñoz-Urrutia, Bernardo Krause and Paola Casanello

P24. The transient blockade of the bradykininin B2 receptor in pregnant guinea-pigs induces a defective trophoblast invasion and vascular remodelling, fetal losses and increased plasma creatinine.
Daniela Schneider, Jenny Corthorn, Rita Ortiz[1], Gloria Valdés

Others

P25. Effects of chronic phase shifts of the photoperiod throughout pregnancy on maternal circadian rhythms.
Halabi D, Mendez N, Pamela Alonso Vazquez, Vergara K, Gutierrez M, Richter HG, Seron-Ferre M, Torres-Farfan C.

P26. Do antenatal glucocorticoids cause cerebral hypoxia after asphyxia?
Christopher K Lear, Miriam Koome, Joanne O. Davidson, Alistair J Gunn, Laura Bennet

P27. Melatonin as a therapy to improve perinatal outcomes in a low resource setting in India.
Suzie Miller, Euan Wallace, James Aridas, Michael Fahey, Atul Malhotra, Vishwajeet Kumar, Graham Jenkin

P28. Conservative management in preterm preeclampsia: some perinatal variables. Hospital Vladimir I. Lenin. January - December 2012.
José Alexander Tamayo Ortiz, Isora Natacha Rodríguez Pérez

P29. The International Center for Andean Studies (INCAS) - A unique natural Lab of Hypobaric Hypoxia.

Emilio A. Herrera, Alberto L. Raggi, Victor H. Parraguez, Aníbal J. Llanos

P30. The Perinatologists Family Tree.
Emilio A. Herrera, Julian T. Parer

16.30-17.00 Coffee break

17.00-18.45 Session II - Preterm Birth, Steroids, Miscellaneous
Chairs: *L Bennet, D Walker*

O1. Maternal dietary creatine supplementation prevents changes in diaphragm muscle function 1 month after asphyxia at birth.
Domenic A LaRosa, Stacey J. Ellery, Helena C Parkington, Rod J. Snow David W. Walker& Hayley Dickinson

O2. Does Neonatal Acute Kidney Injury Following Birth Asphyxia Result in Permanent Nephron Loss and Kidney Dysfunction?
Stacey J. Ellery, Domenic A LaRosa, Michelle M. Kett, Louise Cullen-McEwen, Russell Brown, Rod J. SnowDavid W. Walkerand Hayley Dickinson

O3. The effects of betamethasone on allopregnanolone concentrations and cell death in normally grown and IUGR preterm fetal sheep.
Yawno T, Mortale M, Sutherland AE, Wallace EM, Jenkin G, Walker DW, Miller SL

O4. Antenatal betamethasone effects on transient receptor potential cation channels (trpc) function in sheep arteries.
Jorge P. Figueroa, Jie Zhang, Angela Massmann, Jeong-Heon Lee

O5. Creatine Supplementation Protects the Neonatal Spiny Mouse Following Birth Asphyxia, but does it affect the Mother?
Stacey J. Ellery, Domenic A LaRosa, Michelle M. Kett, Rod J. SnowDavid W. Walker and Hayley Dickinson

O6. The effect of dexamethasone on the preterm fetal sheep responses to acute asphyxia.
Rachael L Robson, Joanne O. Davidson, Alistair Jan Gunn, Laura Bennet

O7. The preterm male lamb displays inadequate respiratory adaptation after birth.
Robert De Matteo, Noreen Ishak, Takushi Hanita, Richard Harding, Foula Sozo

18.45-19.30 Dawes Lecture: A lecture *al alimón* on low oxygen and light-melatonin. (Dr. Aníbal J. Llanos & Dr. María Serón-Ferré, University of Chile, Chile)

20.00-22.30 Welcome reception-dinner (Concert)

Monday 2 September

07.30-08.30 Registration + breakfast

08.30-10.15 Session III - Fetal & Neonatal Brain I
Chairs: *A Gunn, M Herrera-Marschitz*

O8. Synthesis of dehydroepiandrosterone (DHEA) in the brain of the spiny mouse.
Tracey Quinn, Hayley Dickinson, Margie Castillo Melendez, & David W. Walker

O9. Ongoing effects of prenatal stress on perinatal brain development and function.
Greer Bennett, Hannah Palliser, David W. Walker, Jonathan Hirst

O10. Toll-like receptor (TLR) 4 and 7 gene expression in the preterm ovine brain; effects of hypoxia-ischemia (HI) following subacute LPS stimulation.
Simerdeep Dhillon, Laura Bennet, Luke Weaver-Mikaere, Alistair J Gunn, Mhoyra Fraser

O11. Cerebrovascular changes associated with intrauterine growth restriction in newborn lambs.
Margie Castillo Melendez, Tamara Yawno, Euan Wallace, Graham Jenkin, Suzie Miller

O12. Melatonin improves cerebral vascular function in chronically hypoxic neonates.
Roberto Macchiavello, Camilo Montt, Germán Ebensperger, Emilio A. Herrera

O13. A comparison of melatonin and hypothermia for the treatment of the acutely asphyxiated newborn lamb.
James Aridas, Tamara Yawno, Jingang Li, Amy Sutherland, Michael Fahey, Atul Malhotra, Flora Wong, Michael Ditchfield, Alistair Jan Gunn , Euan Wallace, Graham Jenkin, Suzie Miller

O14. Postnatal outcomes in lambs exposed antenatally to fluoxetine.
Tuan Anh Nguyen, Timothy Chow, Wayne Riggs, Dan Rurak

10.15-11.15 Coffee break + poster viewing

11.15-13.00 Session IV - Fetal & Neonatal Brain II
Chairs: *EJ Camm, C Wood*

O15. The $GABA_A$ excitatory-to-inhibitory switch in the hippocampus of perinatal guinea-pigs.
Harold Coleman, Jon Hirst, Helena C Parkington

O16. Exposure to TNF-α and LPS in an *in vitro* ovine model of oligodendrocyte injury: Effects on glutamate and its receptors.
Luke Weaver-Mikaere, Alistair Jan Gunn , Murray Mitchell , Laura Bennet, Mhoyra Fraser

O17. Comparative transcriptomics of the fetal hypothalamic responses to hypoxia versus ischemia.
Charles E. Wood, Eileen I Chang, Elaine M Richards, and Maureen Keller-Wood

O18. Very low regional cerebral oxygen saturation during the postnatal transition of very preterm infants directly after delivery by caesarean section.

Tom Goos, Corinna Binder Andre Kroon, Jeroen Dudink, Hugo van Elteren, Jenny Dankelman, Gerhard Pichler, Berndt Urlesberger, Irwin Reiss

O19. Prevention of the long-term effects elicited by perinatal asphyxia: inhibition of PARP-1 activity as a therapeutic strategy worth to be clinically translated.
Herrera-Marschitz M, Cerfogli C, Neira-Peña T, Rojas-Mancilla E; Perez R, Rivera B, Gutierrez M, Esmar D Espina-Marchant P, Valdes JL, Simola N, Bustamante D, Gebicke-Haerter P, Morales P.

O20. Maternal Allopurinol Administration During Term Labor For Neuroprotection In Case Of Fetal Asphyxia; A Multicenter Randomized Controlled Trial (ALLO trial).
Joepe J. Kaandorp, Manon J. Benders, Ewoud Schuit, Carin M. Rademaker, Martijn A. Oudijk, Martina M. Porath, Sidarto Bambang Oetomo, Maurice G. Wouters, Ruurd M. Elburg, Maureen T. Franssen, Arie F. Bos, Timo R. de Haan, Janine Boon, Inge P. de Boer, Robbert J. Rijnders, Corrie J. Jacobs, Liesbeth C. Scheepers, Danilo A. Gavilanes, Kitty W. Bloemenkamp, Monique Rijken, Gerard H. Visser, Ben Willem J. Mol, Frank van Bel, Jan B. Derks

13.00-14.30 Lunch

14.30-16.15 Session V - Fetal & Neonatal Cardiovascular I
Chairs: *A Llanos, JT Parer*

O21. Dynamical model based simulator of fetal cardiotocography for educational training purposes.
Francisco A. Guerra,, Pablo Moore, Rosario Negron, and Alfredo Illanes

O22. Human fetal endothelium require A_{2A}AR for generating angiogenesis.
Angelo Torres, Jesenia Acurio, Francisco Valenzuela, Patricio. Bertoglia, Carlos Escudero

O23. Mechanisms of hypoxic pulmonary vasoconstriction.
Norbert Weissmann

O24. Hemin Treatment Reverses Pulmonary Arterial Hypertension in High Altitude Neonates by increasing cGMP pathway.
Ebensperger G, Araya CE, Quezada S, González A, Leiva M, Herrera EA, Moraga F,Reyes RV, Díaz M,Silva P , Díaz PV, Llanos AJ

O25. Reactivity of the umbilical vessels of the late chicken embryo.
Riazudin Mohammed, Lilian Kessels, Eduardo Villamor

O26. Antioxidants protect against fetal growth restriction and programming of early adult-onset hypertension in ovine hypoxic pregnancy.
Kirsty Brain, Beth Allison, Youguo Niu, Christine Cross, Nozomi Itani, Andrew Kane, Katie Skeffington, Emilio A. Herrera and Dino A. Giussani

O27. Neonatal Antioxidant Treatment Prevents Impaired Cardiovascular Function at Adulthood Following Neonatal Glucocorticoid Therapy.
Nozomi Itani, Youguo Niu, Emilio A. Herrera, Rhys Evans and Dino A. Giussani

16.45 – 18.30 Session VI – Fetal & Neonatal Cardiovascular II
Chairs*: DA Giussani, N Weissmann*

O28. Role of oxidative stress in the microvascular control of preterm neonates.
Ian Wright, Trevor Mori, Celine Corbisier de Meaultsart, Vicky Clifton

O29. Melatonin rescues endothelial dysfunction during hypoxic development in the chick embryo. *Nozomi Itani, Katie Skeffington, Christian Beck & Dino A. Giussani*

O30. Melatonin modifies the pulmonary antioxidant capacity in neonates gestated under chronic hypoxia.
Flavio Torres, Camilo Montt, Alejandro Gonzalez, Germán Ebensperger, Roberto V. Reyes, Aníbal J. Llanos, Emilio A. Herrera

O31. The effect of chronic inflammation induced by low dose LPS infusion on fetal heart rate variation in preterm fetal sheep.
Christopher K Lear, Joanne O. Davidson, Alistair Jan Gunn, Laura Bennet

O32. Late gestational increases in maternal cortisol in ewes results in changes in gene expression pathways regulating responses to nutrient, cytokines and hypoxia in the septa of ovine fetal hearts.
Maureen Keller-Wood, Elaine M. Richards, Xiaodi Feng, Charles E. Wood, and Keith Walters

O33. Prenatal exposure to hyperoxia modifies the TP receptor-mediated response to H_2O_2 in the ductus arteriosus of the chicken embryo.
Saskia van der Sterren, Lilian Kessels, Francisco Perez-Vizcaino, Angel L Cogolludo, Eduardo Villamor

O34. Partial neuroprotection with selective nNOS inhibition during asphyxia in preterm fetal sheep.
Paul P. Drury, Laura Bennet, Joanne O. Davidson, Sidhartha Tan, Richard Silverman, Arlin Blood, Mhoyra Fraser, Lotte Van den Heuij, Alistair J Gunn

19.00-23.00 Conference dinner (drinks & dancing)

Tuesday 3th September

07.30-08.30 Registration + breakfast

08.30-10.00 Session VII - Placenta
Chairs: *P Casanello, C Escudero*

O35. The Hepcidin/Ferroportin regulatory system and Neonatal Hemochromatosis.
Martina U. Muckenthaler.

O36. How the placenta makes pregnant women vulnerable to influenza (Flu).
Jorge M. Tolosa, Kristy Parsons, Mauro Bendinelli, Peter Wark and Roger Smith

O37. Role of oxidative stress status on the impaired eNOS-dependent relaxation of intrauterine growth restriction-derived placental chorionic arteries.
Daniela Schneider, Bernardo Krause , Paola Casanello

O38. HDAC activity and nitric oxide control the expression of eNOS and arginase-2 in human umbilical artery endothelium in intrauterine growth restriction.
Bernardo Krause , Cherie Hernandez, Daniela Schneider, Paola Casanello

O39. Increased microvascular placental angiogenesis is associated to high tyrosine phosphorylation of vascular endothelial growth factor receptor type 2 in late and early-onset pre-eclampsia.
Carlos *Escudero*[*], *Cristian Celis, Tamara Saez, Sebastian San Martin, Francisco Valenzuela, Patricio. Bertoglia, James .M. Roberts, Jesenia Acurio*

O40. Experimental assessment of human umbilical vein compliance in physiological and IUGR pregnancies.
R. Thomasset, C. Guiot, R. Attini, A Rolfo, A.M. Nuzzo, E. Piccoli, T. Todros

10.00-10.45 Coffee break + poster viewing

10.45-12.30 Session VIII - DOHaD
Chairs: *M Hanson, H Richter*

O41. Intergenerational transmission via paternal line of cardiac disease risk by chronic fetal hypoxia.
Youguo Niu, Beth Allison, Andrew Kane, Ciara Lusby, Emily Camm & Dino A. Giussani

O42. Chronic prenatal hypoxia in the rat affects cognitive function and brain structure in adulthood: intervention by vitamin C.
Emily Camm, Christine Cross, Andrew Kane, Dino A. Giussani

O43. Effects of fetal amniotic exposure to lipopolysaccharide (LPS) on lung structure in adulthood in rats.
Keiji Suzuki, Hidehiro Takahashi, Hiroshi Masaki, Masanori Tamura

O44. Exposure to protein-restriction *in utero* followed by accelerated postnatal growth programmes hypothalamic and hepatic insulin resistance and accelerates metabolic aging.
Lindsey Berends, Y-C Loraine Tung, Peter Voshol, Susan E. Ozanne

O45. Intra-amniotic IGF1 treatment of the growth-restricted fetus alters mRNA expression of key somatotrophic genes in liver and muscle and adult glucose tolerance.
Ana-Mishel Spiroski, Mark Oliver, Travis Gunn, Anne Jaquiery, Jane Harding, Frank Bloomfield

O46. Programming of cardiac dysfunction by maternal diet-induced obesity.
Heather L Blackmore, Denise Fernandez-Twinn, Youguo Niu, Dino A Giussani and Susan E. Ozanne

O47. Gender difference in the influence of birth-weight on metabolic syndrome in 40 to 69 year old Japanese.
Shinji Katsuragi, Yoshihiro Miyamoto, Ryo Suzuki, Chikara Kihira, Tomoaki Ikeda

12.30-13.30 Invited lecture: Obesity a Key Determinant of Maternal and Fetal Health: can we stop the epidemic. (Prof. Dr. Ricardo Uauy, National Prize for Applied Sciences & Technology 2012, INTA, University of Chile, Chile).

13.30-15.00 Lunch

15.30-23.00 Sporting event + dinner

Wednesday 4th September

07.30-08.30 Registration

08.30-9.45 Session IX - Translational & Other
Chairs: *R Reyes, T Todros*

O48. FHR Pattern Management: Reconciliation of the NICHD Category II with the 5-tier System.
Julian T. Parer, Tekoa King, Tomoaki Ikeda

O49. Melatonin is an antenatal antioxidant and potential neuroprotectant in human pregnancy affected by fetal growth restriction.
Nicole Alers, Yen Pham, Jan Loose, Joanne Mockler, Euan Wallace, Suzanne Miller, Graham Jenkin

O50. Prophylactic erythropoietin exacerbates ventilation-induced lung inflammation in preterm lambs.
Graeme Polglase, Mary Tolcos, Valerie Zahra, Megan Wallace, Melissa Siew, Samantha Barton, Jacqueline Melville and Timothy Moss

O51. Erythropoietin through evolution: a speculative view.
Max Gassmann

O52. Ion channels in uterine smooth muscle: new insights into the regulation of contraction.
Helena C Parkington, Harold Coleman, Mary Tonta, Penelope Sheehan

O53. Blockade of connexin hemichannels is neuroprotective after asphyxia in the preterm fetal sheep.
Joanne O. Davidson. Davidson, Paul P. Drury, Louise F. Nicholson, Laura Bennet, Colin R. Green, Alistair Jan Gunn

O54. The effects of human amniotic epithelial stem cells on evolving brain injury in preterm fetal sheep.
Lotte Van den Heuij, Suzie Miller, Graham Jenkin, Mhoyra Fraser, Alistair Jan Gunn , Laura Bennet

9.45-10.15 Coffee break

10.15-11.00 Invited Lecture: The role of maternal melatonin in the programming of daily rhythms in energy metabolism. (Prof. Dr. José Cipolla-Neto, Instituto de Ciências Biomédicas, Universidade de São Paulo, Brazil).

11.00-12.30 Session X - Fetal-maternal circadian rhythm
Chairs: *J Cipolla-Neto, M Serón-Ferré*

O55. The night time party animal: circadian rhythms of the preterm sheep fetus.
Laura Bennet, Joanne O. Davidson, Paul P. Drury, Rachael L Robson, Eleanor Gunn, Alistair Jan Gunn

O56. Gestational chronodisruption has a far-reaching impact on fetal liver genomics.
Carlos Spichiger, Hugo Galdames, Claudia Torres-Farfan, Natalia Mendez, Pamela Alonso Vazquez, Hans Richter

O57. Impact of gestational chronodisruption on adult offspring physiology and therapeutic action of prenatal melatonin.
Mendez N, Vera H, Halabi D, Gavilan D, Spichiger C, Vergara K, Vilches N, Seron-Ferre M, Richter HG, Torres-Farfan C

O58. Absence of maternal melatonin is detrimental to melatonin synthesis in adult offspring.
Fernanda Amaral, Ariane Turati, Julieta Scialfa, José Cipolla-Neto

O59. Altered circadian rhytms of time birth is observed after the February 27th 2010 Chilean earthquake.
Valenzuela FJ, Cumsille P, Bertoglia P, Celis C, Acurio J, Escudero Cristian Celis

O60. Positive and negative effects of a social/chemical lubricant on asphyxial brain injury in preterm fetal sheep.
Paul P. Drury, Joanne O. Davidson, Laura Bennet, Lindsea C Booth, Sidhartha Tan, Mhoyra Fraser, Lotte Van den Heuij, Alistair J Gunn

12.30-13.00 Meeting adjourns – Awards ceremony

13.00-14.30 Farewell Lunch – Closing remarks

DAWES LECTURE

A LECTURE *AL ALIMÓN* ON LOW OXYGEN AND LIGHT-MELATONIN.

Aníbal J Llanos[1,2] and María Serón-Ferré[3]

[1]*Laboratory for Developmental Physiology and Pathophysiology, ICBM, Faculty of Medicine and* [2]*INCAS Universidad de Chile, Santiago, Chile;*[3]*Laboratory of Chronobiology, ICBM, Faculty of Medicine Universidad de Chile, Santiago, Chile.*

Corresponding author's email: allanos@med.uchile.cl; mseron@med.uchile.cl

The bullfighting called *al alimón* is one in which two bullfighters face a bull by holding the two sides of a spread red cape. Although the bullfighting was abolished in Chile in the early XIX century, here in this *arena*, like phantom bullfighters we will lecture *al alimón* grasping either side of a low oxygen and light-melatonin cape.

The fetal and neonatal milieu, in which the fetus and neonate are growing and developing, determine normal or abnormal development and growth. Among crucial stimulus that change markedly from fetus to newborn are oxygen and light-melatonin.

Oxygen, the waste product of photosynthesis, is a key molecule for ATP production. Chronic fetal and neonatal hypoxia is a challenge for normal survival of the fetus, newborn and adult. We studied chronic fetal and neonatal hypoxia in llamas and sheep, the former adapted, the latter acclimatized to the highlands. We found no pulmonary hypertension (PHT) in newborn (NB) llamas with overexpression of the HO-CO pathway, whilst NB sheep had PHT and little HO-CO expression. Moreover, the induction of this pathway in NB sheep reduced the PHT, opening an avenue for translational medicine.

The daily cycles of light/dark, imposed by living in our planet, are transduced to our body circadian clocks by the 24-h rhythm of the neurohormone melatonin. During pregnancy, maternal melatonin relies daily light/dark information to fetal biological clocks and serves a physiological role as preparation for newborn life. The newborn takes several weeks to start its own pineal melatonin production, thus physiological neonatal adaptation takes place in a low melatonin environment. As with hypoxia, melatonin disruption during fetal life has developmental consequences for postnatal life. Presently, we are studying the consequences of altering the low melatonin period in the neonatal transition.

Now the cape is free again, whirling in the mist of time.

Supported by FONDECYT 1090355 and 1090381, CHILE

INVITED LECTURE

Obesity a Key Determinant of Maternal and Fetal Health: can we stop the epidemic.

Ricardo Uauy[1,2], Maria Luisa Garmendia[2], Paola Casanello[1], Camila Corvalan[2], Juan P. Kuzanovic[1].
[1] *Faculty of Medicine, Catholic University of Chile, Santiago, Chile and* [2] *Institute of Nutrition INTA University of Chile Santiago, Chile* Corresponding author's email: druauy@gmail.com

Maternal obesity (pre-pregnancy body mass index higher (BMI) > 30 Kg/m2) is presently a common obstetric risk conditions, affecting over 28% of all pregnancies in Chile. An alarming increase in obesity in women of reproductive age has occurred over the past decades, one in four women are obese at their first prenatal visit. Maternal obesity is of particular concern because of the associated adverse maternal and fetal complications during pregnancy, delivery and postpartum. Pregnant obese women are four times more likely to develop gestational diabetes mellitus (GDM) and twice as likely to develop preeclampsia, compared with normal women. For neonates, maternal obesity is associated with a higher risk of macrosomia (heavy for a given gestation). Women with GDM also have a higher risk of subsequent type 2 diabetes, metabolic syndrome and cardiovascular disease. The offspring of obese women suffer the consequences of maternal obesity beyond intrauterine and perinatal life. The intrauterine environment affects short and long-term metabolic functions in the offspring via physiological and/or epigenetic mechanisms. There is increasing evidence that maternal over-nutrition represents a risk for childhood obesity, early adulthood obesity and insulin resistance, potentially leading to an intergenerational cycle of increasing risk for obesity. Excessive gestational weight gain (GWG) is also associated with adverse maternal and fetal outcomes. However the evidence in this case is weaker compared to that observed pre-pregnancy BMI. Meta-analyses reveal that higher GWG is associated with inadequate birth weight (large or small) for gestational age and with postpartum maternal weight retention.

INVITED LECTURE

The role of maternal melatonin in the programming of daily rhythms in energy metabolism.

José Cipolla-Neto[1].

[1] *Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil*

Corresponding author's email: cipolla@icb.usp.br

The daily production of melatonin in vertebrates is the biological mediator that makes possible the optimum relationship between the cyclic environment and the rhythmic organism.

Melatonin is able to time the interplay between the activity-wakefulness/rest-sleep cycle and the energy acquisition, storing and utilization cycle in order to best cope with the daily and seasonal environmental light-dark cycle.

In mammals melatonin is able to regulate every step in the energy cycle: the acquisition or fuel harvesting, regulating eating behaviour and the balance between energy storage and utilization, regulating insulin action and energy expenditure.

In the adult, the absence of melatonin or its reduction due to the aging process or to environmental agents like shift work and/ or light pollution, induces glucose intolerance, insulin resistance, dyslipidaemia, overweight and sleep and rhythmic disturbances. In this way, the daily energy metabolism rhythm is lost as far as insulin secretion and insulin action, glycolysis/gluconeogenesis balance and energy expenditure are concerned.

Transplacental melatonin signal from mother to foetus is critical not only for determining immediately adaptive physiological responses but, mainly for the priming of predictive adaptive responses that will appear later in life of the offspring. Among these are the daily energy metabolic responses. Rats born to pinealectomized dams show when 4 months old the typical picture of insulin resistance, glucose intolerance and disruption of daily distribution of insulin secretion and action. Treating the pinealectomized mother with melatonin, both during gestation and during lactation, prevent the future metabolic disorder.

The consequences of the present data for the human health will be discussed considering the 24 x 7 organization of the contemporary society, light pollution and social jet lag and the high incidence of obesity and metabolic diseases.

ABSTRACTS

POSTERS

Session I: All posters

Chairs:

Emilio A. Herrera (Universidad de Chile, Chile)

Claudia Torres-Farfan (Universidad Austral de Chile, Chile)

POSTERS

Fetal & Neonatal Cardiovascular

(Posters 01-11)

P01

Pentose phosphate pathway and NADPH oxidase inhibition impairs the response of chicken ductus arteriosus to oxygen.

Evangelia Tserga[1], Marina M. Goorts[1], Eduardo Villamor[1]
[1]*Department of Pediatrics, Maastricht University Medical Center (MUMC+), Maastricht, the Netherlands*
Corresponding author's email: e.villamor@mumc.nl

Introduction: NADPH derived from the pentose phosphate pathway (PPP) is a key system involved in maintaining the function of several important redox and antioxidant defense mechanisms. NADPH oxidases are multicomponent protein complexes containing a catalytic NOX subunit that transfers electrons from NADPH to oxygen, thereby forming reactive oxygen species (ROS). Normoxic contraction of the ductus arteriosus (DA), such as occurs at birth, appears to be dependent upon the increase of ROS in DA smooth muscle cells. In the present study we hypothesized a role for NOX-derived ROS in the signaling pathway of oxygen-induced contraction of the DA. Therefore, we investigated the effects of the inhibition of PPP and NOX in the ex vivo response of chicken DA to oxygen
Materials and Methods: Experiments were performed in myograph-mounted DA rings (pulmonary and aortic sides) isolated from chicken embryos incubated for 19 days (total incubation: 21-d).
Results and Discussion: Exposure to oxygen (21%) induced a sustained contractile response in the pulmonary but relaxation in the aortic side of 19-d DA. Incubation with the PPP inhibitor epiandrosterone or with the NOX inhibitors GKT-136901, VAS2870 and VAS3947 elicited a partial or complete impairment of oxygen-induced contraction. Phenylephrine- and KCl-induced contraction of chicken DA were impaired by epiandrosterone and VAS3947 but not by the other NOX inhibitors. Moreover, VAS3947 evoked an irreversible impairment of the contractility of the vessel. Oxygen-induced relaxation in the aortic part of the DA was not affected by NOX inhibitors.
Conclusion: Our data indicate that PPP and NADPH oxidase activation are events involved in the signaling cascade of normoxic contraction of chicken DA.

P02

Maternal dietary creatine supplementation attenuates changes in isolated heart function 1 month after asphyxia at birth.

Domenic A LaRosa[1], Stacey J. Ellery[1], Victor Suturin[2], Rod J. Snow[3], Helena C Parkington[2], David W. Walker[1,4] & Hayley Dickinson[1].

[1]Ritchie Centre, Monash Institute of Medical Research, Monash University, VIC 3800, Australia; [2]Department of Physiology, Monash University, Victoria 3800 Australia; [3]Centre for Physical Activity and Nutrition, Deakin University, Burwood, Victoria 3125; [4]Department of Obstetrics & Gynaecology, Monash Medical Centre, Clayton Victoria, Australia, 3168.

Corresponding author's email: domenic.larosa@monash.edu

Background: Previously, we showed that a maternal diet supplemented with creatine (Cr) from mid-pregnancy improves offspring survival and protects the newborn brain, diaphragm and skeletal muscle from damage induced by birth asphyxia. Surprisingly we found no acute structural changes in the heart 24h after birth asphyxia. The aim of this study was to determine if heart function was impaired at 1 month after an asphyxic birth despite this absence of structural changes at birth, and if so, whether maternal Cr supplementation protected the heart.

Method: Pregnant spiny mice were fed control or 5% Cr-supplemented diet from 20 days (0.5) gestation. One day before term, fetuses underwent birth by c-section (control) or after in utero asphyxia for 7.5 mins. Pups were then raised by a cross-foster dam for 33 ± 2 days. At necropsy, function of the isolated heart was assessed in vitro using a Langendorff preparation, when left ventricular pressure (LVP) and contractility (dP/dt), heart rate, and coronary flow rate were obtained under baseline conditions, and during a pharmacological challenge induced by increasing doses of Dobutamine.

Results: Relative and absolute heart weights were similar between groups. Under basal conditions there was a clear trend for increased end-diastolic pressure in pups asphyxiated at birth compared to c-section controls, a change not observed after birth asphyxia with Cr supplementation during pregnancy. Dobutamine revealed trends for decreased left ventricular contractility, heart rate and coronary flow rate in post-asphyxial hearts compared to c-section controls.

Conclusions: Despite the absence of acute structural changes after birth asphyxia, these functional data suggest that the hearts of asphyxiated animals may have attenuated diastolic relaxation, and may be limited in their ability to respond to stress. Prenatal creatine supplementation appears to attenuate the changes caused by birth asphyxia.

P03

Pulmonary vascular reactivity responses to pre and postnatal treatment with melatonin in chronic hypoxic newborn sheep.

Marcelino Véliz[1], Alejandro González-Candia[1], Santiago Ramírez [1], Claudio Araya[1], Sebastián Quezada[1], Germán Ebensperger[1], Emilio A. Herrera[1,2].

[1] *Laboratorio de Función y Reactividad Vascular, Programa de Fisiopatología, ICBM, Facultad de Medicina;*[2] *International Center for Andean Studies (INCAS); Universidad de Chile, Chile.*

Corresponding author's address: eherrera@med.uchile.cl

Introduction. Gestation under chronic hypoxia as in high-altitude (HA) causes a reduction of oxygen to the tissues, increased ROS production generating oxidative stress, and favours a pulmonary vasoconstrictor state. This may cause intrauterine growth restriction (IUGR) and neonatal pulmonary hypertension[1]. Melatonin is a neurohormone with important antioxidant properties. During pregnancy melatonin crosses freely the placenta and can increase umbilical vasodilatation[2]. Therefore, we hypothesise that a pre or postnatal treatment with melatonin may improve the birth weight and the neonatal pulmonary vascular reactivity.

Materials and Methods. Fifteen HA lambs were gestated, born and studied at Putre (3,600 m.a.s.l.). Five received maternal oral melatonin (MM, 10mg.kg^{-1}.d^{-1}) in the last third of gestation; five received postnatal oral melatonin (MN, 1mg in 0.5ml.kg^{-1}.d^{-1}) and five received vehicle (CN, 0.5 ml.kg^{-1}.d^{-1}) for 7 days. At day 8 of postnatal treatment (12 d old), the lambs were euthanized with sodium thiopenthone and small resistance pulmonary arteries (PA) were mounted in a wire myograph to assess vascular reactivity.

Results and Discussion. Maternal melatonin (MM) decreased the contractile capacity relative to MN and CN (197.3 ± 7.17 *vs.* 233.2 ± 12.70 *vs*. 391.3 ±12.34, respectively). Furthermore, pre and postnatal melatonin increased the contractile response to serotonin (Fig. 1a). In addition, prenatal melatonin decreased while postnatal increased the muscular dilatation response to a nitric oxide donor (SNP, Fig.1b). In contrast, prenatal treatment decreased the endothelium-dependent maximum dilatation to methacholine. However, postnatal melatonin increased the sensitivity to this agent.

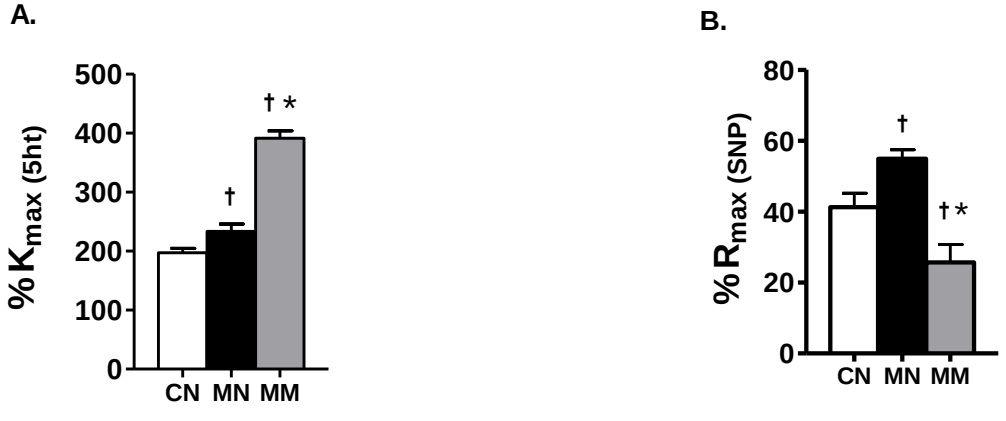


Figure 1. Pulmonary vascular function. Maximal contractile response to 5ht (A, %Kmax), and nitric oxide dependent maximal vasodilatation (B, %Rmax). Values are mean ± SEM in control (CN, white bar), post-natal melatonin (MN, black bar) and prenatal melatonin (MM, gray bar). Significant differences ($p<0.05$): † *vs* CN, ***** MM *vs* MN.

Conclusion. Postnatal melatonin enhanced the vasodilator function in newborn sheep at HA. However, prenatal melatonin diminished the vasodilator responses. In the view of these results, careful should be taken when considering melatonin treatment during pregnancies.

References: 1. Herrera *et al*. Am J Physiol 299, R1676-R1684, 2007

2. Lemley *et al.* Animal Sep;7(9):1500-7, 2013.

Supported by FONDECYT 1110595, 1120655 & 1130424.

P04

Pulmonary vascular responses to hypoxemia and cyclooxygenase blockade in highland and lowland neonatal llamas.

Marcela Díaz[1,3], Germán Ebensperger[1], Emilio A. Herrera[1,2], Roberto V. Reyes[1], Aníbal J. Llanos[1,2]
[1] Laboratory *for Developmental Physiology and Pathophysiology, Faculty of Medicine, Universidad de Chile, Santiago, Chile.*[2] International Center for Andean Studies *(INCAS), Universidad de Chile, Santiago & Putre, Chile.*
[3] *Midwifery School, Faculty of Medicine, Universidad de Chile, Santiago, Chile.*
Corresponding author's email: allanos@med.uchile.cl

Introduction: The llama *(Lama glama*) dwells in the *Alto Andino* developing efficient mechanisms to withstand hypoxia[1], for instance, the absence of pulmonary hypertension (PHT) in both, adults[2] and neonates[3]. In this study, we investigated whether neonatal llamas (NBLL) respond with pulmonary hypertension to an episode of acute hypoxia and COX dependent mechanisms role in the pulmonary vascular function.

Materials and Methods: Five lowland NBLL born at Santiago (580masl) and six highland NBLL, born at Putre (3,600masl) were used. Under general anesthesia, a pulmonary Swan-Ganz catheter was placed. Experiments consisted in 45 min of basal, 15 min of basal plus infusion (either saline or 1.5 mg Kg^{-1} infusion of a COX inhibitor, indomethacin), 60 min of isocapnic hypoxia plus infusion and 60 min of recovery, during basal and recovery periods, NBLL breathed atmospheric air. To induce hypoxia (PO_2 ≈30 mmHg), a mix of air, N_2, and CO_2 was blown into a bag placed over the animal's head. Two-way ANOVA was used for comparing groups (p< 0.05). (CBA Nº0282#097 FAMUCH).

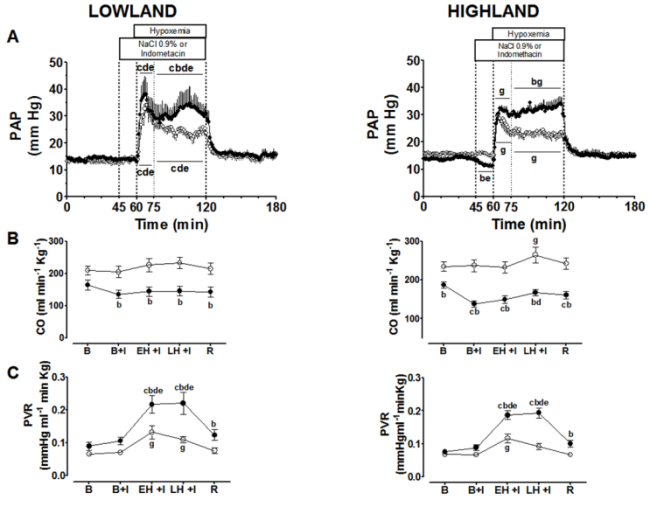


Figure 1. Pulmonary Vascular Responses in lowland and highland newborn llama infused with NaCl 0.9% (○) or Indomethacin (●) A, continuous recording of pulmonary artery pressure (PAP) ; B, cardiac output (CO) and C, pulmonary vascular resistance (PVR). Values are mean + S.E.M. taken at Basal (B), Basal+NaCl 0.9% (B+I), Early Hypoxemia+NaCl 0.9% (EH+I), Late Hypoxemia+NaCl 0.9% (LH+I) and Recovery (R). Significant differences at p<0.05 (ANOVA + Student Newman-Keuls test) are shown as: a vs LA with the same treatment; b vs NaCl 0,9% ; c vs basal; d vs basal + inf; e vs recovery; g vs all except Hypoxia.

Results and Discussion: Lowland and highland NBLL had similar values of pulmonary artery pressure (PAP) and resistance (PVR) basally. Both groups responded to acute hypoxemia with a biphasic increased in PAP and PVR. Indomethacin decreased basal PAP in highland but not in lowland, which could be explained by a decrease function of the vasoconstrictor thromboxane. During hypoxemia there was an increase in PAP in both groups, however the indomethacin group had a greater increase than the saline group, due to a decrease in the vasodilator prostacyclin. In both altitudes, PVR was greater in hypoxemia during indomethacin infusion compared to saline. In contrast, cardiac output decreased with indomethacin during hypoxia (Figure 1).

Conclusion: These results show that COX dependent mechanisms have an important role in modulating the pulmonary circulation of the NBLL.

Supported by FONDECYT 1090355, 1130424 - Chile.

References

1.- Llanos AJ, Riquelme RA, Herrera EA, et al. Evolving in thin air-lessons from the llama fetus in the altiplano. *Respir Physiol Neurobiol* 30 158(2-3):298-306, 2007.

2.- Llanos AJ, Ebensperger G, Herrera EA, et al. Fetal and postnatal pulmonary circulation in the Alto Andino. *Placenta* 32 Suppl 2:S100-3, 2011.

3.- Herrera EA, Reyes RV, Giussani DA, et al. Carbon monoxide: a novel pulmonary artery vasodilator in neonatal llamas of the Andean altiplano. *Cardiovasc Res* 77: 197–201, 2008.

P05

Role of the RhoA/ROCK pathway in neonatal pulmonary hypertension induced by chronic hypoxia during gestation.

Nandy López[1], Germán Ebensperger[1,2], Emilio A. Herrera[1,2], Roberto V. Reyes[1,2], Gloria Calaf[3], Aníbal J. Llanos [1,2].
[1] *Laboratory for Developmental Physiology and Pathophysiology, Program of Pathophysiology, ICBM, Faculty of Medicine, Universidad de Chile, Santiago, Chile* [2] *International Center for Andean Studies (INCAS), Universidad de Chile* [3] *Institute for Advanced Research, Tarapacá University, Arica, Chile.*
Corresponding author's email: allanos@med.uchile.cl

Introduction: RhoA/ROCK pathway has been involved in development and maintenance of hypoxia-induced pulmonary hypertension in adults. Here we determine in pulmonary hypertensive neonatal lambs whose gestation took place in the *Alto Andino*, the effect of chronic hypoxia on the expression of RhoA/ROCK pathway and the cardiopulmonary effects of inhibition of ROCKs with fasudil.
Materials and Methods: We divided ten high altitude newborn lambs (HA) gestated and born at Putre (3,600 m) into two groups: five received oral treatment with fasudil (HAF, 10 mg kg^{-1} day^{-1} for 10 days) and five received the vehicle (HAC). In addition, we studied five lowland neonatal lambs (LAC) from Lluta (50 m) as lowland controls. At the end of the treatment, we subjected the HA to a superimposed episode of hypoxemia (1h air, 1h 10% O_2, 1h air) recording the pulmonary and systemic hemodynamic responses. After the study we euthanized the lambs and studied pulmonary gene expression (qPCR), protein expression and the small pulmonary artery histology (Local bioethical approval CBA #0315 FMUCH).

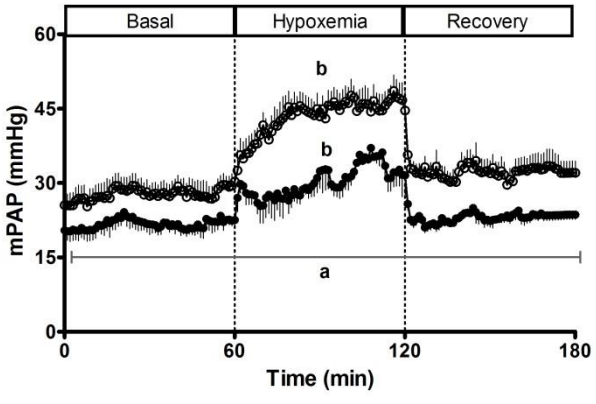


Fig. 1. Mean pulmonary arterial pressure (mPAP) in HAC (open circles) and HAF (solid circles) during acute hypoxia. PAP was recorded continuously during 60 min of normoxia, 60 min of hypoxia and 60 min of recovery. Values expressed as mean ± SEM. Significant differences P<0.05:†vs basal; a *vs* HAC, b *vs* basal.

Results and Discussion: Fasudil decreased pulmonary pressure, basally and under acute hypoxia (HAF: 30.0 ± 0.4 *vs.* HAC: 42.0 ± 0.4mmHg, P<0.05) with no changes in systemic circulation. Fasudil also diminished muscular area of small pulmonary arteries (HAF: 30.0 ± 1.4 *vs.* HAC: 47.4 ± 3.7%, P<0.05) and inhibited the phosphorylation of the myosin light chain phosphatase by 64%. In HAC *vs.* LAC lungs, *in utero* chronic hypoxia doubled protein expression of RhoA and inhibited in 28% ROCKII (P<0.05). Finally, hypoxia induced RhoA and ROCKII mRNA expression in PASMCs of HAC *vs.* LAC neonates (P<0.05).

Conclusion: RhoA/ROCK pathway participates in the development of pulmonary hypertension produced by chronic hypoxia during gestation at high altitude. The inhibition of ROCKs by fasudil constitutes a potential therapy in neonates that underwent chronic hypoxic *in utero.*

References

1.- Llanos AJ, Ebensperger G, Herrera EA, Reyes RV, Pulgar VM, Serón-Ferré M, Díaz M, Parer JT, Giussani DA, Moraga FA, Riquelme RA. Fetal and postnatal pulmonary circulation in the Alto Andino. *Placenta* 32 Suppl 2:S100-3, 2011.
2.- Duong-Quy S, Bei Y, Liu Z, Dinh-Xuan AT. Role of Rho-kinase and its inhibitors in pulmonary hypertension. *Pharmacol Ther*. 137(3):352-64, 2013.
Supported by FONDECYT 3100080 y 1090355, Chile.

P06

Hemin treatment reverses right ventricle hypertrophy, decreases pulmonary arterial pressure and improves postnatal growth in higland newborn lambs.

Santiago Ramírez[1], Marcelino Véliz[1], Sebastián Quezada[1], Alejandro González-Candia[1], Claudio Araya[1], Emilio A. Herrera[1,2], Aníbal J. Llanos[1,2], Marcela Díaz[1], Patricia V. Díaz[1], Germán Ebensperger[1].

[1]Pathophysiology Program, Faculty of Medicine; [2]International Center for Andean Studies; Universidad de Chile, Santiago, Chile.

Corresponding author's email: gebensperger@med.uchile.cl

Introduction. Gestation and birth at high altitudes (HA) induces marked morphometric and hemodynamic changes in newborns, such as growth restriction[1], increased ardiac output (CO), pulmonary arterial pressure (PAP) and resistance (PVR)[2], leading to pulmonary hypertension and cardiopulmonary remodeling, which may derived in right ventricle hypertrophy[3]. Hemin, an Heme Oxigenase enzyme inductor, is known for its antiproliferative effect in smooth muscle cells[4], and for decreases PAP in hypertensive lambs. In this study, we hypothesised that hemin reverts the right ventricle hypertrophy and improves the neonatal growth in chronically hypoxic newborn lambs gestated at high altitudes.

Materials and Methods. 60 newborn lambs were analyzed in a retrospective fashion, 28 of them were gestated, born and studied at low-altitude (LA), 21 at high-altitude (HA), and 11 at high-altitude with daily post-natal Hemin treatment (H-HA,15mg/kg/d for 10 d). All groups were catheterized at 3 d old under general anaesthesia. Thereafter, hemodynamic variables (PAP, SAP, CO) were daily recorded until 15 d old. In addition, heart rate (HR), pulmonary (PVR) and systemic (SVR) vascular resistances were calculated. The neonates were euthanized (Sodium Thiopenthone 100 $mg.kg^{-1}$) at 15 d old, all organs were weighed and a right ventricle hypertrophy index (RVHi) was calculated. All procedures were approved by Local Ehtical Committee.

Results and Discussion. HA induced intrauterine growth restriction and increased the RVHi. In addition, Hemin improved postnatal growth (HA: 4.808 ± 0.205 vs H-HA 6.878 ± 0.234 Kg: Fig. 1), decreased the RVHi in high-altitude (Fig.2), and decreased PAP (HA 31 ± 1 vs H-HA 21 ± 1 mmHg $P < 0.05$) at 15d old.

Conclusions. Postnatal hemin treatment reverts cardiac remodeling and improves postnatal growth in chronic hypoxic neonates. Future studies will focus on the molecular mechanisms behind the cardiovascular effects of this treatment. Hemin arises as a potential effective treatment for pulmonary hypertension in the neonate with chronic hypoxia.

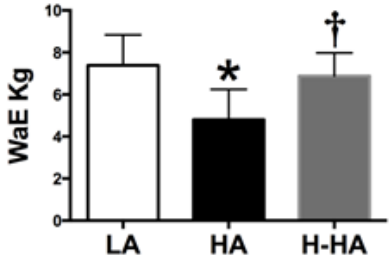


Fig. 1 Weight at euthanasia (WaE). Values are Mean ± SEM. Groups are low-altitude (LA, n=28), high-altitude (HA, n=21) and Hemin-treated high-altitude (H-HA, n=11) newborns. Statistical differences ($P < 0.01$): * vs. LA; † vs HA.

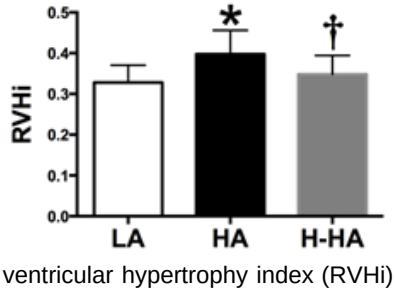


Fig. 2 Right ventricular hypertrophy index (RVHi). Values are Mean ± SEM. Groups are low-altitude (LA, n=28), high-altitude (HA, n=21) and Hemin-treated high-altitude (H-HA, n=11) newborns. Statistical differences ($P < 0.05$): * vs. LA; † vs HA.

1- Ream M *et al*., 2008. Am J Physiol Regul Integr Comp Physiol295(2): R583–R595

2- San-T *et al*., 2013 The Scientific World Journal.

3- McLoughlin P *et al*., 2007J Appl Physiol 103:1451-1453.

4- Chang T *et al.,* 2008. Am J Physiol Heart Circ Physiol 295:H999-H1007,

Supported by Grants CONICYT, FONDECYT 1130424, 1090355, 1110595 y 1120605.

P07

Hemin decreases the remodeling on the pulmonary artery trunk and small pulmonary arteries in high altitude neonatal lambs.

Alexander Riquelme[1,3], Germán Ebensperger[1,2], Gertrudis Cabello[4], Emilio A. Herrera[1,2], Roberto V. Reyes[1,2], Aníbal J. Llanos[1,2].

[1] Laboratorio de Fisiología y Fisiopatología del Desarrollo, Facultad de Medicina, Universidad de Chile, Santiago, Chile

[2] International Center for Andean Studies (INCAS), Universidad de Chile, Santiago & Putre, Chile

[3] Instituto de Estudios de la Salud, Universidad Arturo Prat, Iquique, Chile.

[4] Facultad de Ciencias, Universidad de Tarapacá, Arica, Chile

Corresponding author's email: allanos@med.uchile.cl

Introduction. Neonatal lambs whose gestation took place at the *Alto Andino* develop pulmonary arterial hypertension[1], vascular remodeling with pulmonary artery wall thickening[2]. Hemin, an inducer of the heme oxygenase-1 and 2 (HO-1, HO-2) increases the production of carbon monoxide (CO) reducing the neonatal lamb pulmonary artery pressure[3]. Since CO has also antiproliferative properties, we determine whether hemin reverses the pulmonary vascular remodeling by decreasing the proliferation of smooth muscle cells of the pulmonary arteries.

Materials and Methods. Two groups of pulmonary hypertensive lambs gestated and born in Putre (3,600m) were used, one treated with hemin for 10 days (15 $mg.kg^{-1}$ s.c.; HN, n=5), and the other with vehicle (CN, n=5). At the end of treatment, we euthanized the lambs obtaining the main pulmonary artery and lung for histology and immunohistochemistry, and for gene and protein expression assays. Histological stains were Verhoeff-van Gieson, van Gieson, and hematoxylin-eosin. We used Ki67 immunohistochemistry determination as a proliferation marker. Furthermore, we measured pulmonary RT-PCR and Western blotting for proteins that regulate cell cycle, such as p53 total phosphorylated and cyclin D1.

Results and Discussion. Hemin induced thinning of the muscular layer of the pulmonary artery trunk (HA: 767,6 ± 109,5µm *vs* CN: 1144,0 ± 28,1µm), associated with a decreased density of SMC (HA: 3775 ± 66nucleus/mm2 vs CN: 4320 ± 206nucleus/mm2) and a lower percentage of proliferating cells measured by Ki67 (HA: 7,0 ± 1,1% *vs* CN: 13,3 ± 1,2%). Furthermore, in small pulmonary arteries we found a reduced number of smooth muscle cells and less proliferation. Further, hemin decreased cell proliferation by increasing phosphorylated p53 and decreasing cyclinD1.

Conclusion. Hemin has important antiproliferative effects, inducing thinning of pulmonary trunk and small pulmonary artery in chronically hypoxic neonates. The decrease in cell proliferation is mediated partially by HO1&HO2/CO/p53-phosforylated/cyclin-D1 pathway, arresting the cell cycle.

Supported by FONDECYT 1090355, 1110595 and 1130424 CHILE; GORE-FNDR-BIP 30125349-0.

1.- Herrera et al. Am J Physiol Regul Integr Comp Physiol 292:R2234-R2240, 2007.

2.- Herrera et al. Pediatr Res 63: 169–175, 2008.

3.-Ebensperger G Tesis Doctoral, Universidad de Chile, Facultad de Medicina, Escuela de Postgrado. Santiago, Chile. 139p, 2012.

P08

Hemin treatment attenuates the small pulmonary arteries vasoconstrictor function in hypertensive high altitude neonatal lambs.

[1]Araya CE, [1]Valenzuela JM, [1]Uribe M, [1]Valenzuela A, [1]Quezada S, [1]González A, [1]Leiva M, [1,2]Herrera EA, [3]Moraga F, [1]Reyes RV, [1]Díaz M, [1]Díaz PV, [4]Silva P , [1,2]Llanos AJ, [1]Ebensperger G

[1]Unidad de Fisiología y Fisiopatología Perinatal, Facultad de Medicina, [2]INCAS, Universidad de Chile; [3]Laboratorio de Fisiología, Escuela de Medicina, Universidad Católica del Norte, Coquimbo, Chile; [4]Departamento de Ciencias Biológicas y Fisiológicas, Universidad Peruana Cayetano Heredia, Lima, Perú.

Corresponding author's email: gebensperger@med.uchile.cl

Introduction. High altitude newborn lambs develop pulmonary hypertension[1]. Hemin treatment, a heme-oxygenase inducer, partially reversed pulmonary hypertension and vascular remodeling[2]. We hypothesize that the decrease in PAP after hemin treatment is partially due to a decreased function of main vasoconstrictor (and mitogenic) pathways such as, endothelin, serotonin and thromboxane.

Materials and Methods. Fourteen lambs, whose gestation, birth and experimental procedures took place in high altitude (Putre, 3,600m), were divided in two groups: 7 neonates were treated for 10 days with hemin (15 mg/Kg/day, S.C.) and 7 neonates were treated with vehicle (0.01N NaOH buffered with saline solution) for 10 days and used as controls. After treatment, they underwent euthanasia with an overdose of sodium thiopentone (100 mg•kg^{-1} IV) to study the vascular responses of small pulmonary arteries. Concentration-response curves (CRCs) were analyzed in terms of sensitivity and maximal responses to endothelin-1, thromboxane A2 mimetic (U46619) and serotonin utilizing a wire myograph. Moreover, we determined the mRNA expression to pre-pro endothelin and both endothelin receptors A and B. All procedures were approved by a local ethical committee (CBA # 0561 FMUCH).

Results and Discussion. Hemin induced a decreased maximal contraction to all tested vascoconstrictors (Fig.1; $p<0.05$). Further, there was a decreased ETa & ETb receptors expression in the hemin-treated group ($p<0.05$).

Conclusions: The lower pulmonary arterial pressure in the hemin-treated newborns is partially explained by the decreased function of vasoconstrictor (and mitogenic) agents. Studies are in progress to investigate the molecular mechanisms involved in pulmonary vascular function improvement.

Supported by CONICYT, FONDECYT 1130424, 1090355, 1110595 y 1120605.

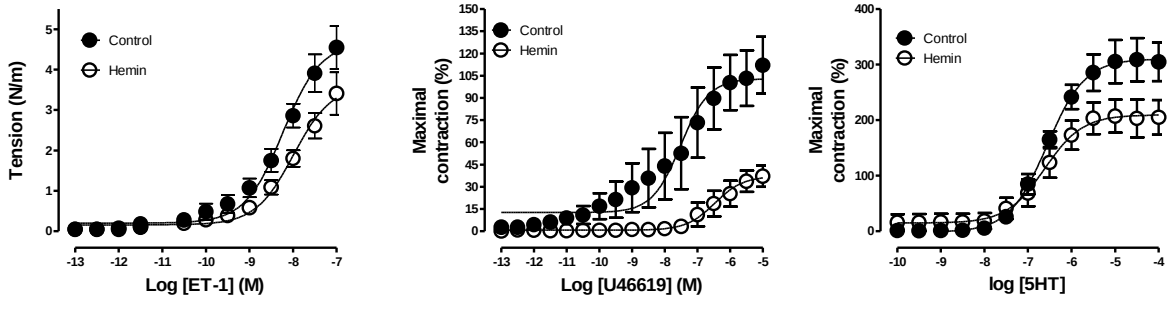


Fig 1. Pulmonary vasoconstrictor function. . Values are mean ± SEM. Groups are control newborns (close circles) and hemin-treated newborns (open circles). Concentration-response curves to (A) endothelin-1, (B) U46619 and (C) and serotonin. Significant differences ($p<0.05$): a *vs. Hemin treated lambs Maximal response;* b *vs. Hemin treated lambs sensitivity*.

References:

(1) Herrera EA. et al. *Am. J. Physiol*. 292(6):R2234-R2240.2007.
(2) Ebensperger G et al. *Fetal and Neonatal Physiological Society Annual Meeting 2009.*
(3) Ebensperger G Doctoral thesis. *2011.*

P09

Prenatal melatonin improves systemic and cerebrovascular function in neonatal lambs gestated under chronic hypoxia.

Alejandro González-Candia[1], Marcelino Véliz[1], Siri-Marie Solbakken[1], Sebastián Quezada[1], Santiago Ramirez[1], Claudio Araya[1], Germán Ebensperger[1], Emilio A. Herrera[1,2].

[1] *Laboratorio de Función y Reactividad Vascular, Programa de Fisiopatología, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile.* [2] *International Center for Andean Studies (INCAS), Universidad de Chile, Putre, Chile.*

Corresponding author's email: eherrera@med.uchile.cl

Introduction. High altitude newborn lambs (HA) present intrauterine growth restriction (IUGR) and pulmonary hypertension (PHT) due to chronic hypoxia[1]. Oxidative stress is enhanced in chronic hypoxia and has a causal role in PHT, leading to pulmonary vascular dysfunction[2]. Melatonin is an endogenous antioxidant that neither the fetus nor the early neonate are able to synthethised[3]. Therefore, we hypothesized that prenatal treatment with melatonin may prevent IUGR, PHT and improve the cardiovascular function in neonates exposed to chronic hypoxic.

Materials and Methods. Ten HA lambs were gestated, born, catheterized and studied at Putre (3,600 m). Five were treated with oral melatonin during the last third of gestation (maternal 10mg.kg^{-1}.d^{-1}) and five treated with vehicle (5ml.kg^{-1}.d^{-1}). At postnatal day 3, neonates were instrumented under general anaesthesia. Thereafter, daily biometry and cardiovascular variables such as cardiac output (CO), heart rate (HR), pulmonary arterial pressure (PAP) and systemic arterial pressures were recorded until 11 days of life.

Results and Discussion. During the first 11 ds after birth, animals treated with melatonin showed a decreased systemic vascular resistance (SVR) and carotid vascular resistance (CVR) (Figure 1A,B,D). In addition, melatonin increased carotid blood flow (CBF/kg) relative to controls (Figure 1C). However, both groups have similar cardiopulmonary variables (CO, HR and PAP).

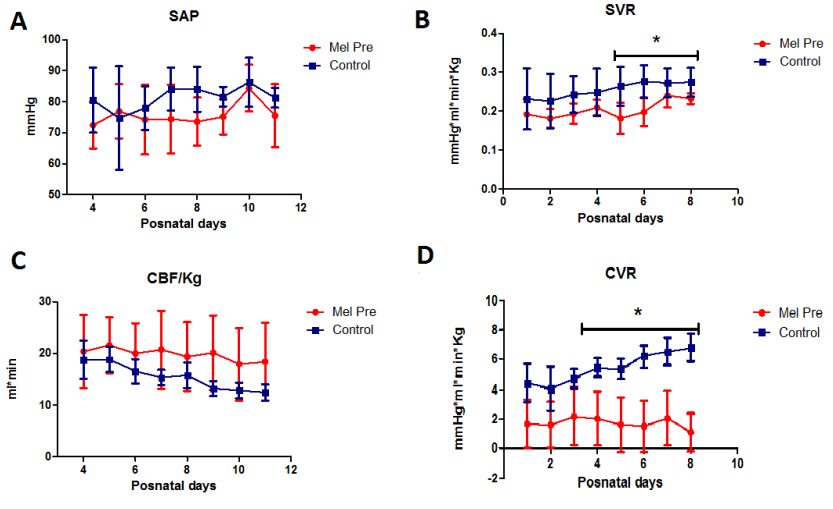


Figure 1. Hemodynamic variables. Systemic arterial pressure (A), Systemic vascular resistance (B), Carotid blood flow (C) and Carotid vascular resistance (CVR). Values are mean ± SEM in control (blue circles) and melatonin prenatal (red squares) neonates.

Conclusion. The premature fall in SAP, SVR and CVR with melatonin indicates that the treatment may improve cardiovascular and cerebrovascular function. Although no significant differences were found in the pulmonary circulation between groups, prenatal melatonin may be beneficial for neonatal systemic vascular function. This work partially supports the potential use of melatonin for clinical intervention against brain hypoxic damage or a developmental origin of cardiovascular disease in complicated pregnancy. However, careful should be taken when considering melatonin treatment during pregnancies.

Supported by FONDECYT 1110595, 1120655 & 1130424.

P10

Administration of 2-aminoethyldiphenylborinate modifies pulmonary vascular remodeling and reactivity in chronic hypoxic newborn lambs.

Daniela Parrau[1,3], Germán Ebensperger[1,2], Emilio A. Herrera[1,2], Ismael Hernandez[1], Débora Santos[1], Sebastián Quezada[1] Aníbal J. Llanos [1,2], Roberto V. Reyes[1,2].

[1]*ICBM, Faculty of Medicine, Universidad de Chile, Santiago, Chile;* [2]*International Center for Andean Studies (INCAS), Universidad de Chile;* [3]*Faculty of Medicine, Universidad Diego Portales.*

Corresponding author's email: vicreyes@med.uchile.cl

Introduction. We demonstrated that 2-aminoethyldiphenylborinate (2-APB) attenuates hypoxic pulmonary vasoconstriction (HPV) in newborn lambs. Further, the inhibition of HPV by 2-APB is stronger in high altitude than in low altitude newborn lambs. The latter is related with a greater pulmonary expression of store operated channels (SOC) under chronic hypoxia[1]. Here, we evaluated if prolonged administration of 2-APB reduces pathologic pulmonary vascular remodeling, vascular reactivity and hypertension in chronic hypoxic newborn lambs.

Materials and Methods. Ten high altitude newborn lambs (HA) gestated and born at Putre (3,600m) underwent catheterization at 2-3 days-old and were divided in two groups: five received treatment with 2-APB (10 $mg.kg^{-1}.day^{-1}$ i.v.) and five received vehicle (DMSO:saline, 1:10). Treatments were administered for 10 days. During treatment, pulmonary artery pressure (PAP) and cardiovascular variables were monitored daily. At 11^{th} day, the cardiovascular response was evaluated *in vivo* in 3h protocol, 1 hour of basal, acute hypoxia and recovery[1]. At 12^{th} day, small pulmonary arteries were isolated to determine vascular reactivity to U46619 (TxA2 agonist), sildenafil (PDE5 blocker) and fasudil (Rhokinase inhibitor) by wire myography. Further, pulmonary artery morphometry, expression of PDE5 and Rhokinase2 were determined in lung (Local bioethical approval CBA #0476 FMUCH).

Results: 2-APB treatment partially reversed pulmonary hypertension, and abolished the increase in PVR induced by acute hypoxia. In addition, 2-APB reduced the pulmonary vascular remodelling and response to U46619, but did not change the TxA2 receptor expression. Both maximal relaxation and sensitivity to sildenafil were greater in treated lambs, but PDE5 expression diminished. Further, 2-APB increased the sensitivity to Fasudil, whilst Rhokinase-2 expression remained unchanged.

Conclusion: 2-APB reduces the pulmonary hypertension of HA lambs and modifies the reactivity of small pulmonary arteries. Agents such as 2-APB, which inhibit SOC among other Ca^{2+}-related signalling mechanisms, could be a valuable tool to treat pulmonary hypertension.

Supported by FONDECYT 1120605,1080663,1110595,1130424 - Chile.

References

1.- Parrau D, Ebensperger G, Herrera EA, Moraga F, Riquelme RA, Ulloa CE, Rojas RT, Silva P, Hernandez I, Ferrada J, Diaz M, Parer JT, Cabello G, Llanos AJ, Reyes RV. Store operated channels in the pulmonary circulation of high and low altitude neonatal lambs. *Am J Physiol Lung Cell Mol Physiol* 304: L540 – L548 (2013).

P11

Post-natal oxygenation at sea level reduces pulmonary hypertension in high altitude newborn lambs.

Caroll Aburto[1], Claudio Araya[1], Ismael Hernandez[1], Roberto V. Reyes[1], Germán Ebensperger[1], Emilio A. Herrera[1,2], Aníbal J. Llanos[1,2].

[1]Pathophysiology Program, Faculty of Medicine; [2]International Center for Andean Studies; Universidad de Chile, Santiago, Chile.

Corresponding author's email: allanos@med.uchile.cl

Introduction. The cardiovascular system experiences important changes in response to hypoxic environments[1,2]. The intensity of these changes depends on the extension and level of lower PO_2 exposition [3]. Specifically, in the perinatal period, chronic hypoxia induces pulmonary hypertension with cardiopulmonary remodeling. We propose that the immediate post-natal oxygenation at sea level can reverse this neonatal condition in newborns fully gestated under chronic hypoxia.

Materials and Methods. 25 newborn lambs were divided in 3 groups: 1, Gestated and delivered in altitude (HHH, 3600 mts; n=9); 2, Gestated and delivered at sea level (LLL, 560 mts; n=11); and 3, Gestated and delivered at altitude and taken to sea level at three days old (HHL, n=5). All groups were catheterized with a Swan-Ganz and a femoral catheter at 4 days old under general anesthesia. At 15 days old, lambs were submitted to three experimental periods of 60 min. each, basal, acute hypoxia ($PO_2 \approx 30$ mmHg) and recovery. Pulmonary arterial pressure (PAP), systemic arterial pressure (SAP) and cardiac output (CO) were recorded. In addition, heart rate (HR), systemic (SVR) and pulmonary (PVR) vascular resistances were calculated. All procedures were approved by a local ethical committee (CBA Nº0282#097 FAMUCH).

Results and Discussion. Post-natal oxygenation decreased PAP in HHL relative to HHH (HHL: 20,55 ± 1,1 $p<0,05$ *vs* HHH: 36,5 ± 1,4; Fig. 1). Further, in response to acute hypoxia, LLL and HHL showed a stronger CO response relative to HHH (LLL: 459 ± 10 *vs* HHL 471 ± 10 *vs* HHH: 375 ± 7, $p<0,05$; Fig. 1).

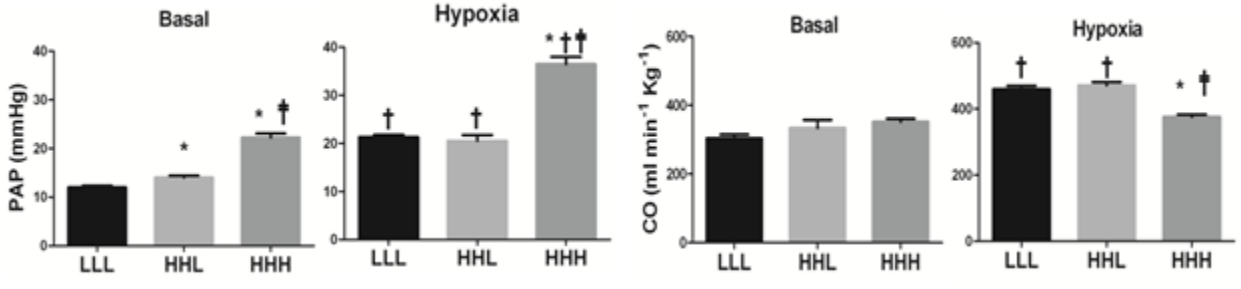


Fig 1. Neonatal cardiopulmonary variables. Values are mean ± SEM. Groups are neonates gestated, born, raised and studied at lowland (LLL), gestated and born at highland and raised and studied at lowland (HHL), and gestated, born, raised and studied at highland (HHH). Significant differences ($p<0.05$): * *vs.* LLL; ‡ vs. HHL; † *vs.* Basal period.

Conclusion: Post-natal oxygenation contributes to the cardiopulmonary function improvement in highland neonates. Futures aims of this study will focus on the structural changes and molecular mechanisms involved in the cardiovascular responses to postnatal oxygenation.

Supported by FONDECYT 1090355, 1120655 & 1110595.

1. Abman SH. *Pediatr Rev* 20: e103–e109, 1999.
2. Herrera EA, Pulgar VM, Riquelme RA, Sanhueza EM, Reyes RV, Ebensperger G, Parer JT, Valdéz EA, Giussani DA, Blanco CE, Hanson MA, Llanos AJ. *Am J Physiol Regul Integr Comp Physiol*. 292(6):R2234-40. 2007.
3. Huicho L. *Respir Physiol Neurobiol* 158(2-3):190-203, 2007.

POSTERS

Fetal & Neonatal Brain

(Posters 12-16)

P12

Repeated cerebral hypoxia-ischemia in the very preterm ovine fetus appears to alter brain development.

Robert De Matteo[1], Mary Tolcos[2], Nadia Hale[2], Amy Shields[2], Richard Harding[1], Takushi Hanita[1]
[1]*Department of Anatomy and Developmental Biology, School of Biomedical Sciences, Monash University, Australia*
[2]*The Ritchie Centre, Monash Institute of Medical Research, Australia*
Email: robert.dematteo@monash.edu

Introduction: Cerebral autoregulation is impaired in extremely preterm infants[1]. Perturbations of cerebral haemodynamics in the preterm infant may lead to hypoxic–ischemic (HI) injury to the developing brain. Our aim was to investigate the effects of repeated HI on the developing brain in the preterm ovine fetus.
Materials and Methods: Pregnant ewes underwent surgery at 101 days of gestation (DG, term ~147 DG) to implant a pair of inflatable cuffs around each carotid artery. At 106 DG, five 10 min carotid artery occlusions (HI group: n=8) were performed at 30 min intervals. Fetal brains were collected 5 days later. Three sham operated fetuses and 5 uncatheterized twin fetuses served as controls (n=8). Haematoxylin and Eosin sections were used for anatomical assessment and volume measurements of brain structures. Immunohistochemistry against Iba-1 and Olig2 was used to detect microglia and oligodendroglia respectively.
Results and Discussion: Cerebrum/body weight ratio was lower in the HI group (1.43 ± 0.12 vs 1.60 ± 0.12 %, $p<0.01$). Compared to controls, the lateral ventricle/cerebrum volume ratio (2.68 ± 0.70 vs 1.59 ± 0.75 %, $p<0.01$) and cerebral white matter/grey matter ratio (74.58 ± 9.78 vs 65.50 ± 6.47 %, $p<0.05$) were higher in the HI group. Oligodendroglial cell density was lower in the HI group (590.3 ± 317.1 vs 1023.6 ± 460.3 /mm^2, $p<0.05$), compared to controls. There was no difference in percentage area of white matter occupied by microglia.
Conclusion: Intermittent brief cerebral ischemia appears to reduce the volume of brain parenchyma and affect the structure of the very preterm ovine developing brain. Further analysis on the characterization of this altered brain development is on-going.

1. Munro, M.J., Walker, A.M., and Barfield, C.P.(2004).Hypotensive extremely low birth weight infants have reduced cerebral blood flow. Pediatrics 114, 1591–1596.

P13

Role of membrane trafficking and adherens junctions during fetal and neonatal brain development: insights from in vivo and in vitro models.

Zahady Velásquez[1], Gabriela Toro[1]; Guillermo Márquez[1], Camilo Muñoz[1], Cristian Parga[1], Loreto Ojeda[1], María Paz Miró[1], Cristian Oliver[1], César Gonzalez[1], Rosa Iris Muñoz[1], Luis Federico Bátiz[1], [2].
[1] *Faculty of Medicine and [2] CISNe, Universidad Austral de Chile, Valdivia, Chile*
Corresponding author's email: federicobatiz@uach.cl

Introduction: Alpha-SNAP is a key protein of the SNARE-mediated membrane trafficking/fusion machinery. A naturally occurring mutation in alpha-SNAP (known as hyh mutation) causes a disruption of the brain-cerebrospinal fluid interface or ventricular zone (VZ) and abnormal brain development. The VZ is sequentially lined by radial glial cells (fetal period) and ependymal cells (neonatal-adult period). *Are defects in N-cadherin-based adherens junctions (AJs) associated with the disruption of the VZ in hyh mutant mice?* On the other hand, *is N-cadherin dysfunction sufficient to provoke VZ disruption/denudation?*
Materials and Methods: The formation of AJs in the VZ was studied in vivo (electron and fluorescence microscopy, biochemical studies in hyh mutant mice) and in vitro (dissociation assays in neurosphere culture system). On the other hand, a novel organotypic culture system from adult bovine collicular recess roof (CRr) explants was used to evaluate the consequences of directly blocking N-cadherin function.
Results and Discussion: In hyh mutants, AJs were immunohistochemically and ultrastructurally anomalous. However, the levels of N-cadherin mRNA and protein were increased in different brain regions of the mutants. Consistently, an abnormal subcellular distribution (subcellular fractionation an immunofluorescence) of N-cadherin was observed in alpha-SNAP mutant brains. Functional experiments in neurospheres demonstrated that N-cadherin adherens junctions of hyh mice are more sensitive to calcium chelators and N-cadherin-blocking peptides. In CRr explants, the blockage of N-cadherin-function led to a progressive ependymal disruption/denudation of CRr ventricular walls. Furthermore, ependymal denudation appears to be a consequence of (i) altered N-cadherin trafficking dynamics and (ii) apoptosis of ependymal cells.
Conclusion: Our results suggest that (1) alpha-SNAP-mediated membrane trafficking/fusion regulates the formation/stability of N-cadherin-based AJs during brain development and (2) N-cadherin function plays a key role in the survival/physiology of ependymal cells. Accordingly, abnormal AJs appear to be a critical element in the pathogenesis of CNS disorders associated with VZ disruption.
Funding: This work was supported by FONDECYT grants 11090373 (to LFB) and 3130756 (to ZV), Chile.

P14

Effect of dopamine or dobutamine treatment on preterm cardiovascular function and brain inflammation following hypoxia-ischaemia.

Nadine Brew[1], Aminath Azhan[1], Irene DenHeijer[1], Ilias Nitsos[1], Suzie Miller[1], Adrian Walker[1], David W. Walker[1] and Flora Wong[1,2,3].
[1]The Ritchie Centre, Monash Institute of Medical Research, Monash University; [2]Monash Newborn, Monash Medical Centre; [3]Department of Pediatrics, Monash University; Melbourne, Australia
Corresponding author's email: flora.wong@monash.edu

Introduction: Systemic hypotension is a common complication in very preterm newborns. To maintain cerebral perfusion, inotropes such as dopamine or dobutamine are often administered, although their effects within the brain are yet to be elucidated.
Aim: To determine the neuropathological and cardiovascular effects of intravenous dopamine or dobutamine in response to hypoxia-ischaemia, in a preterm fetal lamb model.
Methods: At 90days gestation (term=147days) non-occlusive carotid artery and jugular vein catheters and an umbilical occluder were implanted into fetal lambs. Four days post-surgery continuous dopamine (n=7, 10µg/kg/min), dobutamine (n=6, 10µg/kg/min) or saline (n=9) infusion was commenced. Two hours post-infusion, 25mins umbilical cord occlusion (UCO) was performed. Brains were collected 72h later for immunohistochemical analysis. Data presented mean±SE.
Results: Pre-UCO, dopamine and dobutamine significantly elevated fetal heart rate (HR, 220±8 and 220±7bpm) compared to saline infusion (192±7bpm, $p<0.05$) though did not increase mean arterial pressure (MAP). UCO was ceased early in 5/9 saline and 1/6 dopamine-infused lambs due to MAP<10mmHg or asystole. One dobutamine-infused lamb died after UCO. Post-UCO, HR remained elevated in dopamine and dobutamine-infused lambs. There was a significant increase in inflammatory cells (Isolectin-B4, cells/mm^2) in the subcortical white matter of saline (1321±155) and dobutamine-infused (1645±322) lambs, compared to unoperated controls (263±96, $p<0.05$), but no significant difference between dopamine-infused lambs (1090±293) and unoperated controls. There were no differences in inflammatory cell density in the periventricular white matter.
Conclusions: Dopamine and dobutamine sustained MAP during UCO. Dopamine may decrease inflammatory cell infiltration in the subcortical white matter in preterm hypoxic-ischaemic injury.

P15

Potential of autologous umbilical cord blood for the early treatment of hypoxic brain injury.

James Aridas1, Courtney McDonald[1], Tamara Yawno[1], Michael Fahey[1,2], Flora Wong[1,2], Atul Malhotra[1,2], Michael Ditchfield[2,], Madison Paton[1], Margie Castillo Melendez[1], Suzie Miller[1,3], Graham Jenkin[1,3]
[1]The Ritchie Centre, Monash Institute of Medical Research, Monash University, Clayton, Vic, Australia
[2] Monash Childrens, Monash Heatlh, Clatyon, Vic, Australia
[3]Obstetrics and Gynaecology, Monash University, Clayton, Vic, Australia
Corresponding author's email: james.aridas@monash.edu

Introduction: Severe birth asphyxia can result in significant brain inflammation and injury, leading to cerebral palsy in surviving infants. Umbilical cord blood derived cells possess anti-inflammatory properties that have the potential to reduce inflammation and ameliorate neuropathology caused by birth asphyxia.
Methods: Birth asphyxia was induced via umbilical cord occlusion (UCO), before caesarean section delivery, in 20 term lambs (141 days gestation). Eight lambs were delivered by caesarean section without UCO (Controls). Umbilical cord blood (UCB) was obtained at delivery and lambs were resuscitated following standard Australian guidelines. The buffy coat was isolated from the UCB and were labelled with fluorescing iron nanoparticles. A representative sample was analysed for cell type by FACS. Autologous UCB (UCO+CELL; 100±20million, n=8) were administered 12 hours after delivery. Magnetic resonance spectroscopy (MRS) was undertaken at 12 and 72 hours, lambs were euthanased at 72 hours for organ collection and histological analysis.
Results and Discussion: UCO+CELL lambs showed improved muscle tone and suckling response. N-acetyl aspartate:lactate ratio, determined by MRS imaging, was increased in UCO lambs at 12h (0.20±0.05; n=20). At 72h, the ratio was further increased in UCO lambs (0.3±0.19; n=12) compared with controls (12h 0.1±0.07; 72h 0.1±0.06; n=8). By 72h, the ratio in UCO+CELL lambs was not different from that in controls (0.09±0.02). Histological analysis showed fluorescing UCB widely distributed within the cortex, white matter and deep grey matter of UCO+CELL lamb brains. Macrophage and microglia activation (lectin peroxidase) and neuronal cell death was significantly increased in brains of UCO, compared to control lambs, but was reduced to control levels in UCO+CELL lambs, p=0.01.
Conclusion: Administration of autologous UCB to newborn lambs following severe birth asphyxia improves behavioural and biophysical outcomes. We are now investigating which components of cord blood are responsible for the efficacious effects observed.

P16

Pattern of brain injury after prolonged umbilical cord occlusion in near-term fetal sheep.

Paul P. Drury[1], Laura Bennet[1], Joanne O. Davidson[1], Alistair Jan Gunn[1]. [1] *Fetal Physiology and Neuroscience Group, Department of Physiology, The University of Auckland, New Zealand*
Corresponding author's email: aj.gunn@auckland.ac.nz

Introduction The development of neuroprotective strategies ultimately requires validation in robust large animal models of brain injury. The majority of studies at term-equivalent have typically used either relatively mild insults leading to selective neuronal loss or severe ischemic and hypoxic-ischemic paradigms that are pragmatically useful but artificial. It is important to contrast their results with the evolution of injury after severe 'physiological' insults, such as prolonged umbilical cord occlusion, and how the patterns of brain injury in such models compare with clinical outcomes. We hypothesise that severe, near-terminal umbilical cord occlusion would be associated with cortical and sub-cortical injury in near-term fetal sheep.
Methods: 35 chronically instrumented fetal sheep at 125-129 d gestational age (term=147 d) were subjected to either umbilical cord occlusion until mean arterial pressure was <= 8 mmHg (n=15), or sham occlusion (n=5). Surviving fetuses were killed after 72h for histopathology.
Results: 8 fetuses died during recovery due to intractable hypotension. 8 fetuses had periods of status epilepticus with a secondary rise in cortical impedance following occlusion while 14 had mild seizures and no secondary rise in impedance. Assessment with acid-fuschin thionine staining showed severe neuronal loss in hippocampal regions and basal ganglia in all fetuses after occlusion ($p<0.05$) but only mild to moderate cortical injury.
Conclusion: Profound umbilical cord occlusion in near-term fetal sheep was associated with cardiovascular instability, frequent status epilepticus, a secondary rise in cortical impedance, and severe hippocampal and basal ganglia injury, but relatively milder cortical injury. This pattern is highly consistent with the clinical association between sentinel events such as cord prolapse and basal ganglia injury on MRI.

POSTERS

DOHaD

(Posters 17-22)

P17

Effects of perinatal asphyxia on prepulse inhibition in rats.

Cristian Cerfogli[1], Mariana Garín[1],Diego Bustamante[1], Peter Gebicke-Haerter[3], Paola Morales[1], Jose Valdes[2], Mario Herrera-Marschitz[1].

[1] *Millenium Institute BNI-Chile, Program of Molecular & Clinical Pharmacology, ICBM, Medical Faculty, University of Chile, Santiago, Chile.* [2] *Millenium Institute BNI-Chile, program Physiology & Biophysics, ICBM, Medical Faculty, University of Chile, Santiago, Chile.*[3] *Dept. of Psychopharmacology, Central Institute of Mental Health J5, Mannheim, Germany.*

Corresponding author's email: cristiancerfogli@live.cl

Introduction: Delivery is a stressful event menacing the newborn. Perinatal asphyxia (PA) is perhaps the strongest insult that a baby may confront at birth. Several motor and cognitive alterations, including cerebral palsy, seizures, spasticity, ADS, hyperactivity, mental retardation and/or neuropsychiatric syndromes with delayed clinical onset like schizophrenia may occur following PA.

In our lab, an experimental model has been established for investigating in the rat the long-term changes produced by PA; monitoring several molecular, cellular, physiological and behavioural parameters, finding that PA leads to a developmental vulnerability associated with these alterations. Sensorimotor gating deficits have repeatedly been observed in schizophrenia patients, including the lack of prepulse inhibition (PPI). Indeed, in the PPI of startle responses, normal and schizophrenic patients over-react to any stressful stimulation presented without any anticipation. However, if a weak sensory signal anticipates that stressful stimulation (prepulse), that stimulation losses its menacing value. That occurs in normal subjects, but not in patients with schizophrenia. A similar deficit has ben observed in rats suffering of alterations associated to schizophrenia.

Thus, we investigated whether PA produces long-term effects on PPI in PA-exposed. We observed a significant decrease (one-way ANOVA) of the PPI response in control, but not in asphyxia-exposed rats. Also, we observed a lineal correlation between an index of asphyxia severity and the PPI response, greater asphyxia severity, greater the PPI response alteration.

Conclusion: We are evaluating at present whether pharmacological treatments (nicotinamide and selective PARP-1 inhibitors) can reverse the alterations observed in the PPI response. This association in an animal model is of great importance, since it would point out a causal relation between PA and schizophrenia.

Acknowledgements: Supported by FONDECYT-Chile 1120079, 1110263; Millenium Institute (BNI P09-015-F). The excellent technical support from Mr Juan Santibañez and Ms Carmen Almeyda is acknowledged.

P18

The adult innate immune system is programmed by gestational chronodisruption through diminished C3/C4/C9 complement factors.

Jose Sarmiento[1], Pamela Carmona[1], Hugo Folch[1], Carlos Spichiger[1], Hugo Galdames[1], Natalia Mendez[1], Maria Seron-Ferre[2], Claudia Torres-Farfan[1], Hans Richter[1].
*Laboratory of Developmental Chronobiology, Faculty of Medicine, Universidad Austral de Chile, Valdivia, Chile. *Programa de Fisiopatologia, Facultad de Medicina, Universidad de Chile.*
Corresponding author's email: hrichter@uach.cl

Introduction. Gestational chronodisruption (a significant disturbance of circadian rhythms of physiological parameters in pregnancy) has been associated with strong predictors of adult disease, such as increased risk of preterm delivery and low birth weight. Emerging evidence suggests that gestational chronodisruption is associated with altered metabolic, cardiovascular, endocrine and cognitive parameters in the adult offspring. Here, whole-transcriptome profiling was used to test whether gestational chronodisruption modifies immune-related gene expression in the fetal liver, potentially altering the adult immune system.
Materials and Methods. At day 10 of gestation (E10), pregnant rats were randomized in two groups: constant light (LL) and control 12h light/12h dark photoperiod (LD). RNA isolated from E18 liver was subjected to microarray analysis (Affymetrix platform for 28,000 genes). Differential gene expression was validated by qPCR. Integrated transcriptional changes were assessed by gene ontology and pathway analysis. For longitudinal study of liver gene expression and clinical plasma assessment, parallel LL and LD cohorts were raised, with the male offspring being held under LD to be studied at 90 days of age.
Results and Discussion. In the liver from LL fetuses we found that C3, C4, Masp2, C8a, C8b and C9 factors of the complement system were down-regulated, relative to LD fetuses ($p<0.05$). Notably, C3, C4 and C9 transcripts remained significantly down-regulated in the adult offspring, which was accompanied by decreased levels of plasma C3 and C4 proteins (adult males; $p<0.05$ for LD vs. LL). Extensive literature recognizes airway allergic disease as a maladapted Th2/Th17 response to allergens; while different models of allergy suggest that complement factors/receptors regulate the onset of Th2 response during the allergen sensitization phase.
Conclusion. Gestational chronodisruption appears be a novel determinant of long-term immune phenotype and, therefore, it may contribute to increased risk for allergy, asthma, infection and autoimmunity in the adult offspring.
Funding: This work was supported by grants 1110220 from FONDECYT (to HR) and ACT1116 from CONICYT (to CT-F and HR), Chile.

P19

Persistent down-regulation of KChiP2 may contribute to the adult cardiac hypertrophy enforced by gestational chronodisruption.

Pamela Alonso Vazquez, Hugo Galdames, Claudia Torres-Farfan, Carlos Spichiger, Natalia Mendez, Hector Vera, Hans Richter.
*Laboratory of Developmental Chronobiology, Faculty of Medicine, Universidad Austral de Chile, Valdivia, Chile. *Programa de Fisiopatologia, Facultad de Medicina, Universidad de Chile.*
Corresponding author's email: hrichter@uach.cl

Introduction. We recently reported that gestational chronodisruption induces fetal growth restriction and marked effects on fetal adrenal physiology. Here, whole-transcriptome profiling was used to test whether gestational chronodisruption modifies gene expression in the fetal heart, potentially altering cardiac development.
Materials and Methods. At day 10 of gestation (E10), pregnant rats were randomized in two groups: constant light (LL) and control 12h light/12h dark photoperiod (LD). RNA isolated from E18 heart was subjected to microarray analysis (Affymetrix platform for 28,000 genes). Differential gene expression was validated by qPCR. Integrated transcriptional changes were assessed by gene ontology and pathway analysis. For longitudinal study of heart morphology and gene expression, parallel LL and LD cohorts were raised, with the male offspring being held under LD to be studied at 90 days of age.
Results and Discussion. Significant differential expression was found for 383 transcripts in LL relative to LD fetal heart (280 up-regulated and 103 down-regulated); with 42 of them displaying a 1.5-fold or greater change in gene expression. Deregulated markers of cardiovascular disease accounted for alteration of diverse gene networks in LL fetal heart, including local steroidogenesis and vascular calcification, as well as cardiac hypertrophy, stenosis and necrosis/cell death. DNA integrity was also overrepresented, including a 2.1-fold mRNA increase of *Hmga1*, a profuse architectural transcription factor. microRNA analysis revealed up-regulation of miRNAs 218-1 and 501 and concurrent down-regulation of their validated target genes. In addition, persistent transcriptional down-regulation of *Kcnip2* and hypertrophy of the left ventricle were found in the heart from 90 days-old offspring from LL mothers.
Conclusion. The dysregulation of a relevant fraction of the fetal cardiac transcriptome, together with the diversity and complexity of the gene networks altered by gestational chronodisruption, suggest enduring molecular changes which may shape the hypertrophy observed in the left ventricle of adult LL offspring.
Funding: This work was supported by grants 1110220 from FONDECYT (to HR) and ACT1116 from CONICYT (to CT-F and HR), Chile.

P20

Maternal care and adult cognitive flexibility in a rat model of intrauterine growth restriction. Márcio Bonesso Alves[1,2], Roberta Dalle Molle[2], Mina Desai[3], Michael G. Ross[3] and Patricia Pelufo Silveira[1,2]
[1]PPG Neurociências, ICBS and [2]*Faculty of Medicine, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil*
[3]*Dept of Ob/Gyn, Harbor-UCLA Med Ctr, Torrance, CA, USA.*
Corresponding author's email: m.bonesso@gmail.com

Intrauterine growth restriction (IUGR) is characterized when the fetus is unable to reach its growth potential, resulting from a large variety of factors and being associated with several long-term adverse outcomes in both epidemiologic and animal studies. We evaluated the effects of undernutrition during pregnancy in rats (a well established model of IUGR) on maternal care, as well as on cognitive flexibility of their adult offspring.
Methods: From day 10 of gestation and through lactation, Sprague-Dawley rats were provided either an *ad libitum* (AdLib group, n=15), or a 50% food restricted (FR, n=14) diet. At birth, pups were cross-fostered, generating four groups (pregnancy/lactation): AdLib/AdLib (Control); AdLib/FR; FR/FR and FR/AdLib. Maternal behavior was scored from day 2-7 postpartum. Adult cognitive flexibility was measured through the Attentional set-shifting task (ASST) that uses a sweet pellet as the reward for conditioning.
Results: FR/FR and FR/Adlib dams showed significantly less licking/grooming (LG) toward their pups when compared to AdLib/AdLib and AdLib/FR dams ($F_{(3, 22)}$=7,89, p=0,001). At birth, pups of FR dams had reduced body weight (5,32±0,04) as compared to AdLib (6,57±0,05), (p<0,01). Our preliminary data on ASST showed an effect of the group on the reversal discrimination during the intradimensional shift ($F_{(3, 49)}$= 2.886, p=0.047), as well as on the number of errors on that measurement ($F_{(3, 49)}$= 4.085, p=0.012), in which FR/AdLib group needs less trials to reach the completion criteria, with less mistakes than the other 3 groups. No differences were found on the simple, compound, extradimentional shifts and other reversions between the groups.
Discussion: Food restriction during pregnancy impacts maternal care. Moreover, we demonstrate a fetal programming effect, in which surprisingly IUGR improves the performance on the ASST. It is possible that the pleasure and neurochemical alterations induced by the sweet food offered during the task are involved in these findings.
Support: FIPE, CNPq

P21

Maternal obesity and endoplasmic reticulum stress induces insulin resistance in human umbilical vein endothelial cells.

Villalobos-Labra R[1], Westermeier F[1], Sáez PJ[1], Mardones F[2], Kusanovic JP[1], Poblete JA[1], Sobrevia L[3], Casanello P[4], Farias M[1,3]

[1]Division of Obstetrics and Gynecology, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile; [2]Division of Public Health, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile; [3]Cellular and Molecular Physiology Laboratory (CMPL), Center of Medical Research (CIM), Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile; [4]Perinatal Research Laboratory (PRL), Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile.

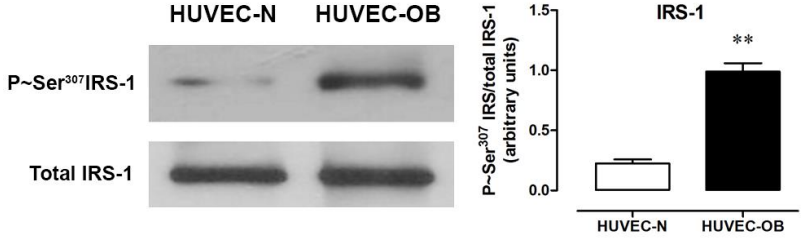
Corresponding author's email: mfarias@med.puc.cl

Introduction: Maternal obesity (MO) has been recognized as a risk factor for maternal and fetal complications, including offspring´s insulin resistance (IR) later in life. Multiple signaling branches of endoplasmic reticulum (ER) stress pathway have been correlated with obesity-related IR at cellular level. Thus, we evaluated the effect of pharmacological induction of ER stress or maternal obesity on endothelial insulin response.

Materials and Methods: Primary cultures of human umbilical vein endothelial cells (HUVEC) were isolated from normal (HUVEC-N) or MO (HUVEC-OB) pregnancies attending to obstetrics clinical service at Pontificia Universidad Católica de Chile Hospital. Then, we evaluated the insulin response and the effect of tunicamycin (inducer of ER stress) and tauroursodeoxycholic acid (TUDCA, chemical chaperone that blocks ER stress) through Western blot analysis to evaluate phosphorylated and total protein levels of IRS-1, Akt and p42/44MAPK.

Results and Discussion: The exposure (0-60 min) of HUVEC-N to physiological levels of insulin (1nM) showed a quickly and maintained increase of P~Akt and P~p44/42mapk, with a maximal response at 1 minute. Conversely, HUVEC-OB showed a reduced and delayed P~Akt and p44/42mapk signals in response to insulin, with a maximal detection at 15 minutes. In addition to this IR state in HUVEC-OB, we found an increased in phosphorylation of IRS-1 on the inactivating residue serine 307 in these cells, compared to HUVEC-N. Moreover, tunicamycin exposure (24 h) induced IR in HUVEC-N, and treatment with TUDCA reversed the IR in HUVEC-OB.

Conclusion: In this study we have shown cellular evidence that MO promotes neonatal IR, possibly through ER stress induction. Since reversal of ER stress with chemical chaperones recovery the functional state of insulin signaling, we propose ER stress as a new target for treatment of endothelial IR.



Western blot analysis of IRS-1 phosphorylation on serine 307 in HUVEC from maternal obesity compared with a normal pregnancy.

P22

Xanthine Oxidase and Programming of Cardiac Dysfunction in Hypoxic Pregnancy: Mechanism and Intervention.

Youguo Niu[1], Andrew Kane[1], Ciara Lusby[1], Beth Allison[1], Joepe Kaandorp[2], Jan B. Derks [2], and Dino A. Giussani[1].
[1]Physiology,Development and Neuroscience, University of Cambridge, UK; [2]University Medical Center, Utrecht, Netherlands.
Corresponding author's email: dag26@cam.ac.uk

Introduction: Hypoxic pregnancy programmes cardiac dysfunction in adulthood (Xue et al. *J Pharm Exp Ther*. 2009; 330: 624; Rueda-Clausen et al. *Card Res*. 2009;81: 713). However, the mechanism remains uncertain, preventing targets for intervention. Recently, we have reported that developmental programming of cardiac dysfunction in hypoxic pregnancy is prevented by maternal treatment with the antioxidant vitamin C (Giussani et al. *PLoS ONE* 2012;7(2):e31017), implicating oxidative stress. However, only very high doses of vitamin C incompatible with human treatment were protective. Here, we investigated an alternative antioxidant strategy: the xanthine oxidase inhibitor allopurinol.

Materials and Methods: Female Wistar rats (n=48) were randomly divided into normoxic (N: 21% O2) or hypoxic (H: 14% O2) pregnancy, with or without maternal treatment with allopurinol (30 mg/Kg in jelly) from days 6-20 of gestation. This experimental model of hypoxia does not affect maternal food intake. At birth, litters were culled to 8 pups (5 males and 3 females) and weighed weekly. At 4 months, following euthanasia, hearts were isolated from 1 male per litter and cardiac function was investigated in a Langendorff preparation.

Results and Discussion: At 4 months, body weight but not heart weight was lower in offspring from hypoxic pregnancy compared to controls. Adult offspring from hypoxic pregnancy showed enhanced myocardial contractility (dP/dt max) with increased left ventricular (LV) beta-adrenergic sensitivity, and reduced coronary flow rate (CFR) with impaired recovery from an ischaemic challenge (Fig. 1 A-D). Maternal treatment with allopurinol in hypoxic pregnancy restored all indices of cardiac dysfunction towards control levels in adult offspring.

Conclusions: The data support a link between xanthine oxidase and developmental programming of cardiac dysfunction in hypoxic pregnancy, providing a potential target for intervention.

BHF, The Royal Society, The BBSRC and Internationalization Fund.

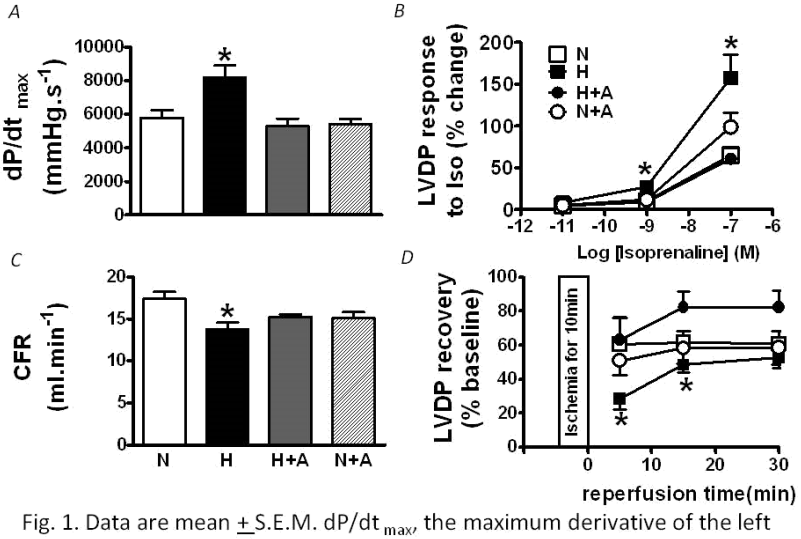


Fig. 1. Data are mean ± S.E.M. dP/dt_{max}, the maximum derivative of the left ventricular pressure; LVDP, left ventricular developed pressure; Iso, isoprenaline. N, n=9; H, n=8; H+A, n=8; N+A, n=9. Significant differences (P<0.05) are, for A, B and D: * *vs.* all; for C * *vs.* N. One-Way ANOVA with Tukey Test.

POSTERS

Placenta

(Posters 23-24)

P23

The increase in oxidative stress in the umbilical vein endothelium from IUGR pregnancies involves changes in GTPCH and DHFR expression.

Ernesto Muñoz-Urrutia[1], Bernardo Krause [1] and Paola Casanello[1].
[1]*Division of Obstetrics & Gynecology and Pediatrics, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile*
Corresponding author's email: pcasane@uc.cl

Introduction: Intrauterine Growth Restriction (IUGR) is associated with chronic fetal hypoxia, lower nitric oxide (NO) synthesis by the endothelial NO synthase (eNOS) in umbilical vein endothelium (HUVEC) and increased placental vascular tone. NO synthesis critically depends of the cofactor tetrahydrobiopterin (BH4), whose levels decrease under oxidative stress, mainly in the presence of peroxynitrite ($ONOO^-$). We studied oxidative stress in IUGR-derived HUVEC as well as the expression of two key enzymes involved in BH4 metabolism, GTP cyclohydrolase (GTPCH), the rate limiting protein of de novo synthesis of BH4; and Dihydrofolate reductase (DHFR), an enzyme in charge of recycling BH4 from its oxidised form, dihydrobiopterin (BH2), in basal conditions and in response to oxidative stress.

Materials and Methods: Normal and IUGR HUVEC were isolated, cultured and exposed to normoxia/hypoxia (5% and 2% O_2, respectively) in presence/absence of 3-morpholinosydnonimine (SIN-1), a peroxynitrite donor (250 µM, 24h). GTPCH and DHFR were determined by qPCR and protein nitration by dot-blot analysis.

Results and Discussion: IUGR-HUVEC showed a higher protein nitration compared to normal-HUVEC. GTPCH basal expression in IUGR-HUVEC was increased without change in DHFR expression. SIN-1 and hypoxia induced GTPCH and DHFR expression in normal-HUVEC without effect on IUGR-HUVEC. The increased *in vitro* oxidative stress in IUGR-HUVEC could be due to an imbalance of BH4 metabolism that consequently regulates NOS activity. These modifications are not further affected by hypoxia or oxidative stress in IUGR-HUVEC.

Conclusions: The chronic exposure of IUGR foetuses to hypoxia and oxidative stress could lead to increased basal levels of oxidative stress of umbilical endothelium *in vitro* and could explain the lack of response to cell stressors. These data suggest an altered BH4 regulation in IUGR-HUVEC which could in part explain the lower NO-dependent vascular relaxation observed in this condition and be early markers of vascular dysfunction observed early in postnatal life.

FONDECYT-1120928, CONICYT AT-24121567. E Muñoz-Urrutia holds a CONICYT PhD fellowship (Chile).

P24

The transient blockade of the bradykininin B2 receptor in pregnant guinea-pigs induces a defective trophoblast invasion and vascular remodelling, fetal losses and increased plasma creatinine.

Daniela Schneider[1], Jenny Corthorn[1, 2], Rita Ortiz[1], Gloria Valdés[1, 2]
[1]*Centro de Investigaciones Médicas, Escuela de Medicina Pontificia Universidad Católica, Santiago, Chile*
[2]*Departamento de Nefrología, Escuela de Medicina Pontificia Universidad Católica, Santiago, Chile*
Corresponding author's email: gvaldes@med.puc.cl

Introduction: The guinea-pig shares with humans spiral artery remodelling, the hemomonochorial placenta(1) and a vasodilator/angiogenic utero-placental repertoire(2). As in HTR-8/SVneo cells bradykinin increase migration and invasion through the bradykinin B2 receptor [B2R](3), the objective of this study was to evaluate whether blocking the B2R during the stage of maximal trophoblast invasion induced preeclampsia-like morphofunctional alterations.
Materials and Methods: Pirbright guinea-pigs on day 20 of pregnancy (D20) were implanted subcutaneously with Alzet pumps delivering from D20 to D34 the non-peptide B2R antagonist Bradyzide 62.5 g/kg/day (BDZG; N=10) or saline (CG; N=12). Systolic pressure was determined on D34, D40 and D60. At sacrifice in D34 or D60 blood and urine were extracted for creatinine and protein determination. Fetuses and placentas were weighed; trophoblasts in utero-placental units were identified with anticytokeratin. Results are expressed as means±SEM. Statistical analysis was performed with Graphpad Prism 5.1, using student and X^2 tests.
Results and Discussion: In comparison with controls transiently antagonizing the B2R provoked 1) reduced spiral arteries with intramural trophoblast (80 vs 100%; P<0.0001) and decidual periarterial trophoblast in D34 (63 vs 100%; P<0.0001); 2) less intraluminal trophoblast in lateral (8±3% vs 64±13%, P<0.01) and myometrial spiral arteries in D60 (20.9±0.6% vs 48.4±4.5%; P<0.0001); 3) increased fetal losses in D34 and D60 (0.82±0.30 vs 0.15±0.10; P<0.05); 4) reduced fetal/maternal %w/w in D34 (0.42±0.02 vs 0.54±0.04%p/p; P<0.01); 5) higher plasma creatinine in D60 (0.48±0.05 vs 0.33±0.02mg/dl; P<0.01). Systolic pressure in BDZG increased on D34 versus CG (70±2 vs 58±4mmHg; P<0.05), but not in D40/D60. No differences were observed in placental weight in D34/D60, and in uricemia and protein/creatinine index in D60.
Conclusion: Transient blockade of B2R produced a defective trophoblast invasion and remodelling of spiral arteries, and increased fetal losses and plasma creatinine, but failed to generate the hypertension and proteinuria of the preeclamptic syndrome.
References: 1. Kaufmann P, Davidoff M. Ad Anat Embryol Cell Biol 1977;53. 2. Valdés G et al. Reprod Biol Endocrinol 2006;6:13. 3. Erices R et al. Reprod Biol Endocrinol 2011;9:97
Financial support Fondecyt 1121161.

POSTERS

Others

(Posters 25-30)

P25

Effects of chronic phase shifts of the photoperiod throughout pregnancy on maternal circadian rhythms.

Halabi D, Mendez N, Pamela Alonso Vazquez, Vergara K, Gutierrez M, Richter HG, Seron-Ferre M, Torres-Farfan C.
*Laboratory of Developmental Chronobiology, Faculty of Medicine, Universidad Austral de Chile, Valdivia, Chile. *Programa de Fisiopatologia, Facultad de Medicina, Universidad de Chile.*
Corresponding author's email: *Claudia.torres@uach.cl*

Introduction: There is compelling evidence supporting that chronic shifts in photoperiod, resultant from shift work, disrupt the adult circadian system contributing to increased risk of disease. However, there is limited information about the impact of this condition during pregnancy on either fetal development or postnatal effects on the offspring. Here we investigated the effects of a model of shift work on the pregnant dam circadian rhythms of heart rate, temperature and activity.
Material and Methods: Female rats (90-105 days old; n = 8) raised and maintained under control photoperiod (LD; 12h light/12h dark) were implanted with a telemetric transponder (ER-4000 Energizer/Receiver, Minimitter surgery Co), seven days before mating. Rats were anesthetized under isoflurane (1.5-2.0%) and the transponder was implanted into the abdominal cavity (intraperitoneal for body temperature and locomotor activity). A separate component was implanted subcutaneously (for heart rate recording). Immediately after surgery the rhythms of heart rate, activity and temperature were continuously recorded under LD. Seven days later the females were mated and separated in two rooms until the end of gestation. A control group (n=4) was maintained in LD photoperiod and a second group was exposed to chronic shifts in photoperiod (CPS, n=4) throughout gestation.
Results: We observed that pregnant females under LD conditions presented circadian rhythms of heart rate, activity and temperature similar than those found before mating, with acrophases during the dark period. In contrast, we observed that pregnant CPS females presented a significant increase in heart rate and that the circadian rhythms of heart rate, activity and temperature were absent after the first shift of photoperiod. In addition, we observed a decrease in litter size (LD: 14.1 ± 0.3 vs CPS: 11.3 ± 1.2) and increased newborn weight at birth (LD 7.07 ± 0.05 g vs CPS 7.89 ± 0.08 g).
Conclusions: The present study brings about one of the first continuous monitoring of maternal circadian rhythms during gestation in the rat. We demonstrated that exposure to chronic shifts in photoperiod through gestation impact maternal circadian rhythms of heart rate, temperature and activity. Actually, we already observed some effects in early newborns that require more studies which, in general, support that gestational chronodisruption is a deleterious signal for fetal development.
Funding: ANILLO ACT-1116; FONDECYT 1110220 & 1120938 (Chile).

P26

Do antenatal glucocorticoids cause cerebral hypoxia after asphyxia?

Christopher K Lear,[1] Miriam Koome,[1] Joanne O. Davidson,[1] Alistair J Gunn,[1] Laura Bennet[1]
[1] Fetal Physiology and Neuroscience group, Department of Physiology, The University of Auckland, New Zealand
Corresponding author's email:l.bennet@auckland.ac.nz

Introduction: We have shown that dexamethasone given after fetal asphyxia induced by umbilical cord occlusion (UCO) is associated with increased neural injury in preterm fetal sheep. Injury was associated with significantly greater cerebral hypoperfusion, in the latent phase of recovery (first 6h), mediated by increased vasoconstriction. Hypoperfusion was not coupled with greater EEG suppression. We examined the hypothesis that greater injury with dexamethasone treatment is due to cerebral hypoxia mediated by uncoupling of cerebral blood flow (CBF) and metabolism. **Methods:** In 0.7 gestation fetal sheep, cortical (parietal) cerebral deoxygenated (Hb) and oxygenated (HBO2) haemoglobin were measured continuously using near-infrared spectroscopy. From these signals we calculated [HBdiff] ([Hb – HBO2]), a measure of true oxygenation, and [THb] ([Hb + HBO2]), a measure of blood volume. 4-5 days post- surgery fetal asphyxia was induced by 25 min of UCO. 15 min after UCO, ewes received an intramuscular injection of either dexamethasone (12mg/3mls, n=3) or saline (n=5). Fetuses were studied for 3 days post-UCO. **Results** All NIRS values fell during the latent phase, with the greatest fall observed in [HBO2]. The fall in [HBO2] was greater in the DEX group ($p<0.05$). There was, however, no difference between groups in [HBdiff]. There were no differences between groups after the latent phase. [HB] remained lower, while [HBO2] rose to above baseline, and [THb] returned to baseline values. **Conclusion:** In contrast to our hypothesis [Hbdiff] values show that dexamethasone treatment was not associated with cerebral hypoxia in the latent phase. The latent phase fall in NIRS values correlated with reduced CBF. Consistent with adult studies, fetal [HBO2] is a more sensitive indicator of changes in CBF[1]. Suppressed [Hb] and elevated [HBO2] after the latent phase is consistent with a greater supply of oxygen vs. demand (luxury perfusion). **Reference** Hoshi Y, J Appl Physiol 2001.

P27

Melatonin as a therapy to improve perinatal outcomes in a low resource setting in India.

Suzie Miller[1,2], Euan Wallace[1,2], James Aridas[1], Michael Fahey[1,3], Atul Malhotra[1,3], Vishwajeet Kumar[4], Graham Jenkin[1,2]

[1] *The Ritchie Centre, Monash Institute of Medical Research, Melbourne, Australia.* [2] *Department of Obstetrics and Gynaecology, Monash University, Melbourne, Australia.* [3] *Monash Children's Hospital, Monash Health, Melbourne, Australia.* [4] *Community Empowerment Lab, Shivgarh, Uttar Pradesh, India*

Corresponding author's email: suzie.miller@monash.edu

Introduction: Every year worldwide up to 4 million babies die within the first month of life and, of these, about a third die within 24 hours of birth. The World Health Organization advises that intrapartum-related neonatal deaths, or 'birth asphyxia', contribute to >20% of neonatal deaths, with the vast majority of these in low resource settings. It is recognized that we must tackle the grim effects of birth asphyxia to make advances towards the Millennium Development Goal (MDG 4) of reducing early childhood deaths. We, and others, are studying the neuroprotective actions of the strong antioxidant melatonin, since oxidative stress, particularly in the young brain, is a key mechanism leading to death or lifelong disability.

Methods, Results and Discussion: This study will be undertaken in two birth settings in India, one rural community and one tertiary in the northern state of Uttar Pradesh. We are planning an RCT to assess whether melatonin administration after compromised births will reduce mortality and developmental impairment. Melatonin will be administered in a transdermal slow-release skin patch, within the first 6 hours after birth. In our birth asphyxia lamb model we have confirmed that melatonin can be effectively delivered via a skin patch, and that it reduces cell death within the brain of the lamb at 72 hours after birth.

Conclusion: Melatonin has many attributes as a neonatal therapy in a low-resource birth setting - it is cheap, readily available, stable at room temperature, safe and easily administered. Its administration requires no special equipment or monitoring. Any rural maternity provider could hold stocks of melatonin patches ready to administer to a newborn with asphyxial symptoms, thereby reducing oxidative stress and preventing or decreasing brain injury. If successful, our work will facilitate the introduction of a new therapy to prevent neonatal death and disability.

P28

Conservative management in preterm preeclampsia: some perinatal variables. Hospital Vladimir I. Lenin. January - December 2012.

José Alexander Tamayo Ortiz[1], Isora Natacha Rodríguez Pérez[1]
[1]Hospital General Docente Universitario Vladimir Ilich Lenin. Holguín. Cuba
Corresponding author's email: atamayo@hpuh.hlg.sld.cu

Introduction: Severe preeclampsia is responsible for many of preterm deliveries. Neonatal mortality related to maternal hypertension ranges from 3.5 and 35 %, being perinatal asphyxia and preterm complications the most common causes of neonatal death. **Objective**: To describe some perinatal variables in patient with preeclampsia under conservative management.
Materials and methods: We conducted a descriptive study in a group of pregnant women with severe preeclampsia admitted to perinatology care unit from January to December 2012 and managed expectantly. We included 30 women counseled for expectant management, with less than 34 weeks of gestational age, and signs of good fetal health. Conservative (expectant) management included careful monitoring, medical treatment to improve maternal health, and induce fetal lung maturation. Women would be induced only when the maternal or fetal condition deteriorates or at 37^{+0} weeks of gestation
Results: Mean Gestational age at diagnostic was 31weeks (SD 2), pregnancy was terminated in 45 % of cases because of maternal deterioration. The mode of delivery was by primitive caesarean in 80% of births. Pregnancy could be prolonged more than 8 days in 70 % of cases and in 72 hours in 4 out of every 5 patients. Mean gestational age at delivery was 34 weeks (SD 2). Newborn weight was more than 1500 gr in 80% of newborns (SD 450gr). Apgar score was 8-9 in 90% of cases.
Conclusion: Conservative management in severe preeclampsia allows obtaining of a newborn in better biofisiological conditions and is recommended in remote from term patients, after proper selection of patients and careful monitoring.

P29

The International Center for Andean Studies (INCAS) - A unique natural Lab of Hypobaric Hypoxia.

Emilio A. Herrera[1,2], Alberto L. Raggi[1,3], Victor H. Parraguez[1,3], Aníbal J. Llanos [1,2].
[1]International Center for Andean Studies; [2]Faculty of Medicine; [3] Faculty of Veterinary Medicine; Universidad de Chile, Santiago, Chile.
Corresponding author's email: eherrera@med.uchile.cl

The *Andean Altiplano* is a 4.000 m high plateau located in the Central Andean Range. It has two types of human population: a permanent one consisting mainly of *quechua* and *aymara* groups, and a intermittent one composed by tourists, mountaineers, military soldiers and mining staff. Further, the *Altiplano* is an exceptional environment that houses high-altitude adapted fauna and flora. All of these offer unique characteristics for the study of humans and animals exposed to chronic or intermittent hypobaric hypoxia.
Thus, 11 years ago, the University of Chile built the Putre Research Station belonging to the International Center for Andean Studies (**INCAS**), aiming to promote and coordinate National and International Scientific activities oriented to preserve, understand and develop the *Altiplano* region.

The Putre Research Station-INCAS is open all year long to be used by Chilean and Foreign researchers interested in any scientific discipline related to high altitude environments. The Center has housed Research projects in diverse areas such as Reproductive adaptations to high-altitude in sheep and llamas, Physiological and molecular adaptations of the pulmonary circulation to hypoxia in newborns, Fetal and postnatal cardiorespiratory control in high-altitude animals, Maternal and fetal cardiovascular variables in ewes adapted and non-adapted to high altitude, Agricultural Programs, and Cosmic radiation, between others.

The Putre Research Station-INCAS has separated House and Laboratory facilities, which can lodge comfortably 4 to 6 researchers. It is located in Putre at 3.600 m, the capital of the Chilean Andean region of Arica and Parinacota, 140 km from Arica at sea level connected by a paved road. Putre has access to all the basic services such as surgery-house, school, restaurants, hotels and diverse shops.

Its flora, fauna, water resources, volcanoes, arid lands, climate conditions, and native and non-native populations, form a natural laboratory with an extremely fragile ecosystem, in which any type of alteration must be carefully studied in order to avoid damages in the environment or system imbalances. We aimed to aid for the study and adequate preservation this environment.

The International Center for Andean Studies (INCAS) is thus, a unique natural Lab of Hypobaric Hypoxia, open to any Research Project. For further information, please check the INCAS website at: www. uchile.cl /incas/.

Supported by FONDECYT 1010636, 1029599, 1050479, 1070405, 1080633, 1100189, 1110595, 1120655, 1130424, 1130181.

P30

The Perinatologists Family Tree.

Emilio A. Herrera[1,2], Julian T. Parer[3].
[1]*International Center for Andean Studies;* [2]*Faculty of Medicine;* [3]University of California San Francisco, USA.
Corresponding author's email: eherrera@med.uchile.cl

Joseph Barcroft (1872-1947) is considered one of the pioneers on fetal researcher of the modern era. He was an extremely innovative and prolific scientist in Blood, Respiratory and Cardiovascular Physiology in King's College Cambridge (1899). In 1922 he led an expedition to the Peruvian Andes for hypobaric hypoxia studies. During this period of his life he published many classical documents on physiological functions, received many honours and got important positions in Europe, culminating in 1935 when he was appointed Knight. In 1932, when aged 60, Barcroft begun the third and perhaps most important chapter of his scientific research— studying the physiology of the developing fetus, pursuing this work right up to his death 15 years later.

The current worldwide research on Perinatal Physiology and Pathophysiology is, without a doubt, part of his legacy. His views created a massive interest in the Researchers and a considerable group of academics followed, extended and enhanced his lines of research, creating a Perinatologists dynasty. In between them, the most relevant personages are: Donald Barron (Yale), Andre Hellegers (Georgetown), William Huckabee (Harvard), James Metcalfe (Harvard & Oregon), Harry Prystowsky (Johns Hopkins), Giacomo Meschia (Yale, University of Colorado), Frederick Battaglia (University of Colorado), Robert Comline (Cambridge), Peter Nathanielsz (Cambridge), Geoffrey Dawes (Oxford), Mont Liggins (NZ), Geoffrey Thorburn (Australia); Jeffrey Robinson (Newcastle & Adelaide), Colin Jones (Oxford); John Patrick (Canada), John Challis (Canada); Gerry Visser & Jalte de Haan (The Netherlands), Edward Hon & EJ Quilligan (Yale & USC); Yuji Murata & Ike Ikenoue (Japan), Abraham Rudolph & Bill Parer (CVRI, UCSF), Jan Nijhuis (Netherlands), Aníbal J. Llanos (Chile), and Larry Longo (Loma Linda).

This is an interactive abstract, not willing to describe the absolute hierarchy of perinatology history, but to create it with the actual actors/actresses. We encourage you to visit this poster and write on it, creating part of the history and The Perinatologists Family Tree.

Supported by many Researchers along history.

Oral Communications

Session II: Preterm Birth, Steroids & Miscellaneous

Chairs:

Laura Bennet (University of Auckland, New Zealand)

David Wlaker (Monash Institute of Medical Research, Australia)

O01

Maternal dietary creatine supplementation prevents changes in diaphragm muscle function 1 month after asphyxia at birth.

Domenic A LaRosa[1], Stacey J. Ellery[1], Helena C Parkington[2], Rod J. Snow[3] David W. Walker[1] & Hayley Dickinson[1].
[1]Ritchie Centre, Monash Institute of Medical Research, Monash University, VIC 3800, Australia; [2]Department of Physiology, Monash University, Victoria 3800 Australia; [3]Centre for Physical Activity and Nutrition, Deakin University, Burwood, Victoria 3125.
Corresponding author's email: domenic.larosa@monash.edu

Introduction: Birth asphyxia is responsible for >900,000 deaths per annum. Using a model of birth asphyxia in the spiny mouse we have shown significant structural and functional deficits in the diaphragm at 24 h after birth, deficits that were largely prevented if the mother had received a 5% creatine (Cr) supplemented diet from mid-pregnancy [1]. However, it is not known if this apparent protection of this crucial respiratory muscle persists postnatally.
Methods: Pregnant spiny mice were fed control or 5% Cr-supplemented diet from 20 days (0.5) gestation. One day before term, fetuses underwent a c-section (control) or asphyxic birth as previously described [1]. Pups were then cross-fostered to a lactating dam for 33 ± 2 days. After necropsy, strips of diaphragm were used to study twitch tension and fatigue *in vitro,* using field stimulation via external electrodes.
Results/Discussion: Single twitch tension parameters (time to peak, relaxation, maximum twitch force) were not different for offspring from control or creatine-fed dams. Maximum tetanic force and muscle fatigue (measured as decrease in maximum force induced by a train of pulses -1x330ms train/sec, 400Hz for 300sec), were significantly reduced in birth asphyxia pups compared to c-section control pups ($p<0.05$); both parameters were significantly increased in birth asphyxia offspring from creatine-fed mothers compared to asphyxia only pups ($p<0.05$) and were not different to controls.
Conclusions: Birth asphyxia induced long-term functional deficits in the diaphragm that become apparent under "stressed" conditions when muscle fatigue is allowed to develop. It can be expected that such offspring may have reduced capacity for exercise that depends on respiratory effort. Creatine supplementation during pregnancy prevents these deficits arising after birth asphyxia.
Reference: DJ Cannata et al. (2010). Maternal creatine supplementation from mid-pregnancy protects the diaphragm of the newborn spiny mouse from intrapartum hypoxia-induced damage. *Ped Res*, 68(5), pp.393–398.

O02

Does Neonatal Acute Kidney Injury Following Birth Asphyxia Result in Permanent Nephron Loss and Kidney Dysfunction?

Stacey J. Ellery[1], Domenic A LaRosa[1], Michelle M. Kett[2], Louise Cullen-McEwen[3], Russell Brown[2], Rod J. Snow[4] David W. Walker[1] and Hayley Dickinson[1]
[1]The Ritchie Centre, Monash Institute of Medical Research, Clayton, Australia.
[2]Department of Physiology, Monash University, Clayton, Australia
[3]Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia
[4]Centre for Physical Activity and Nutrition Research, School of Exercise and Nutrition Sciences, Deakin University, Burwood, Australia
Corresponding author's email: stacey.ellery@monash.edu

Introduction. Intrapartum birth asphyxia results in disturbances to the architecture of the spiny mouse kidney, 24 hours after insult. This outcome was absent when fetal kidneys were preloaded with creatine via maternal dietary supplementation[1]. Whether these disturbances result in permanent loss of nephrons, ensuing renal function deficiencies, and whether the protective capacity of creatine is long lasting, remains the topic of further investigation.
Materials and Methods- Pregnant spiny mice were maintained on normal chow or chow supplemented with 5% w/w creatine from mid-gestation. At term, pups were delivered by caesarean section or subjected to intrauterine asphyxia. Post mortems were conducted at 33 days postnatal age (P33), and kidneys collected for stereological analysis of nephron endowment. Another group of offspring was taken out to 85 days of age, during which time measures of urinary output, osmolality and electrolyte levels (P48 & P72), and conscious glomerular filtration rate (GFR; P85) were undertaken.
Results and Discussion. Birth asphyxia caused decreased growth ($P<0.001$) of male offspring from P20, an outcome not present if the mother had received the creatine supplementation. Food and water intake, urine output, and protein excretion were not different between control and creatine-fed treatment groups. However, at P33 glomerular number was significantly reduced in birth asphyxia males ($P=0.03$), but not females. GFR measured in conscious spiny mice, using transcutaneous detection of FITC-sinistrin was 230 ± 16 µl/min (n=5); preliminary results indicate a high GFR (353 µl/min), in a post-birth asphyxia male (n=1).
Conclusion. Structural disturbances observed 24 hours after birth asphyxia persist to one month of age as a reduction in nephron number, in male offspring only. Ongoing studies including conscious GFR will establish the functional consequences of this finding. Studies will also be completed in offspring from creatine-supplemented dams, to determine the long-term protective capacity of creatine.
References- [1]Ellery, S. J., et al. (2012). Pediatric Research

O03

The effects of betamethasone on allopregnanolone concentrations and cell death in normally grown and IUGR preterm fetal sheep.

Yawno T, Mortale M, Sutherland AE, Wallace EM, Jenkin G, Walker DW, Miller SL.
The Ritchie Centre, Monash Institute of Medical Research, Clayton, Australia.
Corresponding author: david.walker@monash.edu.

Background: Fetal intrauterine growth restriction (IUGR) is associated with brain injury. The neurosteroid allopregnanolone (AP) is a $GABA_A$ receptor agonist that decreases CNS activity, influences brain development, and is neuroprotective in fetal sheep (Yawno et al., Neuroscience, 163:838-47, 2009). IUGR babies are at high risk of preterm birth and are likely to receive antenatal glucocorticoid therapy (e.g. betamethasone) to promote lung maturation. Thus we determined if: i) betamethasone altered fetal brain AP levels and caused brain injury; ii) co-administration of alfaxan (a synthetic analogue of AP) could prevent betamethasone-induced brain injury.
Method: Single umbilical artery ligation (SUAL) at 110 days gestation in sheep was performed to induce fetal growth retardation. Betamethasone (BM; 11.4mg im to ewe) or vehicle (saline) was given on days five (BM1) and six (BM2) following surgery. Alfaxan (20mg/48h i.a. to fetus) was given 30 min prior to BM1. Animals were euthanased on day 7 (i.e., 24 h after BM2), and the fetal brain collected to determine AP concentrations and histopathology.
Results: BM significantly reduced AP concentrations in cortex, PVWM, striatum, hippocampus and cerebellum, and increased pyknotic cell death in the hippocampus, of control and IUGR fetuses (Table). Co-administration of alfaxan with BM treatment significantly ameliorated the increased cell death in both IUGR and control fetuses.

	AP (pmol/ml) – hippocampus	Pyknosis – hippocampus (cells/mm^2)
Control + vehicle	40.34±13.24 (5)	1.70±1.70 (5)
Control + BM	8.07±1.89* (5)	10.03±9.3* (5)
Control + BM + alfaxan	8.88 (1)	5.90±4.09 (5)
IUGR + vehicle	32.94±6.35 (5)	3.40±2.69 (5)
IUGR + BM	7.94±1.90* (5)	22.79±9.4* (5)
IUGR + BM + alfaxan	7.48 (1)	9.30±7.85 (5)

**P<0.05 vs vehicle treated control or IUGR fetuses; (number)*

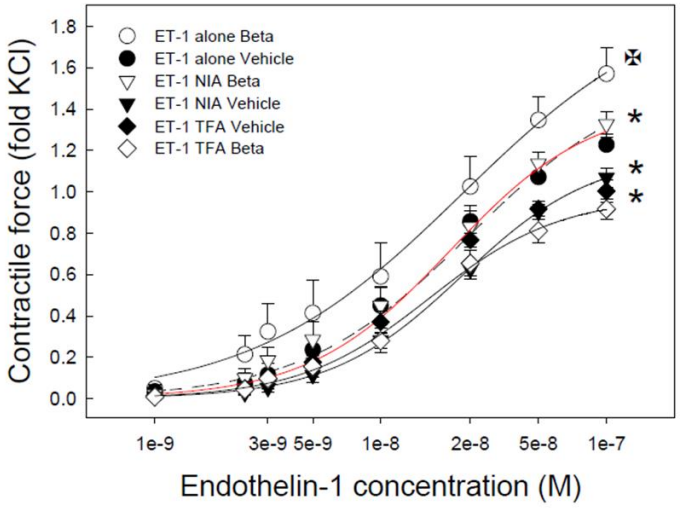
Conclusions: Glucocorticoids given to increase lung maturation also significantly decrease brain neurosteroid levels and induce brain injury. Betamethasone-induced brain injury is reduced by replacing $GABA_A$-active neurosteroids.

O04

Antenatal betamethasone effects on transient receptor potential cation channels (trpc) function in sheep arteries.

Jorge P. Figueroa[1], Jie Zhang[1], Angela Massmann[1], Jeong-Heon Lee[2].
[1] *Wake Forest School of Medicine, Winston-Salem, NC 27157 USA*
[2] *Chonbuk National University Medical School, South Korea*
Corresponding author's email: figueroa@wakehealth.edu

Introduction: The aim of this study was to investigate the mechanisms responsible for the increase response to ET-1 in adult sheep exposed antenataly to betamethasone by studying the role of TRPC channels in the ET-1 effects.
Materials and Methods: Pregnant sheep were treated with BM or vehicle V at 80 dGA. Adult offspring were euthanized at 1.5 yr of age. Contractile force of arteries (300 µm) from the brachial bed in V (n=8) and BM animals (n=10) was studied on a wire myograph. Responses to ET-1 and KCl were assessed in the absence and in presence of TRPC blockers (Tolfenamic acid;TFA) and cADPR inhibitors (niacinamide;NIA). Arteries with and without endothelium were collected and stored at -80ºC. RNA was extracted and cDNAs prepared to measure levels of TRPC4, TRPM2, CD38, ETA, ETB and eNOS. Data are expressed as Mean±SEM and analyzed by ANOVA. * $p<0.05$ vs ET-1 alonet, ✠ $p<0.05$ vs V)
Results and Discussion: ET-1 significantly increased wall tension in both V and BM. Arteries from BM animals displayed a higher sensitivity to ET-1 compared to V treated sheep. NIA and TFA significantly decreased the BM effect on the ET-1 response. Antenatal BM was associated with a significant difference in mRNA abundance for TRPM2 in both males and females and TRPC4 in females only. In contrast we did not find any systematic difference in the mRNA expression for CD38 or ETA.
Conclusion: Our data provide for the first time direct evidence for a mechanism to explain the increase sensitivity to ET-1 following antenatal BM exposure. In both cases TRP ion channels, capable of transporting calcium, are increased by BM. Particularly important is the change in TRPM2 which is known to be activated by cADPR. (HL 68728)
Figure



O05

Creatine Supplementation Protects the Neonatal Spiny Mouse Following Birth Asphyxia, but does it affect the Mother?

Stacey J. Ellery[1], Domenic A LaRosa[1], Michelle M. Kett[2], Rod J. Snow[3] David W. Walker[1] and Hayley Dickinson[1]
[1]The Ritchie Centre, Monash Institute for Medical Research, Melbourne, Australia. [2]Department of Physiology, Monash University, Clayton, Australia 3088.
[3]Centre for Physical Activity and Nutrition Research, School of Exercise and Nutrition Sciences, Deakin University, Burwood, Australia.
Corresponding author's email: stacey.ellery@monash.edu

Introduction. Maternal dietary creatine supplementation and fetal tissue loading has been shown to reduce multi-organ damage in neonates following birth asphyxia[1-3]. However, the effect of this supplementation on the mother is not known.
Materials and Methods. Pregnant spiny mouse were maintained on normal chow or chow supplemented with 5%w/w creatine from mid-gestation (20 days), with weight recorded at 3-day intervals until term (38 days). After 3 and 15 days of supplementation (i.e., at 23 & 35 days gestation) dams underwent assessment of food, water intake and urine output. Creatine content, osmolality and electrolyte levels were measured in the urine samples. At term dams were culled and carcasses collected for Dual-energy X-ray absorptiometry (DEXA; n=6), or the kidneys, brain, liver and skeletal muscle were frozen for molecular analysis of the creatine transporter (CrT) and creatine synthesising enzymes arginine:glycine amidinotransferase (AGAT) and guanidinoaceteate methyltransferase (GAMT; n=8).
Results and Discussion. Weight gain from mid-gestation was similar for creatine-fed and control dams, and DEXA analysis at term showed no change in lean tissue or fat composition. Creatine did not alter food intake, water consumption or urine output. Creatine supplementation increased the rate of urine excretion, however this effect was reduced near term (P_{Diet}<0.03,). Urinary Na^+ (P<0.02) and Cl^- (P<0.04) concentrations were all increased on day 23, but not at day 35 gestation. Renal expression of the creatine synthesising enzyme AGAT (mRNA, P<0.02; protein, P<0.003) was down regulated at term.
Conclusion. Maternal creatine supplementation during the latter half of pregnancy does not alter maternal eating behaviour, weight gain, or body composition. The down-regulation of renal AGAT was expected due to negative feedback of creatine on AGAT gene expression, although it is not yet known when this recovers after cessation of creatine treatment. Change to excretion of electrolytes after 3 days of treatment warrants further investigation.
References- [1]Ellery, S. J., et al. (2012). Pediatric Research. [2]Ireland, Z., et al. (2011) Neuroscience. [3]Cannata, D. J., et al. (2010). Pediatric Research

O06

The effect of dexamethasone on the preterm fetal sheep responses to acute asphyxia.

Rachael L Robson[1], Joanne O. Davidson[1], Alistair Jan Gunn[1], Laura Bennet[1]. [1]*Fetal Physiology and Neuroscience Group, Department of Physiology, University of Auckland, New Zealand.*
Corresponding author's email: l.bennet@auckland.ac.nz

Introduction: We have recently observed that brain injury is significantly increased in preterm fetal sheep when dexamethasone (DEX) is given 4 hours before an acute asphyxial insult. Fletcher et al[1] demonstrated that fetal infusion of DEX during moderate hypoxia caused a persistent bradycardia. We hypothesised that greater bradycardia during asphyxia would cause greater overall hypotension, and that this may be one mechanism leading to greater neural injury.

Methods: Fetal sheep at 98-99d gestation were instrumented to permit continuous monitoring of fetal blood pressure (BP), heart rate (FHR), carotid and femoral blood flow (CaBF & FBF), electroencephalographic (EEG) activity and cortical impedance (to measure extracellular edema). 4-5 days post-surgery, ewes received an i.m. injection of saline (3mls, n=6) or DEX (12mg/3mls, n=6). Asphyxia was induced by 25 min of complete umbilical cord occlusion (UCO) 4 hours post-injection.

Results DEX attenuated the initial hypertension (first 6 min of UCO, ~25% peak rise vs. ~50%, $p<0.05$). Thereafter BP was similar between groups. DEX did not alter the fall in FHR between groups, but did attenuate the initial vasoconstriction, with FBF in the DEX group falling to a nadir of 20% vs. 5% of baseline, $p<0.05$). CaBF was elevated in the DEX group between min 6-11, $p<0.05$). There were no differences in EEG suppression, however, the rise in impedance was slower and the peak attenuated by DEX treatment (25% peak increase vs. 41%, $p<0.05$).

Conclusion: In contrast to our hypothesis, DEX did not cause greater bradycardia or hypotension. While early peripheral vasoconstriction was attenuated, this only affected the early BP rise and did not compromise cerebral perfusion. Intriguingly, the reduced rise in impedance with DEX suggests that there was less cerebral edema and that DEX may protect brain cells during UCO by maintaining cellular metabolism.

References: [1]Fletcher A et al, J Physiol. 2003, 15;549:271-87

O07

The preterm male lamb displays inadequate respiratory adaptation after birth.

Robert De Matteo, Noreen Ishak, Takushi Hanita, Richard Harding, Foula Sozo.
Department of Anatomy and Developmental Biology, School of Biomedical Sciences, Monash University, Melbourne, Australia
Corresponding author's email: robert.dematteo@monash.edu

Introduction: After preterm birth, male infants have a greater incidence of respiratory insufficiency than females. Our objective was to compare cardio-respiratory adaptation in males and females for 8h after preterm birth, using an ovine model of preterm birth in which fewer males survive than females[1].
Materials and Methods: Following surgical preparation of fetal sheep at ~125 days of gestation (DG; term ~147 DG) and administration of maternal betamethasone (5.7mg i.m.) at 131 DG, unanaesthetized lambs (6 male, 8 female) were delivered at 133 DG. Arterial and intrapleural pressures were recorded continuously in spontaneously breathing lambs for 8h after birth and arterial blood was sampled to measure blood gases and metabolites. At 8h, lambs were euthanized and static lung compliance was measured. Bronchoalveolar lavage fluid (BALF) was collected for analysis of total protein concentration and surfactant phospholipid composition. Lung tissue was collected for analysis of the gene expression of *surfactant proteins (SP)-A, -B, -C* and *-D* and the protein expression of SP-A and pro-SP-C.
Results: At 6-8h after preterm birth, males had significantly lower arterial pH and a higher $PaCO_2$ compared to females; mean arterial pressure was not different. Inspiratory effort was significantly greater in males than in females and static lung compliance was 24% lower in males than in females. Total protein concentration in BALF, *SP* gene expression and SP-A protein levels were not different between the sexes. Pro-SP-C was significantly reduced by 24% in males and there were significanlty lower proportions of the surfactant phospholipid species PC 32:1 and PE 36:2 in males compared to females.
Conclusion: We conclude that cardio-respiratory adaptation following preterm birth is less effective in males than females for up to 8 hours. Male lambs have less compliant lungs than females, which is the likely cause of the greater inspiratory effort, CO_2 retention and acidemia. The lower lung compliance of males could be due to sex differences in surfactant composition.

1. De Matteo R, Blasch N, Stokes V, Davis P, Harding R. (2010) Induced preterm birth in sheep: a suitable model for studying the developmental effects of moderately preterm birth. Reprod Sci. 17(8):724-33.

Oral Communications

Session III: Fetal & Neonatal Brain I

Chairs:

Alistair Gunn (University of Auckland, New Zealand)

Mario Herrera-Marschitz (Universidad de Chile, Chile)

O08

Synthesis of dehydroepiandrosterone (DHEA) in the brain of the spiny mouse.

Tracey Quinn[1], Hayley Dickinson[1], Margie Castillo Melendez[1], & David W. Walker[1,2]
[1]The Ritchie Centre, Monash Institute of Medical Research; [2]Department of Obstetrics & Gynaecology, Monash University, Clayton, Melbourne, Australia, 3168.
Corresponding author's email: Tracey Quinn@monash.edu

Introduction: Dehydroepiandrosterone (DHEA) is an androgen with trophic effects on brain growth [1], and anti-glucocorticoid actions that diminish neurotoxicity and oxidative stress [2-5]. We therefore determined if DHEA is produced *de novo* in the developing brain of the spiny mouse (*Acomys caharinus*), a precocial rodent known to synthesize this C19 steroid [6].
Methods: Expression of P450c17 and cytochrome b5 (Cytb5) - the enzyme and accessory protein responsible for the synthesis of DHEA - and the glucocorticoid receptor (GR) were determined in 35 day gestation fetal (term = 39 days), neonatal (day of birth) and adult (80 days old) brains by immunocytochemistry. Double-label immunofluorescence was used to determine co-localisation in neurons (NeuN), astrocytes (GFAP) or oligodendrocytes (CNPase). P450c17 bioactivity was determined using radioimmunoassay of conversion of pregnenolone (PREG) to DHEA by explants of fetal, neonatal and adult brain.
Results: Fetal brain explants produced significantly more DHEA after 48 h in culture (22.46±2.00 ng/mg) than adult brain explants (5.04±2.04 ng/mg; $p<0.0001$, independent ANOVA). The GR, P450c17 and Cytb5 were diffusely expressed in white matter tracts and synaptic boutons in the fetal and neonatal brainstem; P450c17 was also co-expressed in midbrain neurons, a region showing GR neuronal expression only in fetal samples; P450c17 and GR were expressed in pontine nuclei on the day of birth. In contrast, P450c17 expression was detected only in the trigeminal motor nucleus and the corpus callosum of the adult brain.
Conclusion: These results show that the spiny mouse brain can synthesize DHEA in late gestation and the early neonatal period. The predominant expression of P450c17 and Cytb5 in the brain stem and midbrain suggests that DHEA may promote axonal growth of differentiated neurons during development. Local synthesis may protect these regions against glucocorticoid-mediated neurotoxicity, particularly in white matter and pathways associated with the cerebellum and spinal cord.

References

1. **Majewska MD** 1995 Neuronal actions of dehydroepiandrosterone possible roles in brain development, aging, memory, and affect. Ann N Y Acad Sci 774:111-120
2. **Cardounel A, et al.** 1999 Dehydroepiandrosterone protects hippocampal neurons against neurotoxin-induced cell death: Mechanism of action. Proc Soc Exp Biol Med 222(2):145-149
3. **Kimonides VG, et al.** 1998 Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS) protect hippocampal neurons against excitatory amino acid-induced neurotoxicity. Proc Natl Acad Sci U S A 95(4):1852-1857
4. **Kurata K, et al.** 2004 beta-estradiol, dehydroepiandrosterone, and dehydroepiandrosterone sulfate protect against N-methyl-D-aspartate-induced neurotoxicity in rat hippocampal neurons by different mechanisms. J Pharmacol Exp Ther 311(1):237-245
5. **Kimonides VG, et al.** 1999 Dehydroepiandrosterone antagonizes the neurotoxic effects of corticosterone and translocation of stress-activated protein kinase 3 in hippocampal primary cultures. Neuroscience 89(2):429-436
6. **Brown RC, et al.** 2003 Oxidative stress-mediated DHEA formation in Alzheimer's disease pathology. Neurobiol Aging 24(1):57-65
7. **Kimonides et al.** 1998 Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS) protect hippocampal neurons against excitatory amino acid-induced neurotoxicity. Proc Natl Acad Sci U S A 95(4):1852-7
8. **Quinn TA, et al.** 2013 Ontogeny of the adrenal gland in the spiny mouse, with particular reference to production of the steroids cortisol and dehydroepiandrosterone. Endocrinology 154(3):1190-201

O09

Ongoing effects of prenatal stress on perinatal brain development and function.

Greer Bennett[1], Hannah Palliser[1], David W. Walker[2], Jonathan Hirst[2].
[1]Mothers and Babies Research Centre, Hunter Medical Research Centre, Newcastle, Australia. [2]School of Biomedical Science and [3] Richie Centre for Baby Health, Monash University, Clayton, Australia
Corresponding author's email: greer.bennett@uon.edu.au

Introduction: Prenatal stress has been shown to disrupt normal developmental processes. We have previously shown that prenatal stress in late gestation results in altered development of the hippocampus in male fetuses at term. The objectives of this study were to characterise the ongoing effects of prenatal stress on brain development and to determine effects on anxiety and locomotor activity as measures of functional outcomes for the offspring.
Materials and Methods: Stress was induced in pregnant guinea pigs by exposure to strobe light for 2h/day at 50,55, 60 and 65days (term 70days). Dams spontaneously delivered at term and pups were subsequently tested for anxiety and ambulatory/locomotor activity using open field testing at post-natal day (PND) 18. Brains were collected at PND 21 (childhood) for immunohistochemical analysis for markers of myelination (MBP), reactive astrocytes (GFAP) and mature neurons (MAP2).
Results and Discussion: At PND21 offspring of prenatally stressed pregnancies (males n=4, females n=4) showed significantly ($p<0.05$) reduced ambulatory activity compared to controls (males n=5, females n=4). The prenatally stressed offspring also spent less time in the inner zone of the open field ($p<0.01$), indicating a more anxious phenotype. Notably, it was only the male offspring that showed reduced expression of MBP ($p<0.01$) and GFAP ($p<0.05$) in the hippocampus compared to both controls and females at PND21. There was no effect of prenatal stress at PND21 on MAP2 expression.
Conclusion: The present results indicate that male offspring exposed to prenatal stress continue to show reduced levels of neurodevelopmental makers compared to both their control and female counterparts at PND 21. These findings highlight the vulnerability of the male brain early in life to the effects of prenatal stress. Interestingly, both males and females showed altered behaviour at PND 21, indicating that the marked effects of prenatal stress are long lasting.

O10

Toll-like receptor (TLR) 4 and 7 gene expression in the preterm ovine brain; effects of hypoxia-ischemia (HI) following subacute LPS stimulation.

Simerdeep Dhillon[1], Laura Bennet[2], Luke Weaver-Mikaere[1,2], Alistair J Gunn[2], Mhoyra Fraser[1,2].
[1] *Liggins Institute, University of Auckland, New Zealand*
[2] *Department of Physiology, Faculty of Medical Health Sciences, University of Auckland, New Zealand*
Corresponding author's email: m.fraser@auckland,ac,nz

Introduction: Exposure to infection and secondary inflammation both before and after birth is highly associated with premature birth. Although infection has been commonly associated with neural injury, pre-clinical evidence suggests that whereas acute exposure to infection can trigger injury, longer exposure leads to both self-tolerance and variably either protection or sensitisation to injury from other insults. The molecular mechanisms of this variability have not been elucidated. While studies in fetal and newborn animals have highlighted a strong relationship between the Toll-like receptor (TLR)4 agonist, lipopolysaccharide (LPS), and brain injury, very little is known regarding the involvement of other TLRs and which specific TLR signalling attenuates responses to subsequent acute injury. The objective of this study was to determine the effect of LPS and subsequent HI on key inflammatory genes within the fetal brain.
Materials and Methods: In this study, we evaluated gene expression profiles of TLRs 4 and 7, IRF3 and IFNβ in the preterm fetal sheep brain (0.7 gestation; term 145 days) after exposure to sub-acute exposure to LPS and subsequent (24h post-LPS) acute reversible ischemia induced by bilateral carotid occlusion. 5 days after ischemia, brains were collected for qRT-PCR analysis.
Results and Discussion: LPS preconditioning induced a robust increase ($p<0.004$) in TLR 7 mRNA after acute ischemia. This was associated with increased ($p<0.03$) expression of the neuroprotective type I interferon, IFNβ and TLR4 mRNA expression ($p<0.009$), but no change in the interferon regulatory factor (IRF3).
Conclusion: These preliminary data are consistent with the hypothesis that induction of TLR7 is a critical feature of LPS preconditioning. Further studies are required to dissect whether there is a mechanistic link between LPS preconditioning, TLR7 and the amelioration of oligodendrocyte apoptosis.

O11

Cerebrovascular changes associated with intrauterine growth restriction in newborn lambs. Margie Castillo Melendez[1], Tamara Yawno[1], Euan Wallace[1,2], Graham Jenkin[1,2], Suzie Miller[1] .
[1]*The Ritchie Centre, Monash Institute of Medical Research, Monash University, Clayton, Vic, Australia*
[2]*Department of Obstetrics and Gynaecology, Monash University, Clayton, Vic, Australia.*
Corresponding author's email: margie.castillo-melendez@monash.edu

Introduction: Chronic moderate hypoxia induces angiogenic adaptation in the brain, suggesting a modulatory role for oxygen in determining cerebrovascular development. Chronic intrauterine fetal hypoxia, such as occurs in intrauterine growth restriction (IUGR) is likely to lead to a reduction in oxygen delivery to the brain and long-term neurological abnormalities. Thus we investigated cerebrovasculature structure of newborn lambs that were IUGR.
Materials and Methods: Single uterine artery ligation (SUAL) surgery was performed in fetuses at 105d GA (term 145 d) to induce placental insufficiency and IUGR. Fetuses were monitored during pregnancy and ewes delivered naturally at term. Lambs were sacrificed at 24h after birth for brain collection. Immunohistochemistry was performed on 10 µm paraffin-embedded sections with anti-laminin and anti-VEGF antibodies.
Results and Discussion: IUGR brains displayed a significant reduction in the area occupied by laminin-positive staining as well as optical density (staining intensity), in the cortical (CWM) and periventricular white matter (PVWM), and subventricular zone (SVZ). The number of positive blood vessels was significantly reduced in IUGR lambs (n=7) compared with control (n=8) by 32% in CWM ($p=0.009$); 30% in PVWM ($p=0.0002$), and 31% in the SVZ ($p=0.0006$). The perimeter of blood vessels was decreased in all brain regions examined, and reached significance in the CWM. These cerebrovascular changes were associated with a significant reduction in VEGF-A immunoreactivity: decreased by 42% in CWM compared to control ($p<0.0001$); by 28% in PVWM ($p<0.0001$), and by 62% in the SVZ ($p=0.0007$).
Conclusions: Chronic hypoxia associated with IUGR produces profound vascular changes in the white matter of IUGR newborn brains. Furthermore, these changes are associated with reduced levels of the key angiogenic mediator VEGF. Reduction of capillary density could contribute to under-perfusion of white matter brain regions in IUGR. We are now examining whether the antenatal administration of melatonin can moderate these effects within IUGR newborn brains.

O12

Melatonin improves cerebral vascular function in chronically hypoxic neonates.

Roberto Macchiavello1, Camilo Montt1, Germán Ebensperger1, Emilio A. Herrera1,2.
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, Putre, Chile.
Corresponding author's email: eherrera@med.uchile.cl

Introduction. Melatonin is a neurohormone found in several living organism. One of the most important features is its antioxidant capacity, which has placed melatonin as a potential agent for treating/preventing diseases related to oxidative stress and vascular dysfunction. Moreover, melatonin has been proposed as a vasodilator of the cerebral circulation. In this study we assessed if melatonin improves the cerebral vascular function in chronically hypoxic newborn lambs.

Materials and Methods. Ten newborn lambs gestated, born and studied in chronic hypoxia (3,600 masl) were used in this study. Five received oral melatonin (MN, 1mg.kg-1.d-1) and five received vehicle (CN) during 7 days. During treatment, we measured daily hemodynamic variables, carotid blood flow and vascular resistance. Further, these variables were recorded during graded FiO2 changes. Also, middle cerebral artery vascular reactivity (ex vivo) and morphometrics (in vitro) studies were determined.

Results and Discussion. Melatonin-treated neonates presented an increased fractional growth the first 3 days. Further, both groups presented similar hemodynamic systemic variables along the treatment. By the end of treatment, melatonin-treated animals showed a higher carotid blood flow at any arterial PO2. In addition, melatonin-treated animals had an enhanced maximal response to serotonin (CN: 145±9 vs MN: 180±8 %Kmax) and increased methacholine sensitivity (CN: 6.79±0.18 vs MN: 8.35±14 pD2). The vasodilator function was enhanced by nitric oxide-independent mechanisms (Figure 1). Finally, the MCA wall thickness increased with melatonin.

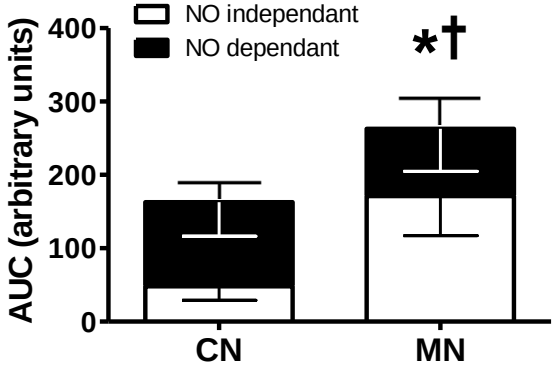


Figure 1. Partial contribution of NO-dependent and NO-independent mechanisms to the endothelial-dependent relaxation. Area under the curve (AUC) for MetCh-induced relaxation (complete bar with positive S.E.M.), the AUC for MetCh-induced relaxation following treatment with LNAME (NO-independent component, white bar with negative S.E.M.), and the remaining AUC after MetCh with LNAME (NO-dependent component, black bar with negative S.E.M). Groups are vehicle (CN) and melatonin (MN) treated. Values are mean ± SEM. Significant differences ($p<0.05$): * vs. CN for total endothelial-dependent relaxation, † vs. CN for NO-independent relaxation .

Conclusion. Post-natal treatment with melatonin modulates the cerebral vascular function, promoting muscular and endothelial reactivity. In addition, melatonin increases the carotid blood flow and determinates morphologic changes that result in better cerebral perfusion in the neonatal period in chronically hypoxic newborns. Melatonin emerges as a realistic potential therapeutic agent in perinatal diseases to treat vascular dysfunction associated with oxidative stress and chronic hypoxia.

Supported by FONDECYT 1110595, 1120655 & 1130424.

O13

A comparison of melatonin and hypothermia for the treatment of the acutely asphyxiated newborn lamb.

James Aridas[1], Tamara Yawno[1], Jingang Li[1], Amy Sutherland[1], Michael Fahey[1,2], Atul Malhotra[1,2], Flora Wong[1,2], Michael Ditchfield[2], Alistair Jan Gunn [4],Euan Wallace[1,3], Graham Jenkin[1,3], Suzie Miller[1,3]

[1] *The Ritchie Centre, Monash Institute of Medical Research, Monash University, Clayton, Vic, Australia.* [2] *Monash Childrens, Monash Health, Clayton, Vic, Australia.*[3]*Obstetrics and Gynaecology, Monash University, Clayton, Vic, Australia.* [4]*Department of Physiology, Auckland University, Auckland, New Zealand*

Corresponding author's email: james.aridas@monash.edu

Background: Hypoxic ischaemic encephalopathy (HIE) describes the clinical syndrome following a severe lack of oxygen to the baby during labour and has significant short- and long-term consequences. Despite the efficacy of hypothermia in reducing mortality and morbidities associated with HIE, nearly 50% of children treated still suffer permanent neurological impairment. HIE is associated with oxidative stress in newborn brains and is reduced by the antioxidant melatonin treatment, suggesting that co-treating HIE newborns with hypothermia and melatonin may provide additive benefit, compared to hypothermia alone.

Aims/Hypothesis: We aimed to determine the effects of melatonin in a newborn lamb birth asphyxia model of HIE with and without hypothermia.

Methods: HIE was induced via umbilical cord occlusion (UCO) at caesarean section in term fetal lambs. Lambs were resuscitated and stabilised after delivery. UCO lambs were allocated to UCO or UCO plus melatonin (UCO+MEL; 60mg/24h), hypothermia (UCO+HYPO; 35°C from 4 to 28h after delivery), or hypothermia+melatonin (UCO+HYPO+MEL). Control (CONTROL) lambs were delivered without UCO. Physiological and behavioural outcomes were observed and MRS was undertaken 72 hours post-delivery, after which lambs were euthanazed and post mortem performed, with brains processed for histology.

Results: Decreased cardiovascular/respiratory drive was observed in UCO+HYPO, compared to UCO±MEL and CONTROL. Cardiovascular/ respiratory indices were not improved by melatonin (UCO+HYPO+MEL). Melatonin groups showed improved behavioural outcomes compared to UCO±HYPO. Brain Lactate:NAA ratio (MRS) was improved in all treated groups (UCO+MEL 0.1±0.0; UCO+HYPO 0.09±0.1; UCO+HYPO+MEL 0.11±0.0) compared to UCO (0.31±0.18). UCO brains showed increased cell death (CA3 hippocampus 109±16cells/mm^2) compared to UCO+MEL (71±19cells/mm^2) and CONT (57±18cells/mm^2; $p<0.05$). Brain histology of hypothermia groups is being undertaken.

Conclusions: Hypothermia is a key treatment therapy for newborns suffering HIE; but 50% of babies still suffer permanent neurological damage. Melatonin is a potential adjuvant therapy to improve neurological outcome for newborns undergoing hypothermia therapy for HIE.

O14

Postnatal outcomes in lambs exposed antenatally to fluoxetine.

Tuan Anh Nguyen[1], Timothy Chow[2], Wayne Riggs[2], Dan Rurak[1]
[1] Department of Obstetrics and Gynecology, University of British Columbia; [2] Faculty of Pharmaceutical Sciences, University of British Columbia, Canada.
Corresponding author's email: drurak@cw.bc.ca

Introduction: Selective serotonin reuptake inhibitors (SSRIs) cause changes in fetal behavioral states and neuroendocrine function in sheep, and alteration in fetal MCA flow characteristics and blunted fetal heart rate variability (HRV) in the human. Yet, the long-term effects of prenatal SSRIs exposure on neurobehavioral development are uncertain. In this study, we investigated postnatal outcomes in lambs exposed to fluoxetine (FX) during late gestation.
Materials and Methods: At ~130 d gestation (term ~ 147 d), a heparin-bonded 5.0 French polyurethane catheter was percutaneously implanted in the ewe's jugular vein under local analgesia. 50mg of FX was given i.v. daily to pregnant ewes (N=5) for 14 days or until birth, with an average exposure time of 12.0±1.3 d. Neonatal rest-activity and feeding behaviors were determined by actiwatch and continuous digital video recording (DVR). Birth weight, daily weight gain, heart rate and tissue sPo_2 (pulse oximeter) and the ECG (surface electrodes) were obtained. Blood samples were also collected at birth in both ewes and lambs and on PNl d 2,5,10,14 in lambs for FX measurement.
Results and Discussion: Following birth, the lamb:ewe FX concentration ratio declined logarithmically. No significant differences in birth weight, daily weight gain, heart rate and sPo_2 were found between control (n=7) and FX-exposed (n=7) lambs. However, prenatally FX-exposed lambs were more active, indicated by significantly faster first time activities and an increase in subsequent activities obtained from actiwatch and DRV observations. This high level of activity still persisted on PN d 5, 10, 14 when FX was no longer detectable. HRV on PN d2 was significantly lower in the FX-exposed lambs than the control lambs.
Conclusion: In conclusion, prenatal FX exposure appears to have behavioral effects in the offspring, which persisted long after FX levels were undetectable. This supports a mechanism of SSRI-induced alteration in fetal brain development with *in utero* exposure to the drugs.
Supported by CIHR and CFRI.

Oral Communications

Session IV: Fetal & Neonatal Brain II

Chairs:

Emily J. Camm (University of Cambridge, UK)

Charles E. Wood (University of Florida, USA)

O15

The $GABA_A$ excitatory-to-inhibitory switch in the hippocampus of perinatal guinea-pigs.

Harold Coleman[1], Jon Hirst[2], Helena C Parkington[1].
[1] *Department of Physiology, Monash University, Melbourne, Vic., Australia*
[2] *Discipline of Pharmacy & Experimental Pharmacology, University of Newcastle, NSW, Australia*
Corresponding author's email: harry.coleman@monash.edu

Introduction: Perinatal trauma can result in neural excitotoxicity and consequent damage to the brain. Potentially, such brain damage might be reduced by prophylactically enhancing the activity of $GABA_A$ receptors since GABA is the main inhibitory neurotransmitter in the brain. The main ion that permeates the $GABA_A$ receptor channel is Cl^- so the direction of the current depends on the intracellular Cl^- concentration. In rat pups, $GABA_A$ is initially excitatory in the hippocampus. At around P8 – P10, $GABA_A$ switches to being inhibitory [1]. The aim of the present study was to determine the nature of $GABA_A$ receptor activation in the perinatal period of a more precocious species, the guinea-pig, particularly whether or when $GABA_A$ switches from being excitatory to being inhibitory.
Materials and Methods: Hippocampal brain slices from guinea-pig pups aged between E50 and P5 were studied electrophysiologically. Recordings were made from CA1 and CA3 pyramidal cells using sharp intracellular microelectrodes filled with 1 M potassium acetate, chosen to cause minimal perturbation to the intracellular Cl^- concentration. $GABA_A$ receptors were activated by electrically stimulating presynaptic axons resulting in postsynaptic potentials (PSPs), or by close application of the $GABA_A$ agonist isoguavacine. Other receptors were blocked when necessary.
Results: In CA1 and CA3 pyramidal neurons from pups at around E55 and earlier, nerve stimulation produced depolarizing PSPs and isoguavacine evoked depolarization that resulted in action potential activity. In contrast, in late gestation nerve stimulation evoked robust hyperpolarizing and therefore inhibitory (I)PSPs and isoguavacine evoked hyperpolarization that suppressed action potential activity.
Conclusion: $GABA_A$ receptors are excitatory up to around E55, and then switch to being inhibitory well before birth (Term; D70). Importantly, this contrasts with the observations in the highly immature rat model where the switch does not occur until well after birth.
Reference:
1. Tyzio R, Holmes GL, Ben-Ari Y, Khazipov R. (2007). Timing of the developmental switch in $GABA_A$ mediated signaling from excitation to inhibition in CA3 rat hippocampus using gramicidin perforated patch and extracellular recordings. *Epilepsia* **48**(Suppl. 5):96–105.

O16

Exposure to TNF-α and LPS in an *in vitro* ovine model of oligodendrocyte injury: Effects on glutamate and its receptors.

Luke Weaver-Mikaere [1, 2] Alistair Jan Gunn [2], Murray Mitchell [1,3], Laura Bennet [2], Mhoyra Fraser [1, 2]
[1] *The Liggins Institute, The University of Auckland, New Zealand.* [2] *The Department of Physiology, The University of Auckland, New Zealand.* [3] *Centre for Clinical Research, The University of Queensland, Australia.*
Corresponding author's email: l.weaver-mikaere@auckland.ac.nz

Introduction: Production of inflammatory mediators, such as the cytokine TNF-α, within the brain of prematurely born infants, is strongly associated with oligodendrocyte (OL) cell death and subsequent hypomyelination. The precise mechanism of injury in neuroinflammation is unclear, however recent evidence suggests that infection can trigger release of TNF-α and subsequent activation of a Cox2-PGE2-dependent release of glutamate from astrocytes. Given that immature OLs express AMPA glutamate receptors (GluRs) and are vulnerable to excess glutamate, this raises the possibility that this mechanism may be a critical pathway to post-infectious injury in the developing brain. We hypothesise that exposure to infectious agents such as TNF-α or bacterial endotoxin (Lipopolysaccharide, LPS) induces white matter injury through combined TNF-α-glutamate activation and upregulation of Ca^{2+} permeable isoforms of the glutamate receptor; AMPA receptors lacking the GluR2 subunit permit excess Ca^{2+} influx.
Materials and Methods: Using primary preterm fetal ovine mixed glial cultures we evaluated whether, and over what time course, a TNF-α-glutamate pathway modulates AMPAR subunit composition, and if this affects OL cell survival.
Results and Discussion: Expression of AMPA receptors was localised to immature OLs and exposure to either TNF-α or LPS resulted in a significant reduction of GluR2 subunit expression at the level of transcription ($p < 0.001$). LPS and TNF-α treatment produced selective OL apoptosis and increased glutamate and PGE2 levels by 72 hours of exposure ($p < 0.001$, $p < 0.01$, respectively), all of which were significantly attenuated by TNF-α inhibition.
Conclusion: Our data of LPS and TNF-α induced changes in glutamate concentration and expression of GluRs *in vitro* suggest that glutamate may contribute to WMI in response to infection/inflammation.

O17

Comparative transcriptomics of the fetal hypothalamic responses to hypoxia versus ischemia. Charles E. Wood[1], Eileen I Chang[1], Elaine M Richards[2], and Maureen Keller-Wood[2]
Departments of Physiology and Functional Genomics[1] and Pharmacodynamics[2], University of Florida Colleges of Medicine[1] and Pharmacy[2]
Corresponding author's email: woodc@ufl.edu

Introduction: Hypoxia stimulates homeostatic responses that are important for survival of the fetus. Responses of the fetal brain to hypoxia are in part dependent upon cellular hypoxia in the peripheral chemoreceptors and in the brain. The present study was undertaken to compare the transcriptomics response to cerebral and carotid body ischemia to that of ventilatory hypoxia to test the hypothesis that additional variables play a role in the stimulation of fetal brain responses to hypoxia.
Materials and Methods: Chronically catheterized fetal sheep (124-132 days gestation, term=147d) were euthanized and hypothalami isolated 1 hr after onset of a 30 min hypoxia (n=3) or normoxia (n=4) or 1 hr after onset of a 10 min occlusion (n=4) or sham occlusion (n=4) of the brachiocephalic artery. mRNA was extracted and analyzed using the ovine Agilent 15.5k array, as used and annotated in this lab. Data analysis using Cytoscape.
Results and Discussion: Hypoxia significantly ($p<0.05$) differentially regulated (DR) 1411 genes. BCO DR 891 genes. Only 237 genes were DR by both stimuli. Gene ontology analysis revealed that genes DR by both stimuli were significantly ($p<0.05$) associated with RNA transcription from RNApol2 promoter, apoptosis, astrocyte migration, enzymatic response to peptides. Hypoxia DR RNA processing+splicing, glucocorticoid biosynthesis, NK cell response. BCO DR prostaglandin, glucocorticoid, chemokine signaling, IgG production, B cell activation, endosome&post-Golgi transport processes. Together, these results demonstrate that the transcriptomics response to hypoxia is more extensive than the response to direct cerebral and chemoreceptor ischemia, and that only a subset of the response to hypoxia is shared with the response to BCO.
Conclusion: We conclude that fetal brain responses to hypoxia are more extensive than can be accounted for by cellular ischemia or carotid chemoreceptor activation alone. We speculate that humoral responses from other tissues are responsible for most of the brain responses to hypoxia.

O18

Very low regional cerebral oxygen saturation during the postnatal transition of very preterm infants directly after delivery by caesarean section.

Tom Goos[1, 2], Corinna Binder[3], Andre Kroon[1], Jeroen Dudink[1, 4], Hugo van Elteren[1], Jenny Dankelman[2], Gerhard Pichler[3], Berndt Urlesberger[3], Irwin Reiss[1].

[1]Department of Paediatrics, Division of Neonatology, Erasmus Medical Centre - Sophia Children's Hospital, Rotterdam, The Netherlands [2]Department of Biomechanical Engineering, Delft University of Technology, Delft, The Netherlands. [3]Department of Paediatrics, Division of Neonatology, Medical University Graz, Graz, Austria. [4]Department of Radiology, Division of Paediatric Intensive Care, Erasmus Medical Centre - Sophia Children's Hospital, Rotterdam, The Netherlands

Corresponding author's email: i.reiss@erasmusmc.nl

Introduction: In order to gain more insight into the adaptation of the regional cerebral oxygen saturation ($rScO_2$) in preterm infants directly after birth, an observational study was performed.

Materials and Methods: In a single-centre pilot study, $rScO_2$ was measured during the resuscitation of very preterm infants (gestational age (GA) ≤30 weeks) delivered by caesarean section.

Infants were resuscitated according to the European Resuscitation Council guidelines. Arterial oxygen saturation (SpO_2) was measured with a Nellcor N600-x pulse oximeter, while $rScO_2$ was measure with an Invos 5100C (Covidien, Mansfield, Massachusetts). Data are presented as median (IQR).

Results and Discussion: Six infants (GA 26 $^4/_7$ weeks (26 $^3/_7$-27 $^1/_4$), birth weight (BW) 888g (693-920)) were the first to be included. In all included infants a $rScO_2$ measurement was obtained within 1:08 minutes after birth. Five infants had an initial $rScO_2$ of 15% or less, one infant 24%. The infants left the resuscitation area after 21:51 (15:27-23:39) minutes, by then the $rScO_2$ had risen to 61% (47-74%). During the resuscitation SpO_2 and Apgar scores were similar to prior observations in our institution [1].

When the results are compared to that of infants with a GA of 30-37 weeks that required respiratory care (N=21), the initial $rScO_2$ is significantly lower [2]. Even in infants with a BW <1500g rScO2 was higher [3]. Two possible explanations for these very low $rScO_2$ could be the different adaptation of the circulation and perfusion of very preterm born neonates, or specifically a difference in perfusion and/or activity in the frontal lobe.

Conclusion: Very preterm infants are born with very low $rScO_2$, which recovers to normal values during resuscitation. The reason for this very low saturation is not yet understood and more investigations are needed.

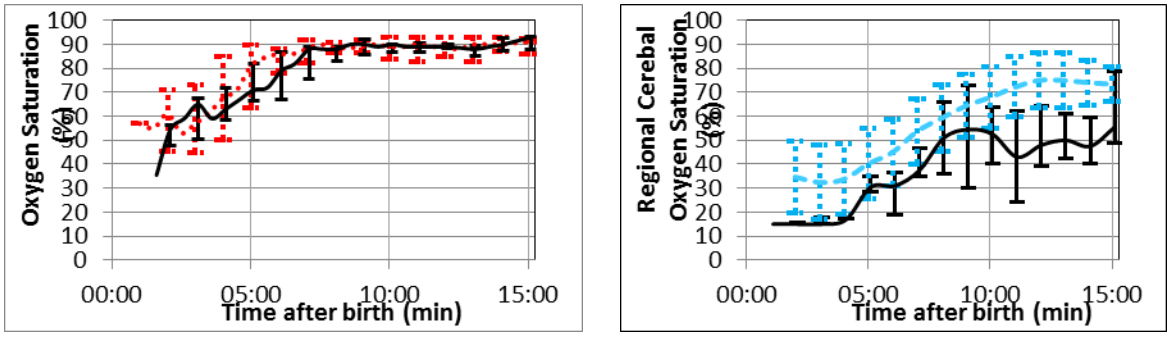


Figure1 A) SpO_2 of the first 15 minutes after birth, the observed infants (solid black) compared to a control group (dotted red) (N=28) [1]. **B)** The $rScO_2$ of the observed infants compared to infants with a GA of 30-37 weeks that required respiratory support (N=21) [2]. (Results are represented as median (IQR)).

References:

1. Goos, T.G., et al., *Observing the resuscitation of very preterm infants: Are we able to follow the oxygen saturation targets?* Resuscitation, 2013
2. Binder, C., et al., *Cerebral and Peripheral Regional Oxygen Saturation during Postnatal Transition in Preterm Neonates.* The Journal of pediatrics, 2013
3. Fuchs, H., et al., *Brain oxygenation monitoring during neonatal resuscitation of very low birth weight infants.* Journal of Perinatology, 2011

O19

Prevention of the long-term effects elicited by perinatal asphyxia: inhibition of PARP-1 activity as a therapeutic strategy worth to be clinically translated.

Herrera-Marschitz M[1,2], Cerfogli C[1,2]; Neira-Peña T[1,2], Rojas-Mancilla E[2]; Perez R[2], Rivera B[2], Gutierrez M[1,2], Esmar D[2], Espina-Marchant P[2], Valdes JL[1], Simola N[4], Bustamante D[2], Gebicke-Haerter P[3,2], Morales P[2].
[1]Millenium Institute BNI-Chile; [2]*Programme of Molecular & Clinical Pharmacology, ICBM*, Faculty of Medicine, University of Chile, Santiago, Chile; [3]Dept. of Psychopharmacology, Central Institute of Mental Health J5, Mannheim, Germany; [4]University of Cagliari, Cagliari, Italy
Correspondence: mh-marschitz@med.uchile.cl

Perinatal asphyxia (PA) is a factor priming the development of the central nervous system, affecting the bioavailability of specific neuronal systems. It has not been clarified yet if that specificity is primarily related to neuronal loss, ectopic neuronal connections, faulty protein expression, epigenetic and/or post-transcriptional modifications or developmental features of the vulnerable systems, by which transient events confer long-term changes in function.
The final outcome of oxygen interruption is death, whenever oxygenation is not promptly re-established. Re-oxygenation triggers a cascade of biochemical events for restoring function, implying oxidative stress, and recovery with defect. A major role for the consequences elicited by PA is played by sentinel proteins, sensing damaged DNA and recruiting the DNA repair machinery, including Poly (ADP-ribose) polymerase-1 (PARP-1). PARP-1 overactivity has shown to worsen the final outcome by further depleting NAD^+ stores, leading to the hypothesis that PARP-1 inhibition is a suitable target for pharmacological treatments lessening the long-term effects produced by PA.
This hypothesis has been studied in our lab with an experimental model of global PA in rats, finding that PARP-1 inhibition with nicotinamide (Vitamin B3) constitutes a powerful pharmacological treatment preventing molecular, cellular and behavioural consequences of hypoxic/ischemic insults occurring at birth. Nicotinamide constitutes a lead for exploring compounds with similar or better pharmacological profiles.

Acknowledgments. Supported by FONDECYT-Chile 1120079, 1110263; Millennium Initiative-2010 BNI (P09-015-F; Chile).

O20

Maternal Allopurinol Administration During Term Labor For Neuroprotection In Case Of Fetal Asphyxia; A Multicenter Randomized Controlled Trial (ALLO trial).

Joepe J. Kaandorp[1], Manon J. Benders[2], Ewoud Schuit[3], Carin M. Rademaker[4], Martijn A. Oudijk[2], Martina M. Porath[5], Sidarto Bambang Oetomo[6], Maurice G. Wouters[7], Ruurd M. Elburg[8], Maureen T. Franssen[9], Arie F. Bos[10], Timo R. de Haan[12], Janine Boon[13], Inge P. de Boer[14], Robbert J. Rijnders[15], Corrie J. Jacobs[16], Liesbeth C. Scheepers[17], Danilo A. Gavilanes[18], Kitty W. Bloemenkamp[19], Monique Rijken[20], Gerard H. Visser[2], Ben Willem J. Mol[11], Frank van Bel[2], Jan B. Derks[2]

1. Dutch Consortium for Studies in Obstetrics, Utrecht; 2. Perinatology, University Medical Center Utrecht, Utrecht; 3. Julius Center for Health Sciences, University Medical Center Utrecht, Utrecht; 4. Clinical Pharmacy, University Medical Center Utrecht, Utrecht; 5. Obstetrics, Maxima Medical Center, Veldhoven; 6. Neonatology, Maxima Medical Center, Veldhoven; 7. Obstetrics, VU University Medical Center, Amsterdam; 8. Neonatology, VU University Medical Center, Amsterdam; 9. Obstetrics, University Medical Center Groningen, Groningen; 10. Neonatology, University Medical Center Groningen, Groningen; 11. Obstetrics, Academic Medical Center, Amsterdam; 12. Neonatology, Academic Medical Center, Amsterdam; 13. Obstetrics, Diakonessenhuis, Utrecht; 14. Neonatology, Diakonessenhuis, Utrecht; 15. Obstetrics, Jeroen Bosch Hospital, Den Bosch; 16. Neonatology, Jeroen Bosch Hospital, Den Bosch; 17. Obstetrics, Maastricht University Medical Center, Maastricht; 18. Neonatology, Maastricht University Medical Center, Maastricht; 19. Obstetrics, Leids University Medical Center, Leiden; 20. Neonatology, Leids University Medical Center, Leiden; The Netherlands.

Corresponding author's email: J.B.Derks@umcutrecht.nl.

Introduction: Hypoxic-ischemic encephalopathy due to perinatal hypoxia-induced free radical formation is an important cause of neurodevelopmental disabilities. Allopurinol (ALLO) reduces the formation of free radicals, which potentially limits hypoxia-induced brain damage. With this trial we aimed to assess whether maternal ALLO treatment during fetal hypoxia would reduce the release of biomarkers associated with neonatal brain damage.

Material and Methods: We performed a randomized double blind placebo controlled multicenter trial (NCT00189007) studying laboring women at term with imminent fetal hypoxia. Fetal distress was suspected in case of an abnormal fetal heart rate, significant ST-changes on fetal ECG or fetal scalp pH<7.20. Women were allocated to receive ALLO 500 mg IV or placebo immediately prior to delivery. Primary endpoint was S100B in cord blood, neuroketal was a secondary endpoint. Both are tissue-specific biomarkers for brain damage. Because S100B followed a very skewed distribution we performed quantile regression to estimate the difference in median between the treatment groups (DiM). For neuroketal we report geometric mean differences (GMD). Because both S100B and neuroketal approached physiological values, we also examined the difference in infants with an S100B or neuroketal value > p75.

Results: Between Oct 2009 and Dec 2011 we randomized 222 women to ALLO (n=111) or placebo (n=111). No significant differences were found between the two groups for both S100B (DiM -7.69 (95%CI -24.9;9.52), RR>p75 0.85 (95%CI 0.53-1.36)) and neuroketal (GMD -7.53 (95%CI -15.5;3.62), RR>p75 0.85 (95%CI 0.51-1.4)). Post-hoc subgroup-analysis however showed a marked gender difference in treatment effect in favor of girls for both S100B (RR>p75 0.37 (95%CI 0.14-0.99)) and neuroketal (GMD-16.4 (95%CI -24.6;-1.64)).

Conclusion: Maternal treatment with ALLO during fetal hypoxia reduces damage to neuronal cells as indicated by brain-tissue-specific chemical biomarkers, but only in girls.

Oral Communications

Session V: Fetal & Neonatal Cardiovascular I

Chairs:

Anibal J Llanos (Universidad de Chile, Chile)

Bill Parer (University of California San Francisco, USA)

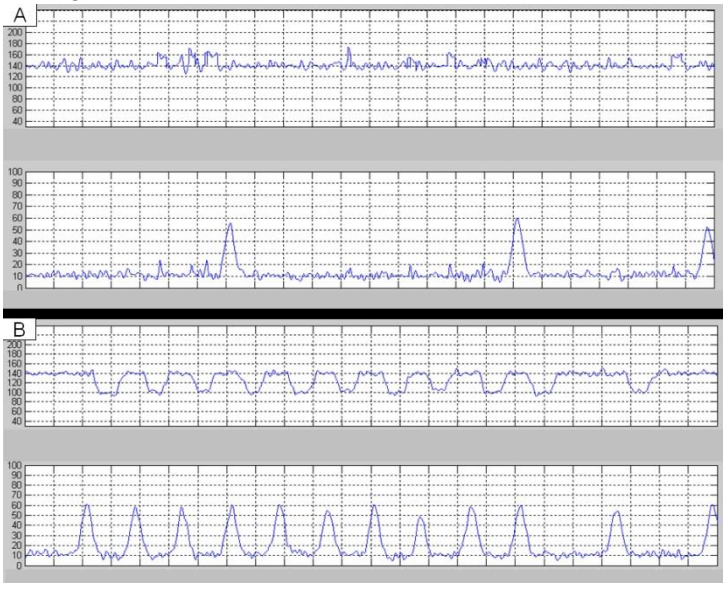
O21

Dynamical model based simulator of fetal cardiotocography for educational training purposes.

Francisco A. Guerra[1, 2,] Pablo Moore[1] , Rosario Negron[1], and Alfredo Illanes[3].
[1] Instituto de Obstetricia y Ginecología, [2] Instituto de Fisiología, [3] Instituto de Electricidad y Electrónica, Universidad Austral de Chile, Valdivia, Chile.
Corresponding author's email: fguerra@uach.cl

Introduction: In medicine, simulator model can be used to assist clinical staff in the interpretation of the fetal cardiotocography (CTG), since they provide a risk free and controllable environment for training. However, currently available obstetric simulators and training programs provide limited realism and most of them simulate only static scenarios without the possibility of changing dynamics during the simulation. Moreover although several mathematical models has been proposed for CU and FHR signal generation separately, few of them put in relationship the dynamics of both signals. Our purpose was to develop a dynamical model based simulator of fetal cardiotocography for training purposes.
Materials and Methods: Based on the physiologic control of the fetal cardiovascular system, a dynamical model based simulator using matlab was develop on five interconnected blocks corresponding to: user's control panel (UCP), a fetal heart rate (FHR) generator (FHR), a uterine contraction (UC) generator, and random fetal movement (FM) generator, plus a memory and a display blocks which allows to store dynamical information about the fetus state through a simple mathematical function.
Results and Discussion: The model provides the main dynamics of a CTG including baseline of the FHR, variability, accelerations, decelerations (early, late and variables). The UCP allows the user to set different simulation parameters including the possibility of generate UC or FM on-line during the simulation. Simulated tracings were analyzed by specialists and evaluated in terms of signal realism and dynamics of the normal changes, periodic and episodic changes of the FHR with no significant differences between real and computer-generated CTG tracings.
Conclusions: This Dynamical model based simulator of fetal cardiotocography fulfills the realism, adding the possibility of changes on line in any part of the tracing generation. The next step will be to integrate this model as a learning object on labor scenarios.

Figure: Two simulated FHR tracing



O22

Human fetal endothelium require A_{2A}AR for generating angiogenesis.

Angelo Torres[1,3], Jesenia Acurio[1], Francisco Valenzuela[1], Patricio. Bertoglia[2], Carlos Escudero[1]

[1]Vascular Physiology Laboratory, Group of Investigation in Tumor Angiogenesis (GIANT), Department of Basic Sciences, University of Bío-Bío, Chillán, Chile. [2]Obstetric and Gynecology Department, Hospital Clinico Herminda Martin, Chillán, Chile and Universidad Católica de la Santísima Concepción, Concepción. [3]Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile.

Corresponding author's email: cescudero@ubiobio.cl

Introduction: We aimed to investigate whether the functional presence of adenosine A_{2A} receptor (A_{2A}AR) is necessary for inducing angiogenesis via the cAMP/NO/VEGF signaling pathway in human umbilical vein endothelial cells (HUVEC).

Materials and Methods: HUVEC isolated from normal pregnancies (n=10) were cultured under standard condition (37 °C, 5% CO_2). Cells were exposed or not to either shRNA-A_{2A}AR or respective control (i.e., shRNA-A)(Santa-Cruz Biotechnology) in presence of polyethylenimine (1 µg/ml). The plasmid eGFP (Addgene) was used for estimating the amount of cell positive to green fluorescent protein (GFP) as transfection control. Knocking down of A_{2A}AR was investigated by semi-quantitative PCR and immunocytochemistry, whereas cell lethality was assayed by counting trypan blue positive cells. Forty-eight hours post-transfection, cells were used for measuring nitrates in culture medium, as well as intracellular cAMP levels or VEGF mRNA levels in presence or absence of A_{2A}AR selective agonist (CGS-21680, 100nM). Tube formation on matrigel was used as functional assay. All experiments were performed in medium supplemented with adenosine deaminase (0.01 UI/ml).

Results and Discussion: Transfection efficiency was ~35%. Transfection with shRNA-A_{2A}AR reduced the expression of A_{2A}AR (protein ~79%, mRNA ~67%), while shRNA-A do not affected it in comparison with cells without transfection. Despite shRNA-A_{2A}AR induce elevated cell death it did not reach statistical significance. Furthermore, knocking down of A_{2A}AR was associated with reduced capacity of tube formation compared with controls. Moreover, cells repressing A_{2A}AR exhibited lower levels of nitrites and VEGF (~30% y ~68%, respectively) than controls. After stimulation with CGS-21680, control cells exhibited elevation in cAMP (~30 fold), nitrites (~1,5 fold) and VEGF (~1,7 fold), whereas these effects were not observed in cells repressing A_{2A}AR.

Conclusion: A_{2A}AR is required for inducing cAMP/NO/VEGF-mediated angiogenesis.

Acknowledge: FONDECYT 1100684, Conicyt Anillo ACT73.

O23

Mechanisms of hypoxic pulmonary vasoconstriction.

Norbert Weissmann

Excellencecluster Cardio-Pulmonary System (ECCPS), Universities of Giessen and Marburg Lung Center (UGMLC), Member of the German Lung Center (DZL) Justus-Liebig-University GIESSEN Aulweg 130 35392 Giessen, Germany
Corresponding author: Norbert.Weissmann@innere.med.uni-giessen.de

Hypoxic pulmonary vasoconstriction (HPV) is an essential physiological mechanism of the lung which adapts blood perfusion to the local ventilation situation. Under conditions of regional alveolar hypoxia HPV reduces blood flow to poorly ventilated and thus hypoxic lung regions. HPV can also be termed normoxic pulmonary vasodilatation as HPV is activated after birth when alveolar ventilation and thus oxygen uptake starts. HPV in this regard helps to prevent blood flow through the lung of the unborn. Disturbances in vasorelaxation after birth may contribute to persistent pulmonary hypertension development of the newborn (PPHN).

Despite intensive research, the underlying mechanisms of HPV have not been fully elucidated yet. On the level of the oxygen sensors reactive oxygen species (ROS) producing systems have been proposed to be the initial part of a reaction chain leading to these physiological and pathophysiological effects. However, there is still discussion whether an increase or a decrease of ROS under hypoxia induces HPV. Also, the downstream targets of ROS signalling are still under investigation. Voltage-dependent potassium channels (Kv-channels) as well as transient receptor potential channels (TRPC) have been found to be essential in this regard.

Deciphering the mechanism underlying HPV is a prerequisite for the development of strategies to treat a disturbed HPV and subsequent hypoxemia as well as the contribution to PPHN and high altitude induced pulmonary hypertension.

O24

Hemin Treatment Reverses Pulmonary Arterial Hypertension in High Altitude Neonates by increasing cGMP pathway.

[1]Ebensperger G, [1]Araya CE, [1]Quezada S, [1]González A, [1]Leiva M, [1,2]Herrera EA, [3]Moraga F, [1]Reyes RV, [1]Díaz M, [4]Silva P , [1]Díaz PV, [1,2]Llanos AJ,

[a]Unidad de Fisiología y Fisiopatología Perinatal, Facultad de Medicina, [b]INCAS, Universidad de Chile; [c]Laboratorio de Fisiología, Escuela de Medicina, Universidad Católica del Norte, Coquimbo, Chile; [d]Departamento de Ciencias Biológicas y Fisiológicas, Universidad Peruana Cayetano Heredia, Lima, Perú.
Corresponding author's email: gebensperger@med.uchile.cl

Introduction. Pregnancy at high altitude yields neonates with pulmonary hypertension[1]. Hemin treatment in high altitude neonatal sheep showed a partial decrease in PAP[2], which may be explained, among other mechanisms, by an increased vasodilator tone dependent on cGMP pathway in small pulmonary arteries[3]. Therefore, we hypothesize that the decrease in PAP in hemin-treated newborn lambs is due to an increased function of the cGMP signaling pathway.
Material & Methods. Fourteen lambs, whose gestation, birth and experimental procedures took place in high altitude (Putre, 3,600m), were divided in two groups: 7 neonates were treated with hemin (15 mg/Kg/day, s.c.) and 7 neonates were treated with vehicle (0.01N NaOH buffered with saline solution) as controls, for 10 days. After treatment, they underwent euthanasia with an overdose of sodium thiopentone (100 mg•kg^{-1} IV) to study the vascular reactivity of small pulmonary artery, utilizing wire myography in response to a NO donor (SNP), BK_{Ca} channel opener (NS 1619), PKG-1 activator (8BrcGMP), sGC activator (YC-1) and inhibitors against PDE5 (Sildenafil) and RhoA kinase (Fasudil). Further, we assesed pulmonary protein expression of sGC, PDE5, BK_{Ca}, PKG-1, and β-actin by immunoblot. All procedures were approved by a local ethical committee (CBA # 0561 FMUCH).
Results and Discussion. Hemin induced an increased maximal relaxation in all the evaluated components of cGMP pathway. Further, there was an increased function of PDE5 and RhoA kinase ($p<0.05$, Fig.1) in hemin neonates. Moreover, the expression of sGC, BKCa channels and PKG-1 was higher in hemin lambs ($p<0.05$).
Conclusions. The lower pulmonary arterial pressure in the hemin-treated newborns is partially explained by the increased function of cGMP signaling pathway. Further studies are in progress to investigate the cGMP levels in pulmonary arteries, the heme oxygenase crosstalk with other pathways and its role in the regulation of the pulmonary vascular tone.

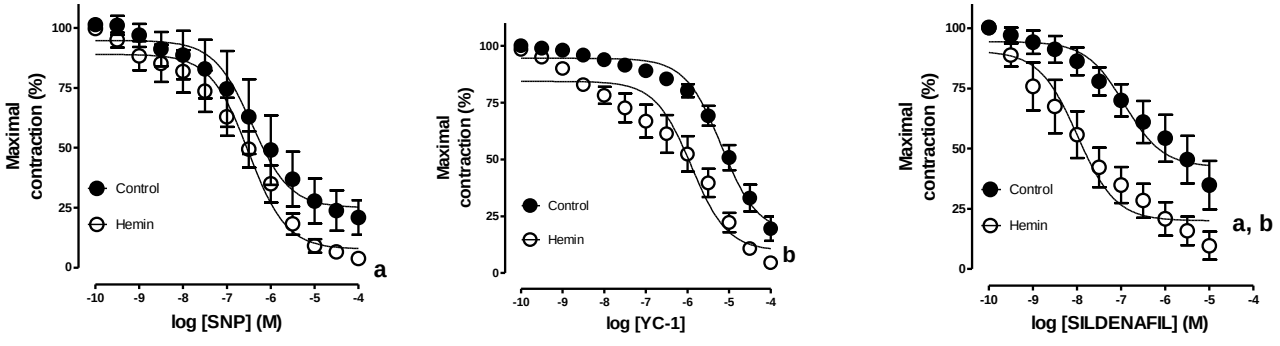


Fig 1. Vascular responses of the cGMP signaling pathway components. Values are mean ± SEM. Groups are controls newborns (close circles) and hemin-treated newborns (open circles). Concentration-response curves to (A) SNP, (B) YC-1 and (C) Sildenafil. Significant differences ($p<0.05$): a *vs. Hemin treated lambs Maximal response;* b *vs. Hemin treated lambs sensivity*.

References:

(1) Herrera EA. et al. *Am. J. Physiol*. 292(6):R2234-R2240.2007.
(2) Ebensperger G et al. *Fetal and Neonatal Physiological Society Annual Meeting 2009.*
(3) Ebensperger G Doctoral thesis. *2011.*

Supported by CONICYT, FONDECYT 1130424, 1090355, 1110595 y 1120605.

O25

Reactivity of the umbilical vessels of the late chicken embryo.

Riazudin Mohammed[1], Lilian Kessels[1], Eduardo Villamor[1]
[1]*Department of Pediatrics, Maastricht University Medical Center (MUMC+), Maastricht, the Netherlands*
Corresponding author's email: e.villamor@mumc.nl

Introduction: The chorioallantoic membrane (CAM) is the gas exchange organ and the yolk sac is the nutritional organ of the late chicken embryo. We hypothesized that the separation of the 'placental' functions would be accompanied by a concomitant functional specialization of the arteries supplying the CAM and the yolk sac.
Materials and Methods: We compared, using wire myography, the reactivity of allantoic and yolk sac arteries from 19d chicken embryos (total incubation period 21days). The contractions induced by KCl, the nonselective adrenergic receptor agonist norepinephrine (NE), the α_1-adrenergic agonist phenylephrine, the α_2-adrenergic agonist oxymetazolin, serotonin, the TP receptor agonist U-46619, and endothelin (ET)-1 and the relaxations induced by acetylcholine (ACh) the NO donor sodium nitroprusside (SNP), the adenylate cyclase activator forskolin and the β-adrenergic agonist isoproterenol were investigated. Glyoxylic acid staining was used to identify catecholaminergic perivascular nerves.
Results and Discussion: The allantoic artery did not show, in its extraembryonic portion, α-adrenergic-mediated contraction or ACh-induced (endothelium-dependent) relaxation, whereas both phenomena were present in the yolk sac artery. Interestingly, both α-adrenergic-mediated contraction and ACh-mediated relaxation were present in the intraembryonic segment of the allantoic artery. β-adrenergic relaxation was present in yolk sac and allantoic arteries but it was significantly higher in the former vessel. Perivascular innervation was absent in the extraembryonic portion of the allantoic artery. ET-1 evoked a more efficacious contraction in the yolk sac than in the allantoic artery, whereas the contractions evoked by 5-HT and U46619 did not significantly differ between the two arteries. The relaxation evoked by SNP did not significantly differ between the two artery types, whereas forskolin induced a higher relaxation in the yolk sac than in the allantoic arteries.
Conclusion: our study demonstrates a different pattern of reactivity in the arteries perfusing the gas exchange and the nutritional organ of the chicken embryo.

O26

Antioxidants protect against fetal growth restriction and programming of early adult-onset hypertension in ovine hypoxic pregnancy.

Kirsty Brain[1], Beth Allison[1], Youguo Niu[1], Christine Cross[1], Nozomi Itani[1], Andrew Kane[1], Katie Skeffington[1], Emilio A. Herrera[2] and Dino A. Giussani[1]

[1] *Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom.* [2] *Faculty of Medicine, Universidad de Chile, Santiago, Chile.*

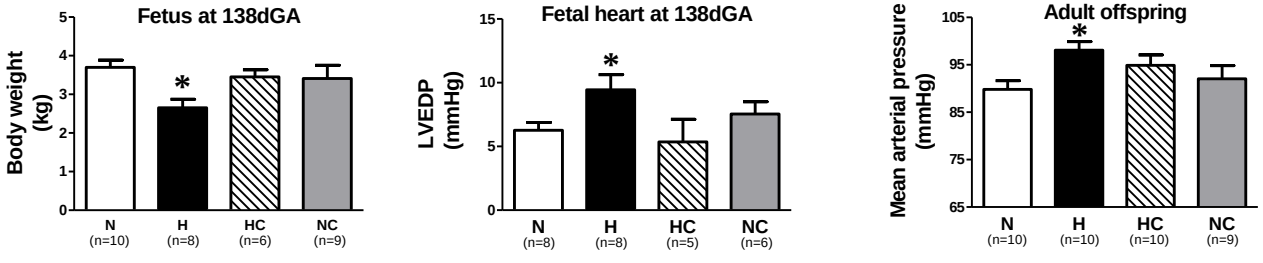
Corresponding author's email: dag26@cam.ac.uk

Introduction: Intrauterine growth restriction (IUGR) is associated with infant death and cardiovascular disease (Barker & Osmond 1988; *Br Med J* 297:134-5). There is no cure for it. One of the most common causes of IUGR is chronic fetal hypoxia, which also programmes cardiovascular dysfunction (Giussani et al. *PLoS One* 2012;7(2):e31017). However, the mechanisms underlying these associations remain unclear. We have developed 4 isobaric hypoxic chambers able to maintain pregnant sheep for the duration of gestation. Using this model, we tested the hypothesis that IUGR and developmental origins of cardiovascular dysfunction in chronic hypoxic pregnancy are secondary to oxidative stress.

Materials and Methods: Chronically catheterised sheep carrying singleton fetuses were exposed to normoxia or hypoxia (10% inspired O_2) ± vitamin C (maternal 200mg.kg^{-1} i.v. daily) for the last third of gestation (105-138 days; term~145 days). At 138 days, in one group of animals, fetuses were delivered and weighed, their tissues collected and cardiac function was investigated in a Langendorff preparation. In another group, lambs were allowed to deliver and *in vivo* experiments were performed at 9 months of age.

Results and Discussion: Maternal P_aO_2 was similarly decreased during chronic hypoxia (*Mat* P_aO_2: N=105.8±1.8; H=47.3±0.6; HC=47.5±0.7; NC=107.1±2.3 mmHg, P<0.05, N *vs.* H). Relative to normoxic controls, growth was reduced, left ventricular end diastolic pressure increased and myocardial contractility and relaxability reduced in chronically-hypoxic fetuses. Adult offspring of hypoxic pregnancy developed significant hypertension (Figure). Treatment of hypoxic pregnancies with maternal vitamin C prevented these adverse effects.

Conclusion: The data offer insight into the mechanism and clinical targets for therapeutic intervention against the programming of cardiovascular disease in risky pregnancy.



Figures: Values are mean±S.E.M. N, normoxic; H, hypoxic; HC, hypoxic with vitamin C; NC, normoxic with vitamin C. Significant differences are: **P*<0.05, (One-Way ANOVA with Tukey Test). LVEDP, left ventricular end diastolic pressure.

O27

Neonatal Antioxidant Treatment Prevents Impaired Cardiovascular Function at Adulthood Following Neonatal Glucocorticoid Therapy.

Nozomi Itani[1], Youguo Niu[1], Emilio A. Herrera[1], Rhys Evans[2] and Dino A. Giussani[1].
[1]Physiology, Development and Neuroscience, University of Cambridge, UK. [2]Physiology, Anatomy and Genetics, University of Oxford, UK.
Corresponding author's email: dag26@cam.ac.uk

Introduction: The benefits of potent steroids, such as dexamethasone, in treating chronic lung disease and cardiovascular shock in preterm infants are established. However, their use in the neonatal intensive care unit (NICU) has raised concerns because of just as potent adverse effects. Clinical studies have shown that perinatal dexamethasone treatment can promote significant cardiac dysfunction in later life (Bensky et al. *Pediatrics* 97(6):818, 1996; Kelly et al, *Pediatrics* 129(5): e1282, 2012). Therefore, there is accumulating interest in modifying NICU dexamethasone therapy to maintain benefits, but prevent adverse effects. The mechanisms underlying the adverse effects of dexamethasone are not understood but oxidative stress may play a role. We investigated whether neonatal antioxidant therapy ameliorates the cardiovascular dysfunction at adulthood induced by neonatal dexamethasone treatment in NICU-relevant doses.
Materials and Methods: Rat pups received daily i.p. injections of a tapering dose of dexamethasone (D; n=8; 0.5, 0.3, 0.1 µg/g) or D with vitamins C and E (DCE; n=8; 200 and 100 mg/kg, respectively) on postnatal days 1-3 (P1-3); vitamins were continued from P4-6. Controls received equal volumes of saline from P1-6 (C; n=8). A fourth group received vitamins alone (CCE; n=8). At P100, hearts were assessed under both working and Langendorff preparations. Peripheral vascular reactivity was determined in femoral arteries via myography.
Results and Discussion: Dexamethasone pups at adulthood had an increased left ventricular (LV) mass (70±2 *vs*. 63±1 %), enhanced LV end diastolic pressure (14±2 *vs*. 8±1 mmHg) and these hearts failed to adapt cardiac output with increased preload or afterload (Fig 1.A,B). Neonatal dexamethasone markedly impaired NO-dependent femoral relaxation at adulthood (Fig 1.C,D); all P<0.05. Combined neonatal dexamethasone with antioxidants prevented the adverse effects.
Conclusions: Neonatal dexamethasone therapy promotes cardiovascular remodelling and dysfunction at adulthood. Combined treatment with antioxidant vitamins is an effective intervention.
Supported by British Heart Foundation and BBSRC.

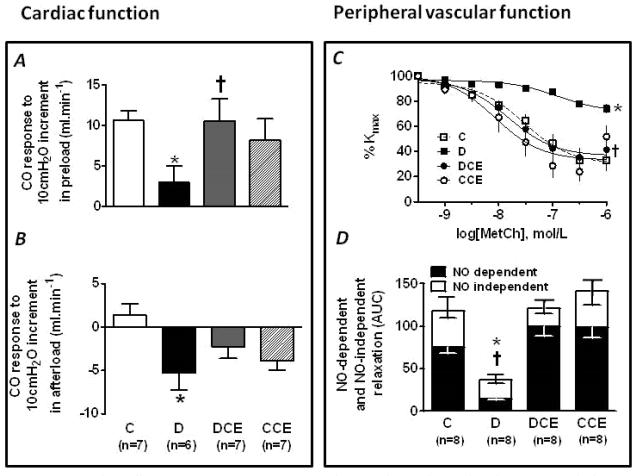


Figure 1. Mean ± SEM. ***A***, cardiac output (CO) response to increase in preload; ***B***, CO response to increase in afterload; ***C***, endothelium-dependent vasodilatation to methacholine (MetCh) in femoral arteries; Significant differences (P<0.05), * *vs* C; † *vs* D; ***D***, NO dependent and independent components in response to Metch. Significant differences (P<0.05), * *vs* C for complete bar; † *vs* C for black bar (one way ANOVA +Tukey test).

Oral Communications

Session VI: Fetal & Neonatal Cardiovascular II

Chairs:

Dino A. Giussani (University of Cambridge, UK)

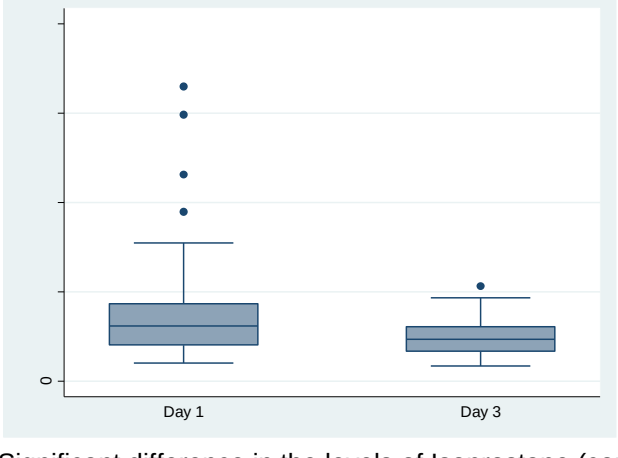
Norbert Weissmann (Universitiy of Giessen & Marburg Lung Center, Germany)

O28

Role of oxidative stress in the microvascular control of preterm neonates.

Ian Wright[1,2], Trevor Mori[3], Celine Corbisier de Meaultsart[1], Vicky Clifton[4].
[1]Mothers and Babies Research Centre, Hunter Medical Research Institute, University of Newcastle. [2]The Neonatal Intensive Care Unit, the John Hunter Children's Hospital, Newcastle, Australia. [3]The Medicine and Pharmacology RPH Unit, University of Western Australia, Crawley, Australia. [4]The Robinson Institute, Lyell McEwin Hospital, University of Adelaide, Australia.
Corresponding author's email: Ian.Wright@newcastle.edu.au

Introduction: Oxidative stress is involved in the development of multiple vascular diseases, such as heart failure and myocardial infarction. Oxidative stress is known to be increased in the newborn period. Our previous studies have shown that microvascular blood flow is dysregulated in newborn preterm infants, conferring a male disadvantage, associated with increased illness severity and hypotension, the risk increasing with increasing prematurity. We hypothesized that oxidative stress might play a role in the microvascular dysfunction in preterm neonates.
Materials and Methods: Levels of isofuran and isoprostane, 2 markers of oxidative stress, were measured in the urine of 190 babies. We investigated different gestations: early preterm (>29 weeks) and preterm (29-36 weeks); and differences by sex; for the first 3 days of life, comparing 6h and 72h. We also studied the correlation between the levels of these markers with the baseline microvascular blood flow, using a Laser Doppler method, as we have previously described.
Results and Discussion: No significant relationship was found between the oxidative stress markers and the sex of the baby. Isofuran had no significant relationship across the gestational or postnatal age groups. Isoprostane showed a significant relationship with postnatal age with a decrease from day 1 to day 3 irrespective of gestation or sex (p=0.0001). No significant correlation was found between isofuran or isoprostane and the baseline microvascular blood flow for gestation, postnatal age or sex.
Conclusion: As with previous groups, we have demonstrated an increase in oxidative stress soon after birth, decreasing by day 3. Contrary to our hypothesis there was no relationship with the demographic markers of microvascular dysfunction (male sex, extreme prematurity), nor with direct measures of microvascular blood flow. We conclude that oxidative stress does not appear to be associated with the microvascular dysfunction that we have demonstrated.



Significant difference in the levels of Isoprostane (corrected for creatinine, pmol/kg/mmol) between day 1 and day 3 of postnatal age, irrespective of gestional age or sex (p=0.0001).

O29

Melatonin rescues endothelial dysfunction during hypoxic development in the chick embryo. Nozomi Itani, Katie Skeffington, Christian Beck & Dino A. Giussani
Department of Physiology Development & Neuroscience, University of Cambridge, UK.
Corresponding author's email: ni231@cam.ac.uk

Introduction: We have proposed the hypothesis that chronic fetal hypoxia programmes cardiovascular dysfunction in adulthood through the generation of oxidative stress *in utero*. In support, hypoxic pregnancy in rats induces oxidative stress in the fetal heart and vessels and maternal treatment with the antioxidant vitamin C prevents the programmed cardiovascular dysfunction (Giussani et al. *PLoS ONE* 7(2):e31017, 2012). However, only high doses of vitamin C incompatible with human treatment were effective. Hence, there is great need to establish alternative more translatable antioxidant strategies. Here, we investigated the effects of melatonin on growth and peripheral vascular function during hypoxic development in the chick embryo.
Materials and Methods: Fertilised eggs were incubated under normoxic (21%) or hypoxic (14±0.5%) conditions from day 0 with or without melatonin treatment from day 13 of incubation. Melatonin ($1mg.kg^{-1}$) or saline vehicle was injected daily into the air cell. At day 19, the embryo was removed and killed by spinal transection. Blood was obtained for haematocrit, the embryo subjected to detailed biometry and third order femoral vessels were mounted on a wire myograph. Dilator reactivity to acetylcholine ($10^{-9.5}$-10^{-5} $mol.L^{-1}$) was investigated ± L-NAME (10^{-5} $mol.L^{-1}$) ± indomethacin (10^{-6} $mol.L^{-1}$) to determine the partial contributions of NO, prostanoids and EDHF to the relaxation.
Results and Discussion: Hypoxic development increased haematocrit (N: 22±2; H: 27±1; HM: 32±1; NM: 26±4%). Hypoxic development promoted severe fetal growth restriction and endothelial dysfunction in femoral vessels (Fig.1). Melatonin treatment during hypoxic incubation rescued the vascular defect via a prostanoid-dependent mechanism, but it did not protect against growth restriction (Fig. 1).
Conclusion: Melatonin rescues peripheral vascular endothelial dysfunction in hypoxic development, providing a possible alternative translatable antioxidant strategy to protect against fetal origins of cardiovascular disease in high risk pregnancy.
Supported by the British Heart Foundation

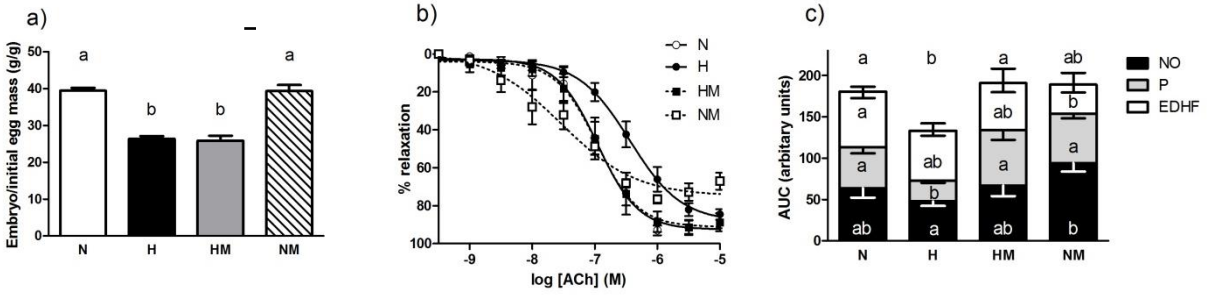


Figure 1. Means + S.E.M. for the a) fetal weight at the end of the incubation period as a percentage of the initial egg mass, b) concentration-response curve to acetylcholine (ACh), and c) nitric oxide (NO) dependent, prostanoid (P) and EDHF-dependent components (area under the curve, AUC) of the endothelial dependent vasorelaxation in femoral resistance arteries. Concentration-response curves were analysed using an antagonist-response best-fit line. The relaxation response was expressed as percentage of the contraction induced by suboptimal pottasium. The contribution of NO-dependent mechanisms to the relaxation induced by ACh was calculated by subtracting the area under the curve (AUC) for Ach - the AUC for ACh + LNAME. The contribution of P-dependent mechanisms was calculated by the AUC for ACh + LNAME - the AUC for ACh + LNAME + Indomethacin. The contribution of EDHF-dependent mechanisms was calculated as the AUC for ACh + LNAME + Indomethacin. Groups are: Normoxia (N), Hypoxia (H), Hypoxia+Melatonin (HM) and Normoxic+Melatonin (NM). n=33, n=38, n=6 and n=6 respectively for a). n=8, n=15, n=6 and n=6 respectively for b) and c). Different letters are significantly different (P<0.05), One-Way ANOVA with Tukey Test.

O30

Melatonin modifies the pulmonary antioxidant capacity in neonates gestated under chronic hypoxia.

Flavio Torres[1], Camilo Montt[1], Alejandro Gonzalez[1], Germán Ebensperger[1], Roberto V. Reyes[1], Aníbal J. Llanos [1,2], Emilio A. Herrera[1,2].

[1]Pathophysiology Program, Faculty of Medicine; [2]International Center for Andean Studies; Universidad de Chile, Santiago, Chile.

Corresponding author's email: eherrera@med.uchile.cl

Introduction. Gestational pathologies that develops under chronic hypoxia such as placental insufficiency, preeclampsia or high altitude pregnancies, may cause pulmonary hypertension in the newborn, a condition that even with proper treatment has a high mortality[1]. One of the most relevant mechanisms involved in this pathology is oxidative stress[2]. Melatonin, the pineal neurohormone, has potent antioxidant properties and has been proposed as therapy in diverse perinatal diseases[3]. Thus, we hypothesize that melatonin reduces the oxidative stress by increasing the lung antioxidant capacity in the pulmonary hypertensive newborn sheep.

Materials and Methods. Ten lambs were gestated, born and studied in high altitude (3,600 m). Five received oral melatonin (MN, 1mg in 0,5 ml·kg^{-1}·d^{-1}) and five received vehicle (CN, 0,5 ml·kg^{-1}·d^{-1}) for 7 days. After treatment, lambs were euthanized and lung tissue was obtained to measure oxidative stress biomarkers (Uric acid, 8-isoprostanes, malondialdehyde) and expression and activity of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx).

Results and Discussion. The oxidative stress biomarkers levels were similar between groups. However, 8-isoprostanes showed a decreasing trend in melatonin group (CN: 54.2±16.3 *vs* MN: 25.9±6.2 pg/mg prot). In contrast, melatonin increases the expression (Figure 1) and activity of CAT and GPx (CAT activity, CN: 3.32±0.24 *vs* MN: 4.19±0.22 U/ug prot; GPx activity, CN: 47,6±16,7 *vs* MN: 74,8±6,5 U/ug prot). Finally, SOD activity was similar between groups, however melatonin increases transcript and protein level of this enzyme.

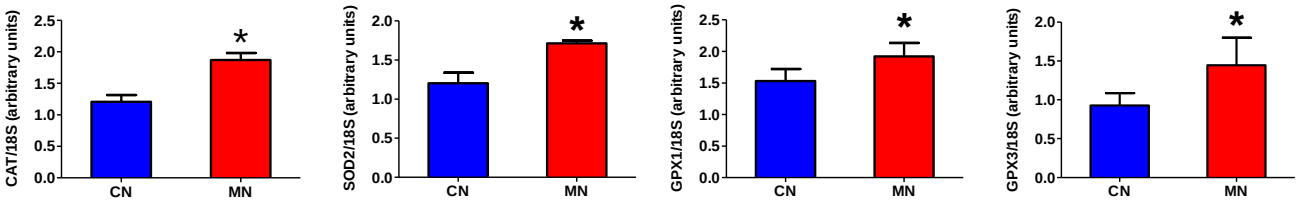


Figure 1. mRNA expression of the antioxidant enzymes, CAT, SOD & GPx1 and GPx3. Groups are control (CN, blue bar, n=5) and melatonin treated (MN, red bar, n=5). Values are mean ± SEM. Significant differences (P<0.05): * vs control (CN).

Conclusion. This study shows that melatonin is able to induce CAT, GPx and SOD protein expression in lung tissue. Although the pulmonary antioxidant capacity was enhanced, there was no evident oxidative stress reduction with melatonin. Further studies will investigate if higher doses or other therapeutic windows may decrease oxidative stress and reverse neonatal pulmonary hypertension at high altitude.

Supported by FONDECYT 1110595, 1120655 & 1130424.

References.

1. Steinhorn R, *Pediatr Crit Care Med.* 11(2): S79–S84, 2010.
2. Tabima DM, *Free Radic Biol Med*. 52(9): p. 1970-86, 2012.
3. Richter HG, *J Pineal Res*. 46(4): p. 357-64, 2009.

O31

The effect of chronic inflammation induced by low dose LPS infusion on fetal heart rate variation in preterm fetal sheep.

Christopher K Lear,[1] Joanne O. Davidson, Alistair Jan Gunn,[1] Laura Bennet[1]
[1]Fetal Physiology and Neuroscience Group, Department of Physiology, The University of Auckland, New Zealand
Corresponding author's email:l.bennet@auckland.ac.nz

Introduction: Preterm labour maybe complicated by exposure of the fetus to infection or inflammation. The effect of such adverse conditions on the indices used to monitor fetal well-being such as fetal heart rate variability (FHRV) are poorly understood. In the current study we evaluated the preterm fetal sheep FHRV, cardiovascular, EEG and body movement responses to the gram negative bacterial product lipopolysaccharide (LPS).
Methods: Chronically instrumented preterm (0.7 gestational age) fetal sheep were exposed to a continuous low-dose LPS infusion (100 ng over 24 h, followed by 250 ng/24 h for 96 h) or saline. Boluses of 1 µg LPS or saline were given at 48, 72, and 96 h.
Results: The initial low dose infusion was not associated with changes in FHRV or behaviour. The first LPS bolus doses induced a rapid transient (~1h) fall of long-term heart rate variability (LTV). This was associated with increased BP ($p<0.05$), a small fall in FHR, and suppression of EEG and body-movements ($p<0.05$). LTV then significantly increased ($p<0.05$, maximal at 3-4 h). Increased LTV was associated with hypotension, tachycardia, and sustained suppression of EEG and body movements. Responses were attenuated with successive boluses. **Conclusion:** Chronic low dose LPS infusions did not change FHRV or behaviour, but superimposed high dose boluses initiated a biphasic pattern of responses: suppression followed by excitation. Suppression of EEG activity and body movements may contribute to initial FHRV suppression. The secondary increase in FHRV is consistent with an earlier report of LPS boluses alone (no prior chronic infusion)[1].Increased FHRV is not associated with increased body movements, but maybe mediated by the effects of LPS or secondary messengers on pacemaker membrane potentials[2]. This may also contribute to FHRV suppression.[2]
References [1]Blad S et al Reprod Sci. 2008, [2]Pappaioannou V et al Current Cardiology Reviews, 2013

O32

Late gestational increases in maternal cortisol in ewes results in changes in gene expression pathways regulating responses to nutrient, cytokines and hypoxia in the septa of ovine fetal hearts.

Maureen Keller-Wood[1], Elaine M. Richards**[1],** Xiaodi Feng[1],Charles E. Wood[2], and Keith Walters[3].
[1] *Dept. of Pharmacodynamics, University of Florida, Gainesville, Florida, USA.* [2] *Dept. of Physiology and Functional Genomics, University of Florida, Gainesville, Florida, USA.* [3] *Georgia Southern University, Statesboro, Georgia, USA*
Corresponding author's email: kellerwd@cop.ufl.edu

Introduction: Maternal Cushing's disease increases fetal and perinatal mortality. Maternal infusion of cortisol (CORT; 1 mg/kg/day) from 115-130d causes enlargement of the ovine fetal heart with cardiomyocyte hyperplasia and increased apoptosis in Purkinje fibers compared to fetal hearts from untreated ewes (CON). Extending the duration of CORT to term (≥25 days) resulted in stillbirth in 3/4 CORT vs live birth in 5/5 CON ewes. Subsequently ewes were sacrificed at 143d or during labor; 6/11 CORT and 8/9 CON had live fetuses at sacrifice.
Materials and Methods: Microarray analysis (ovine Agilent 15.5K array) of septal RNA from surviving 7 CORT and 8 CON hearts revealed genes significantly differentially expressed in CORT vs CON groups. Pathway analyses of these genes were performed using Webgestalt, and with Cytoscape with the plugins Genemania, ClusterOne and Bingo.
Results and Discussion: Webgestalt analysis suggested responses to nutrients and hypoxia were significantly altered in CORT. Modeling with Cytoscape suggested that gene networks for protein translation (p=0.002), regulation of reactive oxygen species (p=0.014), RNA splicing (p=0.025), periodicity (including heart rate, muscle contraction, regulation of endothelial-mesenchymal transition, and T/ B cell regulation; p=0.025), glutamate and dolichol synthesis (p=0.027), lipid glycosylation, actin filament severing (p=0.03), and a large network of immune cell function including T and B cells, macrophages, cytokine-cytokine signaling, glucocorticoid signaling, calcium ion regulation, lipoxygenase, NFκB, and nitric oxide bioavailability, sensory pain perception and protein trimerization (p=0.044) were altered by CORT. Real time PCR analyses validated selected pathways. For example, pyruvate dehydrogenase kinase isoform 4 (PDK4; response to nutrient pathway) and suppressor of cytokine signaling 3 (SOCS3; response to nutrient and/or cytokine signaling pathways) were both significantly increased in CORT.
Conclusions: CORT differentially regulates pathways important for appropriate nutrient signaling and heart function suggesting peripartum fetal death after chronic exposure to cortisol may be linked to cardiac dysfunction.

O33

Prenatal exposure to hyperoxia modifies the TP receptor-mediated response to H_2O_2 in the ductus arteriosus of the chicken embryo.

Saskia van der Sterren[1], Lilian Kessels[1], Francisco Perez-Vizcaino[2], Angel L Cogolludo[2], Eduardo Villamor[1]
[1]*Department of Pediatrics, Maastricht University Medical Center (MUMC+), Maastricht, the Netherlands.* [2]*Department of Pharmacology, School of Medicine, Universidad Complutense de Madrid, Centro de Investigaciones Biomédicas en Red de Enfermedades Respiratorias (CIBERES), Instituto de Investigación Sanitaria Clínico San Carlos (IdISSC), Madrid, Spain.*
Corresponding author's email: e.villamor@mumc.nl

Introduction: Critical time windows exist during development and if environmental changes are experienced in the window of vulnerability, the developmental trajectory of the responding organ may be changed. In the present study we hypothesized that exposure to hyperoxia alters the developmental trajectory of the chicken embryo DA resulting in changes in changes in the response of the vessel to O_2 and/or other vasoactive agents.
Materials and Methods: Hyperoxia was induced by incubating fertilized eggs at 60% O_2 from day 15 to 19 of the 21-d incubation period. DA reactivity (assessed by wire myography), morphometry and mRNA expression of antioxidant enzymes were studied on day 19.
Results and Discussion: Hyperoxic incubation neither affected embryonic growth nor induced signs of DA closure or changed the mRNA expression of superoxide dismutase and catalase. The contractions induced by O_2 (21%), KCl, the inhibitor of K_V channels 4-aminopyridine, phenylephrine, and endothelin-1 and the relaxations induced by acetylcholine (ACh), sodium nitroprusside, isoproterenol, and the Rho kinase inhibitor hydroxyfasudil were similar in DA from embryos incubated under normoxic or hyperoxic conditions. In contrast, hyperoxic incubation impaired the thromboxane prostanoid (TP) receptor-mediated contractions evoked by U46619, 15-E_{2t}-Isoprostane and high concentrations (≥ 3μM) of ACh. Exogenous H_2O_2 evoked endothelium-dependent contraction in the normoxic DA and endothelium-dependent relaxation in the hyperoxic group. The presence of the TP receptor antagonist SQ 29548 unmasked a relaxant response to H_2O_2 in the normoxic DA and the cyclooxygenase (COX) inhibitor indomethacin blocked H_2O_2-induced contraction (in the normoxic group) and relaxation (in the hyperoxic group).
Conclusion: Altogether our functional data suggest that, in the chicken DA, exogenous H_2O_2 induces the release of endothelium-derived COX metabolite(s) with contractile and relaxant properties. Under normal conditions H_2O_2-induced contraction prevails and relaxation is unmasked after pharmacological or functional (i.e. hyperoxia) TP receptor impairment.

O34

Partial neuroprotection with selective nNOS inhibition during asphyxia in preterm fetal sheep.

Paul P. Drury,[1] Laura Bennet,[1] Joanne O. Davidson,[1] Sidhartha Tan,[2] Richard Silverman,[3] Arlin Blood,[4] Mhoyra Fraser,[1] Lotte Van den Heuij,[1] Alistair J Gunn[1]

1 Fetal Physiology and Neuroscience Group, Department of Physiology, University of Auckland, New Zealand, [2]Department of Pediatrics, NorthShore University HealthSystem, Evanston, Chicago, Illinois, USA [4] Department of Chemistry, Northwestern University, Evanston, Chicago, Illinois, USA, [5] Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, California, USA.

Corresponding author's email: aj.gunn@auckland.ac.nz

Introduction: Exposure of preterm infants to hypoxia-ischemia (HI) is associated with death and cerebral palsy. Excessive nitric oxide production during HI has been associated with nitrosative stress, and cell membrane and mitochondrial damage. We tested the hypothesis that the highly selective neuronal nitric oxide synthase (nNOS) inhibitor JI-10, with a Ki of 8 nM, would protect mitochondrial function in preterm fetal sheep after profound asphyxia.

Methods: 12 preterm fetal sheep were chronically instrumented to record blood pressure, fetal heart rate, carotid and femoral blood flow, nuchal muscle activity, EEG, extradural temperature and near-infrared spectroscopy (NIRS, NIRO-500). Asphyxia was induced by 25 min of complete umbilical cord occlusion at 101-104 d gestation. JI-10 was administered 30 min before asphyxia as a 0.022 mg/kg bolus (n=7) or the equivalent volume of saline in controls (n=5). Sheep were killed 7 d after occlusion.

Results: JI-10 had no effect on fetal blood pressure, heart rate, carotid and femoral blood flow, EEG power, spectral edge, nuchal activity or temperature. Low frequency delta and theta power were transiently greater in the JI-10 group from 2-6 h after asphyxia ($p<0.05$). NIRS-defined total haemoglobin, an index of cerebral blood volume, was significantly lower in the Ji-10 group from 1-6 h after asphyxia ($p<0.05$). JI-10 was associated with significant attenuation of the progressive loss of cytochrome oxidase activity after occlusion ($p<0.05$), and significantly later seizure onset compared with saline ($p<0.05$). Histologically, JI-10 was associated with significantly improved numbers of surviving neurons in the caudate nucleus ($p<0.05$) and more, mature oligodendrocytes in periventricular white matter compared to saline ($p<0.05$).

Conclusion: Prophylactic infusion of the selective nNOS inhibitor JI-10 before profound asphyxia was associated with delayed onset of seizures and slower cytochrome oxidase decline and significant neural protection, consistent with protection of mitochondrial function.

Oral Communications

Session VII: Placenta

Chairs:

Paola Casanello (Pontificia Universidad Catolica de Chile, Chile)

Carlos Escudero (Universidad del Bio-Bio, Chile)

O35

The Hepcidin/Ferroportin regulatory system and Neonatal Hemochromatosis.

Martina U. Muckenthaler.
Department of Pediatrics, University of Heidelberg, Molecular Medicine Partnership Unit (EMBL)
Corresponding author's email: martina.muckenthaler@med.uni-heidelberg.de

Imbalances of iron homeostasis account for some of the most common human diseases. Pathologies can result from both iron deficiency or overload and frequently affect the hepcidin/ferroportin regulatory system that maintains systemic iron levels. The small hepatic peptide hormone hepcidin orchestrates systemic iron fluxes and controls plasma iron levels by binding to the iron exporter ferroportin on the surface of iron releasing cells, triggering its degradation and hence reducing iron transfer to transferrin. Hepcidin thus maintains transferrin saturation at physiological levels assuring adequate iron supplies to all cell types.
A major aim of our research is to understand the regulatory circuitry underlying systemic iron homeostasis. We focus on mechanisms that control hepcidin and ferroportin expression employing network/systems-based analyses by integrating transcriptomic and proteomic techniques, mouse models and genome-wide RNAi screens. In my presentation I will review current knowledge about iron transport at the placenta and how the hepcidin/ferroportin regulatory system is impaired in neonatal hemochromatosis, a severe iron overload disorder caused by gestational alloimmune liver disease (GALD).

O36

How the placenta makes pregnant women vulnerable to influenza (Flu).

Jorge M. Tolosa[1,2], Kristy Parsons[3], Mauro Bendinelli[4] Peter Wark[3] and Roger Smith[1]
[1] Mothers and Babies Research Centre. The University of Newcastle and Hunter Medical Research Institute (HMRI). Level 3, Endocrine Unit, John Hunter Hospital, New Lambton Heights, 2305 NSW, Australia. [2] Facultad de Ciencias Médicas, Universidad de Santiago de Chile, Santiago, Chile. [3] Hunter Medical Research Institute and The University of Newcastle. Respiratory Medicine, ohn Hunter Hospital, New Lambton Heights, 2305 NSW, Australia. [4] Retrovirus Centre, Department of Experimental Pathology, University of Pisa, Italy.
Corresponding author's email: Jorge.tolosa@newcastle.edu.au

Introduction. As in previous influenza epidemics and pandemics, pregnant women infected with 2009 pandemic influenza A (H1N1) were disproportionately over-represented among hospitalizations, ICU admissions, and deaths (50% of the pregnant women infected worldwide were hospitalized, of whom 23% were admitted to an ICU and 8% died). Pregnancy requires adaptations of the maternal immune system, both locally and systemically to prevent the rejection of the semi-allogeneic fetus. Recent investigations have shown that the placenta produces and secretes immunosuppressive retroviral envelope proteins and immunomodulating exosomes that may explain the inhibition of innate and adaptive cell-mediated immune responses characteristic of pregnancy. Therefore, to explain the susceptibility of pregnant women to influenza, we hypothesized that the human endogenous retroviral envelope protein syncytin-1 and human placental exosomes suppress maternal innate and adaptive cell mediated immune responses to influenza viruses by altering the function of peripheral blood mononuclear cells (PBMCs).
Materials and Methods. PBMCs were isolated from healthy women, pregnant and non pregnant, vaccinated and non vaccinated against Flu, seeded in 24-well plates (2 x 10^6 cells/well) in 900ul 10% FCS / RPMI medium and infected with influenza viruses and incubated at 35°C/5% CO_2 (H1N1) or 37°C/5% CO_2 (H3N2) for 48 hrs. Supernatants were harvested and tested for cytokines using ELISA and/or Beads Array.
Results and Discussion. We have shown that pregnant women have evidence of impaired antiviral immune responses to influenza virus compared to healthy non-pregnant women, as measured by the release of IFN-α and IFN-γ by PBMCs following exposure to influenza virus. We have also shown that Syncytin-1 has immunosuppressive properties and that Syncytin-1 by itself is able to inhibit some of the antiviral responses in PBMCs.
Conclusion. The release by the placenta of syncytin-1 into the maternal bloodstream may explain the decreased cell-mediated immune responses observed during pregnancy and the severe course of influenza during pregnancy.

O37

Role of oxidative stress status on the impaired eNOS-dependent relaxation of intrauterine growth restriction-derived placental chorionic arteries.

Daniela Schneider[1], Bernardo Krause [1], Paola Casanello[1]

[1] Division of Obstetrics & Gynecology and Pediatrics, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile

Corresponding author's email: pcasane@med.puc.cl

Introduction: Vascular dysfunction in the Intrauterine Growth Restriction (IUGR) placenta associates to impaired eNOS-mediated relaxation due to decreased eNOS-activation, a predominance of arginase activity, along with an enhanced response to vasoconstrictors due to increased levels of oxidative stress. However there is no evidence showing whether oxidative stress modulates eNOS-mediated relaxation in chorionic arteries (CA) from IUGR placentae. We propose that the reduced NOS-dependent relaxation in IUGR chorionic arteries is negatively regulated by oxidative stress.

Methods: CA from control and IUGR placentae were dissected, and vascular rings mounted on a wire-myograph. Isometric force in response to increasing concentrations of calcitonin gene-related peptide (CGRP, 10^{-10}-10^{-6} M) with and without the superoxide dismutase inhibitor (DDC 10^{-5} M), the glutathione peroxidase inhibitor (MS 10^{-3} M), the $ONOO^-$ donor SIN-1 (10^{-5} M) and the antioxidant N-acetyl cysteine (NAC, 10^{-5} M) were determined in vessels pre-contracted with KCl (37.5 mM). Relaxation responses were expressed as percentage of relaxation relative to KCl-induced contraction (%Kmax) and adjusted to concentration-response curves.

Results: CA from IUGR placentae showed a lower relaxation to CGRP (44.3 ± 7.9 %Kmax) compared with control-CA (71.2 ± 5.5 %Kmax) ($p < 0.05$). Pre-incubation with DDC further decreased the relaxation to CGRP in IUGR vessels (15.8 ± 15.1 %Kmax; $p < 0.05$), whilst MS completely blocked the relaxation (-6.4 ± 7.1 %Kmax; $p < 0.001$). Similarly in presence of SIN-1, CGRP induced a concentration dependent constriction in IUGR-CA (-72.6 ± 11.7 %Kmax; $p < 0.0001$). Notably, pre-incubation of IUGR-CA with NAC normalized the response to CGRP (81.0 ± 9.1 %Kmax).

Conclusion: These results show that oxidative stress impaired eNOS-dependent relaxation in IUGR CA. In this context, agents that affects thiol levels (i.e. MS and NAC) have an important effects on vascular reactivity.

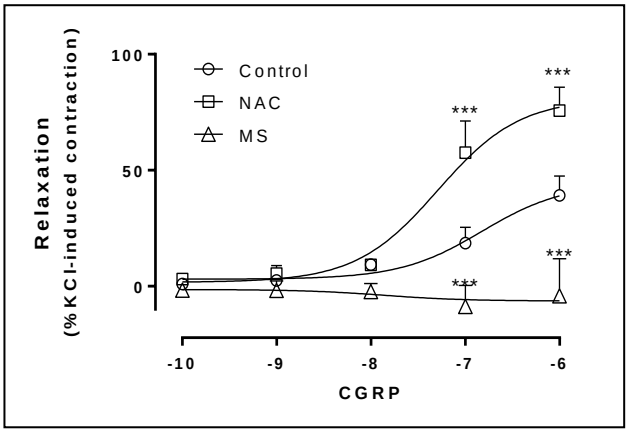


Figure 1. Effects of anti- and pro- oxidants on eNOS-dependent relaxation in IUGR-CA. Concentration-response curves to CGRP in IUGR arteries in absence (circles) and presence of NAC (squares), and MS (triangles). ***p<0.05 versus control, Two-way ANOVA.

References

1. Krause, B.J., Hanson, M.A., and Casanello, P. (2011). Role of Nitric Oxide in Placental Vascular Development and Function. Placenta 32:797-805.
2. Krause, B.J., Carrasco, I., Caniuguir, A., Carvajal, J., Farías, M., Casanello, P. (2013). Endothelial eNOS/arginase imbalance contributes to vascular dysfunction in IUGR umbilical and placental vessels. Placenta 34:20-28.

Funded by Fondecyt 1120928 & 1130801.

O38

HDAC activity and nitric oxide control the expression of eNOS and arginase-2 in human umbilical artery endothelium in intrauterine growth restriction.

Bernardo Krause [1], Cherie Hernandez[1], Daniela Schneider[1], Paola Casanello[1]

[1]*Division of Obstetrics & Gynecology and Pediatrics, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile*

Corresponding author's email: bjkrause@uc.cl

Introduction: Adult vascular dysfunction associated with intrauterine growth restriction (IUGR) is preceded by subtle vascular alterations. Recently we reported that eNOS expression in endothelial cells derived from IUGR-placentae is programmed by altered DNA methylation, and reversed by silencing the DNA methylation machinery. However no reports show the effect of other epigenetic mechanisms on the expression of key genes of the NOS pathway. Here we studied the effect of histone deacetylase (HDAC) activity and NO, which has been reported as an HDAC inhibitor, on the expression of arginase-2 and eNOS in IUGR-umbilical artery endothelium (HUAEC).

Materials and Methods: HUAEC were isolated by collagenase digestion from control and IUGR-umbilical cord and cultured to confluence. Cells were exposed 24 hours to the HDAC inhibitor TSA (0.1 – 10 µM) with or without the NO donor NOC-18 (100 µM) and the NOS inhibitor L-NAME (100 µM). Chromatin accesibility at arginase-2 and eNOS promoter was analyzed with the EpiQ Chromatin Analysis kit (Biorad). Arginase-2 and eNOS mRNA levels were determined by qPCR.

Results: TSA up-regulated arginase-2 and down-regulated eNOS expression in control and IUGR HUAEC. The later effect was not affected by NOC-18 and L-NAME. In control HUAEC the effect of TSA on arginase-2 expression was potentiated by NOC-18, and blocked by L-NAME, without changes in IUGR-HUAEC. In absence of TSA, NOC-18 induced arginase-2 in control and IUGR HUAEC, whilst L-NAME reduced its expression only in control cells.

Conclusion: HDAC activity has a differential effect on eNOS (↑) and arginase-2 (↓) expression in HUAEC. Apparently NO enhances chromatin accessibility at the arginase-2 promoter, which can be potentiated by HDAC inhibition.

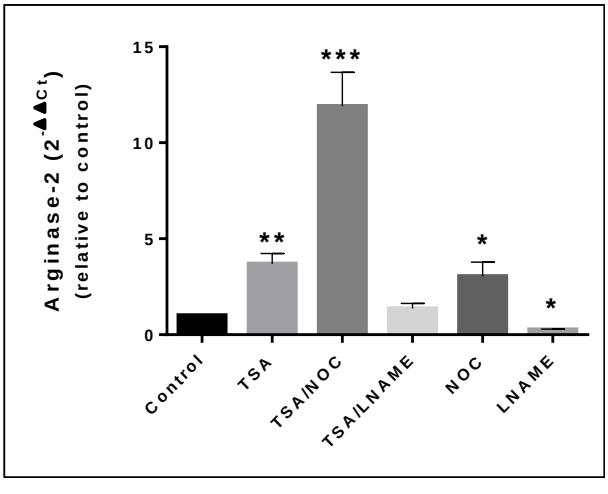


Figure 1. Effect of NO and inhibition of HDAC on arginase-2 expression in control HUAEC. Values are mean ± S.E.M. *p<0.05, **p<0.01, ***p<0.001 versus control, ANOVA.

Funded by FONDECYT 1120928 & 1130801

O39

Increased microvascular placental angiogenesis is associated to high tyrosine phosphorylation of vascular endothelial growth factor receptor type 2 in late and early-onset pre-eclampsia. Carlos Escudero[1*], Cristian Celis[1], Tamara Saez[2], Sebastian San Martin[2], Francisco Valenzuela[1], Patricio. Bertoglia[3], James .M. Roberts[4], Jesenia Acurio[1].

[1]Vascular Physiology Laboratory, Group of Investigation in Tumor Angiogenesis (GIANT), Department of Basic Sciences, University of Bío-Bío, Chillán, Chile. [2]Biomedical Sciences Department, Faculty of Medicine, University of Valparaíso, Valparaíso, Chile. [3]Obstetric and Gynecology Department, Hospital Clinico Herminda Martin, Chillán, Chile and Universidad Católica de la Santísima Concepción, Concepción. [4]Magee Women Research Institute, Department of Obstetrics, Gynecology, Epidemiology and Clinical and Translational Science, University of Pittsburgh, USA

Corresponding author's email: cescudero@ubiobio.cl

Introduction: Placentas from early-onset (EOPE) or late-onset pre-eclampsia (LOPE) exhibit signs of under perfusion, which in turn, may be associated with altered angiogenesis. Both tyrosine 951 (Y951) and Y1175 phosphorylation of the vascular endothelial growth factor receptor 2 (VEGFR2) induced by VEGF triggers the angiogenesis process. Endothelial markers such as CD31, VEGFR2 or IL-8 have been used for estimating angiogenic process in several tissues including placenta. We asked whether vascular density in placental villi was related to Y951 and Y1175 phosphorylation of VEGFR2 in LOPE or EOPE.

Materials and Methods: We obtained placental samples from normal pregnant women (n=22), LOPE (n=13), EOPE (≤ 35 weeks, n=15) and preterm deliveries (n=10). Slices from placental tissue were used for CD31 immunostaining. In addition, we estimated protein concentrations of CD31, VEGF, native and Y951 phosphorylated VEGFR2 by western blot of placental homogenates. Y1175 phosphorylated VEGFR2 was measured by ELISA. VEGF and IL-8 mRNA was determined by quantitative PCR.

Results and Discussion: Vessel density (i.e., CD31 positive area/villi area) in terminal villi and CD31 protein abundance was increased in LOPE and EOPE compared to normal pregnancy, a phenomenon positively correlated with the VEGF protein levels in the LOPE group. mRNA for VEGF and IL-8 were elevated in EOPE compared to normal pregnancy. VEGFR2 protein concentration was not different among the studied groups, whereas Y951 phosphorylation was higher in preterm, LOPE and EOPE compared to normal pregnancy. Moreover, Y1175 phosphorylation was higher in LOPE than normal pregnancy. There are not studies linking high placental microvascular density with Y951 and Y1175 phosphorylation of VEGFR2 as a potential adaptive response during pathological pregnancies.

Conclusion: Increased microvascular placental angiogenesis is related to high Y951 and Y1175 phosphorylation of VEGFR2 in pre-eclamptic pregnancies.

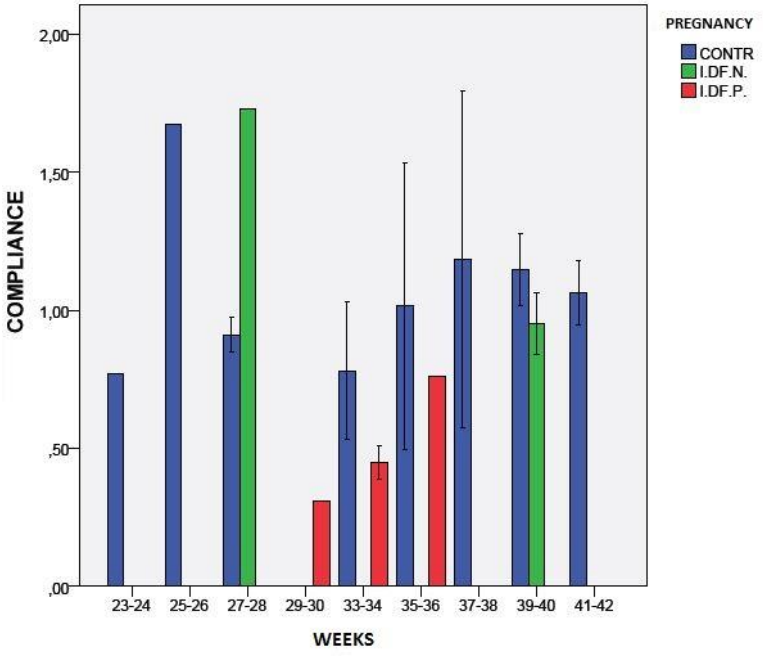
Acknowledge: FONDECYT 1100684, Conicyt Anillo ACT73.

O40

Experimental assessment of human umbilical vein compliance in physiological and IUGR pregnancies.

R. Thomasset[1], C. Guiot[2], R. Attini[1], A Rolfo[1], A.M. Nuzzo[1], E. Piccoli[1], T. Todros[1]
[1]Department of Obstetrics and Gynecology, University of Turin, Turin, Italy [2] Department of Neuroscience, University of Torino, Torino, Italy. CNISM, Torino, Italy.
Corresponding author's email: raphaelthomasset@hotmail.it

Introduction. Epidemiological studies show a correlation between low birth weight and in particular the intra-uterine growth restriction (IUGR) and cardiovascular diseases of the adult. One of the key aspects of cardiovascular disease is the reduction of vessels compliance. Our hypothesis is that umbilical vein (UV) of IUGR fetuses has a lower compliance than that of normal fetuses.
Materials and methods. The UV of 31 normal and 10 IUGR fetuses, 6 of them with pathological and 4 with normal umbilical artery (UA) doppler were studied. Twin pregnancies were excluded. A 7cm long piece of cord was mounted on an apparatus allowing to assess its characteristic pressure-volume curve of UV. Saline was pumped inside the UV and, at the same time, the pressure and the volume were recorded. For each UV, mesurements were carried out three times.
Results. In IUGR, compliance was minor than in controls of the same gestational age. The reduction of compliance is observed only in cords of fetuses with pathological UA doppler, but not in fetuses with normal doppler.
Conclusions. The study was conducted on a small number of cases, although it shows that there is a significant difference in UV compliance between IUGR and normal fetuses. These data highlight how the intrauterine growth restriction, typically assessed by pathological UA doppler, impact not only the fetus and the placenta dimension, but also the structure of the umbilical cord.



Graphic: graphical representation of compliance (µl/mmHg*cm) where weeks are grouped, standard error intervals are viewed where the number of cases is more than one
I.DF.N: iugr with normal umbilical artery doppler
I.DF.P: iugr with abnormal umbilical artery Doppler

Oral Communications

Session VIII: DOHaD

Chairs:

Mark Hanson (University of Southampton, UK)

Hans Richter (Universidad Austral de Chile, Chile)

O41

Intergenerational transmission via paternal line of cardiac disease risk by chronic fetal hypoxia.

Youguo Niu, Beth Allison, Andrew Kane, Ciara Lusby, Emily Camm & Dino A. Giussani
Department of Physiology Development & Neuroscience, University of Cambridge, UK.
Corresponding author's email: dag26@cam.ac.uk

Introduction: The environment during development can induce intergenerational non-genomically determined phentotypic changes in mammals. Studies have focussed on intergenerational transmission by maternal behaviour, altered nutrition or excess glucocorticoids (Zambrano et al. *J Phys* 2005; 566:225; Torrens et al. *BJN* 2008; 100:760; Drake et al. *AJP* 2005; 288: R34; Iqbal et al. *Endo* 2012;153(7):3295; Long et al. *AJOG* 2012;207(3):203.e1-8; Francis et al. *Science*1999; 286:1155). Whether transmission occurs via the father or mother is also of interest. While maternal transmission can arise from effects on the maternal environment, and/or inheritance of nuclear and mitochondrial DNA, and/or epigenetic mechanisms mediated by female germ cells, paternal transmission via sperm occurs solely due to genetic, epigenetic and small noncoding RNA mechanisms, permitting epigenetic hypotheses to be tested more accurately. The most common consequence of complicated pregnancy is chronic fetal hypoxia. However, whether generational inheritance of disease risk can be induced by chronic fetal hypoxia via either parental line is completely unknown.

Materials and Methods: Pregnant Wistar rats (n=24, F0) underwent normoxic (N: 21% O2) or hypoxic (H: 14% O2) pregnancy from days 6-20 of gestation. This model does not affect maternal food intake. At 12 weeks, F1 males and females were mated with partners from outside the colony to produce an F2 generation from both paternal and maternal lineages, which did not experience hypoxia. In both F1 and F2, cardiac function was assessed via Langendorff preparations at 4 months of age in male offspring.

Results and Discussion: F1 offspring of hypoxic pregnancy showed reduced basal coronary flow and impaired cardiac recovery from ischaemia-reperfusion (Fig. 1). These defects were transmitted to F2 via the paternal but not via the maternal line (Fig.1).

Conclusion: We show paternal intergenerational programming of cardiac dysfunction in rats following pregnancy complicated by chronic fetal hypoxia.

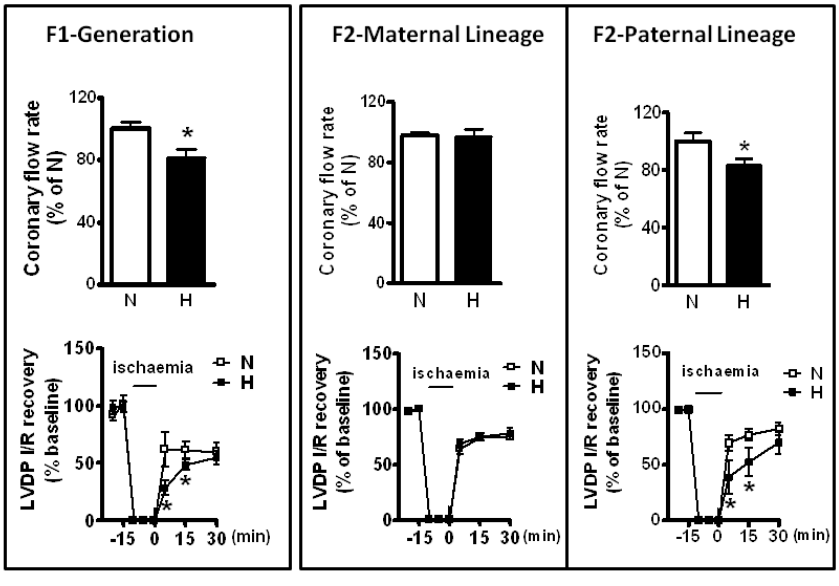


Figure 1. Cardiovascular function (mean±SEM) in Male F1(n=9 for N, n=8 for H) and F2 offspring via maternal (n=8 for N, n=8 for H) or paternal lineage (n=9 for N, n=9 for H). **P*<0.05, H *vs.* N, (Student's unpaired *t* test).

Supported by the British Heart Foundation

O42

Chronic prenatal hypoxia in the rat affects cognitive function and brain structure in adulthood: intervention by vitamin C.

Emily Camm, Christine Cross, Andrew Kane, Dino A. Giussani
[1] *Department of Physiology, Development & Neuroscience, University of Cambridge, United Kingdom*
Corresponding author's email: ejc68@cam.ac.uk

Introduction: The majority of the international research effort on developmental programming to date has focused on alterations in maternal nutrition or stress, and their contributions to cardiometabolic diseases (Gluckman et al. *N Engl J Med* **359**, 61-73, 2008). Few studies have examined programming of adult neurological disease. Even fewer have investigated the effects of prenatal hypoxia as a programming stimulus, and currently there are no interventional studies in this area. Here, we investigated effects of hypoxic pregnancy with and without antioxidant therapy in programming alterations in brain structure and function in adult rats.

Materials and Methods: Wistar dams underwent normoxic (N, 21% O_2) or hypoxic (H, 13% O_2) pregnancy from days 6-21 +/- vitamin C (0.5g.100ml^{-1} in drinking water). At 3.5 months, Morris water maze and open field testing was performed to assess cognitive function and behaviour. Following testing, brain tissue was assessed histologically and stereologically for growth and development and injury.

Results and Discussion: Relative to controls, offspring from hypoxic pregnancy spent less time searching in the quadrant that had previously contained a submerged platform in the water maze, suggesting impaired memory retention ($P<0.05$, Fig. 1A). They also showed reduced hippocampal neuronal number, volume fraction of blood vessels and synaptophysin-positive puncta (pre-synaptic marker; all $P<0.05$, Fig. 1B-D). These effects were independent of alterations in cerebral or neuronal soma volumes ($P>0.05$). Maternal vitamin C improved memory retention ($P<0.05$) and restored neuronal number, and volume fraction of blood vessels and synaptophysin-positive puncta in the hippocampus (Fig. 1A-D).

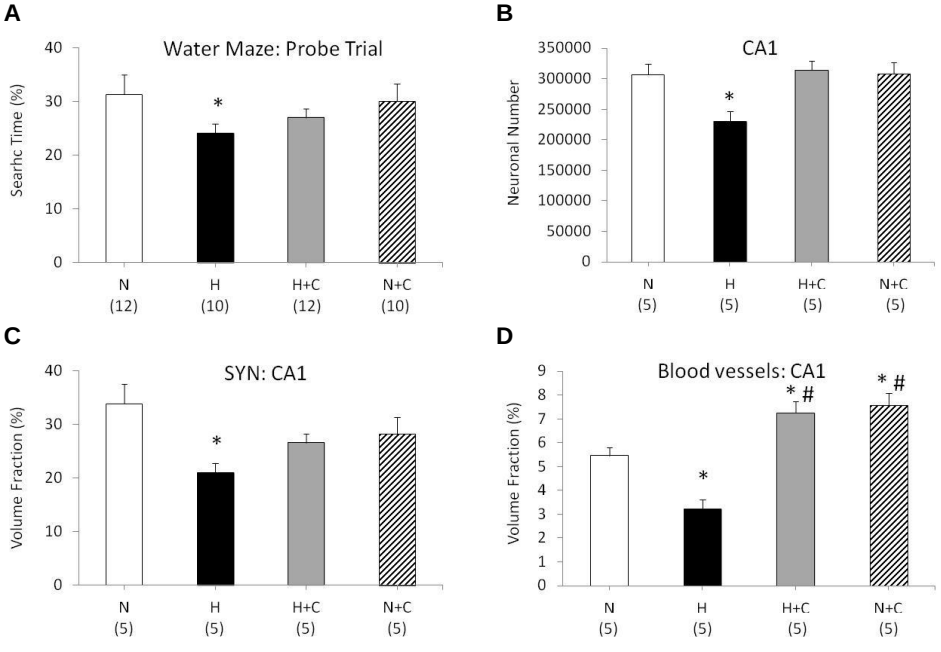


Figure 1. Values are mean ± SEM. (A): search time in quadrant that previoiusly contained platform in water maze. (B-D): neuronal number (B), volume fraction of synpatophysin (SYN) puncta (C) and blood vessels (D) in the CA1 region of the hippocampus in offspring of normoxic (N, white), hypoxic (H, black), hypoxic + vitamin C (H+C, grey) or normoxic + vitamin C (N+C, stripe) pregnancies. *P<0.05 *vs.* normoxic, # P<0.05 *vs.* hypoxic (One-way ANOVA + Tukey's test).

Conclusion: Programmed alterations in cerebral structure and function by prenatal hypoxia can be ameliorated by maternal antioxidant treatment. The data support a role for oxidative stress linking intrauterine insults with programming neurological disease, giving insight to mechanism and intervention.

Supported by the British Heart Foundation

O43

Effects of fetal amniotic exposure to lipopolysaccharide (LPS) on lung structure in adulthood in rats.

Keiji Suzuki[1], Hidehiro Takahashi[2], Hiroshi Masaki[3], Masanori Tamura[2].
[1] Department of Pediatrics, Faculty of Medicine, *Tokai University, Isehara, Kanagawa, Japan* [2] Center for Maternal, Fetal and Neonatal Medicine, Saitama Medical Center, Kawagoe, Saitama, Japan. [3] Perinatal Center, St Marianna University School of Medicine, Yokohama, Kanagawa, Japan
Corresponding author's email: dkswnd@tokai-u.jp

Introduction: Chorioamnionitis is known to be associated with impairment of developing organs such as brains and lungs. To study effects of antenatal intra-amniotic injection of potent proinflammatory agent, lipopolysaccharide (LPS) on fetal and neonatal growth and development of the lungs.
Materials and Methods: At 20 d gestation, pregnant SD rats were anesthetized and the uterus exposed under general anesthesia. The uterine wall was punctured and 0.1 μg or 1 μg *E. coli* endotoxin (O55:B5), or saline (0.1mL) was injected into each amniotic sac (LPS-L, LPS-H and SAL groups, respectively). No fetal surgery was performed for the NTX group. At 22 d (term), the fetuses were delivered vaginally or abdominally. The newborn pups were breast fed and nursed by their own or foster mother. At 8 weeks, the pups were euthanized and the lungs harvested, perfused, pressure-fixed (10cmH_2O) through the airways and pulmonary arteries and processed for morphometric analyses.
Results: The mortality rate was higher in the LPS groups compared to the SAL or NTX group. LPS-exposed pups weighed lighter at birth. At 8 wks however, only male LPS-H pups still weighed lighter and had proportionally lighter lungs. At 8 wks, LPS-H females had lower alveolar surface density (Sv-alv), higher mean alveolar volume (Valv), and lower numerical density of alveoli (Nv-alv). Also in males, Valv tended to be higher and Nv-alv tended to be lower in the LPS-H group.

	treatment group	Sv-alv (mm^{-1})	Valv (μm^3)	Nv-alv (mm^{-3})
Male	NTX	814+/-145	34900+/-24000	46100+/-18000
	SAL	670+/-54	16600+/-2500	44100+/-5200
	LPS-L	691+/-71	13400+/-3300	52600+/-8500
	LPS-H	724+/-54	35300+/-6100	25600+/-5600
	p	*0.7*	*0.2*	*0.2*
Female	NTX	844+/-138	20200+/-7400	54200+/-23700
	SAL	801+/-29	17700+/-3100	46600+/-6600
	LPS-L	725+/-30	16900+/-1500	42100+/-3400
	LPS-H	627+/-43	33300+/-1200	21100+/-600
	p	*0.03*	*0.009*	*0.005*

Conclusion: Antenatal intra-amniotic LPS resulted in higher perinatal mortality in the offspring. In surviving pups, only high dose (1 μg) LPS influenced on postnatal body and lung growth. Fetal amniotic exposure to high dose LPS had a long term influence on lung development, and resuted in lungs with fewer and larger alveoli in adulthood.

O44

Exposure to protein-restriction *in utero* followed by accelerated postnatal growth programmes hypothalamic and hepatic insulin resistance and accelerates metabolic aging.

Lindsey Berends[1], Y-C Loraine Tung[1], Peter Voshol[1], Susan E. Ozanne[1].
[1] *Wellcome Trust – MRC Institute of Metabolic Science, University of Cambridge, Cambridge, UK*
Corresponding author's email: lmb81@cam.ac.uk

Introduction: Substantial evidence from human and animal studies indicates that the early-life nutritional environment is an important determinant of metabolic disease susceptibility. In particular, rapid postnatal growth following *in utero* growth restriction is important for later risk of developing obesity, Type-2 diabetes and features of the metabolic syndrome – for which insulin resistance is a central feature. Using a well-established maternal protein-restriction rodent model, our aim was to determine the programming effects of accelerated postnatal growth on peripheral and central insulin sensitivity in early and late adulthood.
Methods: Male "recuperated" (R) mice [born to low protein (8%) fed dams and suckled by control fed (20%) dams] and controls (C) (offspring born to and suckled by control dams) were studied at 3 and 12 months of age. Whole-body and tissue-specific insulin sensitivity was determined using dual-tracer hyperinsulinemic-euglycemic clamp technology. Central insulin sensitivity was assessed by measuring the anorectic effects of intracerebroventricular (ICV) insulin.
Results and Discussion: Recuperated mice underwent accelerated postnatal growth becoming significantly heavier than controls at weaning ($p<0.001$). Adiposity was increased in recuperated ($p<0.05$) and older mice ($p<0.001$). Recuperated mice had larger adipocytes ($p<0.001$). GTT area under the curve in 3mR was higher than 3mC ($p<0.05$) but comparable to 12m mice (maternal diet-age interaction, $p=0.015$). Glucose infusion rate (GIR; an indicator of whole-body insulin sensitivity) was reduced in recuperated mice ($p<0.05$); hepatic insulin sensitivity was also reduced in recuperated mice ($p<0.05$). Compared to 3mC, GIR and hepatic insulin sensitivity were impaired to a similar degree in 3mR and 12mC and worse still in 12mR. In 3mC, ICV insulin reduced food intake acutely ($p<0.01$ for both) but had no effect in 3mR or 12m groups.
Conclusion: Early-life exposure to mismatched nutrition programmes early-onset metabolic dysfunction that is exaggerated with age. Hepatic and hypothalamic insulin resistance are important components of this risk.

O45

Intra-amniotic IGF1 treatment of the growth-restricted fetus alters mRNA expression of key somatotrophic genes in liver and muscle and adult glucose tolerance.

Ana-Mishel Spiroski[1,2], Mark Oliver[1,2], Travis Gunn[1], Anne Jaquiery[1,2,3], Jane Harding[1], Frank Bloomfield[1,2,3]
[1]Liggins Institute, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand. [2]Gravida: National Centre for Growth and Development, c/o Liggins Institute, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand. [3]Department of Paediatrics: Child and Youth Health, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand
Corresponding author's email: am.spiroski@auckland.ac.nz

Introduction: Fetal growth restriction (FGR) is associated with adult metabolic disease. Intra-amniotic insulin-like growth factor (IGF)-1 treatment of FGR in sheep increases *in utero* growth, but effects on postnatal metabolism are unknown.
Materials and Methods: FGR was induced in sheep by uteroplacental embolisation. FGR fetuses received 360 µg IGF1 (FGR-IGF1; n=27) or saline (FGR-Saline; n=23) intra-amniotically once weekly for five weeks from 107 d (term, 148 d). Controls (n=31) were un-operated and un-embolised. One week after birth lambs underwent percutaneous liver and muscle biopsies. mRNA expression was measured by qPCR and calculated as fold change (99% confidence intervals) relative to 3 housekeeping genes. Animals underwent an intravenous glucose tolerance test (GTT) at 18 months. Data were analysed by ANOVA with Tukey's *post hoc* test and are mean±SEM.
Results and Discussion: Hepatic IGF acid labile subunit (*IGFALS*) mRNA expression was ~34% greater in FGR-Saline animals than Controls; FGR-IGF1 decreased *IGFALS* mRNA in males by 45% vs. FGR-Saline. Hepatic IGF binding protein (IGFBP)-1 (*IGFBP1*) mRNA was 61% and 156% greater in FGR-Saline and FGR-IGF1 females than Controls; in males, *IGFBP1* and *IGFBP3* were ~60% and ~50% lower in FGR-Saline and FGR-IGF1 than Controls. FGR-Saline males had 61% greater insulin-regulated glucose transporter 4 (*SLC2A4)* mRNA in muscle than Controls, whereas FGR-IGF1 had 83% and 194% greater *SLC2A4* compared with FGR-Saline and Controls, respectively. Glucose area under the curve in the first 15 minutes of the GTT was greater in FGR-Saline males compared with Controls, with FGR-IGF1 intermediate (38.4±2.7 vs. 44.0±2.7 vs. 39.8±2.7 mmol·L·min^{-1}; p<0.05); there was no difference amongst females.
Conclusion: Intra-amniotic IGF1 treatment of FGR has sex-specific effects on early postnatal regulation of the somatotrophic axis and glucose handling in adulthood. More detailed interrogation of adult metabolic state is required to determine whether this potential therapy has adverse long-term effects.

O46

Programming of cardiac dysfunction by maternal diet-induced obesity.

Heather L Blackmore[1], Denise Fernandez-Twinn[1], Youguo Niu[2], Dino A Giussani[2] and Susan E. Ozanne[1].
[1]*University of Cambridge Metabolic Research Laboratories and MRC Metabolic Diseases Unit, Wellcome Trust MRC-Institute of Metabolic Science, Level 4, Box 289, Addenbrookes Treatment Centre, Addenbrookes Hospital, Cambridge, UK.* [2]*University of Cambridge, Physiology, Development and Neuroscience, Tennis Court Road, Cambridge UK.*
Corresponding author's email: hlb46@cam.ac.uk

Introduction: Human and animal studies show that exposure to an adverse environment *in utero* and early postnatal life can increase offspring susceptibility to cardiovascular disease. With the prevalence of obesity increasing worldwide, *in utero* exposure of offspring to maternal obesity is increasingly common. This study investigated the effect of maternal diet-induced obesity on cardiovascular function in young adult mouse offspring.
Methods: Obesity was induced in female mice by feeding a diet high in sugar and fat 6 weeks prior to mating, throughout pregnancy and lactation. Control females were fed a chow diet throughout life. Male offspring of both groups were weaned onto chow and cardiac function studied in one per litter at 12 weeks. Chronotropic and inotropic responses to sympathetic (Isoprenaline) and parasympathetic (Carbachol) agonists as well as recovery to an episode of ischaemia/reperfusion (I/R) were investigated in a Langendorff preparation. Cardiac perfusate was collected after I/R to assess cardiac damage marker, Creatinine Kinase (CK).
Results and Discussion: Independent of changes in body weight between groups, offspring of obese dams had impaired systolic and diastolic function (Figure 1A-D), attenuated chronotropic and inotropic responsiveness to Carbachol (both $p<0.001$) and enhanced inotropic responsiveness to Isoprenaline ($p<0.01$), highlighting cardiac sympathetic dominance (Figure 1E). Further, LVDP recovery to I/R was diminished in hearts of offspring of maternal obesity ($p<0.05$) and this was associated with increased CK. (Figure 1F).
Conclusion: Offspring of obese mothers have impaired cardiac function at 12 weeks of age, predictive of heart failure. These data have important health implications for future generations exposed to obesity in early life.

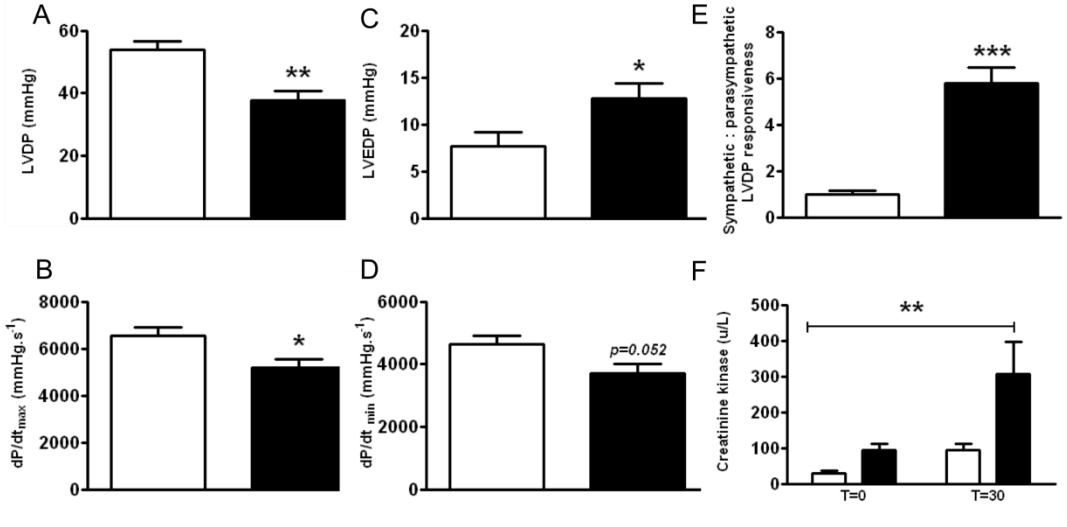


Figure 1: Left ventricular developed (A) and end diastolic (C) pressures. Myocardial contractility (B) and relaxability (D) Index of sympathetic dominance (E) and Perfusate CK levels following 10 minutes ischaemia (T=0) and 30 minutes after reperfusion (T=30).

O47

Gender difference in the influence of birth-weight on metabolic syndrome in 40 to 69 year old Japanese.

Shinji Katsuragi[1], Yoshihiro Miyamoto[2], Ryo Suzuki[1], Chikara Kihira[3], Tomoaki Ikeda[3].
[1] *Obstetrics and Gynecology, Sakakibara Heart Institute, Tokyo, Japan.* [2] *Internal Medicine, National Cerebral and Cardiovascular Center, Osaka, Japan.* [3] *Obstetrics and Gynecology, Mie University, Mie, Japan*
Corresponding author's email: skatsura12@yahoo.co.jp

Introduction: We investigated gender difference in the influence of birth-weight on metabolic syndrome in Japan.
Materials and Methods: The relationship between birth weight and the following parameters was investigated in 1241 subjects aged 40-69 years (males 521, females 720): waist≥90 cm (males) and≥80 cm (females), blood pressure (SBP/DBP 130/85 mm Hg and/or current use of antihypertensives), fasting blood glucose≥110 mg/dl and/or current use of insulin or oral diabetes medication, triglyceride≥150 mg/dl and/or current use of cholesterol-lowering medication, HDL cholesterol<40 mg/dl. Subjects were classified based on birth weight in the maternal notebook (<2500, 2500-3500, <3500 g) and on the examinee's memory ("light", "medium" and "heavy" body weight).
Results and Discussion: The maternal notebook correlated well with the examinee memory ($r=0.73$; $p<0.025$). "Light" body weight was a risk for hypertension (OR 2.0; 95%CI 1.02-4.09), hypertriglyceridemia (OR 3.0; 95%CI 1.06-9.77), low HDL cholesterol (OR 3.80; 95%CI 1.08-4.09) in females when controlling for age, BMI, smoking, alcohol. The percentage of "light", "medium" and "heavy" females fulfilling the glucose criteria was 14.1%, 11.6% and 4.8% ($p<0.05$) respectively.
Conclusion: Females with a light birth weight had greater risk for metabolic syndrome than heavy ones for blood pressure, HDL-cholesterol, and glucose level. These phenomena were not observed in males.

Oral Communications

Session IX: Translational & Other

Chairs:

Tullia Todros (University of Turin, Italy)

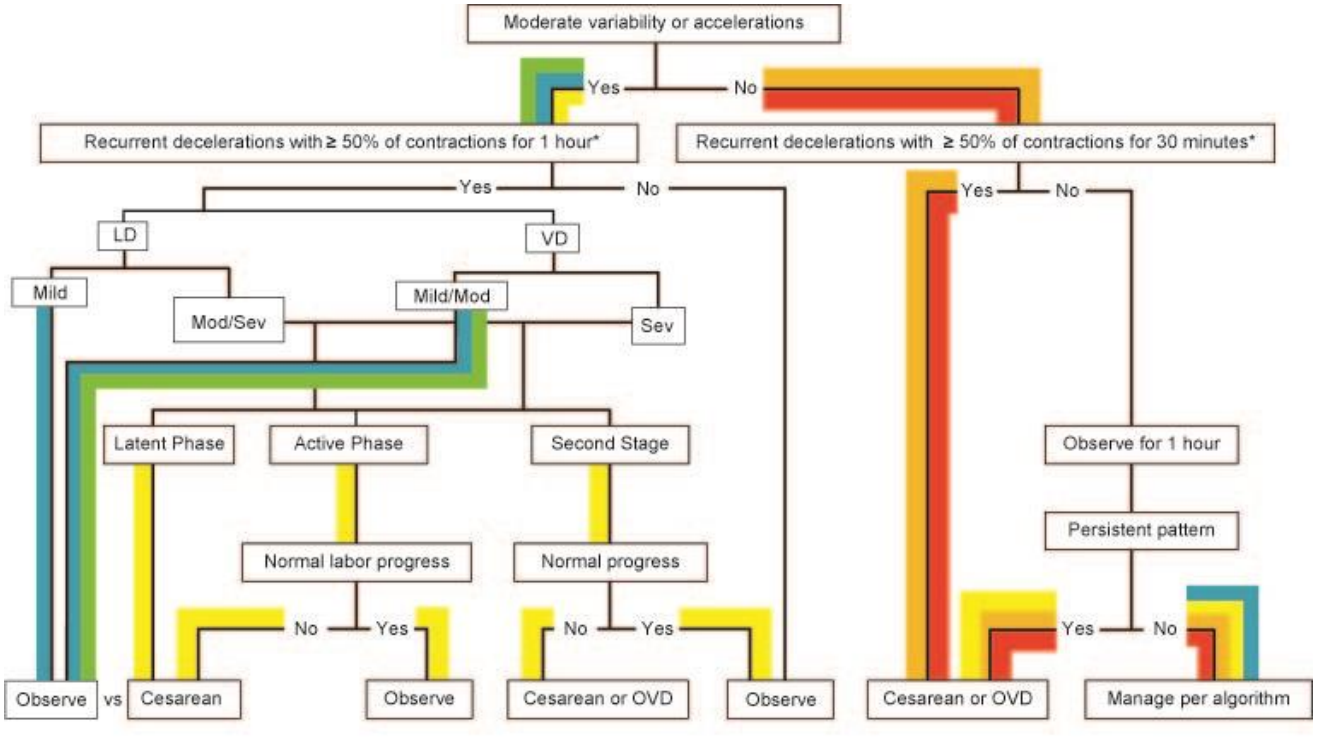
Roberto Reyes (Universidad de Chile, Chile)

O48

FHR Pattern Management: Reconciliation of the NICHD Category II with the 5-tier System.

Julian T. Parer[1], Tekoa King[2], Tomoaki Ikeda[2]
[1]University of California San Francisco, USA and [2]Mie University, Japan.

Background: The NICHD Category II is extremely heterogeneous, occurs in more than 85% of labours, and has not been found useful for making management decisions. The 5-tier system was felt to be too complex, although a recent app (FHR 5-tier) has made it simpler. A consensus algorithm (Clark et al, AJOG 2013) has attempted simplification, although this algorithm omits distinctions between minimal and undetectable variability, late and variable decelerations, and the severity of decelerations, baseline changes and prolonged decelerations.
Aim: To incorporate into Cat II missing FHR characteristics which are associated with developing fetal acidemia to aid management decisions, without losing simplicity.
Results: The modified Clark algorithm (Figure) now contains late vs variable decelerations, clarification (quantitation) of "significant" decelerations, and incorporation of the colour codes of the 5-tier system.
Comment: The simple flow of the decision tree is preserved, while allowing more options for withholding intervention in institutions where logistics allow rapid action in cases of rapid FHR pattern deterioration. This should still allow avoidance of fetal metabolic acidemia, without excessive operative intervention. These algorithms still require testing for validity and effectiveness.



* That have not resolved with appropriate conservative corrective measures, which may include supplemental oxygen, maternal position changes, intravenous fluid administration, correction of hypotension, reduction or discontinuation of uterine stimulation, administration of uterine relaxant, amnioinfusion, and/or changes in second stage breathing and pushing techniques.

LD = Late decelerations; VD = Variable decelerations

O49

Melatonin is an antenatal antioxidant and potential neuroprotectant in human pregnancy affected by fetal growth restriction.

Nicole Alers[1,2], Yen Pham[1], Jan Loose[1], Joanne Mockler[1,2,3], Euan Wallace[1,2,3], Suzanne Miller[1,2], Graham Jenkin[1,2]
[1] *The Ritchie Centre, Monash Institute of Medical Research, Monash University, Clayton, Victoria, Australia.*[2] *Department of Obstetrics and Gynecology, Southern Clinical School, Monash University, Clayton, Victoria, Australia.*[3] *Monash Health, Monash Women's, Monash Medical Centre, Clayton, Victoria, Australia.*
Corresponding author's address: nicole.alers@monash.edu

Introduction: Fetal growth restriction (FGR) is the failure to reach full growth potential. It is a serious complication of pregnancy, associated with increased risks of perinatal mortality and major short- and long-term morbidity, including neurodevelopmental impairment. Placental dysfunction is the major cause of FGR; leading to chronic fetal hypoxia and increased fetal and placental oxidative stress. We are investigating whether maternal antenatal administration of melatonin, a strong antioxidant and neuroprotectant, will reduce fetal oxidative stress, mitigate brain injury and improve neurodevelopmental outcomes of FGR neonates.
Materials & Methods: From the time of diagnosis of FGR until birth, melatonin (4mg twice daily) was administered orally to mothers. Pregnancy outcome, fetal growth and maternal and fetal well-being were monitored. Weekly maternal blood and, postnatal,umbilical cord blood and placental tissues were obtained to determine malondialdehyde (MDA) and 8-isoprostane (8-IP); markers of oxidative stress. Gestation-matched FGR controls were used for comparisons. Neonates are being assessed for well-being and developmental and neurological outcome.
Results & Discussion: To date, 9 women have completed the study. The administration of melatonin increased daytime melatonin levels 330 fold, from 24.7±7.4 pg/ml, without clinical maternal or fetal adverse effects other than transient daytime tiredness. In 5 women, feto-placental Dopplers improved; including normalization of abnormal uterine artery flow velocity, and/or improvements in ductus venosus, waveforms. MDA and 8-IP were lower in FGR melatonin treated, than in FGR control, placentae (MDA: 11.1±3.1 vs 20.4±3.9 nmol/mg, p=0.09; 8-IP: 44.4±8.0 pg/mg vs 123.7±67.8 nmol/mg, p=0.28). The interval between diagnosis of FGR and birth was greater in melatonin treated FGR than in FGR controls (35±12 vs 26±5 days, p=0.34).
Conclusions: Preliminary results indicate that melatonin treatment in FGR improves uteroplacental Doppler waveforms and prolongs pregnancy. Placental oxidative stress is decreased after maternal treatment with melatonin. Melatonin could be an efficacious antenatal neuroprotectant in FGR pregnancies.

O50

Prophylactic erythropoietin exacerbates ventilation-induced lung inflammation in preterm lambs.

Graeme Polglase[1,2], Mary Tolcos[1], Valerie Zahra[1], Megan Wallace[1,2], Melissa Siew[1], Samantha Barton[1], Jacqueline Melville[1] and Timothy Moss[1,2.]
[1]The Ritchie Centre, Monash Institute of Medical Research, Melbourne, Australia
[2]Department of Obstetrics and Gynaecology, Monash University, Melbourne, Australia.
Corresponding author's email: graeme.polglase@monash.edu

Introduction: Lung inflammation resulting from inadvertent ventilation-induced lung injury (VILI) of preterm neonates during the immediate neonatal period likely contributes to the pathogenesis of bronchopulmonary dysplasia (BPD). Erythropoietin (EPO) has been suggested to reduce BPD in preterm infants. We hypothesised that prophylactic administration of EPO would reduce the initial lung inflammation caused by VILI.
Methods and Materials: Lambs at 126 days of gestation were delivered and ventilated with a high tidal volume lung injury strategy for 15 minutes. Lambs were subsequently ventilated for 1 3/4 hours prior to tissue collection (*Ventilation*; n=7). *Ventilation+EPO* lambs (n=8) were treated identically to *Ventilation* lambs but received intravenous administration of EPO (5000 IU/kg) at 5 minutes. Physiological variables were recorded continuously. Unventilated controls (UVC; n=8) were humanely killed at birth. Samples of the right upper lung lobe were inflation fixed and sections were stained with Hemotoxylin & Eosin. Lung injury, inflammation, haemorrhage, epithelial sloughing and airway wall thickness were scored histologically. Samples of lung from the right lower lobe were frozen for measurement of mRNA expression for pro-inflammatory cytokines and early markers of lung injury using qRT-PCR. Data were compared by repeated measures ANOVA or Kruskal-Wallis ANOVA.
Results and Discussion: Ventilation pressures and volumes were not different between groups.
Ventilation+EPO lambs had higher FiO_2, SaO_2 and oxygenation during the first 10 minutes than *Ventilation* lambs ($p<0.001$ for all). There were no physiological differences after this time.
Histological scores of lung injury ($p=0.012$), airway wall thickness ($p=0.051$), inflammation ($p=0.010$) and haemorrhage ($p=0.005$) were higher in *Ventilation+EPO* lambs compared to *Ventilation* lambs. Ventilation increased lung inflammation and early markers of lung injury, with EPO administration exacerbating lung inflammation and markers of lung injury (Table 1).
Conclusion: Contrary to our hypothesis, prophylactic EPO exacerbates the pulmonary inflammatory response to VILI, which may increase the incidence and severity of long-term respiratory disease. More studies are required before EPO administration is used for lung protection in preterm infants.

Table 1:

	UVC	*Ventilation*	*Ventilation+EPO*
Interleukin (IL)-1β	1.0±0.1	10.7±2.9*	33.9±11.3*#
IL-6	1.0±0.1	34.4±9.4*	130.5±51.0*#
IL-8	1.0±0.2	23.1±7.1*	56.7±23.1*#
Early growth response gene-1	1.0±0.2	9.6±3.4*	11.3±3.2*
Connective tissue growth factor	1.0±0.1	5.8±0.7*	15.8±9.6*#
Cysteine-rich angiogenic inducer-61	1.0±0.2	14.3±3.7*	11.3±2.3*

*indicates significant difference to UVC. #indicates significant difference to *Ventilation*.

O51

Erythropoietin through evolution: a speculative view.

Max Gassmann

Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Switzerland, and Universidad Peruana Cayetano Heredia (UPCH)

Corresponding author's email: maxg@access.uzh.ch

Reduction in oxygen availability - as occurring after blood loss or at high altitude - induces erythropoietin (Epo) gene expression that in turn elevates red blood cell production and hence the blood's O_2 content. Interestingly - and in contrast to Quechuas/Aymaras - Tibetans only moderately increase their hematocrit at high altitude. Obviously, evolution has selected a blunted erythropoietic response for the Tibetan population (and some high altitude mammals). On the other hand, at sea level, the hematocrit of man and mammals is set well below the maximal possible oxygen uptake capacity. We found that the optimal hematocrit in acutely and chronically Epo-treated mice is 0.58 and 0.68, respectively. However, these high hematocrit values are seldom reached in men and mammals. By doing so, the blood viscosity is kept low, thereby reducing potential cardiovascular risks.

As already hypothesized by Carlos "Choclo" Monge, most men and mammals are "sea level design" and if so, one might speculate that oxygen-dependent Epo signal did not originally evolved to increase erythropoiesis. Indeed, it is well accepted that Epo exerts a neuroprotective function when the central nervous system (CNS) is challenged with reduced oxygen supply. The fact that Epo/Epo receptor-like proteins are expressed in very low organisms including insects provides further (speculative) evidence that Epo might have evolved as a factor that influences/protects the CNS. As such, we observed that increased Epo levels in the mouse brain augmented exercise performance in a non-erythroid manner. In other words: a single dose of recombinant human Epo demonstrated an unexpected improvement in maximal exercise performance that was independent of total hemoglobin mass, whole blood volume and cardiovascular parameters.

O52

Ion channels in uterine smooth muscle: new insights into the regulation of contraction.

Helena C Parkington[1], Harold Coleman[1], Mary Tonta[1], Penelope Sheehan[2].
[1] *Department of Physiology, Monash University, Clayton, Melbourne, Australia.* [2] *Royal Women's Hospital, Parkville, Melbourne, Australia*
Corresponding author's email: helena.parkington@monash.edu

Introduction: Uterine contractions are highly dependent upon electrical activity and hence ion channel activity. Ionic conductances responsible for determining the amplitude and duration of the action potential (AP) and for the co-ordinated spread of activity in labour remain unresolved.
Materials and Methods: Myometrium was obtained from women and guinea-pigs before and during labour. Electrical activity was recorded simultaneously with contraction in whole tissue using sharp microelectrodes. Ion channels were studied in collagenase-isolated smooth muscle cells using patch-clamp electrophysiology. Immunohistochemistry and western blotting were used to probe ion channel location and levels.
Results and Discussion: Myometrial APs consisted of a spike followed by a plateau of depolarization. Voltage-gated L- and T-type Ca^{2+} channels were responsible for the upstroke of the spike and for Ca^{2+} delivery for contraction. Spike amplitude was markedly enhanced upon blockade of "big" conductance BK_{Ca} K channels. The density of these channels was reduced in labour and is likely responsible for the larger spike amplitude that we observed in labour. The AP plateau was markedly prolonged following blockade of hERG K channels. The levels of this channel and its current were markedly enhanced in women with high BMI and may explain the high incidence of failure to progress in labour in these women. Finally, when two strips of myometrium were connected via a thread, contraction in one strip resulted in depolarization and AP firing in the other strip. Patch-clamp electrophysiology revealed the presence of depolarizing cation channels that were activated when myometrium was stretched. These channels have complex multi-level characteristics.
Conclusion: We identified a novel mechanism of contraction spread in myometrium. Contraction of a local region of myometrium activates stretch-sensitive mechanisms that ensure spread of activity to adjacent regions. We identified ion channels responsible for a more robust AP. Together, these mechanisms ensure forceful, co-ordinated contractions in labour.

O53

Blockade of connexin hemichannels is neuroprotective after asphyxia in the preterm fetal sheep.

Joanne O. Davidson,[1] Paul P. Drury,[1] Louise F. Nicholson,[2] Laura Bennet,[1] Colin R. Green,[3] Alistair Jan Gunn [1]
[1] Fetal Physiology and Neuroscience group, *Department of Physiology, The University of Auckland, Auckland, New Zealand*
[2] *Department of Anatomy with Radiology, The University of Auckland, Auckland, New Zealand*
[3] *Department of Ophthalmology, The University of Auckland, Auckland, New Zealand*
Corresponding author's email: aj.gunn@auckland.ac.nz

Introduction: Preterm infants have a high rate of neuronal and white matter damage, that is at least in part related to hypoxia-ischemia. There is now compelling evidence that early loss of the vulnerable oligodendrocytes in white matter is followed by impaired maturation. Abnormal opening of connexin hemichannels has been implicated in the spread of injury after brain ischaemia at term but it is unknown whether they also play a role in the immature brain, before the onset of myelination. In this study, we tested the hypothesis that blockade of connexin hemichannels would improve recovery of brain activity and reduce cell loss after asphyxia in preterm fetal sheep.
Materials and Methods: Asphyxia was induced by 25 min of complete umbilical cord occlusion in preterm fetal sheep (103-104 d gestational age). Connexin hemichannels were blocked by intracerebroventricular infusion of mimetic peptide starting 90 min after asphyxia, at a concentration of 50 μM/h, for one hour followed by 50 μM/24h for 24 hours (occlusion-peptide group, n=6) or vehicle infusion for controls (occlusion-vehicle group, n=7). **Results:** Peptide infusion was associated with earlier recovery of electroencephalographic power after asphyxia compared to occlusion-vehicle ($p<0.05$). 7 days after occlusion, peptide infusion was associated with reduced neuronal loss in the caudate and putamen ($p<0.05$), but not in the hippocampus ($p>0.05$), with an increase in total oligodendrocyte numbers in the intragyral and periventricular white matter, and marked improvement in numbers of mature oligodendrocytes in the intragyral white matter, compared to occlusion-vehicle ($p<0.05$), with increased proliferation ($p<0.05$).
Conclusion: Blockade of connexin hemichannels was neuroprotective in preterm fetal sheep after asphyxia, with evidence of reduced maturational delay of oligodendrocytes.

O54

The effects of human amniotic epithelial stem cells on evolving brain injury in preterm fetal sheep.

Lotte Van den Heuij[1], Suzie Miller[2], Graham Jenkin[2], Mhoyra Fraser[1], Alistair Jan Gunn [1], Laura Bennet[1].
[1] Fetal Physiology and Neuroscience Group, Dept Physiology, The University of Auckland, New Zealand, [2] Ritchie Centre, Monash Institute for Medical Research, Monash University, Australia
Corresponding author's email address: l.bennet@auckland.ac.nz

Introduction: Premature infants are at significant risk of asphyxia and consequent brain injury, but there are no effective neuroprotection treatments for these infants. Studies in adults animals show that stem-cells may be neuroprotective. Human amniotic epithelial cells (hAECs) are non-tumorigenic and non-immunogenic and have been shown to reduce ischemic brain lesions and improve functional outcomes after stroke. In the current study we evaluated the effects of a single hAEC bolus infusion at two different time points on the development of neural injury in preterm fetal sheep.
Methods: Preterm fetal sheep (103-4 days gestation) were continuously monitored before, during and for 7 days after a intracerebroventricular (icv) administration of either hAECs (1*106 cells/1ml, given as a 25min infusion) or vehicle. Treatments started 2 or 24 h after asphyxia induced by 25min of complete umbilical cord occlusion (UCO). Blood samples were taken for fetal blood gas and cytokine analysis by ELISA. Brains were histologically assessed post-mortem.
Results: hAEC treatment was associated with a reduction in macrophage infiltration into intragyral white matter (IGWM) in the 2 h group. However, hAEC treatment did not reduce oligodendrocytes loss in the periventricular or IGWM or neuronal loss in the striatum and did not increase proliferation. There were no differences in blood gases and IL-6 did not differ between groups, but IL-10 was higher ($p<0.05$) in the 2h group between 6-48h post-asphyxia. hAEC treatment suppressed EEG activity which was associated with reduced cerebral blood flow. Seizures onset was delayed and duration of the period of seizures was shorter in the 2h group.
Conclusions: A single icv bolus of hAECs at 2 and 24h was not associated with reduced injury at 7d in the areas assessed, despite effects on IL-10, EEG, blood flow and seizures in the 2h group. Evaluation of repeated treatments is now being undertaken.

Oral Communications

Session X: Fetal-maternal circadian rhythm

Chairs:

Jose Cipolla-Neto (University of Sao Paulo, Brazil)

Maria Seron-Ferre (Universidad de Chile, Chile)

O55

The night time party animal: circadian rhythms of the preterm sheep fetus.

Laura Bennet, Joanne O. Davidson, Paul P. Drury, Rachael L Robson, Eleanor Gunn, Alistair Jan Gunn [1]
[1] *Fetal Physiology and Neuroscience group, Department of Physiology, The University of Auckland, Auckland, New Zealand*
Email: l.bennet @auckland.ac.nz

Introduction. Adverse events occurring *in utero* can lead to many adverse outcomes for the fetus, and after birth, ranging from frank injury to more subtle reprogramming of organ development and functional control. To understand the mechanisms of action, to establish periods of susceptibility, and to determine the efficacy of treatments, requires that we understand how normal fetal behaviour develops. Circadian rhythms are important for the normal development of behaviour, but preterm fetal circadian behavioural rhythms remain surprisingly poorly understood. The objective of this study was to characterise the neural, cardiovascular and behavioural circadian patterns of the preterm fetal sheep at 0.7 gestation; ~ 27-30 week human in terms of neural maturation.
Methods. Sheep fetuses at 97-98d were instrumented to measure continuous changes in fetal EEG, body movement amplitude, and the incidence and amplitude of breathing movements (FBMs), carotid blood flow (CaBF), mean arterial blood pressure (MAP), fetal heart rate (FHR). Cerebral cytochrome oxidase (CytOx) was measured using near infrared spectroscopy. Recordings commenced 5d after surgery. Ewes were fed ad-libitum and housed with companion sheep in temperature controlled rooms (~17-18C), with a 12h day-night light cycle (06.30-18.30h daytime).
Results. CytOx was low during the day, and markedly elevated at night. All variables, except MAP, increased from mid-late afternoon/early evening, peaking around 12-2am and falling thereafter, with a marked nadir in measurements seen between 8-12pm. MAP incrementally increased throughout the recordings as a function of growth, but in contrast to the other variables MAP fell at night, and markedly rose during the day.
Conclusion. The preterm fetus has clear circadian rhythms in behaviour, with fetal activity prioritised to primarily night time hours, while the MAP data suggests growth appears to occur predominantly during the day. Increased CytOx at night may reflect increased ATP production needed to support increased neural activity.

O56

Gestational chronodisruption has a far-reaching impact on fetal liver genomics.

Carlos Spichiger, Hugo Galdames, Claudia Torres-Farfan, Natalia Mendez, Pamela Alonso Vazquez, Hans Richter.
Laboratory of Developmental Chronobiology, Faculty of Medicine, Universidad Austral de Chile, Valdivia, Chile. Corresponding author's email: hrichter@uach.cl

Introduction. We recently reported that alteration of the photoperiod along pregnancy (gestational chronodisruption) induces fetal growth restriction and marked effects on fetal adrenal physiology. Using a functional genomics approach, here we investigated whether gestational chronodisruption modifies the expression of metabolic disease markers in the fetal liver.
Materials and Methods. At day E10 dams were randomized in two groups (n=5 each): normal photoperiod (LD; 12h light/12h dark) and chronodisruption (LL; constant light). At E18 the mothers were euthanatized to isolate total RNA from the fetal liver, which was subjected to high stringency transcriptome analysis (Affymetrix Rat Gene 1.1 ST GeneChip for 28,000 rat genes; SAM algorithm; FDR≤10%). Selected differential transcripts were validated independently by qPCR. Integrated transcriptional changes were analyzed using 'Database for Annotation, Visualization, and Integrated Discovery' and 'Ingenuity Pathway Analysis'.
Results and Discussion. Chronodisruption did not modify food/water intake, body weight gain nor plasma corticosterone in the dams; however, it suppressed maternal plasma melatonin and induced fetal growth retardation. Transcriptome profiling revealed significant changes in the expression level of 3.431 genes in LL relative to LD fetal liver (1.960 up-regulated and 1.471 down-regulated; 17% of the whole transcriptome). Several selected transcripts were validated by real-time PCR. Gene ontology and pathway analysis indicated modification of diverse regulatory networks in the LL fetal liver, including: organogenesis, hematopoiesis, coagulation cascade, complement system and carbohydrate and lipid metabolism. Epigenetic modification may also be important in hepatic developmental adaptation to LL, given that the profuse microRNA Mir122 was significantly down-regulated in the LL fetal liver.
Conclusion. Gestational chronodisruption impinges upon a large set of fetal liver gene networks, likely giving rise to enduring metabolic maladaptation. Indeed, a number of transcripts deregulated in the fetal liver by gestational chronodisruption, are associated with postnatal risk of metabolic syndrome, fatty liver, insulin resistance and immunological disorders.

Funding: This work was supported by grants 1110220 from FONDECYT (to HR) and ACT1116 from CONICYT (to CT-F and HR), Chile.

O57

Impact of gestational chronodisruption on adult offspring physiology and therapeutic action of prenatal melatonin.

Mendez N, Vera H, Halabi D, Gavilan D, Spichiger C, Vergara K, Vilches N, Seron-Ferre M, Richter HG, Torres-Farfan C.

Laboratory of Developmental Chronobiology, Faculty of Medicine, Universidad Austral de Chile, Valdivia, Chile.

Corresponding author's email: *claudia.torres@uach.cl*

Introduction: Recently we reported that gestational chronodisruption induces fetal growth restriction and marked effects on fetal adrenal physiology, supporting that gestational chronodisruption may act as a deleterious signal for fetal development. Here, we examined the long term effects of gestational chronodisruption on heart, liver, hippocampus and components of the endocrine system during adult life.

Material and Methods: At day 10 of gestation, rats were randomized in three groups (n=5 each): normal photoperiod (LD), chronodisruption (LL; which suppresses maternal/fetal circulating melatonin) and chronodisruption supplemented with 2.0 µg/mL melatonin in drinking water (LL+Mel). Immediately after delivery the mother and their pups returned to LD photoperiod until 90 days of age; time at which we investigated different physiological and behavior parameters.

Results: We did not observed differences in newborn weight either immediately after birth or through life. However, altered phenotypes were observed in adulthood: left ventricle hypertrophy, reduced plasma corticosterone and melatonin, altered intraperitoneal glucose tolerance response and spatial memory deficit. Notably, melatonin replacement had a normalizing effect on overall alteration produced by gestational chronodisruption at both, fetal and adult stages.

In the hippocampus of adult LL offspring, we observed complete lack of day/night differences in transcription of clock genes and NMDA receptor subunits, together with a significant deficit of spatial memory; however, hippocampal gene expression and cognitive function were equivalent in LL+Mel and control LD photoperiod.

Conclusions: Our work offers the potential for therapeutic effects of prenatal melatonin administration against developmental origin of cardiovascular, endocrine, immune and cognitive disease in pregnancy subjected to chronodisruption. In translation, the present results might have far-reaching consequences for the offspring of pregnant shift working women.

Funding: ANILLO ACT-1116; FONDECYT 1110220 & 1120938 (Chile).

O58

Absence of maternal melatonin is detrimental to melatonin synthesis in adult offspring. Fernanda Amaral[1], Ariane Turati[1], Julieta Scialfa[1], José Cipolla-Neto[1].
[1] Department of Physiology and Biophysics, *University of São Paulo, São Paulo, Brasil*
Corresponding author's email: fgamaral@icb.usp.br

The pineal gland and its hormone melatonin are critically involved in the synchronization of a series of physiological phenomena that are fundamental for homeostasis, including the energy metabolism. Pinealectomy leads to glucose peripheral intolerance, in addition to a desynchronization of insulin synthesis and of liver, muscle and adipose tissue metabolic processes. The wide-spread use of artificial light during the night and the shift work regimens are known to positive correlate with increased incidence of metabolic diseases. Besides that, light at night is detrimental to melatonin synthesis. Recent data from our research group showed that rats born to pinealectomized (PINX) dams present glucose intolerance and hepatic insulin resistance, among other metabolic impairments, but the melatonin synthesis status in these animals remains unknown. Considering that, we aimed to evaluate melatonin synthesis in the offspring of PINX dams. Female Wistar rats were assigned to Control, PINX, PINX-Mel (0.5 mg/Kg of melatonin daily added to the night drinking water during gestation and lactation), PMG (melatonin administration exclusively during gestation) and PML (melatonin administration exclusively during lactation) groups and mated with control male rats. We found that both male and female rats born to PINX dams presented melatonin synthesis decrease at ZT18 when compared to rats born to Control dams. Interestingly, the melatonin synthesis recovery in the offspring was gender specific. Melatonin had to be given to the mother during gestation to be able to restore melatonin synthesis in the female offspring. On the other hand, melatonin synthesis in the male offspring was restored only when the mothers received melatonin both during gestation and lactation. These results point to differential melatonin synthesis programming in male and female offspring and the observed melatonin synthesis decrease due to maternal melatonin absence could contribute to the energy metabolism impairment described in these animals.

O59

Altered circadian rhytms of time birth is observed after the February 27th 2010 Chilean earthquake.

Valenzuela FJ[1], Cumsille P[2], Bertoglia P[3], Celis C[1], Acurio J[1], Escudero Cristian Celis[1,2].
[1]Vascular Physiology Laboratory, Group of Investigation in Tumor Angiogenesis (GIANT), Department of Basic Sciences, Universidad del Bío-Bío, Chillán, Chile. [2]Grupo de Investigación en Matematicas (GMA), Basic Sciences Department, Universidad del Bio Bio, Chillan, Chile. [3]Obstetric and Gynecology Department, Herminda Martin Clinical Hospital, Chillan, Chile.
Corresponding author's email: fvalenzuela@ubiobio.cl

Introduction: Circadian system is organized as a master clock located in the hypothalamus and peripheral clocks located in almost every tissue of the body. Alteration of the internal temporal order such as shift work or stress is associated with pathologies such as cancer and preterm birth. In human, time birth showed a circadian rhythm with a peak at second middle of night and early in the morning. We hypothesize stress produced by earthquake modify the circadian system during normal and preterm pregnancy.

Materials and Methods: We analysed total deliveries that occurred in 2009 (n=3.609) and 2010 (n=3.279) in the Clinical Hospital Herminda Martin (Chillan, Chile). Local Ethical Committee approved this study. Preterm birth was defined by <37 week of gestation and inclusion criteria was, normal deliveries (eutocia), and absence of pharmacological treatment. Analyses were performed by One-way ANOVA, Newman-Keuls post-test and mathematical approximation using the approach of cosine function and least squares.
Results and Discussion: A total number of preterm delivery (n=276, 1097 in 2009 and 2010, respectively) was founded in the records. Circadian rhythm of delivery was observed in normal pregnancy during 2009 years with a peak between 21-24 hours and fit a theoretical cosine function ($R^2=0.55$). This pattern was altered after 2010 earthquake, showing a minor amplitude oscillation and peak between 13-16 hours ($P<0.05$). On the other hand, the circadian pattern was not observed in preterm deliveries occurred either on 2009 or 2010, however a higher number of preterm births occurred during the winter of 2010. Additionally, we observed change in the relationship between female/male percentage in premature birth during 2010 year (34.6 and 65.4, respectively).
Conclusion: The early initiation of labor occurred on 2010 and change of relationship between female/male might be associated to stressful condition generated by the 27th February earthquake, resulting in altered function of circadian system during the pregnancy.

Supported by CONICYT 79112027 and FONDECYT 1100684, DIUBB 122109 GI/EF.

O60

Positive and negative effects of a social/chemical lubricant on asphyxial brain injury in preterm fetal sheep.

[1]Paul P. Drury,[1] Joanne O. Davidson, [1] Laura Bennet, [1] Lindsea C Booth, [1] Sidhartha Tan, [2] Mhoyra Fraser, [1] Lotte Van den Heuij, [1] Alistair J Gunn.
1Department of Physiology, University of Auckland, New Zealand. 2Department of Pediatrics, NorthShore University HealthSystem, Evanston, Chicago, Illinois, USA.
Corresponding author's email: aj.gunn@auckland.ac.nz

Background: Melatonin is a naturally occurring indolamine with mild anti-oxidant properties that is neuroprotective in perinatal animals. There is limited information on its effects on preterm brain injury. We tested the hypothesis that prophylactic maternal low-dose melatonin is neuroprotective following profound asphyxia in fetal sheep at 0.7 of gestation, and the secondary hypothesis that the vehicle, low-dose ethanol, is deleterious.
Methods: In this study, 23 chronically instrumented fetal sheep received 25 min of complete umbilical cord occlusion at 101-104 d gestation (term is 147 d). Melatonin was administered to the ewe 15 min before occlusion (0.1mg/kg bolus followed by 0.1 mg/kg/h for 6 h, n=8), or the equivalent volume of vehicle (2% ethanol, n=7), or saline (n=8), or maternal saline plus sham occlusion (n=8). Fetuses were killed after 7 d recovery in-utero.
Results: Fetal blood pressure, heart rate, nuchal activity and temperature were similar between groups. Vehicle infusion was associated with improved neuronal survival in the caudate nucleus, but greater neuronal loss in regions of the hippocampus, with reduced proliferation and increased amoeboid microglia in white matter ($p<0.05$). Maternal melatonin infusion was associated with faster recovery of fetal EEG, prolonged reduction in carotid blood flow, similar neuronal survival to vehicle, improved numbers of mature oligodendrocytes and reduced microglial activation in white matter ($p<0.05$).
Conclusion: Prophylactic maternal melatonin treatment is partially protective but its effects are partly confounded by ethanol used to dissolve melatonin.

AUTHOR INDEX

DELEGATES LIST

Name	University-Institution	Country	Email
Aburto, Caroll	Universidad de Chile	Chile	caroll.aburto@gmail.com
Alonso, Pamela	Universidad Austral de Chile	Chile	pamelalonso.1@gmail.com
Amaral, Fernanda	University of Sao Paulo	Brazil	fgamaral@gmail.com
Araya Quijada, Claudio E	Universidad de Chile	Chile	gebensperger@med.uchile.cl
Aridas, James	Monash Institute of Medical Research	Australia	james.aridas@monash.edu
Batiz, Federico	Universidad Austral de Chile	Chile	fedebatiz@gmail.com
Bennet, Laura	The University Auckland	New Zealand	l.bennet@auckland.ac.nz
Bennett, Greer	University of Newcastle	Australia	greer.bennett@gmail.com
Berends, Lindsey	University of Cambridge	UK	lmb81@cam.ac.uk
Berry, Max	University of Otago	New Zealand	max.berry@otago.ac.nz
Blackmore, Heather	University of Cambridge	UK	hlb46@cam.ac.uk
Bonesso, Marcio	Hospital de Clínicas de Porto Alegre	Brazil	m.bonesso@gmail.com
Brain, Kirsty	University of Cambridge	UK	klb61@cam.ac.uk
Camm, Emily	University of Cambridge	UK	ejc68@cam.ac.uk
Carmona, Pamela	Universidad Austral de Chile	Chile	pamelacarmona@uach.cl
Casanello, Paola	Pontificia Universidad Católica de Chile	Chile	paolacasanello@gmail.com
Castillo-Melendez, Margie	Monash Institute of Medical Research	Australia	Margie.Castillo_Melendez@monash.edu
Cerfogli, Cristian	Universidad de Chile	Chile	cristiancerfogli@live.cl
Cipolla-Neto, José	University of Sao Paulo	Brazil	cipolla@icb.usp.br
Coleman, Harold	Monash Institute of Medical Research	Australia	harry.coleman@monash.edu
Davidson, Joanne	The University of Auckland	New Zealand	
De Matteo, Robert	Monash Institute of Medical Research	Australia	Robert.dematteo@monash.edu
Derks, Jan	Unversity Medical Center Utrecht	The Netherlands	J.B.Derks@umcutrecht.nl
Diaz, Marcela	Universidad de Chile	Chile	mdiaz@med.uchile.cl
Drury, Paul	The University of Auckland	New Zealand	
Ebensperger D., Germán	Universidad de Chile	Chile	gebensperger@med.uchile.cl
Escudero, Carlos	Universidad Bio-Bio	Chile	cescudero@ubiobio.cl
Ferrer Márquez, Fernando	Pontificia Universidad Católica de Chile	Chile	fernandoferrerm@gmail.com
Figueroa, Jorge	Wake Forest, Bapist Health, NC	USA	Figueroa@wakehealth.edu
Fraser, Mhoyra	University of Auckland, Liggins Institute	New Zealand	m.fraser@auckland.ac.nz
Gassman, Max	University of Zurich	Switzerland	maxg@access.uzh.ch
Gavilan, Diego	Universidad Austral de Chile	Chile	dgavilanlenam@gmail.com
Giussani, Dino A.	University of Cambridge	UK	dag26@cam.ac.uk
González-Candia, Alejandro	Universidad de Chile	Chile	alejjobg@gmail.com
Guerra, Francisco	Universidad Austral de Chile	Chile	fguerra@uach.cl

Gunn, Alistair	The University Auckland	New Zealand	aj.gunn@auckland.ac.nz
Gutierrez, Mariana	Universidad Austral de Chile	Chile	mariangutierr@gmail.com
Halabi, Diego	Universidad Austral de Chile	Chile	diego.halabi@uach.cl
Hanson, Mark	University of Southampton	UK	m.hanson@soton.ac.uk
Herrera, Emilio A.	Universidad de Chile	Chile	eherrera@med.uchile.cl
Herrera-Marschitz, Mario	Universidad de Chile	Chile	mh-marschitz@med.uchile.cl
Ikeda,Tomoaki	Universidad Austral de Chile	Chile	t-ikeda@clin.medic.mie-u.ac.jp
Itani, Nozomi	University of Cambridge	UK	ni231@cam.ac.uk
Jenkin, Graham	Monash Institute of Medical Research	Australia	Graham.jenkin@monash.edu
Katsuragi, Shinji	Sakakibara Heart Institute	Japan	skatsura12@yahoo.co.jp
Keller-Wood, Maureen	University of Florida	USA	kellerwd@cop.ufl.edu
Krause Leyton, Bernardo	Pontificia U. Católica de Chile	Chile	bjkrause@uc.cl
Larosa, Domenic	Monash Institute of Medical Research	Australia	domenic.larosa@monash.edu
Lear, Christopher	The University Auckland	New Zealand	l.bennet@auckland.ac.nz
Llanos, Anibal J	Universidad de Chile	Chile	allanos@med.uchile.cl
Macchiavello, Roberto	Universidad de Chile	Chile	r.macchiavello@hotmail.com
Mendez, Natalia	Universidad Austral de Chile	Chile	natamendezc@gmail.com
Miller, Suzie	Monash Institute of Medical Research	Australia	suzie.miller@monash.edu
Muckenthaler, Martina	University of Heidelberg	Germany	martina.muckenthaler@med.uni-heidelberg.de
Muñoz-Urrutia, Ernesto	Pontificia Universidad Católica de Chile	Chile	ernestobq@gmail.com
Nijhuis, Jan G.	Maastricht Univ. Med Center (MUMC)	The Netherlands	jg.nijhuis@mumc.nl
Parer, Julian (Bill)	University of California San Francisco	USA	parerb@obgyn.ucsf.edu
Parkington, Helena	Monash Institute of Medical Research	Australia	helena.parkington@monash.edu
Parrau, Daniela	Universidad de Chile y UDP	Chile	dparrau@gmail.com
Polglase, Graeme	Monash Institute of Medical Research	Australia	Graeme.polglase@monash.edu
Quezada, Sebastián	Universidad de Chile	Chile	seba.quezada.r@gmail.com
Ramirez, Santiago	Universidad de Chile	Chile	gebensperger@med.uchile.cl
Reiss, Irwin	Erasmus MC-Sophia Children`s Hospital	The Netherlands	i.reiss@erasmusmc.nl
Reyes, Roberto	Universidad de Chile	Chile	vicreyes@med.uchile.cl
Richter, Hans	Universidad Austral de Chile	Chile	hrichter@uach.cl
Riquelme, Alexander	Universidad de Chile	Chile	alexander.riquelme.herrera@gmail.com
Robson, Rachael	The University Auckland	New Zealand	
Rurak, Dan	University of British Columbia	Canada	drurak@cw.bc.ca
Schneider, Daniela	Pontificia U. Católica de Chile	Chile	bjkrause@uc.cl
Seron-Ferre, Maria	Universidad de Chile	Chile	mseron@med.uchile.cl
Spichiger, Carlos	Universidad Austral de Chile	Chile	cspichiger@uach.cl
Spiroski, Ana Mishel	U. of Auckland, Liggins Institute	New Zealand	am.spiroski@auckland.ac.nz
Stacey, Ellery	Monash Institute of Medical Research	Australia	stacey.ellery@monash.edu

Suzuki, Keiji	Tokai University School of Medicine	Japan	dkswnd@tokai-u.jp
Thomasset, Raphael	University of Turin	Italia	raphaelthomasset@hotmail.it
Todros,Tullia	University of Turin	Italy	tulia.todros@
Tolosa González, Jorge M.	The University of Newcastle	Australia	Jorge.tolosa@newcastle.edu.ac
Torres, Flavio	Universidad de Chile	Chile	flaviotorres@ug.uchile.cl
Torres-Farfan, Claudia	Universidad Austral de Chile	Chile	claudia.torres@uach.cl
Uauy, Ricardo	Universidad de Chile	Chile	druauy@gmail.com
Uribe Araneda, Mauricio	Universidad de Chile	Chile	mauriciouribe@ug.uchile.cl
Valenzuela, Francisco	Universidad Bio-Bio	Chile	fvalenzuela@ubiobio.cl
Valenzuela, José Maria	Universidad de Chile	Chile	jm_valenzuela@ug.uchile.cl
Van Den Heuij, Lotte	The University of Auckland	New Zealand	
Velasquez, Zahady	Universidad Austral de Chile	Chile	zahady@gmail.com
Veliz, Marcelino	Universidad de Chile	Chile	maveliz@ug.uchile.cl
Vera, Hector	Universidad Austral de Chile	Chile	hveragcl@gmail.com
Villalobos, Roberto	P. Universidad Católica de Chile	Chile	roberto.e.villalobos@gmail.com
Villamor, Eduardo	Maastricht Univ. Med Center (MUMC)	The Netherlands	E.Villamor@mumc.nl
Walker, David	Monash Institute of Medical Research	Australia	david.walker@monash.edu
Weaver-Mikaere, Luke	U. of Auckland, Liggins Institute	New Zealand	mikaere@auckland.ac.nz
Weissmann, Norbert	ECCPS, University of Giessen	Germany	Norbert.Weissmann@innere.med.uni-giessen.de
Wood, Charles	University of Florida	USA	woodc@ufi.edu
Wright, Ian	University of Wollongon	Australia	Ian.Wright@hnehealth.nsw.gov.au
Zimmermann, Luc	Maastricht Univ. Med Center (MUMC)	The Netherlands	luc.zimmermqann@mumc.nl