Fetal and Neonatal Physiological Society

2016 Cambridge

43rd Annual Meeting of the Fetal and Neonatal Physiological Society 2016

17th to 20th
September 2016
University of Cambridge, UK

Fetal and Neonatal Physiological Society

2016 Cambridge

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The Physiological Society

The Journal of Physiology

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Professor Dino Giussani

Meeting Organiser

Mrs Kristin Giussani

Local Organising Committee

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Dr Youguo Niu
Dr Ana-Mishel Spiroski
Dr Nozomi Itani
Miss Katie Skeffington
Mr Christian Beck
Miss Shani Austin-Williams
Mr Alan Cattell

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Professor Abigail Fowden, University of Cambridge
Professor Anne Ferguson-Smith, University of Cambridge
Professor Graham Burton, University of Cambridge
Professor Gordon Smith, University of Cambridge
Professor Sue Ozanne, University of Cambridge
Dr Alison Forhead, University of Cambridge
Dr Amanda Sferruzzi-Perri, University of Cambridge
Dr Erica Watson, University of Cambridge
Dr Topun Austin, University of Cambridge
Dr Miguel Constancia, University of Cambridge
Dr Catherine Aiken, University of Cambridge

Welcome

Dino A. Giussani
Meeting Director

September 2016

I am both proud and delighted to welcome you all to Cambridge for this 43rd International Annual Meeting of the Fetal and Neonatal Physiological Society. Cambridge is renowned, worldwide, as a seat of learning and it is entirely appropriate that this should be the venue for us to gather and exchange ideas on a range of topics relating to pregnancy, maternal and infant health. In addition, the meeting celebrates the centenary of Sir Joseph Barcroft's research. An exciting scientific programme has been put together by the Organising Committee and this will be held in the Physiological Laboratory, where Barcroft taught and worked. We were extremely gratified to note the record number of very high standard abstracts that were submitted. As a result, I am confident that our Meeting will be both informative as well as enjoyable.

For those among you who have not previously visited Cambridge, I urge you to explore as much as possible. Situated in the rural East of England, and with its unique setting on the banks of the River Cam, Cambridge is one of the most beautiful cities in England. The University, which comprises 31 separate colleges, incorporates some magnificent architecture - Kings College Chapel being a prime example. Cambridge has always enjoyed a reputation for being at the forefront of scientific research, and today it is also considered as a thriving centre for hi-tech industries.

I hope that all delegates leave at the end of the Meeting with excellent memories and a strong wish to visit again. In closing, I would like to express my sincere thanks to Kristin Giussani, the Meeting Organiser and to my colleagues on the Local Organising Committee, whose input was invaluable. Many thanks also to our sponsors, whose generosity has contributed greatly to the quality of this event. I would also like to thank Dr Zhongchao Wang, Mr Christian Beck and Mr Roberto Ichingolo for their invaluable help with the website.

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 June-September, as determined by the Organizational Committee. Approximately half of the meetings will be held in Europe.

Any residual funds from the prior meeting shall be passed on to the coordinator for the next meeting. Audit will not be required if the residual funds are less than 10,000 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.

In Memoriam Dr. Julian T. (Bill) Parer (1934-2016)

With great sadness I report to the Society that Professor Julian T (Bill) Parer, MD, PhD passed away on August 3 2016 while hiking with his dog on Mt. Tamalpais, near Phoenix Lake in Marin, California.

Bill was born in Australia as the third of 4 boys to Stan and Irene Parer. He obtained a Bachelor of Agricultural Science degree from Melbourne University, a Master of Rural Science degree from the University of New England (Armidale, New South Wales). Bill then moved to the United States, where he received his PhD from Oregon State University in 1965 and an MD from the University of Washington in Seattle in 1971. He studied maternal and fetal physiology under the mentorship of Dr. James Metcalfe of the University of Oregon, and trained under Drs. Edward J. Quilligan and Edward Hon at the University of Southern California. After completing his OBGYN residency at the University of Southern California, Bill joined the faculty of the Department of OBGYN and Reproductive Sciences at the University of California, San Francisco in 1974 as a perinatologist and a researcher, reaching the rank of Professor in 1982. He was the Director of the Division of Maternal-Fetal Medicine at UCSF, and for 33 years was in charge of the Maternal-Fetal Medicine Fellowship at UCSF.

Bill was a leading academic in the field of perinatal biology and medicine and a member of the FNPS for the past 40 years. Bill served as President of the Society from 2002-2004 and as Scribe from 2005-2007. Bill's research focused on fetal physiology, with an emphasis on fetal responses to hypoxia and asphyxia, oxygen transport in pregnancy, fetal heart rate monitoring, and on the effects of high altitude on pregnancy and programming of pulmonary hypertension. Bill had long standing collaborations with Dr. Tomoaki Ikeda, University of Mie, Japan, and Dr Anibal Llanos of the Universidad de Chile. His pursuits resulted in more than 230 manuscripts and book chapters. He also cared for thousands of high-risk pregnant women in the San Francisco Bay Area and beyond, providing perinatal care and unique services such as intrauterine fetal transfusion and abdominal cerclage. Bill created and directed a continuing medical education course for 40 years on antepartum and intrapartum management, which attracted attendees from around the globe.

Bill was truly passionate about his clinical and scientific work, and served as a role model to many of us in the field and in our Society who were fortunate to work with him and/or to be trained by him. In addition to his highly significant scientific contributions, we will all remember Bill sitting in the front row at meetings, wearing shorts and a baseball cap, making incisive contributions while being extremely supportive to young investigators. He was an accomplished mountaineer and avid hiker, a fun-loving person and one of our greatest friends.

Sincerely,

Dino A. Giussani
FNPS President

Minutes of the Fetal Neonatal Physiological Society Annual General Meeting

Vancouver, Canada

Wednesday, August 12, 2015

Present: Laura Bennett, Jan Derks, Rob Galinsky (ECR member), Dino Giussani (President), Tomoaki Ikeda, Suzie Miller, Tim Moss (minute taker), Jan Nijhuis, Bill Parer, Luc Zimmerman.
Apologies: Emilio Herrera (Scribe), Dan Rurak.

1. **Minutes of the last meeting accepted.**
   Matters arising: all new board member invitees agreed (Dr Suzie Miller (Australia), Dr Jan Derks (Netherlands), Dr Charles Ducsay (USA) and Rob Galinsky (New Zeland)); Lucy Green notified of turnover.

2. **Vote of thanks to the organisers.**
   Thank you Dan.
   Some discontent from members about requirement to pay for drinks. Expectation is that the meeting is a single-cost event.
   Location and timing of meeting needs consideration to maximise attendance.
   Meeting location should be chosen to optimise activities for accompanying persons and ease of access to social functions/activities.

3. **FNPS Website.**
   Update status. Links to web pages of other Societies and Organisations on our FNPS website.Christian Beck is working on the website and it will go live in 2 weeks. Hosting by Cambridge permits no cost.Dino has been approached by other organisations (e.g. SIDS) about linking to FNPS site. Board agree on a case-by-case basis.

4. **FNPS abstract archives digitisation.**
   A discussion took place of feasibility. Dino Giussani is to find out procedure if done at Cambridge.

5. **Availability of conference abstracts/proceedings online.**
   PDF of conference program to be uploaded to FNPS website so abstracts are available online.

6. **FNPS Bank account.**
   Baseline account for FNPS administrative costs. Previous agreement about 10 Euro from each registration to go towards FNPS account. Dino will ask Dan about return from FNPS 2016.

7. **Future meetings.**
   2016. Cambridge, UK. Perhaps to coincide with the centenary celebration of some of Joseph Barcroft's initial findings on Fetal Physiology. September 17-20 (Saturday-Tuesday). Cost similar to 2014 & 2015, all inclusive (4 nights; meals, entertainment). Include celebration of Barcroft and publication of *J Physiol* special edition. Comment about Ritchie Centre colloquium in Cambridge to coincide with next year's

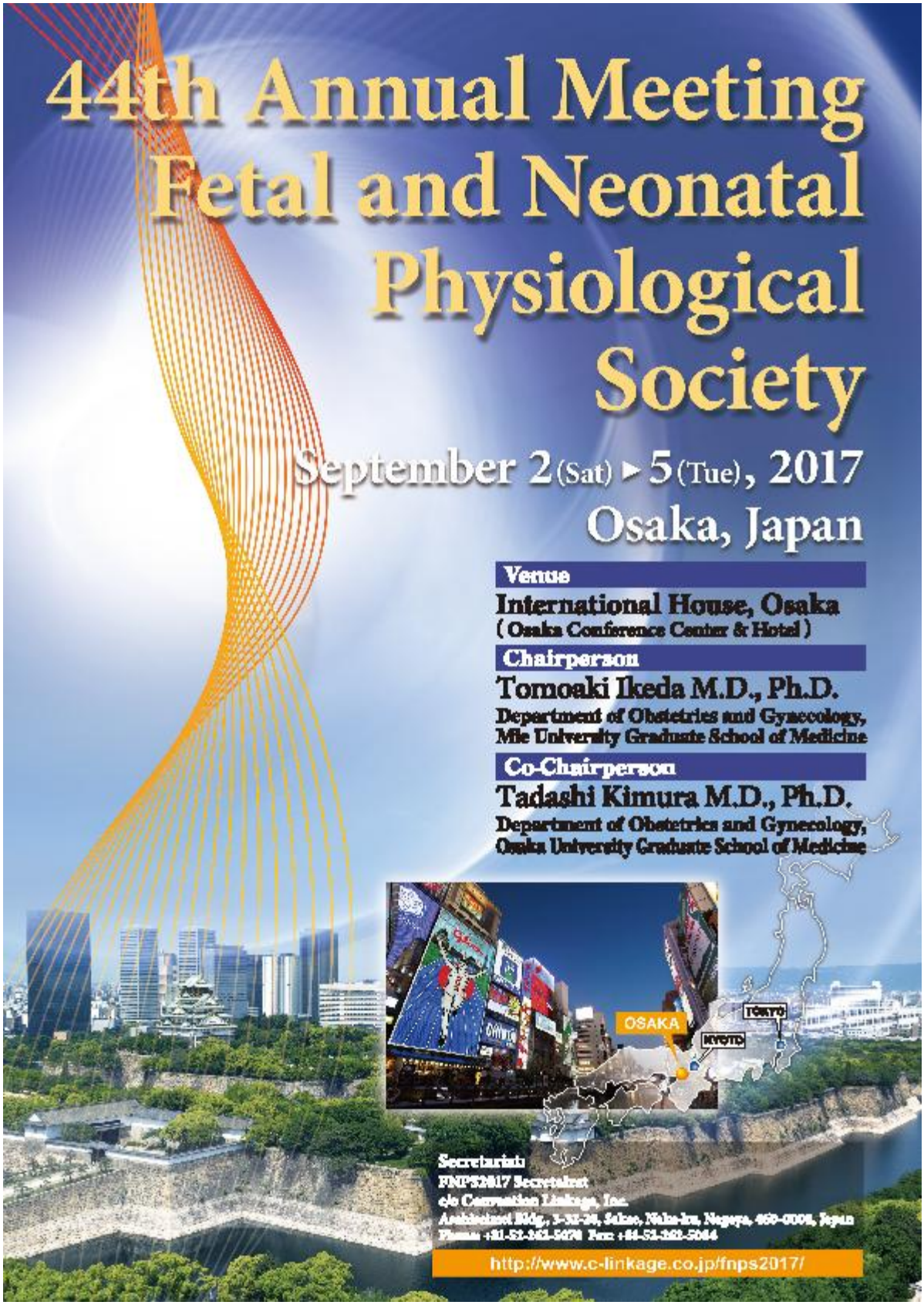
FNPS meeting.Thursday and Friday preceding FNPS. St John's College.

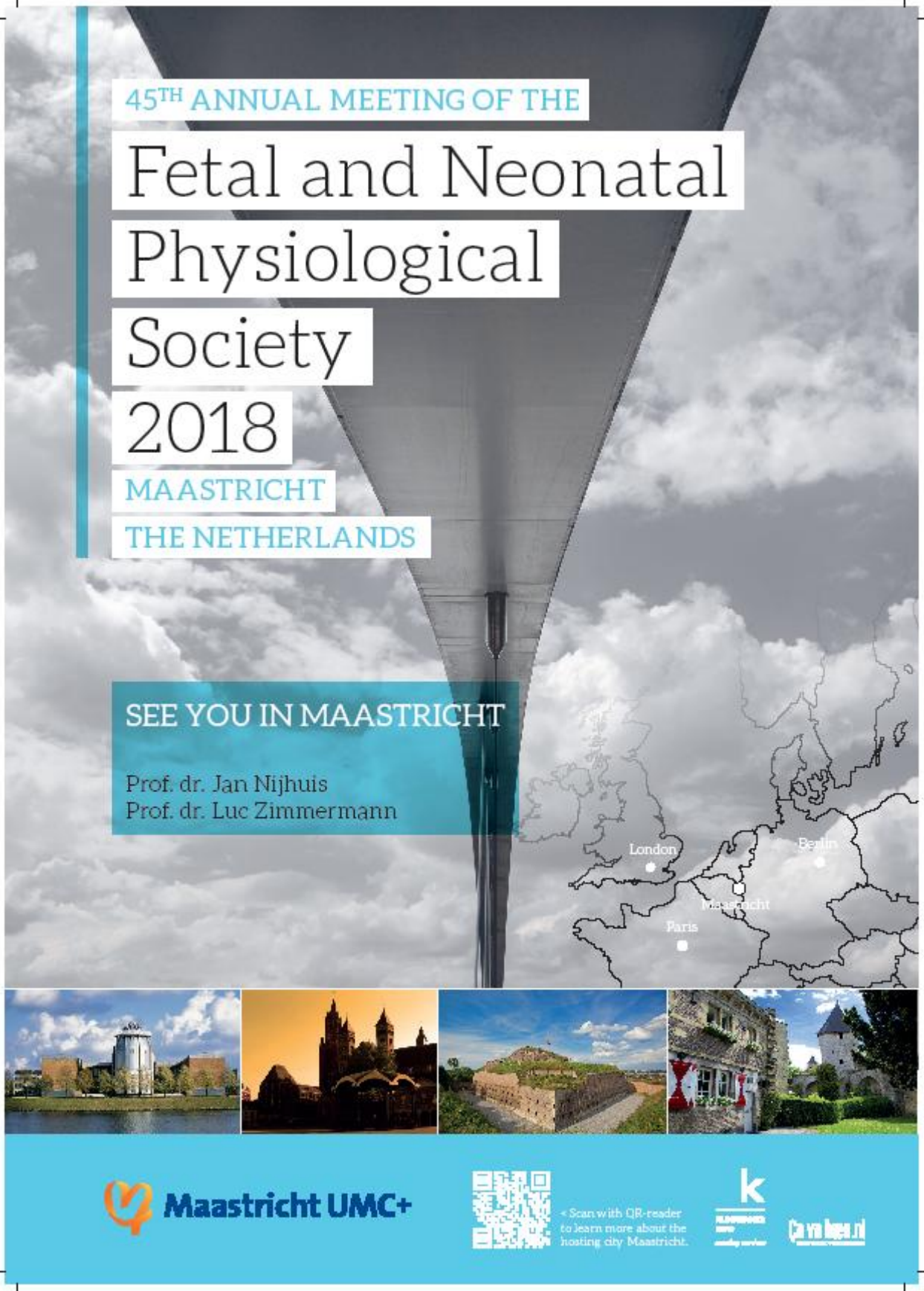
2017 Osaka, Japan. September 2-5.
2018 Maastricht, The Netherlands. Festschrift for Jan.
2019 New Zealand. September. Laura is investigating options.

8. **Prizes.**
FNPS 2015, Tania Gunn & Bo Gennser Memorial Prizes Nomination of eligibility for prizes should occur at time of abstract suggestion.

Respectfully submitted,

Tim Moss (minute taker) & Dr Emilio Herrera (FNPS Scribe)


44th Annual Meeting
Fetal and Neonatal
Physiological
Society
September 2 (Sat) ▶ 5 (Tue), 2017
Osaka, Japan
Venue
International House, Osaka
(Osaka Conference Center & Hotel)
Chairperson
Tomoaki Ikeda M.D., Ph.D.
Department of Obstetrics and Gynecology,
Mie University Graduate School of Medicine
Co-Chairperson
Tadashi Kimura M.D., Ph.D.
Department of Obstetrics and Gynecology,
Osaka University Graduate School of Medicine
OSAKA
TOKYO
KYOTO
Secretariat
FNPS2017 Secretariat
c/o Convention Linkage, Inc.
http://www.c-linkage.co.jp/fnps2017/


45TH ANNUAL MEETING OF THE
Fetal and Neonatal Physiological Society 2018
MAASTRICHT
THE NETHERLANDS
SEE YOU IN MAASTRICHT
Prof. dr. Jan Nijhuis
Prof. dr. Luc Zimmermann
London
Berlin
Paris
Maastricht UMC+
< Scan with QR-reader to learn more about the hosting city Maastricht.

Previous Meetings of the FNPS

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, UK
2007	Sendai, Japan
2008	Maastricht, The Netherlands
2009	Lake Arrowhead, USA
2010	Winchester, UK
2011	Palm Cove, Australia
2012	Utrecht, The Netherlands
2013	Puerto Varas, Chile
2014	St Vincent, Italy
2015	Vancouver, Canada

FNPS Cambridge Social Events

The following programme has been formulated to provide you with a real flavour of English cultures and customs and, particularly, a sample of traditional Cambridge life. We hope you participate fully and enjoy the variety of events.

Saturday 17th September
Welcome Reception and Dinner

6.30 – 7.30 pm: Welcome Champagne Reception
7.30 pm: Dinner
Pembroke College (Trumpington Street)
Dress code: Casual Smart

This reception party will give delegates an ideal opportunity to meet up with friends and colleagues prior to the start of the meeting.

Monday 19th September
Sporting Event

3.30 – 6.30 pm
The Granta Pub (Newnham Road, Cambridge)
Dress code: Very casual, weatherproofs advised

An afternoon of fun and frivolity, the highlight of which will be the Punting Event. Why not test your ability and take part? Teams will depart at intervals and be punting over a pre-determined course. There will also be a river trivia. The winning team will receive a prize. In the event of a tie break, the teams will be asked specific questions about the history of what they will have seen along the way.

If you prefer to stay on dry land, you will be able to watch the beginning and end of the action from the patio at the Granta Pub where the punts will set off and finish. Cash bar. Entertainment by ***Freddie & Friends New Orleans Jazzmen.***

FNPS Cambridge Social Events (continued)

Monday 19th September
BBQ and Céilidh
7.00 pm
Caius Harvey Court Gardens (West Road)
Dress code: Casual

When the teams return, their efforts will be rewarded as we meet up for the evening at this lovely venue within Gonville and Caius College Gardens. There will be a traditional hog roast and delicious vegetarian options. If you've always wanted to try traditional English ales, this is your chance! The cash bar will have a variety of speciality ales available as well as standard drinks.

Later in the evening 

will keep you entertained and we very much hope you will all participate in the dancing. Céilidh (pronounced 'Kaylee') is the traditional Gaelic social dance in Ireland and Scotland. Before discos and nightclubs, there were Céilidhs in most town and village halls on Friday or Saturday. The basic dance steps can soon be learned, particularly after sampling the speciality ales!!

Tuesday 20th September
Champagne Reception and Gala Banquet
7.15 – 8.00 pm: Champagne Reception
8.00 pm: Gala Banquet
Gonville and Caius College (Trinity Street)
Dress code: Lounge suit

This promises to be the social highlight of the meeting and is definitely an evening not to be missed. Experience all the grandeur and formality of a Cambridge college while savouring a superb 4-course feast and fine wines. Entertainment will be supplied by Cameo Opera (MUSIC FOR EVERY OCCASION; For details please contact Matthew Craven or Judith Buckle, Peete House, Teynham Street, Sittingbourne. ME9 9EX; email: MLCRAVEN@BTINTERNET.COM).

Activities for accompanying persons

A formal daytime programme for accompanying persons has not been organised because there is an overwhelming variety of available activities within Cambridge itself and its immediate surroundings. These include walking tours of the city, a 'Behind the Scenes' look at the Cambridge Arts Theatre, a Ghost Tour and an open topped bus which you can hop on and off as you wish. For details or further suggestions, please contact our registration desk. Please find tourist leaflets in your conference bags.

Notes for Speakers

Oral presentations

Keynote lectures and oral presentations will take place in the Main Lecture Theatre at the Physiological Laboratory, University of Cambridge, Downing Site.

All presentations will be given in Microsoft Powerpoint on a PC. Speakers are asked to submit their presentations on a memory stick to Dr Youguo Niu (known as 'Niu') in the Library Reading Room (C15) in the Physiology Department, at least one day before their presentation (Saturday: 16:00 – 18:00; Sunday: 10:30 – 11:00, 16:15 – 17:30; Monday: 10:30 – 11:00, 14:30 – 15:00).

Oral presentations will be 10 minutes in length followed by a 5 minute discussion. Due to the time schedule, speakers must ensure their presentation does not exceed this time limit.

There will be several prizes for the best oral and poster communications by PhD students and Post-Doctoral fellows, which will be presented at the Gonville & Caius Gala Banquet.

Poster presentations

Posters can be displayed throughout the meeting. However, there will be two 1.5 h formal poster sessions (Sunday 18th September 16.15 to 17.30 h and Tuesday 20th September 11.00 to 12.30 h). These will be held in the Experimental Classrooms H21 and G15.

Presenters are asked to ensure that their posters are put on display during the morning of Sunday 18th September.

All poster presenters should be in attendance during the specified poster session, as there will be formal assessment by senior members of the Society.

Speaker ready room

The Library Reading Room (C15) will be available with facilities for presenters to check their Powerpoint presentations.

General Information

Registration

Registration will take place at the Physiological Laboratory at the University of Cambridge, Downing Site from 14.00–18.00 h on Saturday 17th September. The Registration Desk will be located in Experimental Classroom H21. It will be manned throughout the meeting and should be the first port of call for delegates and accompanying persons requiring any assistance.

Internet access

Wireless internet access will be available, free of charge. You will be provided with an individual Ticket ID number and a password. This ticket is valid from 17-09-2016 to 20-09-2016. Connect to the 'UniOfCam' network ESSID and then open your web browser. Try to visit any http web site (such as http://www.cam.ac.uk/) and use your ticket when prompted.

For University Wireless Service help, please visit http://www.ucs.cam.ac.uk/wireless/ or for general IT support, visit http://www.ucs.cam.ac.uk/.

If you still have problems connecting after reading the aforementioned documentation, email network-support@ucs.cam.ac.uk to create a support query with the University Computing Service.

Trade exhibitors

Trade exhibitors will be located in Experimental Classroom H21. Please take the time to visit them as they have been generous sponsors of this meeting.

Refreshments

Morning and afternoon coffee/tea will be served in the Experimental Classroom H21. Lunch will be served in the Dining Hall of Emmanuel College.

Medical assistance

If medical assistance is required, please contact the registration desk or speak to a member of the Organising Committee.

FNPS Prizes

The FNPS takes great pride in encouraging young investigators to present their work and to reward them for their great effort. Traditionally, the FNPS awards 5 prizes. This year, to celebrate the work of our great colleague and friend, Bill Parer, there will be 6 prizes and two of them will be named in his memory.

- 2016 FNPS Dr. Julian T. (Bill) Parer Award for best oral presentation delivered by a PhD student
- 2016 FNPS Dr. Julian T. (Bill) Parer Award for best oral presentation delivered by a Post Doc
- 2016 Tania Gunn Prize for best oral presentation delivered by PhD student
- 2016 Tania Gunn Prize for best oral presentation delivered by a Post Doc
- 2016 Bo Gennser Prize for best poster presentation delivered by a PhD student
- 2016 FNPS Prize for best poster presentation delivered by a Post Doc

Programme Outline

Saturday Sept 17th

14:00 - 18:00	Book into accommodation	Harvey Court and Stephen Hawking Building (West Road)
14:00 - 18:00	Registration	Physiological Laboratory, Downing Site
18:30 - 19:30	Welcome Champagne Reception	Pembroke College (Trumpington Street)
19:30	Welcome Dinner	Pembroke College

Sunday Sept 18th

07:30 - 08:30	Breakfast	Harvey Court, West Road
08:45	Welcome to Conference	Main Physiology Lecture Theatre
09:00	**Oral Session I:** **Developmental Neuroscience A** Chair: Prof. John Challis	Main Physiology Lecture Theatre
09:00 - 09:30	Keynote Lecture 1: Prof. Stephen Matthews	
09:30 - 10:30	**4 talks**	
10:30 - 11:00	Refreshment break	Experimental Class Rooms
11:00	**Oral Session II:** **Developmental Neuroscience B** Chair: Prof. Peter Nathanielsz	Main Physiology Lecture Theatre
11:00 - 11:30	Keynote Lecture 2: Prof. Carina Mallard	
11:30 – 12:15	**3 talks**	
12:30 - 13:30	Lunch	Emmanuel College (St Andrew's Street)
13.30 - 14:00	**Commemoration Photograph**	Emmanuel College
14:15	**Oral Session III:** **Developmental Programming** Sponsored by DOHaD Chair: Prof. Laura Bennet	Main Physiology Lecture Theatre
14:15 - 14:45	Keynote Lecture 3: Dr Mary-Elizabeth Patti	
14:45 - 16:15	**6 talks**	
16:15 - 17:30	Refreshment break/ Poster Session I	Experimental Class Rooms
17:30	**Oral Session IV:** **Clinical Fetal and Neonatal Physiology** Chair: Prof. Bryan Richardson	Main Physiology Lecture Theatre
17:30 - 18:00	Keynote Lecture 4: Dr Graeme Polglase	
18:00 - 19:00	**4 talks**	
19:00 -19:15	Tribute Professor Julian T. (Bill) parer	Main Physiology Lecture Theatre
19:15	Free evening in Cambridge	

Programme Outline(continued)

Monday Sept 19th

08:00 - 08:45	Breakfast	Harvey Court, West Road
09:00	**Oral Session V:**	Main Physiology Lecture Theatre
	Developmental Cardiovascular Physiology A	
	Chair: Prof. Eugenie Lumbers	
09:00 - 09:30	Keynote Lecture 5:	
	Prof. Sandra Davidge	
09:30 - 10:30	**4 talks**	
10:30 - 11:00	Refreshment break	Experimental Class Rooms
11:00	**Oral Session VI:**	Main Physiology Lecture Theatre
	Developmental Cardiovascular Physiology B	
	Chair: Prof. Frank Bloomfield	
11:00 - 12:15	**5 talks**	
12:30 - 13:30	Lunch	Emmanuel College
13:45	**Oral Session VII**:	Main Physiology Lecture Theatre
	Developmental Hypoxia	
	Chair: Prof. Carlos Blanco	
13.45 - 14.30	**3 talks**	
14:30 - 15:00	Business Meeting	Main Physiology Lecture Theatre
15:30 - 18:30	Sporting Event	River Cam/The Granta Pub+Dixie Band
19:00 - Late	BBQ & Cèilidh	Caius Fellows Garden (West Road)

Tuesday Sept 20th

08:00 - 08:45	Breakfast	Harvey Court, West Road
09:00	**Oral Session VIII:**	Main Physiology Lecture Theatre
	Utero-Placental Studies A	
	Chair: Prof. Abby Fowden	
09:00 - 09:30	Keynote Lecture 6	
	Prof. Paola Casanello	
09:30 - 11:00	**6 talks**	
11:00 - 12:30	Refreshment break/	Experimental Class Rooms
	Poster Session II	
12:30 – 13:30	Lunch/Board Meeting 1	Emmanuel College
13:45	**Oral Session IX**	Main Physiology Lecture Theatre
	Utero-Placental Studies/Parturition Physiology B	
	Chair: Prof. Donald Peebles	
13:45 – 15:00	**5 talks**	
15:00 – 15:30	Refreshment break/Board Meeting 2	Experimental Class Rooms
15:30	**Oral Session X:**	Main Physiology Lecture Theatre
	Developmental Metabolism	
	Chair: Prof. Julie Owens	
15:30 – 16:00	Keynote Lecture 7:	
	Prof. Sue Ozanne	
16:00 - 17:00	**4 talks**	

Programme Outline(continued)

17:00 – 18:00	**Dawes Lecture** Chair: Prof. Dino Giussani Keynote Lecture: Dr Andrew Murray		Main Physiology Lecture Theatre
19:15 – 20:00	Champagne Reception		Gonville & Caius College, Trinity Steet
20:00 - Late	Gala Banquet with Entertainment		Gonville & Caius College, Trinity Steet 'Cameo Opera'

Programme

Programme

All lectures will be held in the Main Physiology Lecture Theatre.
Presenting authors are underlined.

Saturday 17th September

14:00 – 18:00	Book into accommodation Harvey Court and Stephen Hawking Building (access via Porter's Lodge on West Road; see map on back cover)
14:00 – 18:00	Registration Physiological Laboratory, Downing Site (see map on back cover)
18:30 – 19:30	Welcome Champagne Reception Pembroke College (access via Porter's Lodge on Trumpington Street; see map on back cover)
19:30	Welcome Dinner Pembroke College

Sunday 18th September

07:30 – 08:30	Breakfast Harvey Court (West Road)
08:45	Welcome to Conference by Professor Dino Giussani Main Physiology Lecture Theatre (Downing Site)

Oral Session I: Developmental Neuroscience A
Chair: Professor John Challis, Australia

09:00 – 09:30	**Keynote Lecture 1** **Stephen Matthews:** Glucocorticoids and programming of the fetal brain: multigenerational outcomes.
09:30– 09:45	Cho, H.T., Wassink, G., Mathai, S., Dhillon, S.K., van den Heuij, L.G., Davidson, J.O., Bennet, L., Gunn, A.J., & **Fraser, M**. The TLR7 agonist Gardiquimod protects oligodendrocytes from damage after asphyxia in the preterm fetal sheep.
09:45–10:00	**Hanita, T**., Matsuda, T., Usuda, H., Kitanishi, R., Saito, M., Watanabe, S., Kobayashi, Y. Magnetic resonance imaging is useful for detecting acute phase of cerebral white matter injury in preterm ovine foetus.

10:00–10:15 **Sortica da Costa,C.,** Placek, M.M., Czosnyka, M., Smielewski, P., Cabella, B, Kasprowicz, M., Austin, T. Complexity of Brain Signals is Associated with Outcome in Preterm Infants.

10:15–10:30 **Wassink, G**., Davidson, J.O., Fraser, M., Bennet, L., Gunn, A.J. Non-Additive Neuroprotection with Delayed Hypothermia and Recombinant Human Erythropoietin after Cerebral Ischemia in Near-Term Fetal Sheep.

10:30–11:00 Refreshment break
Experimental Class Rooms

Oral Session II: Developmental Neuroscience B
Chair: Professor Peter Nathanielsz, USA

11:00–11:30 **Keynote Lecture 2**
Carina Mallard: Effects of intrauterine growth restriction on brain development.

11:30–11:45 Nakamaura, S., Walker, D., **Wong F.Y.** Development of neurovascular coupling in the fetal sheep and newborn lamb

11:45–12:00 **Zarate, M. A.,** Chang, E., Rodriguez, M., Triplett, E. & Wood, C Identification of bacteria in brain cortex and placenta of fetuses exposed to hypoxic hypoxia (HH).

12:00–12:15 **Miller, S.L.,** McDonald, C., Bush, L., Thomson, S., Calalang, M., Sutherland, A.E., Jenkin, G., Castillo-Melendez, M. Neuroprotective effects of umbilical cord blood cells in fetal growth restriction.

12:30–13:30 Lunch
Emmanuel College

13:30–14:00 Commemoration Photograph
Emmanuel College

Oral Session III: Developmental Programming
Chair: Professor Laura Bennet, New Zealand
Sponsored by DOHaD

14:15–14:45 **Keynote Lecture 3**
Mary-Elizabeth Patti: Intergenerational impact of paternal metabolism on metabolic disease risk

14:45–15:00 Ibáñez-Chávez, C.A.,Vazquez, M., Reyes-Castro, L.A., Vega-García, C.C., Bautista-Carbajal, C.,Gerow K., Nathanielsz, P.W.,**Zambrano, E.** Fat cell size distribution in male offspring (F1) of obese mothers: effects of maternal and offspring exercise (Ex) intervention.

15:00–15:15 **Jones, L. E.,** Newland, P.L., Cagampang, F.R., Poore, K.R., Cleal, J.K., Green, L.R. The effect of a maternal vitamin D depleted diet during gestation on the behaviour and activity of young adult mouse offspring.

15:15–15:30 Herrera, E.A., Cifuentes-Zúñiga, F., Figueroa, E., Villanueva, C., Hernández, C., Alegría, R., Arroyo, V., Farías, M., Uauy, R., Casanello, P., **Krause, B.J.** N-acetyl cysteine, a glutathione precursor reverses vascular dysfunction and endothelial epigenetic programming in IUGR guinea pigs.

15:30–15:45 **Skeffington, K.L*.,** Botting, K.J*., Niu, Y., Allison, B.J., Brain, K.L., Itani, N., Beck, C., Logan, A., Murray, A.J., Murphy, M.P., & Giussani, D.A. **Co-first authors*. The mitochondria-targeted antioxidant MitoQ prevents the programming of cardiovascular dysfunction by developmental hypoxia in sheep.

15:45–16:00 Maki, Y., Elias, A.A., Matushewski, B., Nygard, K., Regnault, T.R.H., **Richardson, B.S.** Maternal nutrient restriction (MNR) in guinea pigs leads to fetal growth restriction (FGR) with sex-related increases in tissue hypoxia.

16:00–16:15 **Franke, K.,** Gaser, Ch., de Rooij, S.R., Schwab, M. & Roseboom, T.J. In-vivo evidence for premature brain aging in old men prenatally exposed to the Dutch Famine.

16:15–17:30 Refreshment break / Poster Session I
The Main Physiology Lecture Theatre / Experimental Class Rooms

Oral Session IV: Clinical Fetal and Neonatal Physiology
Chair: Professor Bryan Richardson, Canada

17:30–18:00 **Keynote Lecture 4**
Graeme Polglase: Preterm lung injury in the delivery room: implications for multi-organ injury

18:00–18:15 **Shaw, C.J.**, Rivens, I., Civale, J., Botting, K.J., Niu, Y., ter Haar, G., Giussani, D.A., Lees, C.C. High Intensity Focused Ultrasound (HIFU): A method of non-invasive placental vascular occlusion.

18:15–18:30	**Kubo, M**., Nii, M., Maeda, Y., Shimura, M., Magawa, S., Kawamura, T., Tanaka, H., Murabayashi, N., Osato, K., Umekawa, T., Kamimoto, Y. and Ikeda, T. A case-control study on effect of a novel treatment administering tadalafil for fetal growth restriction.
18:30–18:45	**Antolic, A.,** Wood, C.E. & Keller-Wood, M. Fetal ECG and Heart Rate are Altered in the Perinatal Period Following Exposure to Chronic Maternal Hypercortisolemia.
18:45–19:00	**Horne, R.S.C.,** Fung, A., McNeil, S., Fyfe, K.L., Odoi, S., and Wong, F.Y. The longitudinal effects of persistent apnoea during sleep on cerebral oxygenation in ex-preterm infants.
19:00–19:15	Tribute to Professor Julian T. (Bill) Parer Main Physiology Lecture Theatre
19:15	Free evening in Cambridge

Monday 19th September

08:00 – 08:45	Breakfast Harvey Court, West Road

Oral Session V: Developmental Cardiovascular Physiology A
Chair: Professor Eugenie Lumbers, Australia

09:00 – 09:30	**Keynote Lecture 5** **Sandra Davidge:** Pregnancy complications and programming of cardiovascular dysfunction in adult offspring.
09:30– 09:45	**Astorga, C.R.**, Gonzalez-Candia, A., Candia, A., Figueroa, E.G., Villanueva, C.A., Ebensperger, G., Reyes, R. V., Llanos, A.J., Herrera, E.A. Vascular morphological and anti-proliferative changes induced by melatonin treatment in chronic hypoxic newborn lamb with pulmonary hypertension.
09:45–10:00	Vaughan,O.R., Rossi, C.A., Ginsberg,Y., Krishnan,T.I., Barker,H., White, A., and **David, A.L.**

10:00–10:15 **Ashley, B. ,** Hussain, S., Thomas, H. N. B., van Rijn, B. B., Cagampang, F. R., Cleal, J. K**.** Maternal obesity during pregnancy alters microRNAs that regulate circadian clock genes in the fetal mouse heart.

10:15–10:30 Darby, J.R.**, Berry, M.,** Dyson, R., Gray, C. and Morrison, J.L. Preterm birth coupled with antenatal glucocorticoid treatment increases cardiac MR and 11β-HSD1 in adult life.

10:30–11:00 Refreshment break
Experimental Class Rooms

Oral Session VI: Developmental Cardiovascular Physiology B
Chair: Professor Frank Bloomfield, New Zealand

11:00–11:15 **William (Bill) Pearce** and Thorpe, R. B. In fetal cerebral arteries, chronic hypoxia attenuates NO- and cGMP-induced vasorelaxation by inhibiting PKG colocalization with BK channel proteins.

11:15–11:30 **Gardner, D.S.,** Karamitri, A., Kwong, W.Y., Emes, R.E., and Sinclai,r K.D. Cardiovascular and metabolic health of aged adult offspring derived from mothers deficient in B-vitamins during the periconceptional period.

11.30–11:45 **Darby, J. R.,** Regnault, T.R.H., and Morrison, J.L.Sex dependant cardiac effects of a postnatal Western diet: exacerbated by low birth weight?

11:45–12:00 **Vrselja, A.,** Ahmadi-Noorbakhsh, A., Noble, P. B., Pillow, J. J., and Black, M. J. The effect of intrauterine inflammation on left ventricular cardiomyocyte growth and maturation in preterm lambs.

12:00–12:15 **Lear, C.A.,** Galinsky, R., Wassink, G., Yamaguchi, K., Davidson, J.O., Westgate, J.A., Bennet, L., Gunn, A.J. The myths and physiology surrounding intrapartum decelerations – the critical role of the peripheral chemoreflex.

12:30–13:30 Lunch
Emmanuel College

Oral Session VII: Developmental Hypoxia
Chair: Professor Carlos Blanco, Ireland

13:45–14:00 **Galli, G. L. J.**, Crossley, J., Elsey, R., Dzialowski, E., Shiels, H. A., and Crossley, D. A. Developmental Programming of Mitochondrial Function by Hypoxia; A Cold-Blooded Perspective.

14:00–14:15	**Aiken, C.E.,** Tarry-Adkins, J.L., Spiroski, A.M., Nuzzo, A.M., Giussani, D.A., and Ozanne, S.E. Gestational hypoxia induces NOX2-mediated oxidative stress and accelerated ageing in the developing ovary.
14:15–14:30	**Stark, M.J.,** Hodyl, N.A., Andersen, C.C. Compensatory alterations in fetal fractional oxygen extraction are dependant upon both gestational age and birth weight centile.
14:30–15:00	Business Meeting The Main Physiology Lecture Theatre
15:00–15:30	Free time
15:30–18:30	Sporting Event River Cam / The Granta Pub + Dixie Band (Newnham Road)
19:00–late	BBQ & Cèilidh Caius Fellows Garden (West Road)

Tuesday 20th September

08:00 – 08:45	Breakfast Harvey Court, West Road

Oral Session VIII: Utero-Placenta Studies A
Chair: Professor Abby Fowden, UK

09:00 – 09:30	**Keynote Lecture 6** **Paola Casanello**: Epigenetic programming of vascular function by fetal growth trajectory: the placenta as the black box.
09:30– 09:45	**Lumbers, E.R.,** Wang, Y., Delforce, S.J., Morris, B.J., Marques, F.Z., Broughton-Pipkin F., Roberts C.T., Pringle K.G. Factors regulating the expression of the prorenin receptor-prorenin angiotensin pathway.
09:45–10:00	**Constancia, M.,** Fowden, A., Burton, G., Georgopoulou, A., Sferruzzi-Perri, A. & Sandovici, I. IGF2 is essential for the expansion of the fetoplacental vasculature during late gestation development.

10:00–10:15 **Kalisch-Smith, J.I.,** Simmons, D.G., Pantaleon, M., Moritz, K.M. Periconceptional alcohol exposure reduces trophoblast giant cell differentiation and outgrowth capacity in the rat.

10:15–10:30 **Nuzzo, A.M.,** Barrile, R., Mele, P., Eva, C. E., Todros, T. Rolfo, A. Therapeutic Effect of Human Placental-Derived Mesenchymal Stromal Cells on a Lipopolysaccharide Induced Mouse Model of Preeclampsia.

10:30–10:45 Simner, C.L., Lewis, R.M., Cooper, C., Harvey, N.C., and **Cleal, J.K.** Placental uptake and metabolism of vitamin D.

10:45–11:00 **Sferruzzi-Perri, A.N**., López-Tello, J., Fowden, A.L., Constancia, M. Maternal and fetal genomes interplay through phosphoinositol 3-kinase (pi3k)-p110α signalling to modify placental resource allocation to fetal growth.

11:00 –12:30 Refreshment break / Poster Session II
Experimental Class Rooms

12:30–13:30 Lunch/Board Meeting 1
Emmanuel College

Oral Session IX: Utero-Placenta/ Partuirtional Physiology B
Chair: Professor Donald Peebles, UK

13:45–14:00 **Georgieva, A.,** Ugwumadu, A., Papageorghiou, A., and Redman, C.W.G. Significance of the first hour of the fetal heart rate monitoring during labour: nonreactive vs. reactive initial trace.

14:00–14:15 Smith, A.M., Ghnenis, A.B., Odhiambo, J.F., **Nathanielsz, P.W.,** Ford, S.P. A heretical view: Placental 11β-HSD2 may not perform a placental protective function but rather act to provide substrate for fetal peripheral cortisol synthesis.

14:15–14:30 Partap, U., Sovio, U., **Smith, G.C.S.**. Fetal Growth and the Risk of Spontaneous Preterm Birth (sPTB) in a Prospective Cohort Study of Nulliparous Women.

14:30–14:45 **Acharya, G.,** Widnes, C., Wilsgaard, T., and Odibo, A.O. Sexual dimorphism in feto-placental circulation during the second half of pregnancy: a longitudinal study.

14:45–15:00 **Parkington, H. C.,** Tonta, M. A., Stevenson, J., Goundar, C., Sheehan, P. M., Tare, M., Coleman, H. A., and Brennecke, S. P. Changes in myometrial potassium channel function and expression in dysfunctional human labour.

15:00–15:30 Refreshment break/Board Meeting 2
Experimental Class Rooms

Oral Session X: Developmental Metabolism
Chair: Professor Julie Owens, Australia

15:30–16:00 **Keynote Lecture 7**
Sue Ozanne: Eating for two during pregnancy-Programming by maternal diet-induced obesity.

16:00–16:15 **Lomas-Soria, C.**, Bautista, C.J., Reyes-Castro, L.A., Vega, C., Rodríguez-González, G.L., Cox, L., Nathanielsz, P.W. and Zambrano E. Maternal obesity (MO) programs dysfunction of the oxidative phosphorylation pathway producing oxidative stress (OS) and leads to hepatic steatosis in offspring (F1) male rats exacerbated by aging.

16:15–16:30 **Dickinson, H.,** Ellery, S.J., Della Gatta P., Lappas, M., Murthi, P., Snow, R.J. and Walker, D. Creatine metabolism in human pregnancy.

16:30–16:45 **Harris, S.E.,** De Blasio, M. J., Wooding, F.B.P., Blache, D., Meredith, D., Fowden, A.L. and Forhead, A. J. Hypothyroidism induces hyperplasia of unilocular adipocytes in perirenal adipose tissue of the ovine fetus.

16:45–17:00 **Wesolowski, S.R.,** Brown, L.D., Rozance, P.J., Wilkening, R.B. & Hay, W.W. Decreased substrate oxidation and increased lactate parallel hepatic glucose production in fetal sheep with intrauterine growth restriction.

Dawes Lecture
Chair: Professor Dino Giussani, United Kingdom

17:00–18:00 **Dawes Lecture**
Murray, A.J.
Life at the limit– Studies of human energy metabolism at extreme high altitude.

18:00–19:15 Free time

19:15–20:00 Champagne Reception
Gonville & Caius College (Trinity Street)

20:00–Late Gala Banquet with Entertainment (Cameo Opera)
Gonville & Caius College (Trinity Street)

Posters

Posters

Posters will be displayed in the Experimental Classroom H21 and G15 on two Poster Sessions. Presenting authors are underlined.

Poster Session I
Sunday 18th September (16:15–17:30)

Developmental Neuroscience(1)

Poster 1 A novel non-invasive MRI biomarker revealing premature brain aging in the young adult female baboon resulting from developmental programming
Franke, K., Clarke, G.D., Dahnke, R., Gaser, Ch., Li, C., Schwab, M., Nathanielsz, P.W.

Poster 2 Antenatal maternal glucocorticoid treatment produces premature brain aging in the male middle-aged baboon offspring as revealed by *in-vivo* MRI
Franke, K., Clarke, G.D., Dahnke, R., Gaser, Ch., Li, C., Schwab, M., Nathanielsz, P.W.

Poster 3 Sensorimotor gating deficits following pre and postnatal stress in guinea pig offspring
McInerney, K.M., Palliser, H.K., Shaw, J.C. and **Hirst, J.J.**

Poster 4 Differential expression of neurogenesis mediators by physiological and preeclamptic placenta-derived mesenchymal stromal cells
Barrile, R., Nuzzo, A.M., Leonardi, R., Mele, P., Eva, C., Todros T., Rolfo, A.

Poster 5 The Role of Neonatal Dexamethasone Exposure on Adult Psychiatric Phenotypes in a Rodent Model
Yates, N. J., Martin-Iverson, M. T., Robertson, D., and Rodger, J.

Poster 6 Creatine Protects the Fetal Brain from the Effects of Severe *In Utero* Hypoxia
Dickinson,H., Rajakaruna, S., Ellery, S., Muccini, A., Hale, N., Snow, R., Walker, D.

Developmental Programming

Poster 7 The effect of maternal obesity in mice on pup anxiety and mother-pup interactions in the first week of life
Rasool, A., Green, L.R., Teeling, J.L., Cagampang, F.R., Poore, K.R.

Poster 8 Prenatal high fat diet exposure in mice primes offspring for increased neuroinflammation and altered hypothalamic stress markers
Rasool,A., Green, L.R., Teeling, J.L., Cagampang, F.R., **Poore, K.R.**

Poster 9 Maternal protein restriction (MPR) during pregnancy accelerates aging of sperm quality in male rat offspring (OFF)
Rodríguez-González, G.L., Reyes-Castro, L.A., Vega, C.C., Boeck L., Ibáñez, C., Nathanielsz, P.W., Larrea, F. and Zambrano, E.

Poster 10 Maternal obesity (MO) during pregnancy and lactation increases oxidative stress is the neonatal rat testis
Rodríguez-González, G.L., Nava, B.M., Reyes-Castro, L.A., Lomas-Soria, C, Nathanielsz, P.W., Larrea, F. and Zambrano, E.

Poster 11 Maternal obesity (MO) up-regulates the protein associated with stress in the late gestation baboon fetal frontal cortex
Yang, S., Li, J., Nathanielsz, P.W., and **Li, C.**

Poster 12 Intrauterine Growth Restriction Produces Accelerated Cardiac Aging In Male And Female Adult Baboons
Kuo A.H., Li C., Li J. , Huber H. , **Nathanielsz P.W.** , and Clarke G.D.

Clinical Fetal and Neonatal Physiology

Poster 13 Retinal microvascular plasticity in a premature neonate
Kandasamy ,Y.,Hartley, L.,Smith ,R., Wright ,I.M.

Poster 14 Perinatal prognosis using of the 5-tier system of assessing fetal heart rate tracing
Murabayashi, N., Kubo, M., Nii, M., Maeda, Y., Shimura, M., Magawa, S., Kawamura, T., Tanaka, K., Tanaka, H., Osato, K., Umekawa, T., Kamimoto, Y., Parer, J., and Ikeda, T.

Poster 15 Magnesium sulfate reduced fetal ventricular tachycardia and Torsa de Pointes in congenital long QT syndrome
Maeda, Y., Kawamura, T., Ono, R., Suzuki, R., Kawabata, I., Yoshida, A., Katsuragi, S., Ikeda, T.

Poster 16 A data-driven system for continuous fetal monitoring in labour: the Oxford prototype
Georgieva, A., Papageorghiou, A., and Redman, C.W.G.

Poster 17 The three cases of urinary tract obstruction with bladder rapture
Magawa, S., kamimoto, Y., Tanaka, H., Murabayashi, N., and Ikeda, T.

Poster 18 Fetal behavioural state in late gestation is affected by maternal sleep position
Stone, P.R., Thompson, J.M.D., Stewart, A.W., McIntyre, J., Burgess, W., Gunn, A.J., Lear, C., Bennet, L., Mitchell, E.A.

Poster 19 Renal function in the first month of life in Australian Indigenous and non-Indigenous preterm neonates
Black, M. J., Davison, B., Chatfield, M., Ryan, D., Sutherland M.R., Diwakarla, S., Hoy, W.E., Singh, G.

Developmental Cardiovascular Physiology

Poster 20 The effect of moderate preterm birth on the structure and growth of cardiomyocytes in the right ventricle of adult sheep
Black, M. J., Mrocki, M., Nguyen, V., Bensley, J., Polglase, G.R.

Poster 21 miR-133a and miR-15 family target gene expression in the fetus and 6 month_old sheep heart in response to myocardial infarction
Lock, M.C., Soo, J.Y., Darby, J.R, Brooks, D., Porrello, E.,Tellam, R., **Morrison, J.L.**

Poster 22 The redistribution of cardiac output by vasopressin infusion in the premature fetal sheep
Watanabe, S., Matsuda, T., Usuda, H., Kitanishi, R., Saito, M., Hanita, T. and Kobayashi, Y.

Poster 23 The effect of cinaciguat (bay-582667) on the cardiopulmonary circulation in hypoxic neonatal lambs at high-altitude
Beñaldo, F.A., Araya-Quijada, C., Ebensperger, G., Guzmán-Silva, C.P., Castillo-Galan, S., Serón-Ferré, M., **Herrera, E.A**., Reyes, R.V., Moraga, F., Parer, B., Llanos, A.J.

Poster 24 Antenatal melatonin modulates cellular pro-oxidant sources in newborn lambs with pulmonary hypertension
Gonzalez-Candia, A., Castillo, R.L., Carrasco-Pozo, C., Ebensperger, G., Reyes, R.V., Llanos, A.J, **Herrera, E.A.**

Poster 25 Can fetal heart rate variability identify the phases of injury after asphyxia in preterm fetal sheep?
Yamaguchi, K., Lear, C.A., Gunn, A.J., Ikeda, T. and Bennet, L.

Poster Session II
Tuesday 20th September (11:00–12:30)

Developmental Neuroscience(2)

Poster 26 Umbilical cord blood derived mesenchymal stem/stromal cells protect against preterm white matter brain injury following hypoxia-ischemia
Li, J., Yawno, T., Sutherland, A., Gurung, S., Paton, M., McDonald, C., Tiwari, A., Jain, K., Miller,S.L., and **Jenkin, G.**

Poster 27

Krishnan, T., Vaughan, O.R., Hristova, M., Rossi, C.A., and David, A.L.

Poster 28 Effects of maternal obesity on cognitive function in the adult offspring in the rat
Coleman, H. A., Abdulwahid, A. A. & Parkington, H. C.

Poster 29 Maternal protein restriction around conception alters the foetal mouse brain by increasing neuronal differentiation during gestation, and is associated with adult memory deficits
Gould, J. M., Pearson-Farr, J., Smith, P.J., Airey, C.J., Airey, L., Fleming, T.P., Willaime-Morawek, S.

Poster 30 Impact of hypercapnia on neurovascular coupling in the fetal sheep and newborn lamb
Nakamaura, S., Walker, D., **Wong, F.Y.**

Poster 31 Development of mitochondrial function in the cortex and cerebellum of the ovine fetus: role of thyroid hormones
Davies, K. L., Forhead, A. J., Murray, A. J., Fowden, A. L., **Camm, E. J.**

Developmental Hypoxia

Poster 32 Chronic Fetal Hypoxia and Programming of Cardiovascular Dysfunction:
The role of Xanthine Oxidase
Beck, C., Skeffington, K.L., Itani, N., Botting, K.L., Giussani, D.A.

Poster 33 Intergenerational transmission of protection against heart disease via the maternal mitochondria
Niu, Y., Kane, A.D., Allen, S., Ashmore, T., Camm, E.J., Murray, A.J., Ozanne, S.E., Ferguson-Smith, A.C., Giussani, D.A.

Poster 34 Mitochondrial-Derived Oxidative Stress and the Developmental Programming of Cardiac Dysfunction – Studies in the Chicken
Skeffington, K.L., Beck, C., Itani, N., Niu, Y., Shaw, C.J., Murphy, M.P. and Giussani, D.A.

Poster 35 Developmental programming of pulmonary hypertension by chronic prenatal hypoxia
Spiroski, A.M., Shaw, C.J., Camm, E.J., Ashmore, T.J., Sutherland, M.R., Nuzzo, A.M., Eastwell, E.R., and Giussani, D.A.

Posters

Utero-Placenta Studies

Poster 36 Treatment Administering Tadalafil for Severe Preeclampsia with Fetal Growth Restriction
Tanaka, H., Kubo, M., Nii, M., Maeda, Y., Shimura, M., Magawa, S., Kawamura, T., Tanaka, K., Murabayashi, N., Osato, K., Umekawa, T., Kamimoto, Y., and Ikeda, T.

Poster 37 The mouse dam fails to metabolically adapt to the pregnant state in response to a deficiency of Igf2 inplacental endocrine cells
López-Tello, J. & **Sferruzzi-Perri, A.N.**

Poster 38 Labour-associated proinflammatory genes in the amnion are marked by bivalent epigenetic histone modifications
Zakar, T. Mitchell, C. M., Hirst, J. J., Mitchell, M. M., & Murray, H.

Poster 39 Enlarged uterus in amniotic fluid embolism and C1 esterase inhibitor
Kawamura, T., Katsuragi, S., Hiroaki, T., Hirata, T., Yamawaki, T., Maeda, Y., Ikeda, T.

Poster 40

Dauzat, C., Bansard,C., Nguyen, A., Bentz,S., Spézia, F., Forster, R., Lees, M.**, David, A.L.**

Developmental Metabolism

Poster 41 Maternal fructose-sweetened beverage intake renders offspring physiologically-sensitive to further fructose intake
Gray, C., Coppi, A.A., Lobo-Ladd ,A.A.B., Lobo-Ladd, F.V., Gardiner, S.M. and **Gardner D.S.**

Poster 42 Maternal low protein intake early in gestation specifically impacts hepatic glucose metabolism in adult offspring of sheep
Gardner, D.S., Karamitri, A., Rhodes, P., Glaab, E., Rhind, S.M. and Dunford, L.J.

Poster 43 Perinatal changes in mitochondrial function in ovine fetal skeletal muscle
Davies, K.L., Camm, E.J., Forhead, A.J., Murray, A.J. and Fowden, A.L.

Poster 44 Lipid accumulation in the primate fetal liver with maternal obesity may be regulated by novel epigenetic mechanisms
Puppala, S., Li, C., Glenn, J.P., Quinn, A., Palarczyk, J., Dick, Jr. E.J., Nathanielsz, P.W., **Cox L.A.**

Posters

Poster 45 Similarities and differences in outcomes in the term fetal baboon pancreas to challenges of maternal under nutrition and obesity
Li, J., Tursun, A., Nathanielsz, P.W., and **Li, C.**

Poster 46 Maternal nutrient restriction (MNR) or excess (MNE) increases oxidative stress in multiple fetal baboon tissues at 0.9G: do all roads lead through Rome?
Li**,** J., Yang, S., Tursun, A., **Nathanielsz**, P.W., and Li, C.

Poster 47 Offspring (F1) sex differences in postnatal growth characteristics in a Nonhuman Primate Model of Programming by Maternal Obesity (MO)
Jenkins, S.L., Li, J., Li, C., the late McDonald, T.J., Huber, H.F., Cox, L.A., and **Nathanielsz, P.W.**

Poster 48 Liver transcriptome outcomes differ in adult male and female rat offspring exposed to maternal obesity (MO) in pregnancy and lactation
Lomas-Soria, C., Ibañez-Chávez, C.A., Reyes-Castro, L.A., Vega, C., Bautista, C.J., Rodríguez-González, G.L., Cox, L., Nathanielsz, P.W., and Zambrano, E.

Poster 49 Impaired post-partum weight loss associated with inflammation and lipid peroxidation in rats exposed to low-protein diet *in utero*
Aiken C.E., Tarry-Adkins, J.L. and Ozanne, S.E.

Poster 50 Differential effect of lower vitamin D status on fetal hind limb skeletal muscle and bone structure in sheep
Jones, L.E., Al-Rawi, S., Lanham, S.A., Hanson, M.A., Cooper, C., Harvey, N.C., Calder, P.C., Fraser, W.D., Poore, K.R., Oreffo, R.O.C., **Green, L.R.**

Poster 51 Effect of preterm birth on mRNA expression of drug transporters in guinea pig liver
Soo, J., **Berry, M.,** Dyson, R., Gray, C. and Morrison, J.L.

Poster 52 Earthquake and perinatal medicine in Japan-Disaster liaison in paediatrics and perinatal medicine (DLPPM)
Unno, N., Tsuruwa, M., Itoh, T., Tsuda, N., Nishigaya, Y., Hattori, K., Sugawara, J., Suzuki, M., Nakai, A., Wada, K., Nakamura, T. and Masuzaki, H.

Oral Session I

Developmental Neuroscience A

Keynote Speaker: Stephen Matthews
Chair: John Challis

Keynote Speaker and Session Chair

Professor Stephen G. Matthews

Stephen G. Matthews is Professor of Physiology, Obstetrics and Gynecology and Medicine at the University of Toronto. He is the Director of Research at the Fraser Mustard Institute for Human Development. Professor Matthews received his PhD from the University of Cambridge, UK. He was appointed to the University of Toronto in 1996, 1996, becoming Full Professor in 2003. He served as Chair of the Department of Physiology 2007-2014. Professor Matthews' research is focused towards understanding how alterations in the fetal environment affect developmental trajectories trajectories leading to permanent modification of neurologic and endocrine function. His His recent research has established that the effects of such environmental manipulation manipulation can extend across multiple generations. With a focus on epigenetics, his research team is determining the molecular mechanisms by which such 'programming' can occur. In a parallel program of study, his group is investigating drug and hormone transport mechanisms in the placenta and fetal brain, with a focus on identifying the impact of infection (including Zika and malaria) and on developing novel treatments that modulate drug transport at these two sites. Professor Matthews is committed to translating fundamental research. He was founding co-director of of the MAVAN program, which follows neurocognitive development in children following adverse early experience, and and has been involved in a number of large international clinical trials. He has published over 195 scientific papers and and chapters, and his research has won a number of international prizes including the Mortyn Jones Memorial Medal (2006), the SGI President's Achievement Award (2012) and the 2014 Cannell Lectureship (APOG). Professor Matthews is regularly invited to present his work around the world and is involved in a number of international research research initiatives.

Website: http://www.physiology.utoronto.ca/content/stephen-matthews

Professor John RG Challis

Professor John Challis is currently Executive Director of the Western Australian Health Translation Network. John was previously the Pro Vice-Chancellor for Health and Medical Research at the University of Western Australia and also holds the title of of University Professor Emeritus at the University of Toronto, and Adjunct Professor at the University of British Columbia and at Simon Fraser University.

John completed his training at the Universities of Cambridge, University of California, California, San Diego and Harvard Medical School and held a Junior Research Fellowship at Wolfson College, University of Oxford, before moving to McGill University, Montreal, Canada. More recently he served as Chair of the Department Department of Physiology at the University of Toronto, and later as Vice President Research and Associate Provost of that University. He was the inaugural Scientific Scientific Director of the Canadian Institutes for Health Research, Institute of Human Development, Child and Youth Health and served as President and CEO of the Michael Smith Foundation for Health Research in Vancouver BC.

He has published more than 500 peer review papers and chapters in his research areas of pregnancy, preterm birth and developmental aspects of health and disease and trained more than 100 graduate students and postdoctoral fellows, and held in excess of $25 million peer reviewed research funding.

Abstract

1

Glucocorticoids and programming of the fetal brain: multigenerational outcomes

Matthews, S.G.

Departments of Physiology, Ob-Gyn and Medicine, Fraser Mustard Institute for Human Development, University of Toronto

The developing fetal brain is responsive to endocrine, nutritional and chemical cues. Glucocorticoids (GC) are critical for normal brain development and are tightly regulated at low levels for most of pregnancy in the fetus. Levels increase rapidly near term; a surge that is critical for lung development and maturation of other organ systems including the brain. We have shown that there are profound epigenetic changes in the developing hippocampus at the time of the GC surge. Fetal GC levels can be elevated earlier in gestation as a result of maternal or fetal stress. In addition, a substantial number of human fetuses are exposed to high levels of synthetic glucocorticoid (sGC), in cases of threatened preterm birth. Fetal overexposure to GC can result in life-long changes in the regulation of hypothalamic-pituitary-adrenal (HPA) function and behaviours in the offspring of several species. In animal models, these programming effects are associated with profound modification of the hippocampal and hypothalamic transcriptomes and epigenomes. Further, prenatal exposure to GC can have transgenerational effects on growth, HPA function and behaviours. Importantly, effects of GC on endocrine function and behaviour appear to be paternally transmitted.

Abstract

2

The TLR7 agonist Gardiquimod protects oligodendrocytes from damage after asphyxia in the preterm fetal sheep

Cho, H.T., Wassink, G., Mathai, S., Dhillon, S.K., van den Heuij, L.G., Davidson, J.O., Bennet, L., Gunn, A.J., and **Fraser, M.**

Department of Physiology, University of Auckland, Auckland, New Zealand

Introduction: Toll-like receptors (TLRs), key regulators of innate immunity, are involved in brain injury both after infectious and non-infectious insults. While TLR activation, in particular that of TLR4, appears detrimental to the immature brain, our recent studies have identified a candidate TLR pathway for protection involving upregulation of cerebral TLR7 (1). In the present study, we examined the potential of the TLR7 agonist, Gardiquimod (GDQ) to ameliorate cell loss after acute profound asphyxia in preterm fetal sheep.
Methods: Fetal sheep at 0.7 gestation (days 103-104; term ~ 145 days) received a continuous intracerebroventricular (icv) infusion of GDQ (1.11 mg/hour; GDQ-occlusion, n = 5) or vehicle (vehicle-occlusion, n = 9; vehicle sham-occlusion, n = 9) at a rate of 11.1µl/minute for 3 hours commencing 60 minutes after the end of a 25 minute umbilical cord occlusion (UCO). After 3 days recovery in utero, sheep were killed.
Results: In the periventricular and intragyral white matter, GDQ improved survival of immature and mature oligodendrocytes (CNPase) and total oligodendrocytes (Oligo-2) and reduced apoptosis (cleaved Caspase-3) and astroglial activation (GFAP) ($P < 0.05$). In regions of the grey matter, GDQ improved neuronal survival ($P < 0.05$) in the caudate nucleus, but not the putamen or the CA1/2, CA3, CA4, DG regions of the hippocampus and the thalamic nucleus.
Conclusions: In conclusion, our studies provide the first evidence that in the preterm fetal sheep therapeutic manipulation through TLR7 signalling can reduce white matter damage, offering the potential to preserve myelination in a physiological manner. Further studies, are needed to identify TLR7 signalling downstream mechanisms of protection and to assess the impact of GDQ on the development of the normal and injured preterm brain.

References:

Dhillon SK, Gunn AJ, Jung Y, Mathai S, Bennet L, Fraser M 2015 Developmental Neuroscience 37(6):497-514.

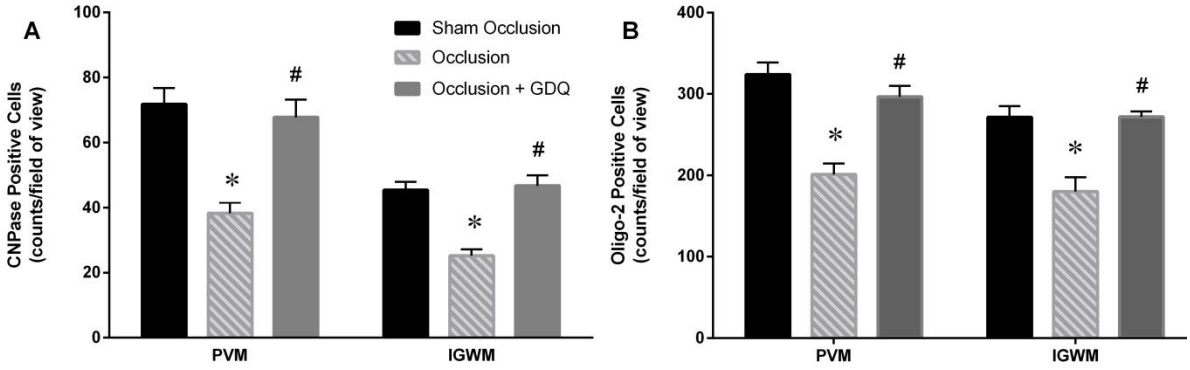


Figure 1. The effect of ICV administration of GDQ on the number of (A) CNPase positive (immature and mature) and (B) Oligo-2 positive (total) oligodendrocyte cells in the periventricular (PVWM) and intragyral white matter (IGWM) after 72 h of recovery from 25 min of umbilical occlusion. Cell numbers are mean ± SEM.∗ $p< 0.05$: vs. sham occlusion. # $p<0.05$: vs. occlusion.

Abstract

3

Magnetic resonance imaging is useful for detecting acute phase of cerebral white matter injury in preterm ovine foetus

Hanita, T.[1], Matsuda, T.[1], Usuda, H.[1], Kitanishi, R.[1], Saito, M.[1], Watanabe, S.[1], Kobayashi, Y.[2]

1 Center for Perinatal and Neonatal Medicine, Tohoku University Hospital, Sendai, Miyagi 2 Department of Veterinary Pathology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan

Introduction: Cerebral white matter injury (WMI) is the most common cause of chronic neurological disability in preterm infants. Although magnetic resonance imaging (MRI) is commonly used as a means to diagnose WMI, histopathological features of MRI signal abnormalities are not well understood. The aim of the current study was to determine the correlation between MRI signal abnormalities and histologically defined WMI in preterm ovine foetus.

Methods: Chronically instrumented ovine foetuses (n=17), following induction of intrauterine inflammation, haemodynamic insult, or sham surgery, underwent MRI within two hours after euthanasia. The gestational age and the body weight at MRI were 108.7 ± 5.1 days and 1.51 ± 0.50 kg, respectively (mean ± SEM). Subsequently, brains were perfusion-fixed, removed, and processed for histopathological examinations. WMI was determined histologically on the level of the frontal lobe, the anterior basal ganglia, the mammillary bodies, and the occipital lobe. Whether MRI could detect the histologically defined WMI lesions was statistically analysed.

Results: Histologically, 9 foetuses had WMI lesions, whereas 8 foetuses had no WMI lesions in the brain. MRI showed signal abnormalities (high intensity lesions on T1-weighted images and low intensity lesions on T2-weighted images) in all the foetuses with WMI, whereas no signal abnormalities were detected in any foetuses without WMI ($p < 0.01$). While 87 lesions of coagulation necrosis were determined histologically, 80 lesions were detected with MRI (92.0%).

Conclusions: It was suggested that WMI induced prenatally by intrauterine inflammation or haemodynamic insult could be accurately diagnosed with MRI at birth.

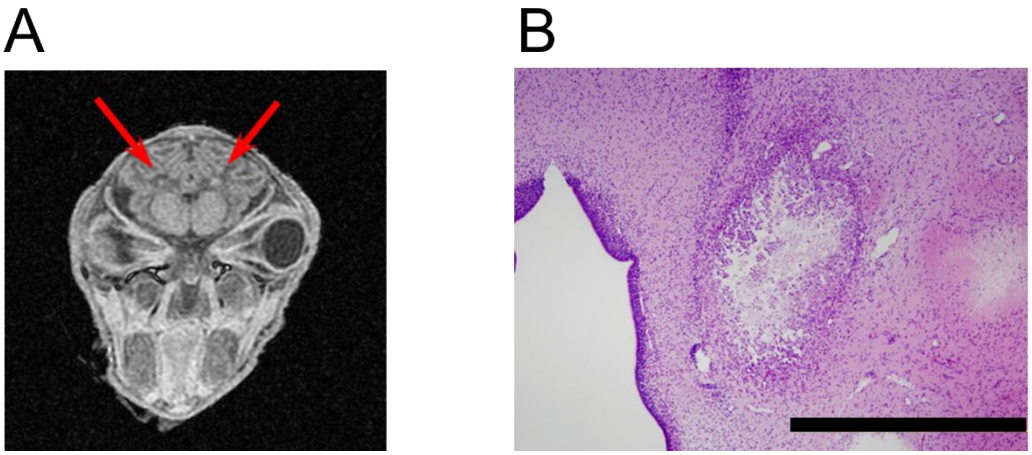


Figure 1. MR Image and micrograph of WMI.
(A) MR image (T1WI) of WMI on the level of anterior basal ganglia. The red arrows signify the lesions of WMI. (B) Micrograph (haematoxylin-eosin staining) of the left lesion in the Figure 1A. Coagulation necrosis is detected in the middle of the picture. The black bar signifies 20 μm.

Abstract

4

Complexity of Brain Signals is Associated with Outcome in Preterm Infants

Sortica da Costa,C[1], Placek, M.M.[2], Czosnyka, M.[3], Smielewski, P.[3], Cabella, B[3], Kasprowicz, M.[2], Austin, T.[1]

[1]The Rosie Hospital, Cambridge University Hospitals, Foundation Trust, UK; [2]Department of Biomedical Engineering, Wroclaw University of Technology, Wroclaw, Poland; [3]Department of Clinical Neurosciences, Academic Neurosurgical Unit, Addenbrooke's Hospital, University of Cambridge, Cambridge

Introduction: A healthy biological system has the ability to react and adapt to minute changes in its environment, a property that characterize complex control systems. Decreased complexity has been associated to aging and poor outcome. We applied Multiscale Entropy analysis to assess the complexity of systemic signals and the complexity of various cerebral near-infrared spectroscopy derived parameters. We further correlated the complexity index (CoI) of brain and systemic signals with outcome.

Methods: Prospective observational study of 61 preterm infants with a median (range) gestation age (GA) of 26 (23 - 31) weeks with an indwelling arterial catheter. All infants were studied before 24 hours of age, following parental consent. A NIRS sensor (Hamamatsu Photonics, KK, Japan) was placed on the infant's temporoparietal area of the head. NIRS signals as oxygenated haemoglobin (HbO_2), deoxygenated haemoglobin (Hb), tissue oxygenation index (TOI) and tissue haemoglobin index (THI) and systemic signals as continuous mean arterial blood pressure (MABP), heart rate (HR) and arterial saturation (SaO_2) were collected using ICM+ software.

Results: Lower CoI-HbO_2, CoI-Hb and CoI-TOI were observed in those infants who developed intraventricular haemorrhage (IVH) compared to those who did not (P=0.002, P=0.010 and P=0.003 respectively). Mean CoI-HbO_2, CoI-Hb and CoI-THI were lower in those infants who died compared to those who survived (P=0.002, P=0.004 and P=0.003, respectively). Complexity of HbO_2 was an independent predictor of IVH (P=0.010) and mortality (P=0.047). CoI-MABP was the only complexity index of systemic signals associated with outcome.

Conclusions: This is the first study to apply Multiscale Entropy to assess the complexity of cerebral near-infrared signals in preterm infants. The results from our cohort revealed that decreased complexity of brain signals recorded within the first 24 hours of life was associated with mortality and brain injury in this population. Furthermore, the complexity index of brain signals had better correlation with outcome than the complexity index of systemic physiological signals.

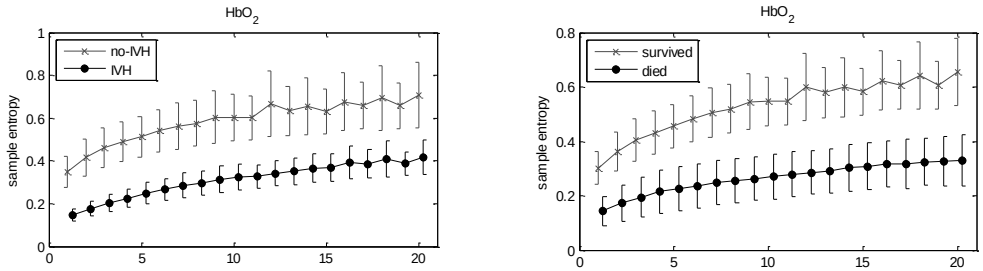


Figure 1. Example of average Mutilscale Entropy plots of HbO_2 for the whole cohort of patients.

Abstract

5

Non-Additive Neuroprotection with Delayed Hypothermia and Recombinant Human Erythropoietin after Cerebral Ischemia in Near-Term Fetal Sheep

Wassink G., Davidson J.O., Fraser M., Bennet L., Gunn A.J.

Department of Physiology, Faculty of Medical and Health Sciences, University of Auckland, New Zealand

Introduction: Current hypothermia protocols for term infants affected by hypoxic-ischemic encephalopathy are incompletely neuroprotective. In neonatal animals, recombinant human erythropoietin (rh-EPO) is neuroprotective through non-hematopoietic mechanisms. However, it remains unclear whether combined treatment with delayed cerebral hypothermia plus rh-EPO can further improve neural outcomes.
Methods: Term-equivalent fetal sheep (at 0.8 gestation) received 30 min of global cerebral ischemia. From 3 to 72 hours after ischemia fetuses received either normothermia plus vehicle infusion (ischemia-control, n=8), or cerebral hypothermia (ischemia-hypo, n=8), or continuous rh-EPO infusion (ischemia-EPO, 5000 IU/kg loading dose, then 5000 IU/kg every 6 hours, n=8), or combination treatment with cerebral hypothermia *plus* rh-EPO (ischemia-EPO-hypo, n=8). Post-mortem was performed 7 days after cerebral ischemia.
Results: Cerebral ischemia was associated with marked neuronal loss and induction of microglia in the parasagittal cortex. Hypothermia was associated with reduced neuronal loss ($p<0.001$) and microglial induction ($p<0.01$) in the parasagittal cortex, with greater overall recovery of EEG power and spectral edge frequency from 48 hours onwards ($p<0.001$). Ischemia-EPO was associated with improved neuronal survival ($p<0.05$), reduced induction of Iba1-positive microglia ($p<0.001$), and faster recovery of spectral edge frequency but not EEG power from 120 hours onwards, compared to ischemia-control ($p<0.05$). Ischemia-EPO-hypo was not significantly different from ischemia-hypo for any outcome.
Conclusion: These preliminary findings suggest that delayed cerebral hypothermia and recombinant human erythropoietin are independently neuroprotective, but that delayed induction of combined hypothermia and rh-EPO after global cerebral ischemia is not associated with additive neuroprotection in near-term fetal sheep.

Notes for Oral Session I

Oral Session II

Developmental Neuroscience B

Keynote Speaker: Carina Mallard
Chair: Peter Nathanielsz

Keynote Speaker and Session Chair

Professor Carina Mallard

Carina Mallard directs the Perinatal Research group at The Institute for Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Sweden.
Dr Mallard received her PhD from University of Auckland in 1995 and undertook postdoctoral training at University of Melbourne as a SIDS Research Fellow. In 1999 she received a Swedish Research Council Award.
The goal of her research is to understand mechanisms that lead to brain damage in preterm infants and to develop neuroprotective therapies for this vulnerable population.

Professor Peter W Nathanielsz

Dr..Peter Nathanielsz obtained his scientific training and Bachelor's and Doctoral degree and PhD from University of Cambridge in England where he developed a lasting interest in understanding how the fetus develops. After a period on the faculty at University of Cambridge, he assumed the position as Director of the Laboratory of Fetal Physiology at the University of California, Los Angeles, where he taught for six years. Following that he was on the Faculty at the College of Veterinary Medicine, Cornell University, as the Director of the Laboratory for Pregnancy and Newborn Research fro 20 years. He moved to New York University School of Medicine where he was Director of the Center for Women's Health Research from 2002 – 2004. In 2004 he moved to to the University of Texas Health Science Center at San Antonio and formed the Center for Pregnancy and Newborn Research (CPNR). In 2015 he received the Distinguished Professor of Life Course Health, University of Wyoming. He remains in San Antonio to maintain his group's non-human primate research program on life course health at Southwest National Primate Research Center where he is a Core Scientist. Dr. Nathanielsz' books ***Life in the womb: the origin of health and disease*** and ***Life before birth*** has been translated into 12 languages. They were written to inform the general, non-specialist reader about fetal development and developmental programming.

Abstract

6

Effects of intrauterine growth restriction on brain development

Mallard, C.

Perinatal Center, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Sweden

Intrauterine growth restriction (IUGR), broadly describes the fetus that does not grow to its genetic potential and is associated with an increased risk of preterm birth and perinatal death. Furthermore, IUGR is strongly linked to neurodevelopmental deficits in surviving infants. Although the aetiology of IUGR is not fully understood, placental insufficiency is considered an important underlying factor, where it leads to fetal hypoxemia and reduced nutrient availability.

The spectrum of brain abnormalities associated with IUGR in the human is heterogeneous, probably reflecting the timing and severity of the in utero compromise. Late-onset IUGR is the most common form, where the compromise to the fetus becomes apparent in the third trimester of pregnancy. In IUGR, fetal growth is often asymmetric with a relative sparing of brain growth in relation to the growth of the body. However, despite the relative sparing of brain growth, IUGR is often associated with both brain structural and functional deficits.

In this seminar I will review experimental evidence that demonstrate the effects of IUGR on fetal brain development and potential underlying mechanisms.

Abstract

7

Development of neurovascular coupling in the fetal sheep and newborn lamb

Nakamaura S[1,2], Walker D.[2], **Wong F.Y.[1,3,4]**

[1]Department of Paediatrics, Faculty of Medicine, Kagawa University, Kagawa, Japan. [2] The Ritchie Centre, Hudson Institute of Medical Research, Melbourne, [3] Department of Paediatrics, Monash University, Melbourne, [4] Monash Newborn, Monash Medical Centre, Melbourne, VIC, Australia

Introduction: Neurovascular coupling (NVC) is an important mechanism that provides increased cerebral blood flow and cerebral oxygenation in response to increased neural activity. NVC has been extensively studied in animal and clinical studies, in both adult and paediatric populations. To date, there is no animal model of neurovascular coupling available for the fetal and preterm brain, and there is limited understanding of the perinatal development of NVC. We aimed to characterise the development of NVC in the fetal and newborn sheep brain by measuring changes in cerebral oxy- and deoxy-haemoglobin (ΔoxyHb and ΔdeoxyHb) after neural activation, using near infrared spectroscopy (NIRS)

Methods: Under isoflurane anaesthesia, scalp EEG electrodes and NIRS optodes were positioned bilaterally over the somatosensory cortex of fetal (128-132 days gestation; n=7) and neonatal (4-8 days old; n=8) sheep, and the left median nerve was stimulated via a cuff by trains of electrical pulses (3-8mA, 2msec) of 3 different durations (1.8, 4.8 & 7.8s). The somatosensory evoked potential (SEP), ΔoxyHb and ΔdeoxyHb were recorded.

Results: In the fetuses, 1.8s stimulation always increased ΔoxyHb in the contralateral cortex (a positive functional response, figure 1), whereas more prolonged stimulation (4.8s, 7.8s) mostly produced either a sustained decrease in contralateral ΔoxyHb (negative response), or a biphasic response in which ΔoxyHb showed an initial reduction followed by a later rise (4.8s: 86%; 7.8s: 75% of all stimulations). In contrast, in neonatal lambs, 7.8s stimulation mostly produced positive response with increase in contralateral ΔoxyHb (63% of all stimulations). The SEP pattern was unaltered by duration of stimuli.

Conclusions: Prolonged somatosensory stimulation induced decreased cerebral oxygenation in the fetal brain, but increased cerebral oxygenation in the neonatal brain. Our results suggest immature NVC in the fetal brain, where oxygen delivery may not match the increased cerebral oxygen consumption that occurs with neural activation.

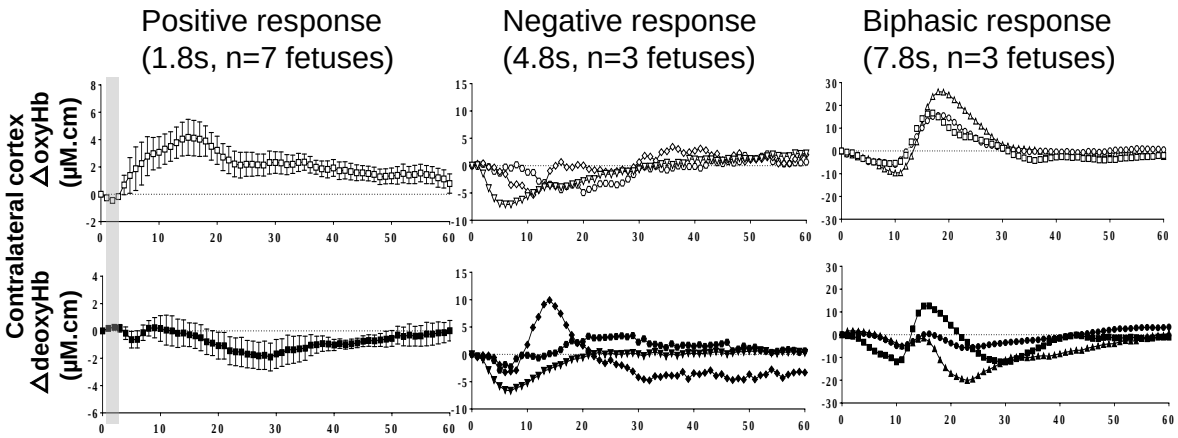


Figure 1. Cerebral functional response patterns: In the fetal sheep, a positive functional response with increased oxyHb in the contralateral cortex, was produced after the median nerve stimulation for 1.8s. Prolonged stimulations at 4.8s and 7.8s mostly produced either the negative or biphasic functional responses.

Abstract

8

Identification of bacteria in brain cortex and placenta of fetuses exposed to hypoxic hypoxia (HH)

Zarate, M. A. [1], Chang, E. [1], Rodriguez, M. [2], Triplett, E. [2] & Wood, C. [1]

[1]Department of Physiology and Functional Genomics, University of Florida College of Medicine, Gainesville, FL, USA. [2]Department of Microbiology & Cell Science, University of Florida Institute of Food and Agricultural Sciences, Gainesville, FL, USA

Introduction: Our lab has previously reported that fetal HH produces a robust inflammatory response characterized by an upregulation of inflammatory markers, and aggregation of macrophages in different brain regions, and other peripheral organs. Based on the interpretation of these results, we hypothesize that bacterial colonization is the main cause for this inflammatory cascade and cells infiltration in the brain cortex of fetuses subjected to HH.
Methods: We used a total of 16 brain cortex and placenta samples of fetuses exposed to HH or normoxia (n=8 per group). Paraffin embedded brain cortex tissues were sectioned (5 um) and stained using the Gram technique for bacterial detection. Placenta and brain cortex snapped frozen samples were culture in brain heart infusion media, and collected for Gram staining and Sanger sequencing. DNA was extracted from brain cortex tissues for metagenomics sequencing.
Results: We detected Gram (+) and, mostly, gram (-) populations on bacteria in the histological brain cortex sections on the HH group, and this was validated (same morphology) by Gram staining of the harvested live bacterial colonies obtained from the cultured samples. HH placenta tissues also showed bacterial colonies with very similar characteristics than the ones obtained from the HH brain samples. Sanger and metagenomics sequencing confirmed that predominant bacteria found in brain and placenta of fetuses subjected to HH belong to the *Staphylococcus* and *Shigella* genera. Other types of bacteria found involved *Enterobacter*, *Escherichia*, and *Pseudomona*. Normoxic brain and placenta tissues did not show any type of bacterial populations, and had negative results from Sanger or metagenomics sequencing.
Conclusions: We conclude that HH induces bacterial invasion in the fetal cerebral cortex and placenta, particularly *Staphylococcus* and *Shigella* strains. This might lead to the activation of the immune system (macrophage aggregation), and inflammatory pathways previously observed in our work. The origin of this bacteria species and how they reach the fetal environment remains unclear and it might involve a maternal component.

Animal ID#	Condition	Growth (using 100 mg tissue)
593A	Hypoxia	+
593B	Hypoxia	+
512A	Normoxia	-
512B	Normoxia	-
691A	Normoxia	+
691B	Normoxia	-
622A	Hypoxia	-
622B	Hypoxia	+
625A	Hypoxia	-
625B	Hypoxia	+
726A	Normoxia	-
726B	Normoxia	-
769A	Hypoxia	+
769B	Hypoxia	+
761F	Normoxia	-
538A	Hypoxia	+
538B	Hypoxia	+

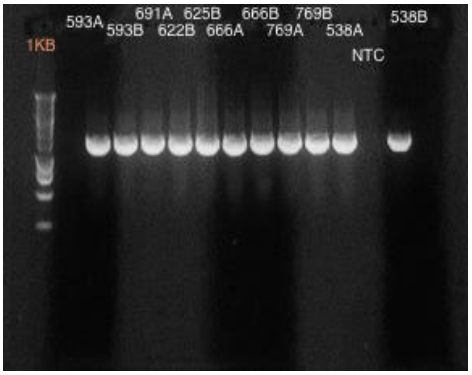


Figure 1. Bacterial Identification in fetal brain samples. (Left) Identification of bacterial culture growth from fetuses subjected to hypoxic hypoxia or control 18 hours after incubation. (Right) DNA extracted from fetal cerebral cortices showed positive bacterial 16S DNA amplification by PCR.

Abstract

9

Neuroprotective effects of umbilical cord blood cells in fetal growth restriction

Miller, S.L.[1,2], McDonald, C.[1], Bush, L.[1], Thomson, S.[2,3], Calalang, M.[3], Sutherland, A.E.[1], Jenkin, G.[1,2,3], Castillo-Melendez, M[1].

[1]The Ritchie Centre, Hudson Institute of Medical Research, [2]Dept Obstetrics & Gynaecology, and [3]Monash Newborn, Monash University, Clayton, Victoria, Australia

Introduction: Fetal growth restriction (FGR) is a serious complication of pregnancy linked to high rates of stillbirth, preterm delivery and poor neurodevelopmental outcomes including cerebral palsy. As a novel therapy, umbilical cord blood (UCB) stem cells could improve the structure and function of the brain. We investigated the effects of UCB cells in a fetal ovine model of FGR.

Methods: Umbilical cord blood from term healthy sheep pregnancies was collected and cryopreserved. We induced placental insufficiency and FGR in sheep at 88d gestation via single umbilical artery ligation. UCB mononuclear cells ($15x10^6$ cells per estimated fetal weight) were administered iv to the fetus at 125d gestation, and the fetal brain collected for analysis at 135d gestation. We determined cell death (caspase-3) and oxidative DNA cell damage (8-OHD) in the white matter using immunohistochemistry. We also determined the cell types affected via double label for either caspase-3 or 8-OHD with NeuN (neurons), CNPase (oligodendrocytes) or glucose transporter-1 (GLUT-1, endothelial cells).

Results: Cell death was increased throughout the white matter (eg corpus callosum, CC; periventricular, PVWM; subcortical white matter, scWM) in FGR brains compared with control, control+UCB, and FGR+UCB ($P<0.0001$). Significant apoptotic cell death was also evident in the dentate gyrus (DG) and subventricular zone (SVZ) of FGR brains ($P<0.0001$), two important proliferative areas. Within grey matter, increased caspase-3 was seen in the cortex and hippocampus ($P<0.005$). FGR+UCB therapy resulted in decreased cell death (to basal levels) throughout all brain regions examined. Double label showed moderate co-staining of caspase-3 with neurons (cortex and CA3), and high colocalisation in oligodendrocytes (PVWM & scWM) and in endothelial cells throughout the brain.

Conclusions: UCB therapy in FGR protects against apoptotic cell death in both grey and white matter regions of the brain. UCB therapy was found to protect blood vessels by preventing apoptosis in mature endothelial cells. In addition, UCB treatment has neuroprotective effects on two active neurogenic zones of the brain (SVZ and DG). Because the SVZ and DG contain resident stem cells, UCB therapy may enhance brain plasticity by preventing apoptosis of multi-potent neural precursors cells.

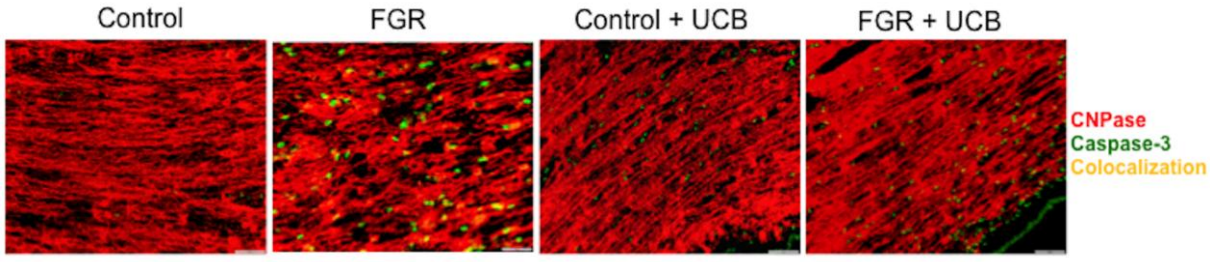


Figure 1. The effects of UCB cell therapy on cell death within the white matter. Shown are representative images of co-staining for oligodendrocytes and mature myelin (CNPase, red) together with apoptotic cell death (caspase-3, green). Cell death was upregulated in the white matter of the FGR brain, and prevented with administration of UCB stem cells.

Notes for Oral Session II

Notes for Oral Session II

Oral Session III

Developmental Programming (Sponsored by DOHaD)

Keynote Speaker: Mary-Elizabeth Patti
Chair: Laura Bennet

Keynote Speaker and Session Chair

Mary-Elizabeth Patti MD

Dr. Patti received her medical degree from Jefferson Medical College *magna cum laude*. She completed clinical residency training in internal medicine at the University of Pittsburgh, and clinical and research training in the joint Harvard Medical School endocrinology fellowship program. She is currently Investigator at Joslin Diabetes Center, Co-Director of the Advanced Genetics and Genomics and Enrichment Cores, Director of the Joslin Hypoglycemia Clinic, and Assistant Professor of Medicine, Harvard Medical School. She was elected to the American Society of Clinical Investigation in 2009 and to Fellowship in the American College of Physicians in 2014. She has served in numerous leadership roles within the American Diabetes Association, including as chair of the annual meeting for several years, and as organizer of diabetes-focused Keystone Symposia. She has authored over 100 peer-reviewed manuscripts.

Dr. Patti's laboratory focuses on identification of molecular mechanisms by which environmental or nutritional risk factors confer risk for prediabetes. Her laboratory studies utilize both cellular and animal models to study how insulin resistance and nutrition during early life can affect the metabolic function of stem cells and tissues critical for insulin sensitivity and glucose tolerance.

Professor Laura Bennet

Chair of Perinatal Physiology
Dept Physiology
The University of Auckland

Professor Laura Bennet is co-director of the Fetal Physiology and Neuroscience Group in the department of Physiology, at the University of Auckland. She is a fetal systems physiologist with a specialist interest in preterm fetal and neonatal cardiovascular and neurophysiological adaptations to common in utero insults such as hypoxia and infection, the effect of common therapeutic treatments for preterm labour such as glucocorticoids on these adaptations, and the development of treatment strategies and biomarkers to prevent injury and identify the at risk baby. Current neuroprotection studies include the evaluation of stem-cells to ameliorate inflammation and promote neural plasticity after injury.

Abstract

10

Intergenerational impact of paternal metabolism on metabolic disease risk

Patti, M.E.

Joslin Diabetes Center and Harvard Medical School, Boston, MA, USA

Introduction: Common metabolic diseases, including diabetes and obesity, are the result of interactions between genes and environment. It is well-recognized that the intrauterine environment is an important modifier of this risk. Thus, fetuses carried by women who are obese, diabetic or suffer from suboptimal nutrition are at increased risk of insulin resistance, obesity, type 2 diabetes (T2D), and cardiovascular disease as adults. Emerging data from murine models indicate that paternal metabolism, including obesity, diabetes, and prior nutritional exposures, also influence disease risk in subsequent generations. First generation *male* offspring of mothers undernourished (UN) during pregnancy (designated F1-UN) are at risk of metabolic disease themselves, & produce second-generation (F2) & third-generation (F3) offspring that develop increased adiposity & impaired glucose tolerance as adults. Such disease phenotypes in F2/F3 mice indicate F1 males can transmit heritable, non-genetic, traits created by this early *in utero* exposure to his offspring & grandoffspring.

We hypothesize that these paternal effects are mediated via epigenetic mechanisms perturbing not only germ cells but also the function of critical stem cell populations. In previous studies, we demonstrated that in utero exposure to undernutrition alters locus-specific DNA methylation in spermatozoa of adult males. Moreover, these changes are associated with persistent transcriptional dysregulation in multiple tissues of offspring mice.

Methods: To determine whether phenotypes resulting from intrauterine exposures are cell autonomous, we harvested murine embryonic fibroblasts (MEFs) from both first-generation embryos (directly exposed to altered nutrition of mother) and second-generation embryos (offspring of father exposed to altered nutrition during his intrauterine life). Proliferation, gene expression, and metabolism were analyzed.

Results: Proliferation is reduced in MEFs harvested from both first and second generation embryos, with additional defects in cell cycle progression and metabolism observed in first generation MEFs.

Conclusions: Even transient nutritional exposures or the legacy of prior paternal exposures can result in persistent cell autonomous defects in proliferation. Such defects may contribute to multi-organ phenotypes linked to metabolic disease risk.

Abstract

11

Fat cell size distribution in male offspring (F1) of obese mothers: effects of maternal and offspring exercise (Ex) intervention

Ibáñez-Chávez, C.A.[1], Vazquez, M., Reyes-Castro, L.A.[1], Vega-García, C.C.[1], Bautista-Carbajal, C.[1], Gerow K.[2], Nathanielsz, P.W.[2], **Zambrano, E.**[1].

[1]Reproductive Biology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, DF, Mexico, 14000 and [2] Wyoming Pregnancy and Life Course Health Center, University of Wyoming, Laramie Wyoming, United States 82071

Introduction: Animal studies show that maternal obesity (MO) is associated with increased offspring (F1) adiposity. There are few data on effects of maternal (F0) or F1 exercise (Ex) intervention on F1 adipose tissue. Since increased fat cell size plays an important role in adverse metabolic effects programmed by MO we analysed F1 fat cell size distribution.

Methods: Founder generation (F0) female Wistar rats were fed from weaning through pregnancy and lactation with chow (C) or high energy obesogenic diet (MO), and bred at PND 120. Half of the C and MO females wheel-ran (Ex) 30 min/day, 5x a week from PND 90 until the end ofpregnancy. Male F1(n=8 litter/group) were weaned to C diet at PND 21 (C,CF0-Ex, MO and MO-F0Ex). One male F1 of C and MO litters wheel-ran 30 min/day 5x a week from PND 50 to 110 (C-F1Ex and MO-F1Ex). All F1 euthanized at PND 110, total adipose tissue was excised and fat cell size was determined as cross sectional area in retroperitoneal adipose tissue depot and characterized by the gamma probability density function in all F1 groups.

Results: At PND 110, body weight was similar among groups. The amount of fat was similar between C and MO and lower in CF0-Ex and MO-F1Ex compared to MO. Greater fat cell size was found in all MO groups and more variability in MO and MOF0-Ex (Fig. 1A, B). Maternal exercise reduces fat cell size in MO (MOFO-Ex) but not in C (CF0-Ex) groups. F1 exercise reduces size and variability in MO (MOF1-Ex) and variability in C (CF1-Ex). Differences were observed in kurtosis and symmetry of the distribution when data were fitted by gamma distribution (Fig 1C, D)

Conclusions: MO programs adipose tissue hypertrophy and greater fat cell size variability, F0 and F1 exercise intervention have different beneficial effects related to fat cell size distribution.

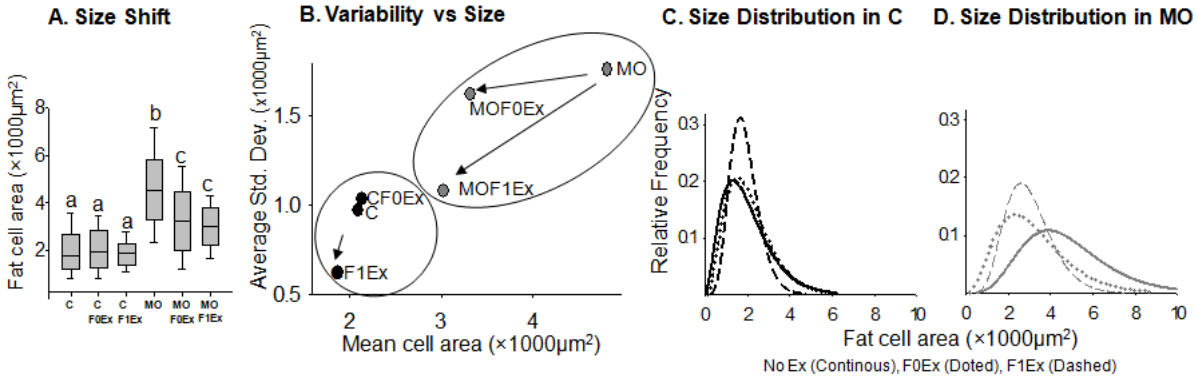


Figure 1. Fat cell size in C and MO F1 with exercise (Ex) intervention in F0 and F1. A. Median and interquartile range as bars and vertical boxes, statistical differences by Kruskall-Wallis test ($p<0.05$), **B.** Variability among groups as average standard deviation vs. mean of fat cell area, **C-D.** Size Distribution modelled by gamma distribution (goodness of fit $p<0.05$).

Abstract

12

The effect of a maternal vitamin D depleted diet during gestation on the behaviour and activity of young adult mouse offspring

Jones, L. E[1]., Newland, P.L[2]., Cagampang, F.R[1,2]., Poore, K.R[1]., Cleal, J.K[1,2]., Green, L.R[1,2].

[1]Inst. Developmental Sciences, University of Southampton, UK, [2] Biological Sciences, University of Southampton, UK

Introduction: The fetus is reliant on maternal vitamin D, and vitamin D deficiency (VDD) affects a substantial proportion of the human population. Maternal VDD is linked to altered offspring brain development (1) and to impaired skeletal muscle structure and function (2, 3). In mice, we tested the idea that a maternal VDD diet would impair activity in young adult offspring.

Methods: Female C57BL/6J mice were fed a control (C; 1 IU/g vitamin D_3) or VDD (0 IU/g vitamin D_3) diet 6 weeks prior to mating and throughout pregnancy and lactation. Offspring were weaned onto the C diet creating two diet groups: C/C and VDD/C (n=5 and n=7 per group, respectively). Open-field activity was assessed for 5 minutes in female 15wk offspring. Distance travelled, time ambulatory (period in which a mouse crossed more than 3 photo-beams in any two seconds), average velocity, vertical counts (number of times offspring reared onto hind limbs) and number of jumps were recorded. Data are mean ± SEM and were analysed by independent t-test.

Results: VDD/C female offspring displayed a reduction in distance travelled (P<0.001) and time spent in ambulatory behaviour (P<0.01) compared to C/C offspring, but average velocity was not different between groups. Vertical counts and the number of jumps in this time period were significantly lower in the VDD/C compared to C/C offspring (P<0.01).

Conclusions: The observed reduction in offspring activity with a maternal VDD diet may reflect altered anxiety or hindlimb strength. Underlying mechanisms could include impaired brain development and neurological function, and altered skeletal muscle fibre type composition, respectively. These changes in response to a maternal VDD diet may have implications for offspring age-related degeneration in neurological function and skeletal muscle strength.

Supported by The Gerald Kerkut Charitable Trust.

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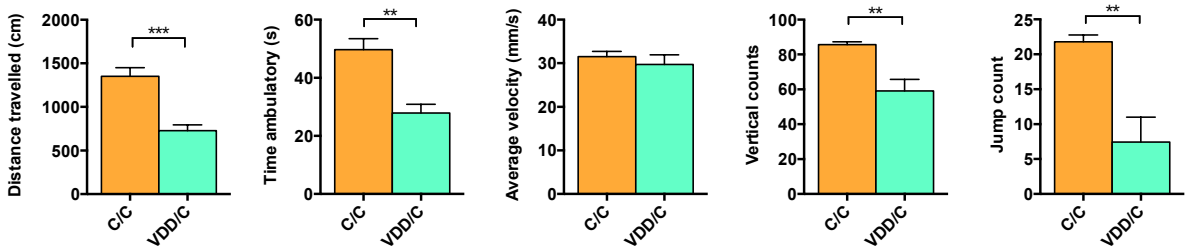

Figure 1. Open-field behaviour tests. Distance travelled, time ambulatory, average velocity, vertical counts and number of jumps of female 15wk offspring subjected to either a control (C) or vitamin D deficient (VDD) maternal diet. Data are mean ± SEM and statistically analysed by independent t-test. *** P<0.001, ** P<0.01.

Abstract

13

N-acetyl cysteine, a glutathione precursor reverses vascular dysfunction and endothelial epigenetic programming in IUGR guinea pigs

Herrera, E.A., Cifuentes-Zúñiga, F., Figueroa, E., Villanueva, C., Hernández, C., Alegría, R., Arroyo, V., Farías, M., Uauy, R., Casanello, P., **Krause, B.J.**

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[2]Departament of Neonatology, Division of Pediatrics & [3]Division of Obstetrics & Gynecology Faculty of Medicine, Pontificia Universidad Católica de Chile. Marcoleta 391, Santiago 8330024, Santiago, Chile

Introduction: In humans, intrauterine growth restriction (IUGR) is associated with vascular dysfunction, oxidative stress and signs of endothelial programming of the umbilical vessels. We aim to determine the effects of maternal antioxidant treatment with N-acetyl cysteine (NAC) on fetal endothelial function and eNOS programming in IUGR guinea pigs.
Methods: Pregnant guinea pigs were submitted at mid gestation to a surgery for implanting ameroid constrictors at both uterine arteries (IUGR), or a sham intervention (Control). Half of the sows of each group received NAC (500 mg × $Kg_{body\ weight}^{-1}$ × day^{-1}) in the drinking water starting at day 34 until day 60. Fetal biometry and umbilical resistance (RI) and pulsatility (PI) indexes were followed by ultrasound in awake sows throughout gestation. At term (~ 60 days) fetuses and their placentae were extracted by C-section, dissected and weight. Umbilical arteries and fetal aorta were isolated to assessed vascular function by wire-myography. Primary cultures of endothelial cells from fetal aorta, femoral and umbilical arteries were carried out to determine the levels of eNOS mRNA by qPCR and analyze the DNA methylation of 12 CpG sites in *Nos3* promoter by pyrosequencing.
Results: Doppler ultrasound measurements showed that NAC reduced placental vascular resistance in IUGR and this effect was associated with a recovery in fetal weight and increasing fetal-to-placental ratio at term (~40%) compared with untreated IUGR. Additionally, NAC restored eNOS-dependent relaxation determined by wire myography in aorta and umbilical arteries, and normalized eNOS mRNA levels in primary cultures of endothelial cells (EC) from aorta, femoral and umbilical arteries. Pyrosequencing analysis showed that IUGR-derived EC have a decreased methylation (~30%) at CpG -170 (from the TSS) and this epigenetic signature was absent in fetuses treated with NAC.

Conclusions: These data show that IUGR-EC have common molecular markers of programming in umbilical and systemic arteries. Notably, maternal treatment with NAC restores fetal growth by increasing placental efficiency and reversing the functional and epigenetic programming of the endothelium in IUGR guinea pigs.

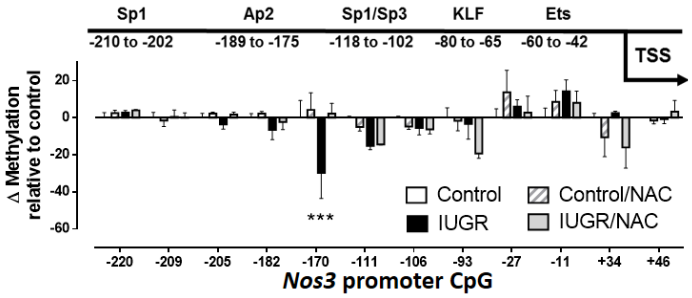

Figure 1. Level of DNA methylation in the *Nos3* promoter of guinea pig fetal aorta. Schematic representation of guinea pig *Nos3* and changes in DNA methylation levels relative to control in CpGs present in the *Nos3* promoter of fetal aorta endothelial cells. Values as Mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001 vs untreated-control, ANOVA.

Abstract

14

The mitochondria-targeted antioxidant MitoQ prevents the programming of cardiovascular dysfunction by developmental hypoxia in sheep

Skeffington, K.L.[1*], Botting. K.J.[1*], Niu, Y.[1], Allison, B.J.[1], Brain, K.L.[1], Itani, N.[1], Beck, C.[1], Logan, A.[2], Murray, A. J.[1], Murphy, M.P.[2], & Giussani, D.A.[1]; * *Co-first authors*

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2. Medical Research Council, Mitochondrial Biology Unit, Cambridge, UK

Introduction: Chronic fetal hypoxia programmes cardiovascular dysfunction via oxidative stress (1). In rodents, maternal treatment of hypoxic pregnancies with the antioxidant vitamin C is protective, however, only at concentration incompatible with human clinical translation (2,3). Here, we show in sheep that MitoQ is a suitable alternative therapeutic candidate.
Methods: Singleton pregnant ewes with an indwelling femoral artery and vein catheter (surgically implanted at 100 days gestation; term ~145) were exposed to normoxia (N) or hypoxia (H; 10% O_2) with or without maternal MitoQ treatment (Q; 1.2 mg/kg/day i.v. in saline) during the last third of gestation (105-138 days; *n*= N:10, H:10, HQ:7, NQ:9). After natural delivery, offspring were maintained until 9 months, and then chronically instrumented with vascular catheters and a femoral flow probe to determine *in vivo* cardiovascular function followed by *ex vivo* peripheral endothelial function (wire myography). Data were analysed by 2-way ANOVA with repeated measures, as appropriate.

Results: Offspring of hypoxic pregnancy were smaller at birth (N: 3.4±0.2; H: 3.0±0.1; HQ: 3.0±0.2; NQ: 3.4±0.2kg, P<0.05) and hypertensive at adulthood (A). Maternal MitoQ in hypoxic pregnancy restored the programmed hypertension in adult offspring. Adult offspring from MitoQ pregnancies showed increased *in vivo* NO bioavailability evidenced by a greater fall in femoral vascular conductance to NO blockade with LNAME (100 mg/kg i.a.; B) and by *in vitro* restoration of the programmed impaired femoral artery dilator sensitivity to SNP (C).
Conclusions: Maternal MitoQ treatment in pregnancy complicated by chronic fetal hypoxia protects against programmed cardiovascular dysfunction in adulthood. The mechanism underlying this protection involves programmed increases in NO bioavailability and sensitivity in the cardiovascular system of the adult offspring.
Supported by the *British Heart Foundation*

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Kane AD, Herrera EA, Camm EJ, Giussani DA (2013). Vitamin C prevents intrauterine programming of in vivo cardiovascular dysfunction in the rat. *Circ J*. 77(10), 2604-11

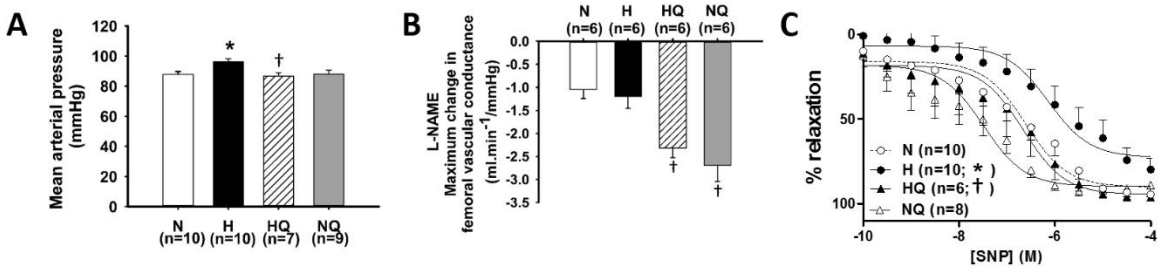

Figure 1. Mean arterial pressure in adulthood (A), femoral vascular conductance in response to LNAME bolus (B) and *in vitro* femoral artery relaxation to increasing SNP concentration (C). P<0.05; 2-way ANOVA for effect of Hypoxia Hypoxia (*) and MitoQ (†).

Abstract

15

Maternal nutrient restriction (MNR) in guinea pigs leads to fetal growth restriction (FGR) with sex-related increases in tissue hypoxia

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Introduction: Maternal undernourishment can be causative for FGR with altered placental development and reduced nutrient transport for glucose, amino acids and lipids. However, whether oxygenation is also decreased as seen with placental insufficiency-related FGR, remains unknown. We have determined the extent to which MNR in guinea pigs as a causative factor for FGR impacts immunoreactivity (IR) for Hypoxyprobe-1 (HP-1), a widely used marker of tissue hypoxia.
Methods: Guinea pigs were fed ad libitum (Control) or 70% of the control diet pre-pregnancy switching to 90% at mid-pregnancy (MNR). Near term, HP-1 was injected into pregnant sows with fetuses then necropsied for body/organ weights, and brain, liver, kidney and placental tissues were assessed for HP-1 IR. Statistical significance was assumed for $p<.05$.
Results: FGR-MNR fetuses (8males/8females) were 36% smaller, while their brains and livers were 12% and 40% smaller, respectively, in comparison to the appropriate for gestational age (AGA)-Control fetuses (8males/8females). HP-1 IR in the male and female AGA-Controls was similar across the brain regions studied and throughout the liver, but increased in the renal proximal convoluted tubules vs the glomeruli, and in the placenta labyrinth lobules peripherally vs centrally, indicating regional differences in basal oxygenation. HP-1 IR was increased in the FGR-MNR fetuses by 2-4X in the brain and more so in males than females, by ~4X in the liver and proximal convoluted tubules and ~15X in the glomeruli but with no sex differences evident, and with no changes in the placenta.
Conclusions: MNR in guinea pigs results in asymmetric FGR with increased HP-1 IR as an index of local tissue hypoxia in the brain which was more so in males than females, and in the liver and kidneys which was similar for males and females, but with no changes in the placenta. As such, chronic hypoxia is likely to be an important signaling mechanism for the decreased fetal growth seen with maternal undernourishment and programming of related adverse outcomes, and appears to be largely post placental in nature. Moreover, there are sex-related differences in the brain which may contribute to the sex-specific expression of adverse neurodevelopment in FGR offspring.

Abstract

16

In-vivo evidence for premature brain aging in old men prenatally exposed to the Dutch Famine

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Introduction: Decreased fetal nutrient delivery is widespread in both developing and industrialized countries, leading to aberrant developmental programming, finally resulting in negative impacts on the general lifespan, lifelong health, disease outcomes, brain development, and cognitive functions. However, the effects of maternal nutrient restriction (MNR) during gestation on structural brain aging in late life have not been explored yet. We hypothesized that offspring exposed to MNR would show altered brain structure resembling premature brain aging.

Methods: Utilizing the MRI subsample (*n*=118) of the Dutch famine birth cohort, this study implements an innovative, non-invasive *in vivo* MRI biomarker using pattern recognition to analyze structural brain aging and to subsequently evaluate the effects of MNR during early gestation on individual brain aging in the adult offspring (mean age 67 years).

Results: We observed increased *BrainAGE* scores of more than 4 years ($p<0.05$) in adult male offspring who were exposed to the Dutch famine in early gestation as compared to offspring born before the famine. Additionally, advanced brain aging corresponded to worse performances in cognitive tests as well as increased depression scores.

Conclusions: Future work will have to further explore the effects of a variety of developmental and environmental factors affecting early development on neuroanatomical maturation and degeneration, thus potentially facilitating early treatment and/or preventative interventions.

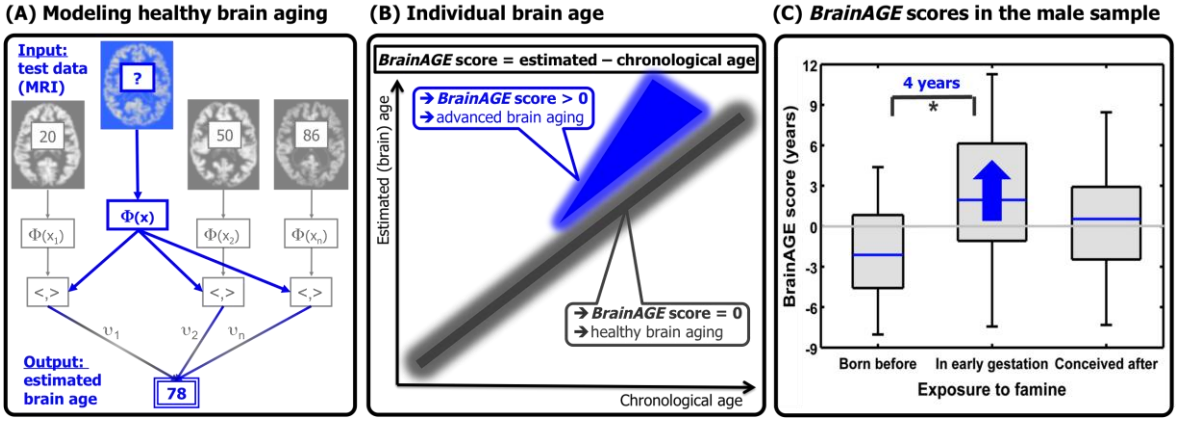

Figure 1. Depiction of the *BrainAGE* concept and study results. (A) The model of healthy brain aging is trained with chronological age and segmented structural MRI data of a training sample (gray). Subsequently, the individual brain age of a new test subject is estimated (blue). **(B)** The difference between estimated and chronological age results in the individual *BrainAGE* score. **(C)** *BrainAGE* scores were significantly increased in old men, who were exposed to the Dutch famine in early gestation (*$p<0.05$).

Notes for Oral Session III

Oral Session IV

Clinical Fetal and Neotal Physiology

Keynote Speaker: Graeme Polglase
Chair: Bryan Richardson

Keynote Speaker and Session Chair

Associate Professor Graeme R. Polglase

Associate Professor Polglase graduated with first class BSc (Hons) Physiology and Doctor of Philosophy at Monash University under the mentorship of Professors Stuart Hooper, Richard Harding and Colin Morley and Dr Megan Wallace. He undertook a postdoctoral fellowship at the School of Women's and Infants' Health at The University of Western Australia with Professor John Newnham, Professor Alan Jobe and Dr Timothy Moss. In 2010 he was recruited back to The Ritchie Centre, Monash University University and now Hudson Institute of Medical Research where he currently is. In 2016 2016 he was awarded an NHMRC Career Development Fellowship and National Heart Foundation of Australia Future Leaders Fellowship, and the prestigious Heart Foundation Paul Korner Innovation Award. He has published over 110 research articles articles and has secured over $15 million in grant funding. He is an Associate Editor for for Frontiers in Pediatrics: Neonatology, and regularly reviews for a number of clinical and physiological journals. A/Prof Polglase heads the Perinatal Transition Research Group which is working to improve improve respiratory, cardiovascular, and neurological outcomes of infants born preterm: the single greatest cause of neonatal morbidity and mortality. His findings continue to expand understanding of how key events during fetal development, birth, and post delivery influence the pulmonary, cardiovascular, and cerebral circulation lead to organ inflammation and injury to improve outcomes. As the single greatest cause of neonatal morbidity and mortality, he hopes this work will improve outcomes for some of our tiniest patients.

Professor Bryan Richardson

Dr. Bryan Richardson is a Professor in the Departments of Obstetrics and Gynaecology, Physiology and Pharmacology, and Pediatrics at The University of Western Ontario, and a Scientist in the Fetal and Newborn Health Program of the Children's Health Research Institute in London, Ontario.

He has had longstanding support from the MRC/CIHR, initially as an MRC Fellow and and subsequently a Scholar, and with continuous national funding for over 25 years, most recently as a member of the CIHR Group in Fetal Growth Restriction: Mechanisms and Outcomes. He is currently investigating the impact of maternal undernourishment leading to fetal growth restriction with chronic hypoxia on later development focusing on the brain.

Dr. Richardson is recognized for his research contributions internationally having published 143 peer-reviewed medical articles, and 20 book chapters/symposia proceedings. He has supervised and mentored 41 research trainees, many of whom subsequently have taken up academic faculty positions here in Canada and in Japan. Japan. He was the first WYETH AYERST Canada/CIHR Clinical Research Chair in Women's Health for Perinatology, Perinatology, and subsequently a recipient of a Canada Research Chair (Tier 1) in Fetal and Neonatal Health and Development (2004-2011).

As further indication of his stature in the area of perinatal physiology and clinical practice, Dr. Richardson was voted to the Executive Council of the Perinatal Research Society, and subsequently served as its President. He also served on the Executive Council of the Fetal and Neonatal Physiological Society, and on the inaugural Advisory Board for the CIHR Institute of Human Development, Child and Youth Health during which time he led the research agenda for Healthy Pregnancies.

Dr. Richardson has been a reviewer for several scientific medical journals in the fields of Obstetrics/Gynaecology and Perinatal Physiology, and presently serves on the editorial boards for *Early Human Development* and *Reproductive Sciences*. He additionally has served as a committee member for several granting agencies including March of Dimes, Dimes, and the MRC/CIHR Clinical Investigation A Committee. Dr. Richardson also served as Academic Chair of Western's Department of Obstetrics and Gynaecology from 2004 until 2012, overseeing a period of substantial growth growth and promoting academic excellence and funding partnerships.

Abstract

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Preterm lung injury in the delivery room: implications for multi-organ injury

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The initiation of respiratory support in the delivery room is one of the most important but least controlled interventions a preterm infant will face. Tidal volumes (V_T) used in the neonatal intensive care unit are carefully measured and adjusted. However, the V_Ts that an infant receives during resuscitation are usually unmonitored and highly variable, and up to 80% receive excessively high VTs. It is now well established that exposure of the lungs to high V_Ts elicits a profound pro-inflammatory response and up-regulation of key lung-injury genes, resulting in structural remodelling and long term adverse consequences including bronchopulmonary dysplasia. However this response is not isolated to the lungs. Studies in preterm lambs and infants have demonstrated that the initial respiratory support results in a profound systemic inflammatory cascade, which has downstream effects on multiple organs, particularly the brain (Figure 1). Therefore any therapeutic strategies that can reduce lung inflammation and injury at birth are likely to also have neuroprotective benefits.

We have investigated a number of strategies to reduce the regional inflammatory response of the lung to respiratory support. This includes the use of protective ventilation strategies including prophylactic surfactant, sustained inflations and different ventilation modalities. Pharmacologically, we have investigated a number of inflammatory cytokine blockers, and potential clinical therapies including Erythropoietin, Nanoparticles and human amnion epithelial cells. These therapies to date have had variable impact on lung inflammation and injury and may amplify the systemic inflammatory cascade resulting in increased brain inflammation and injury.

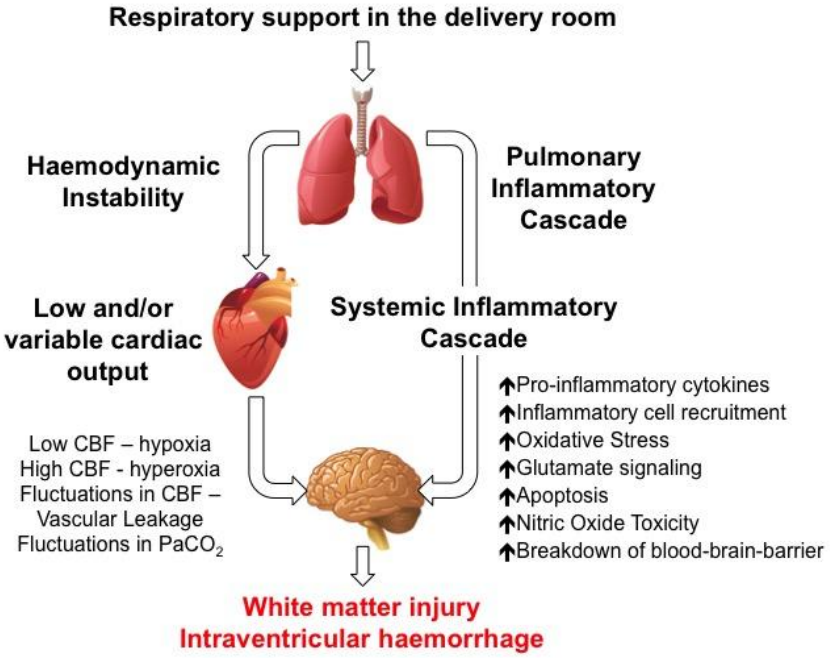

Figure 1. Mechanisms underlying how the initial respiratory support in the delivery room can lead to down-stream inflammation and injury, particularly to the preterm brain.
(Barton et al. Front Pediatr. 2015; 3: 97).

Abstract

18

High Intensity Focused Ultrasound (HIFU): A method of non-invasive placental vascular occlusion

Shaw, C.J.[1,2], Rivens, I.[3], Civale, J.[3], Botting, K.J.[1], Niu, Y.[1], ter Haar, G.[3], Giussani, D.A.[1], Lees, C.C.2. [1]*Department of Physiology, Development and Neuroscience, University of Cambridge, UK;* [2] *Institute of Developmental and Reproductive Biology, Imperial College London, UK;* [3]*Joint Department of Physics, Institute of Cancer Research, London, UK*

Introduction: HIFU can non-invasively occlude blood vessels, thus may represent a lower risk method of placental vascular occlusion in complicated monochorionic pregnancy, compared to current invasive techniques. We have shown HIFU can safely and effectively occlude placental vasculature in the instrumented fetal sheep model when applied through the uterus with short term follow-up (Shaw et al. *Sci. Transl. Med.* in press). To further develop this technique, we applied HIFU through intact maternal abdominal skin and assessed subsequent fetal and maternal recovery up to term.

Methods: Twelve pregnant sheep were anaesthetised at 116±2d gestational age (term ~148d) and HIFU (n=6) or no (n=6) exposures of up to 6 placental vessels were performed through intact maternal abdominal skin, targeted with colour Doppler. HIFU exposures were 1.66 MHz, 5s duration, free field $_{(IPSTA)}$ 2000-5000 W.cm^{-2}. Ewes and fetuses were subsequently surgically instrumented with vascular catheters and femoral flow probes and recovered from anaesthetic. A bespoke wireless data acquisition system (CamDAS), which records continuous maternal and fetal cardiovascular data, and daily blood sampling were used to assess recovery for 20 days.

Results: Based on a comparison of pre and post treatment colour Doppler imaging, 92% (33/36) of placental vessels were occluded in a single HIFU exposure series; the remaining 3/36 vessels were occluded following a retreatment. All fetuses survived to the end of the 20 day follow-up period, and no differences in maternal or fetal blood pressure, heart rate, metabolic status or oxygenation was observed between treatment groups (fig 1a-d). The expected ontogenic changes in blood pressure, heart rate and femoral blood flow in the fetus occurred in both groups (fig 1b). There were no significant adverse events.

Conclusions: HIFU appears to be an effective method of placental vascular occlusion, and may be translatable to human pregnancy. Based on these findings, it is well tolerated by the mother and fetus.

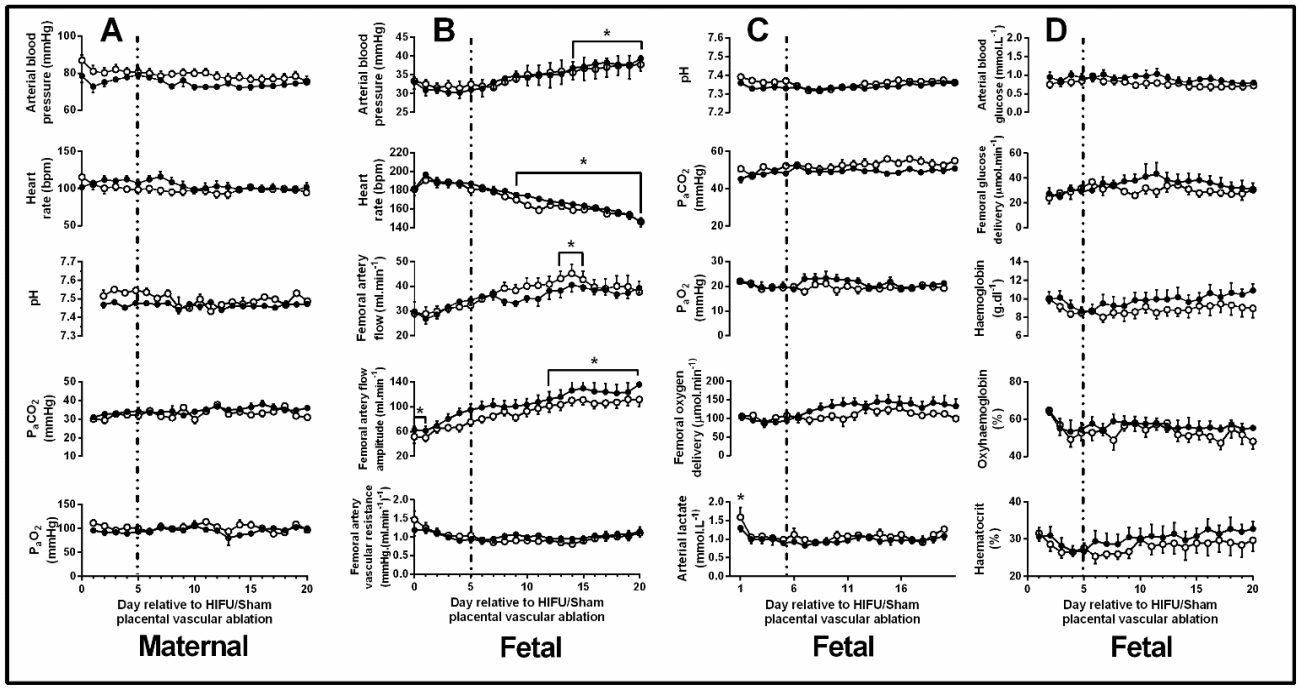

Figure 1. Maternal and fetal cardiovascular and metabolic recovery from HIFU/sham placental vascular occlusion. Values represent daily mean ± SEM (closed circle: HIFU, n=6; open circle: sham, n=6) for maternal and fetal cardiovascular and metabolic values during the 20 days of post occlusion follow-up. Recovery from surgery and anaesthesia was judged to be complete on day 5 (dashed line) and this forms forms the baseline. Significant differences: * $p < 0.05$ time vs. baseline (day 5), repeated measures 2 way ANOVA with post hoc Tukey's test. test.

Abstract

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A case-control study on effect of a novel treatment administering tadalafil for fetal growth restriction

Kubo, M., Nii, M., Maeda, Y., Shimura, M., Magawa, S., Kawamura, T., Tanaka, H., Murabayashi, N., Osato, K., Umekawa, T., Kamimoto, Y. and Ikeda, T.

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Introduction: In fetal growth restriction (FGR), clinicians are often forced to end the pregnancy for prematurity on the fetus with an associated high risk of adverse neonatal outcome. We used PDE-5 inhibitor, Tadalafil successfully in some cases of FGR. The aim of this retrospective case control study was to assess Tadalafil treatment in pregnant women with FGR in terms of maternal and neonatal outcomes.
Methods: We retrospectively analyzed 11 singleton pregnant women with FGR who received Tadalafil treatment along with conventional management for FGR (Tadalafil treatment group) compared with 14 control singleton pregnant women who only received the conventional management for FGR (conventional management group). The conventional management for FGR was performed on the basis of the guidelines for obstetrical practice in Japan.
Results: Fetal growth velocity from enrollment to birth were significantly higher in the Tadalafil treatment group than in the conventional management group (17.7 (95%CI: 10.6-23.0) versus 12.8 (95%CI: 0-17.2) g/day, respectively; P<0.05).
Conclusions: Tadalafil may be a novel treatment for FGR with growth arrest.

Table1 Maternal and perinatal outcomes of pregnancies with FGR treated with and without tadalafil.

	Conventional management group (n=14)	Conventional management with tadalafil group (n=11)	P value
Fetal growth velocity (g/day)*	12.8 (0, 17.2)	17.6 (10.5, 23.0)	*<0.05*[a]
Eligibility-to-delivery interval (days)	28.0 (9.8, 41.8)	42.0 (21, 68)	0.06[a]
Gestational age at birth (days)	233.0 (206.8, 259.5)	258.0 (248, 263)	0.06[a]
(Median expressed as weeks and days)	(33 weeks + 2 days)	(36 weeks + 6 days)	
Cesarean section, n (%)			
Birth weight (g)	1384 (870.3, 1949)	1990 (1488, 2168)	*<0.05*[a]
Male, n (%)	6 (46.2)	3 (27.3)	0.34[b]
Placental weight (g)	302.5 (213.8, 425)	406.0 (315, 425)	0.15[a]
Apgar score (1 min)			
Apgar score (5 min)			
Umbilical artery pH			

Data were shown as median (interquartile range). a: Mann–Whitney test. b: Chi-square test.

*Fetal growth velocity (g/day) was calculated as defined in the methods section.

Table 1. Maternal and perinatal outcomes of pregnancies with FGR treated with and without tadalafil. Fetal growth velocity were significantly higher in the Tadalafil treatment group than in the conventional management group.

Abstract

20

Fetal ECG and heart rate are altered in the perinatal period following exposure to chronic maternal hypercortisolemia

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[1]University of Florida Departments of Pharmacodynamics and Physiology and [2]Functional Genomics

Introduction: Experimental data from animal studies and clinical data from human studies indicate that the late gestation fetal heart may be particularly susceptible to either precocious or persistent glucocorticoid signalling. Our group has found a marked increase in perinatal death, cardiac structural changes, and an increase in apoptosis of cardiac Purkinje fibers in an ovine model of chronic stress in late pregnancy. Thus, we investigated the effect of cortisol on fetal blood pressure and ECG.

Methods: In this model, the ewe is infused with 1 mg·kg-1·day-1 cortisol from 115d gestation to term (CORT). Four ewes with singleton fetuses were assign to CORT at 115 days of gestation. Five ewes were not treated. DSI radiotelemetry devices were implanted into the fetuses at 119±1d for continuous monitoring of ECG, HR, and aortic and amniotic fluid pressures through delivery. We used DSI software to analyze 24h means of MAP and HR after surgery and over the 14d before birth, 1h means in ECG and HRV parameters on each day for 14d before birth, and 1h means during the last 24h of fetal life and 10m means in the final 1h before birth of HR, MAP, and ECG parameters. Data were analyzed using two-way ANOVA corrected for repeated measures across time with comparison of individual time points by t-test with Bonferroni adjustment.

Results: Maternal cortisol infusion had no significant effect on MAP, HR, or parameters of HRV (LF:HF) in the period from -14d to birth. CORT significantly prolonged the fetal P and P-R intervals of the ECG over the last 24h and 1h before birth. CORT also significantly reduced fetal HR in the last hour before birth, and increased P:PR interval and Tpe:QTcf ratio (interval from the peak to the end of the T wave relative to the corrected QT interval) in the hour before birth.

Conclusions: Although higher doses of glucocorticoids induce fetal hypertension, this increase in CORT did not increase MAP. Nevertheless, CORT produced pathophysiological changes in the cardiac conduction system. P-R elongation also occurred during recovery from surgery, suggesting the maladaptive effects of CORT become evident during periods of cardiac stress.

	-14 Days to Birth		24 Hours Before Birth		1 Hour Before Birth	
	Control	Cortisol	Control	Cortisol	Control	Cortisol
MAP (mmHg)	51.4±2.9	44.4±3.3	56.3±3.7	49.2±4.2	58.0±3.7	50.3±4.2
HR (BPM)	164±6	160±7	154±7	149±7	162±8	135±9*
HRV (LF:HF)	3.28±0.31	3.16±0.35	-	-	-	-
P Interval (msec)	30±2	35±2	33±2	43±3*	33±3	51±3*
P-R Interval (msec)	78±2	86±3	75±3	90±3*	73±4	96±5*
P:PR	0.38±0.02	0.42±0.02	0.44±0.03	0.50±0.03	0.45±0.01	0.53±0.01*
Tpe:QTcf	0.14±0.01	0.11±0.01	0.09±0.02	0.12±0.02	0.09±0.01	0.14±0.02*

Table 1. Fetal measurements of mean aortic pressure (MAP) heart rate (HR), heart rate variability (HRV; LF:HF, low to high frequency power) and the ECG parameters P interval, P-R interval, P to PR ratio and Tpe to QTcf ratio. Values are mean±SEM; *, P<0.05.

Abstract

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The longitudinal effects of persistent apnoea during sleep on cerebral oxygenation in ex-preterm infants

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Background: While apnoea of prematurity in preterm infants is usually resolved by near-term equivalent age, apnoea of shorter durations may persist during infancy. The aim of our study was to assess the incidence and impact of persistent apnoea on heart rate (HR), oxygen saturation (SpO_2) and brain tissue oxygenation index (TOI) over the first 6 months after term equivalent age.
Methods: 24 preterm infants 13M/11F born between 27-36 weeks of gestational age were studied with daytime polysomnography at 2-4 weeks, 2-3 months and 5-6 months post-term corrected age. Apnoeas lasting ≥3s were included and % changes in HR, SpO_2 and TOI (NIRO-200 Hamamatsu) from baseline were analysed.
Results: 253 apnoeas were recorded at 2-4 weeks, 203 at 2-3 months and 148 at 5-6 months. Infants had between 5-27 apnoeas at 2-4 weeks, 1-27 at 2-4 months and 1-16 at 5-6 months with an average of 3 per hour at all three studies. Apnoea duration was significantly longer at 2-4 weeks (mean ± sem, 4.6 ± 1.4 s) compared to 5-6 months (4.4 ± 0.9 s, $p<0.05$). There was no effect of gestational age, sleep state or sleep position on apnoea duration, nadir HR, nadir SpO_2 or nadir TOI. At 2-4 weeks nadirs in HR, SpO_2 and TOI were all positively correlated with apnoea duration ($R=0.402$, $p<0.001$; $R=0.314$, $p<0.001$; $R=0.137$, $p<0.05$ respectively). At 2-3 months nadirs in HR and TOI were correlated with apnoea duration ($R=0.283$, $p<0.001$; $R=0.195$, $R= 0.220$ $p<0.01$ respectively) and at 5-6 months only HR nadir was positively correlated with apnoea duration ($R=0.240$, $p<0.01$). At 2-4 weeks the nadirs in HR (-6.9 ± 0.6 bpm), and TOI (-2.7 ± 0.2%) were significantly less than at 2-3 months (nadir HR: -9.5 ± 0.7 bpm, $p<0.01$ and nadir TOI: -4.6 ± 0.3 %, $p<0.001$) and at 5-6 months (nadir HR -9.8 ± 0.8 bpm, $p<0.05$, and nadir TOI -5.5 ± 0.6%, $p<0.001$).
Conclusions: In ex-preterm infants apnoeas were frequent and apnoea duration was correlated with falls in HR, SpO_2 and TOI over the first 6 months after term equivalent age. These short apnoeas were associated with decreases in cerebral oxygenation, which were more marked at 2-3 months and 5-6 months than at 2-4 weeks. Although events were short they may contribute to the adverse neurocognitive outcomes which are common in ex-preterm children.

Notes for Oral Session IV

Oral Session V

Developmental Cardiovascular Physiology A

Keynote Speaker: Sandra Davidge
Chair: Eugenie Lumbers

Keynote Speaker and Session Chair

Professor Sandra T. Davidge

Professor Sandy Davidge received her PhD from the University of Vermont in 1993 and continued her training as a postdoctoral fellow at the Magee Women's Research Institute Institute in Pittsburgh, Pennsylvania under the mentorship of Professor Jim Roberts. She She then moved to Edmonton, Alberta Canada in 1996 for her first faculty position at the University of Alberta. Professor Davidge is currently the Director of the Women and Children's Health Research Institute, Professor in the Departments of Obstetrics & Gynecology and Physiology at the University of Alberta and holds a Tier 1 Canada Research Chair in Maternal and Perinatal Cardiovascular Health. She is the President-elect (2016) for the Society of Reproductive Investigations (formally SGI) and past President of the North American Perinatal Research Society (2006-2007). She serves on numerous scientific committees and editorial boards in the United States and Canada. Professor Davidge's research has been recognized for Excellence in Research with the the President's Scientific Achievement Award from the international Society Gynecologic Investigation (2003) and was elected Fellow in the Canadian Academy of Health Sciences (2014). Her research program encompasses studying vascular function as it relates to: 1) complications in pregnancy (preeclampsia and maternal aging) and 2) developmental origins of adult cardiovascular diseases. Both preeclampsia and aging are associated with vascular endothelial dysfunction and oxidative stress; hence her research program focuses on common vasoactive mediators altered by pro-oxidants in these conditions that are unique to the reproductive stage of women. The Davidge laboratory laboratory combines their expertise in pregnancy research and aging to understand long-term consequences of an adverse pregnancy on cardiovascular health of the offspring as they age. Ultimately, the goal is to develop preventative/therapeutic strategies for cardiovascular complications, particularly as it relates to maternal and perinatal perinatal health. Professor Davidge has published over 200 peer-reviewed manuscripts and review articles in these areas and is funded by multiple operating grants from the Canadian Institutes for Health Research.

Emeritus Scientia Professor Eugenie R Lumbers AM FAA Dist FRSN

Eugenie Lumbers graduated in medicine (MBBS) in 1965, gained a doctorate in medicine in 1971 and in science in 1986. She was a CJ Martin Fellow and Junior Research Fellow at Wolfson College, Oxford, where she studied with Dr Joan Mott at the the Nuffield Institute for Medical Research. In 1974 she joined the School of Physiology and Pharmacology at UNSW and gained a personal chair in 1988. She became a Scientia Professor in 1998, a Fellow of the Australian Academy of Science in 2002, a Distinguished Fellow of the Royal Society of NSW in 2011, and a member of the Order of of Australia in 2012. She has had a national and international profile since her discovery discovery of inactive renin (prorenin). Eugenie made significant discoveries in adult and fetal cardiovascular physiology on inhibition of the vagal component of the cardiac baroreflex by angiotensin, the fetal cardiovascular effects of cortisol and through her work in fetal cardiovascular physiology and renal function. She extended this research into the study of the fetal origins of adult disease with particular reference to the impact of of maternal renal dysfunction on fetal renal development. From 2003-2007 Eugenie retired and went sailing. In 2008 she began work at University of Newcastle on the role(s) of the human intrauterine renin angiotensin system (RAS). Her current projects include the impact of the early RAS on placental development and pregnancy outcome, its role in preterm birth and the role of the prorenin receptor and the RAS in the growth and spread of endometrial and cervical cancer. She has also maintained an active role in studying the impact of maternal health in Indigenous women on pregnancy outcome and infant welfare.

Eugenie holds professorial appointments at UNSW (Emeritus Professor), University of Newcastle and University of Queensland. She is married to Bill Forbes. They have 3 children and 5 grandchildren.

Abstract

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Pregnancy complications and programming of cardiovascular dysfunction in adult offspring

Davidge, S.T.

Women and Children's Health Research Institute, Departments of Obstetrics & Gynecology and Physiology, University of Alberta, Edmonton, Alberta Canada

Abundant evidence exists that a compromised prenatal (and early postnatal) environment leads to an increased risk of hypertension and cardiovascular (CV) disease later in life. Our laboratory is focused on understanding the pathophysiological outcomes for offspring born from compromised *in utero* environments including prenatal hypoxia and maternal aging. We have focused on these pregnancy complications as fetal hypoxia is one of the most common consequences of complicated pregnancies worldwide and advanced maternal age is becoming increasingly common, and is associated with increased maternal and fetal morbidity and mortality. Our studies show that adult offspring born from these complicated pregnancies have impaired endothelial-dependent vascular function, enhanced cardiac susceptibility to ischemia and reperfusion injury and indices of the metabolic syndrome when exposed to a high fat diet. Our laboratory has been focused on identifying plausible mechanisms and implementing early life interventions to protect against adverse programming. Our data show that simultaneous resveratrol supplementation when exposed to a high-fat diet in early life prevented cardiac dysfunction after ischemia/reperfusion injury in adult male and female offspring exposed to prenatal hypoxia. We have also recently demonstrated a beneficial effect of resveratrol supplementation in later life at a time of known CV dysfunction in both male and female hypoxic exposed offspring. However, resveratrol increased cardiac p-AMPK and SOD2 levels in only female offspring. We have also assessed a non-pharmacological interventional approach using exercise. Exercise is an effective preventive intervention for CV diseases; however, it may be detrimental in conditions of compromised health. With exercise training, we showed improved baseline cardiac performance in male control offspring but a reduced baseline cardiac performance in offspring exposed to prenatal hypoxia. Interestingly, exercise decreased superoxide generation in control offspring while the polar opposite effect occurred in offspring exposed to prenatal hypoxia; thus for male offspring exposed to prenatal hypoxia, exercise may be a secondary stressor on cardiac function.

Perspectives: There are several promising avenues of research into the mechanisms involved in the programming of CV diseases and potential treatment strategies, however understanding mechanisms and the impact of secondary stressors/co-morbidities is critical. Nevertheless, the use of early postnatal intervention with antioxidants or resveratrol to improve health outcomes in adult offspring might prove to be beneficial in the reversal and/or prevention of CV disease originating from early life beginnings.

Abstract

23

Vascular morphological and anti-proliferative changes induced by melatonin treatment in chronic hypoxic newborn lamb with pulmonary hypertension

Astorga, C.R.[1,2], Gonzalez-Candia, A.[1]., Candia, A.[1], Figueroa, E.G.[1], Villanueva C.A.[1], Ebensperger, G.[1], Reyes, R. V.[1], Llanos, A.J.[1,3], Herrera, E.A.[1,3*].

[1]*Pathophysiology Program, Faculty of Medicine University of Chile, Santiago, Chile;* [2]*Facultad de Medicina, Universidad de los Andes;* [3]*International Center for Andean Studies*
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Introduction. Chronic hypoxia during gestation lead to neonatal pulmonary hypertension (NPH)[1]. Vascular remodeling is one of the main hallmarks of this disease and includes decreases in media/lumen ratio and increases smooth muscle cells (SMCS) proliferation[2,3]. These morphological changes have been associated with oxidative stress[4]. Melatonin is a neurohormone with important antioxidant properties[5]**.** Therefore, we studied the vascular anti-remodeling effects of melatonin in NPH induced by hypoxia.
Methods. Twelve neonatal lambs were gestated in chronic hypobaric hypoxia and divided into two groups: 6 control (CN, EtOH 1.4%, 0.5 $ml.kg^{-1}.d^{-1}$ oral**)** and 6 melatonin treated (MN,1 $mg.kg^{-1}.d^{-1}$ melatonin in EtOH 1,4%, 0,5 $ml.kg^{-1}.d^{-1}$ oral) between days 4-11 of life. At 12 days old lambs were euthanized and pulmonary samples collected for histomorphometric study and determination of vascular remodeling markers such as α-actin and myosin heavy chain (MHC). Proliferation were studied by immunohistochemistry (Ki67+ cells) and western blotting (p21 and Cyclin D1 expression). Bioethics Committee approved this study.
Results and Discussion. Melatonin treatment increases luminal area/vascular area ratio. Also, decreases α-actin expression and ki67+ cells (%) without alters myosin heavy chain (MHC) expression. There were no changes in p21 and there is a trend in decreased expression of the Cyclin D1 that is why it is possible that this morphological and anti-proliferative changes have been regulated by others keys mediators.
Conclusion. Postnatal melatonin treatment alters vascular remodeling by increasing luminal area/vascular area ratio and decreasing SMCs proliferation.

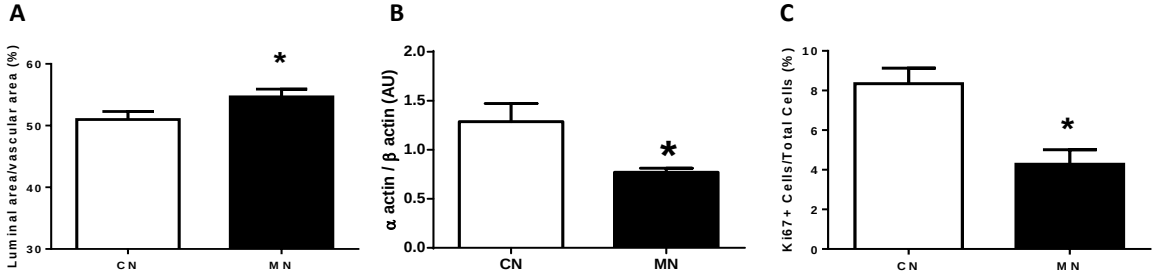

Figure 1: Vascular morphological and anti-proliferative changes induced by melatonin treatment in PNH. **A.** Luminal area/vascular area, **B.** α-actin expression **C.** Ki67+ cells/total cells. Average ± SEM for control (CN), and postnatal melatonin treated neonates (MN). Significant differences (t-test, $p<0.05$): * vs CN.

Funding: FONDECYT 1130424, 11130232, 1140647 & 1151119.
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Abstract

24

Vaughan, O.R., Rossi, C.A., Ginsberg,Y., Krishnan,T.I., Barker,H., White, A., and **David, A.L.**

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Abstract

25

Maternal obesity during pregnancy alters microRNAs that regulate circadian clock genes in the fetal mouse heart

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Human Development and Health, Institute of Developmental Sciences, University of Southampton Faculty of Medicine, Southampton General Hospital (mailpoint 887), Southampton SO16 6YD, UK

Introduction: Maternal obesity and diabetes are prevalent during pregnancy and have long-term consequences on the offspring's health. Metformin (MET) is prescribed to treat gestational diabetes with unknown impact on the fetus. In pregnant mice, maternal obesity results in altered blood pressure and heart size in the adult offspring. Whether this originates during fetal development is unknown. A potential underlying mechanism involves microRNAs which are implicated in cardiac differentiation, remodelling and disease. We investigated whether high fat (HF) diet-induced maternal obesity with or without MET alters microRNA expression and expression of the target mRNAs within the fetal heart.

Methods: Six weeks before conception and during pregnancy, female C57/BL6J mice were fed HF (45% kcal fat) or control (C, 7% kcal fat) diet with half of each group given MET in drinking water (250mg/kg bodyweight/day). This generated four groups (n=4 per group): C, C+MET, HF and HF+MET. On day 16 of pregnancy dams were killed and fetal hearts collected. MicroRNA expression was measured using small RNA sequencing (Illumina miRNA-Seq). Differential expression analysis identified up and down-regulated microRNAs and pathway analysis identified their target genes. Gene expression levels of *Clock*, *Bmal1*, *Per1, Cry1, Per2, Cry2*, *Sirt3* and *Fto* were measured by qRT-PCR. Data were analysed using two-way ANOVA.

Results: 33 microRNAs in HF and 48 microRNAs in C+MET fetal hearts were expressed differentially compared to controls. Pathway analysis identified circadian rhythm as a potential microRNA regulated gene pathway altered by both maternal obesity and MET. Maternal obesity reduced fetal heart mRNA expression of the clock genes *Per2*, *Cry1* and *Cry2* vs lean mothers and MET reduced expression of *Cry1* and *Fto* vs control mothers ($p<0.05$).

Conclusion: This study identified microRNAs that alter the circadian rhythm pathway in fetal hearts in response to maternal obesity and MET. The circadian genes *Per2, Cry1* and *Cry2* are targets of these microRNAs. This suggests that maternal obesity during fetal development could alter the expression of microRNAs and their target genes in fetal heart. This may underlie the associated cardiovascular dysfunction in postnatal life.

This work is supported by the BBSRC & Diabetes UK

Abstract

26

Preterm birth coupled with antenatal glucocorticoid treatment increases cardiac MR and 11β-HSD1 in adult life

Darby, J.R.[1], **Berry, M.** [2,3]*, Dyson, R.[2], Gray, C.,[2] and Morrison, J.L.[1*]

*[1]Early Origins of Adult Health Research Group, School of Pharmacy & Medical Sciences, Sansom Institute for Health Research, University of South Australia, Adelaide, SA, Australia. [2]Centre for Translational Physiology, University of Otago, Wellington, New Zealand. [3]Department of Paediatrics and Child Health, University of Otago, Wellington, New Zealand. *=equal contribution*

Introduction: Globally ~15 million babies are born preterm each year. The short-term morbidity associated with preterm birth is well described and, although the long-term cardiometabolic health risks in adults born preterm are recognised, their mechanistic basis is unclear. Herein, we used a guinea pig model of medically induced moderate preterm birth, designed to closely relate to human preterm pregnancy by preserving maternal-infant bonding and antenatal corticosteroid exposure.

Methods: Betamethasone (1 mg/kg) was administered to pregnant guinea pigs 48 and 24h before induction of preterm labour (62d gestation; term=69d; n=37), to mimic the gold-standard of care for women at risk of preterm delivery. The control group consisted of term-born pups with no betamethasone exposure (n=34). Cardiac tissue was collected at 28d (childhood) and 9 months (adulthood). qRT-PCR was used to determine the mRNA expression of molecules involved in the regulation of cardiac development. Data was analysed using a 3 way ANOVA (factors: preterm, age, sex).

Results: Preterm birth did not alter the mRNA expression of the glucocorticoid receptor (GR) or 11β-HSD2, which converts cortisol to cortisone, at either age. However, at 9 months of age, pups that were born preterm had an increase in the mRNA expression of the mineralocorticoid receptor (MR; Figure 1) and 11β-HSD1, the cortisone to cortisol converting enzyme, compared to those born at term. MR is associated with fibrosis, however, there were no changes in the mRNA expression TGF-β and MMP-2. Furthermore, these changes were consistent across both sexes.

Conclusions: In this study, we have used an animal model that is closely comparable to human preterm birth to show show that adults born preterm have altered cardiac mRNA expression of MR and 11β-HSD1. MR signalling has previously been implicated in cardiac hypertrophy and the onset of fibrosis independent of increased blood pressure. Consequently, the finding that adults born preterm have increased cardiac mRNA expression of MR may shed some light on the molecular pathways involved in the cardiac changes underlying cardiometabolic risk found in preterm-born preterm-born adults. Further histological analysis to determine whether there is increased fibrosis in the myocardium is is underway.

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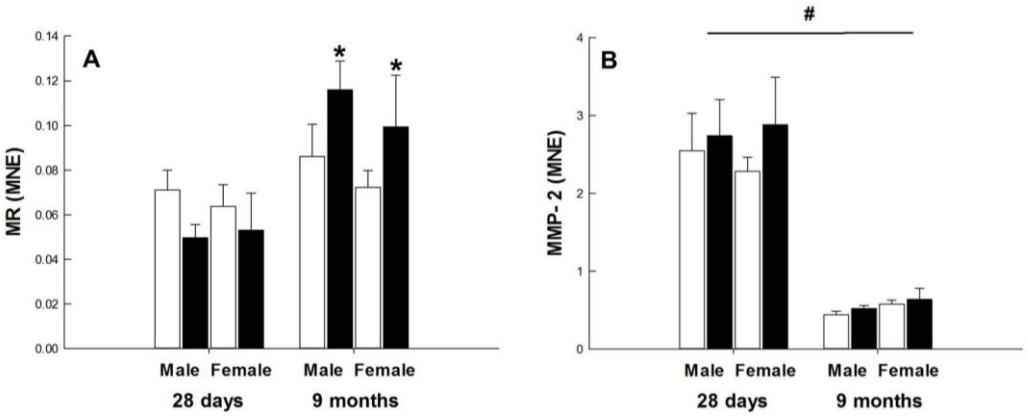

Figure 1. Increased mRNA expression of the mineralocorticoid receptor (MR, A) and no change in matrix metalloproteinase-2 (MMP-2; B) in preterm born offspring at 28d and 9 months after birth. Open bars, term; black bars, preterm. *, preterm effect; #, age effect.

Notes for Oral Session V

Oral Session VI

Developmental Cardiovascular Physiology B

Chair: Frank Bloomfield

Session Chair

Professor Frank Bloomfield

Professor Bloomfield studied medicine at the University of Manchester, taking a First Class BSc (Hons) in Experimental Immunology and Oncology during an Intercalated year before graduating with MBChB in 1990. He then trained as a paediatrician in the North-West of England gaining his MRCP in 1994 before emigrating to Auckland, New Zealand. He completed his training as a neonatal paediatrician in Auckland, being awarded his FRACP in 2000, and also studied for a PhD under the mentorship of Professor Jane Harding, graduating in 2000. A post-doc in Toronto, Canada, with Professor John Challis followed between 2000 and 2002, where he also worked as a neonatologist on the neonatal emergency retrieval team at the Hospital for Sick Children. Frank returned to Auckland and the Liggins Institute in 2002 and has worked as a clinical academic, combining research with a clinical role as a Neonatal specialist at National Women's Hospital. He currently is Director of the Liggins Institute, Immediate Past President of the Perinatal Society of Australia and New Zealand, Council member of the Perinatal Research Society of the USA and a member of NZ's National Maternity Monitoring Group, which has oversight for quality of all maternity services in New Zealand. Frank has been secured >NZ$28 million in research funding to support studies spanning large animal physiology, laboratory studies and clinical research into fetal and neonatal growth, nutrition and development.

Abstract

27

In fetal cerebral arteries, chronic hypoxia attenuates NO- and cGMP-induced vasorelaxation by inhibiting PKG colocalization with BK channel proteins

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Center for Perinatal Biology, Loma Linda University School of Medicine
Loma Linda, California, 92350 USA

Introduction: Chronic hypoxia attenuates nitric oxide (NO) and cGMP induced vasorelaxation in fetal cerebral arteries. Because cGMP-dependent protein kinase (PKG) mediates much of NO and cGMP signaling in vascular smooth muscle, we tested the hypothesis that hypoxia diminishes the ability of PKG to regulate target proteins that cause vasorelaxation. Prominent among proteins that influence vascular tone is the large conductance calcium-sensitive potassium (BK) channel, which is a substrate for PKG and is responsive to phosphorylation on multiple serine/threonine residues. Given the importance of this channel in the regulation of vascular tone, we also examined whether hypoxia attenuates PKG and BK channel protein abundances and PKG kinase activity.
Methods: Middle cerebral arteries were harvested from normoxic and hypoxic (altitude of 3,820 m for 110 days) fetal and adult sheep. In fresh arteries we assessed effects of 8-pCPT-cGMP and iberiotoxin on 5-HT dose-response relations. From artery homogenates we measured PKG activity and abundances, and abundances of BK channel α- and β1-subunits. We quantified protein colocalization using confocal microscopy of fixed 5 μm sections following double immunostaining for various protein pairs.
Results: The BK inhibitor iberiotoxin attenuated vasorelaxation induced by 8-pCPT-cGMP in normoxic but not hypoxic arteries in both age groups. Neither expression nor activity of PKG-I were significantly affected by chronic hypoxia in fetal or adult arteries. The spatial proximities of PKG with BK channel α- and β1-proteins were strongly dissociated by hypoxia (**Fig.1**).
Conclusions: These results support the hypothesis that in fetal lamb cerebral arteries, hypoxia reduces the ability of PKG to attenuate vasoconstriction in large part through suppression of the ability of PKG to associate with and thereby activate BK channels.

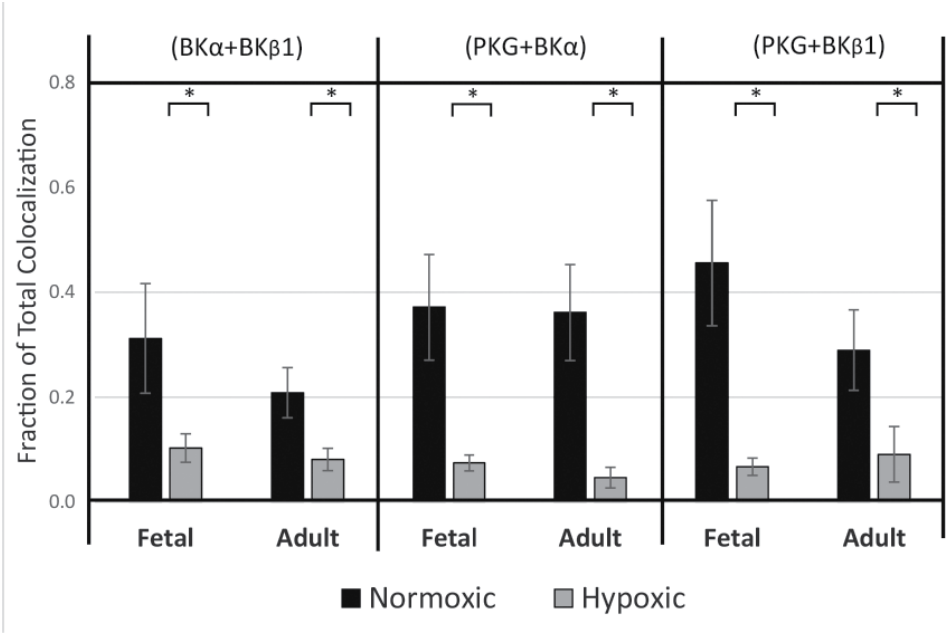

Figure 1. Chronic hypoxia attenuated colocalization among BK, BK, and PKG. PKG. Confocal colocalization colocalization expressed as a fraction of all colocalized pixels reveled that chronic hypoxia significantly reduced associations among all three protein pairs (BKα with BKβ1, BKβ1, PKG with BKα, and PKG with BKβ1) in both fetal and adult arteries. These data suggest that hypoxia reduces activation of BK channels by decreasing their spatial proximity to, and phosphorylation by, PKG. *=P<0.05). Error bars indicate SEM for n≥6 for all groups.

Abstract

28

Cardiovascular and metabolic health of aged adult offspring derived from mothers deficient in B-vitamins during the periconceptional period

Gardner, D.S.,[1] Karamitri, A.[1,2], Kwong, W.Y.[2], Emes, R.E.[1] and Sinclair, K.D.[2]

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Introduction: Adult health can be substantially influenced by events that occur during pregnancy, particularly the period around conception. We have shown that suboptimal B-vitamin nutrition around conception makes young adult offspring more obese, hypertensive and insulin resistant[1]. All these measured variables are markers of increased susceptibility to metabolic syndrome, which tends to develop with advanced age. In this study, we revisit the same population of animals as previously reported[1], but at the more advanced age of 6 years (c.f. 1.5 - 2 years) to determine whether their health has deteriorated to such an extent that clinical signs of disease are evident (e.g. further increases in blood pressure, or glucose intolerance and insulin resistance or development of osteoporosis).

Methods: In all sheep, we characterised resting and stimulated cardiovascular function by telemetry, metabolic competence by GTT and ITT, body composition by DXA and, after euthanasia, conducted genome-wide analyses of methylated DNA (MBD-Seq) using in-house protocols.

Results: In offspring at 2-years of age, we demonstrated that physiologically relevant reductions in dietary supply of one-carbon metabolites can modify DNA methylation to influence offspring health and physiology (e.g. higher blood pressure in males)[1]. With advancing age, we find that such 'epigenetic-programming' does not always produce unidirectional adverse outcomes. Whilst age deteriorated some physiological functions in control animals (e.g. DBP increased from ~ 86±2 to 108±1), the phenotype of those exposed to a methyl-deficient diet did not appear to have deteriorated; DBP remained stable at 96±2 & 91±1 mm Hg at 2 and 6 years, respectively with no marked change in glucose or insulin handling (see Figure). Nevertheless, aged sheep exposed to a methyl-deficient diet retained an 'epigenetic imprint' of maternal diet.

Conclusions: For the first time in a large animal model, we report that the direction of change of an adverse 'epigenetically-programmed' phenotype as individuals age is not necessarily unidirectional towards increased non-communicable disease.

1. Sinclair KD, et al. *PNAS* **104,** p19351 (2007).

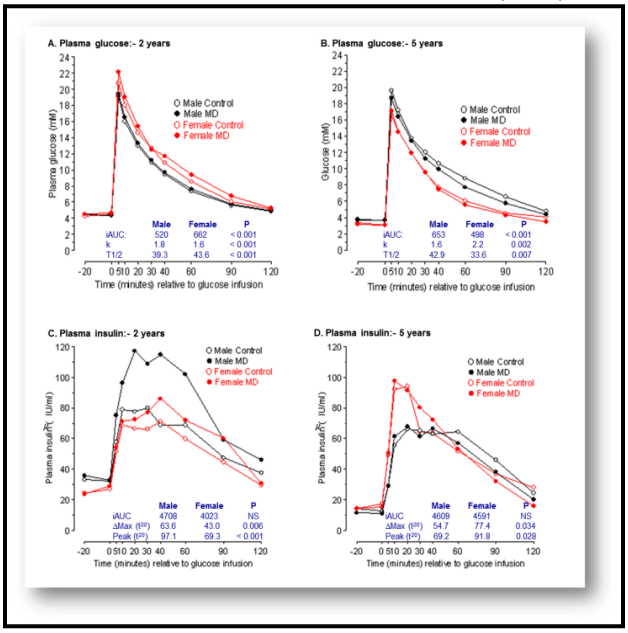

Figure 1. Glucose and Insulin dynamics during a GTT. Glucose and Insulin were measured in plasma obtained by venepuncture at timed intervals after an *i.v.* bolus of glucose (time zero; 0.4g/kg BW^{-1}). Data are; Control (Male, n=7; Female, n=10) and MD-Diet (Male, n=6; Female, n=10). iAUC, incremental area-under-response curve. *K*=rate constant (mM/min) and T½=half-life for clearance of glucose; MD, Methyl-deficient periconceptional diet. Statistics conducted using RM-ANOVA (Genstat, VSNi, UK).

Abstract

29

Sex dependant cardiac effects of a postnatal Western diet: exacerbated by low birth weight?

Darby, J. R[1]., Regnault, T.R.H. [2,*] & Morrison, J.L.[1,*]

[1]Early Origins of Adult Health Research Group, School of Pharmacy & Medical Sciences, Sansom Institute for Health Research, University of South Australia, Adelaide, SA, Australia
[2]Department of Obstetrics and Gynaecology, Western University, 1151 Richmond Street, London, ON, Canada

Introduction: Low birth weight (LBW)/intrauterine growth restriction (IUGR) as a result of an adverse *in utero* environment is a risk factor for the onset of cardiovascular disease (CVD) in adult life. These LBW offspring experience accelerated catch up growth after birth and exhibit metabolic inflexibility when consuming a high saturated fat and high sugar Western diet (WD), which is abundant in our society today. We assessed the independent and possibly additive effects of placental insufficiency induced LBW and a postnatal WD on the heart.
Methods: LBW was induced by uterine artery ablation in guinea pigs at ~32 days gestation (term=68 days). Resulting male and female pups were classified into normal birth weight (NBW; >85g), or LBW (<85g) groups, and weaned onto either a control diet (CD) or WD. Left ventricle tissue samples were collected at 150 days after birth and qRT-PCR was used to determine the mRNA expression of key cardiac growth and metabolic genes. There was an effect of sex (3 way ANOVA) and thus, data was split by sex and a 2 way ANOVA performed.
Results: LBW-WD males had increased cardiac IGF-2 mRNA expression and WD males had increased IGF-2R mRNA expression independent of birth weight. In addition, MHC-α and MMP-2 mRNA expression was also increased in WD males, and effect greater in LBW-WD males. WD fed males also displayed increased CPT-1β and PGC-1α (Figure 1) mRNA expression and again these effects were more pronounced in LBW-WD. Basal glucose transporter GLUT-1 mRNA expression was decreased in LBW-WD males, but not in NBW-CD or WD males. Interestingly, there was no effect of diet on the expression of these molecules in females; however, LBW females had decreased expression of both CPT-1β and PGC-1α irrespective of diet.
Conclusions: There appears to be a sexual dimorphism between the mRNA expression changes in cardiac growth and metabolic pathways as a result of both birth weight and postnatal WD. WD changes the expression of signalling molecules in males and LBW may enhance these effects. The lack of changes in the female may suggest a detrimental detrimental failure to mount an appropriate metabolic response to the WD.

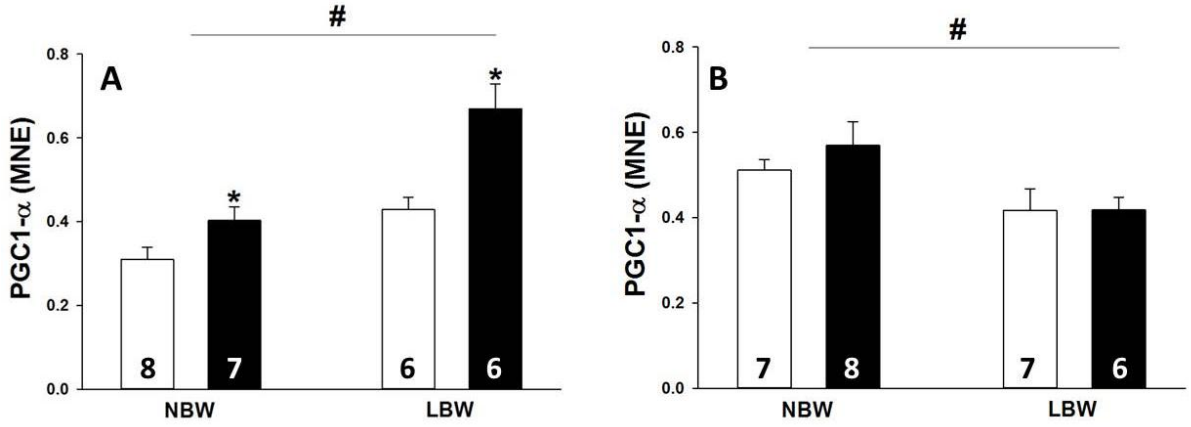

Figure 1. Differential effect of diet and birth weight on the mRNA expression of PGC1-α in males (A) and females (B). (B). Open bars, CD; black bars, WD. *, diet effect; #, birth weight effect.

Abstract

30

The effect of intrauterine inflammation on left ventricular cardiomyocyte growth and maturation in preterm lambs

Vrselja, A.[1], Ahmadi-Noorbakhsh, A.[2], Noble, P. B.[2], Pillow, J. J.[2,3], & Black., M. J.[1]

[1]Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia; [2]School of Anatomy, Physiology & Human Biology; and [3]Centre for Neonatal Research and Education, School of Paediatrics and Child Health, University of Western Australia, Perth, Australia

Introduction: Cardiomyocytes, the functional units of the heart, undergo a process of maturation and differentiation during late gestation whereby they progressively lose their proliferative capacity. Preterm birth and exposure to intrauterine inflammation (a common antecedent of preterm birth) have the potential to impact the proliferation and maturation of cardiomyocytes and ultimately the functionality of the heart. We hypothesised that normal cardiac development and maturation is modified by prematurity and exposure to intrauterine inflammation.

Methods: Date-mated merino ewes were randomised antenatally to receive either an intra-amniotic injection of lipopolysaccharide (LPS) (4mg, *Escherichia coli*), to induce an intrauterine inflammatory response, or saline, as an experimental control, 48 hours before preterm delivery. Lambs were delivered preterm at 128d gestational age (GA) (term is 150d GA) and managed postnatally in the Preclinical Intensive Care Research Unit (PICRU) at the University of Western Australia. Postnatal care of lambs followed contemporary clinical management. At 7d postnatal age (135d postconceptional age) lambs were euthanised and the hearts excised and perfusion fixed. Fetal control lambs were delivered at 135d GA and euthanised immediately. Cardiac tissue was randomly and systematically sampled for assessment of cardiac morphology, cardiomyocyte number, nuclearity and proliferation, and myocardial extracellular matrix deposition.

Results: Antenatal LPS exposure did not result in gross morphological changes of the preterm heart at 7d postnatal age, including left ventricular wall thickness ($P > 0.05$). The number of cardiomyocyte nuclei in the preterm heart at 7d postnatal age was not influenced by antenatal LPS exposure ($P > 0.05$). The proportion of proliferating cardiomyocyte nuclei did not differ between preterm lambs antenatally exposed to LPS or saline ($P > 0.05$).

Conclusions: The findings suggest that antenatal LPS exposure does not lead to alterations in cardiomyocyte growth in the preterm lamb heart at 7d postnatal age. However, analysis of cardiomyocyte nuclearity (yet to be examined) may show differences in cardiomyocyte maturation following intrauterine inflammation, which would in turn, impact cardiomyocyte endowment.

Abstract

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The myths and physiology surrounding intrapartum decelerations – the critical role of the peripheral chemoreflex

Lear, C. A., Galinsky, R., Wassink, G., Yamaguchi, K., Davidson, J.O., Westgate, J.A., Bennet, L., Gunn, A.J.
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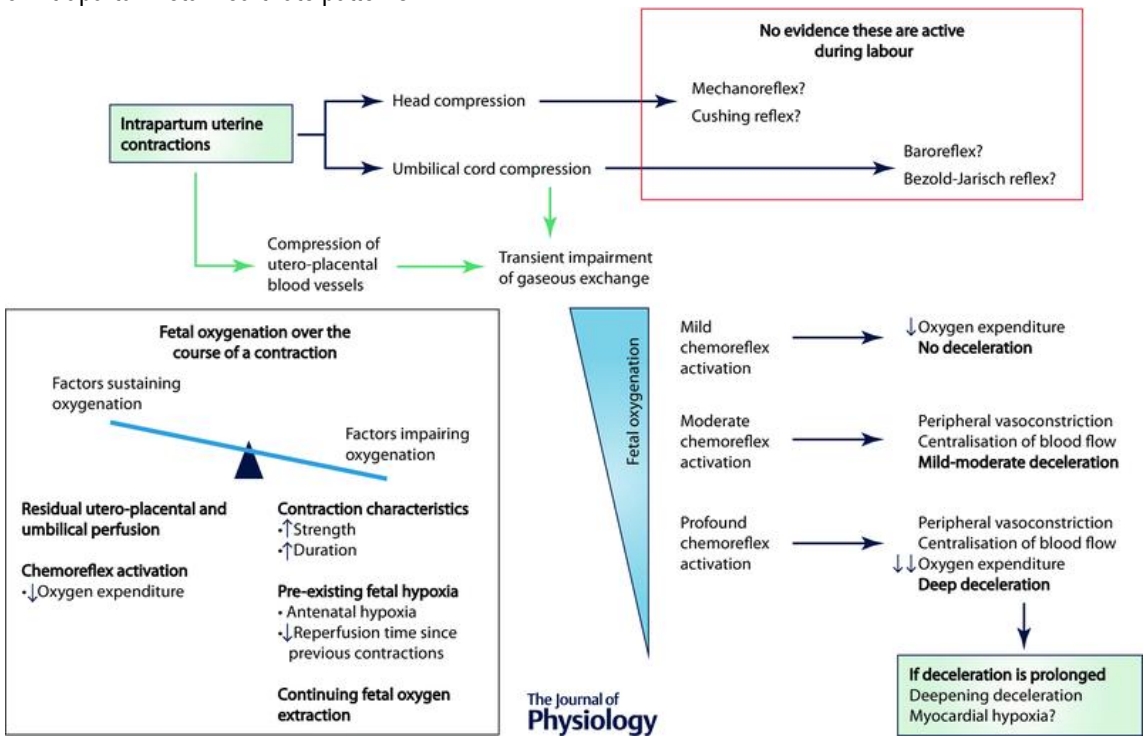
A distinctive pattern of recurrent rapid falls in fetal heart rate, called decelerations, are commonly associated with uterine contractions during labour. These brief decelerations are mediated by vagal activation. The reflex triggering this vagal response has been variably attributed to a mechanoreceptor response to fetal head compression, to baroreflex activation following increased blood pressure during umbilical cord compression, and/or a Bezold-Jarisch reflex response to reduced venous return from the placenta. Although these complex explanations are still widespread today, there is no consistent evidence that they are common during labour. Instead, the only mechanism mechanism that has been systematically investigated, proven to be reliably active during labour and, crucially, capable of producing rapid decelerations is the peripheral chemoreflex. The peripheral chemoreflex is triggered by transient periods of asphyxia that are a normal phenomenon associated with all uterine contractions. This should not not cause concern as the healthy fetus has a remarkable ability to adapt to these repeated but short periods of asphyxia. This means that the healthy fetus is typically not at risk of hypotension and injury during uncomplicated labour even during repeated brief decelerations. The physiologically incorrect theories surrounding decelerations that that ignore the natural occurrence of repeated asphyxia likely gained widespread support to help explain why many babies are born healthy despite repeated decelerations during labour. We propose that a unified and physiological understanding of intrapartum decelerations that accepts the true nature of labour is critical to improve interpretation of of intrapartum fetal heart rate patterns.



The role of the peripheral chemoreflex in intrapartum decelerations.

Notes for Oral Session VI

Oral Session VII

Developmental Hypoxia

Chair: Carlos Blanco

Session Chair

Professor Carlos Blanco

Professor Carlos Blanco of Dutch and Argentinean nationality has spent most of his career in the Netherlands (Director of Neonatology, Division leader of Perinatal Medicine, Chairman Department of Paediatrics) of the University Hospital at the University of Maastricht. He has been a visiting researcher Department of Physiology at Oxford and Cambridge University. Professor Blanco was Director of the NCRC for 6 years where he developed a successful funding strategy (investment of €25 millions) to support pediatric research in Ireland. He is widely published (H index 28). His research interests lie in the area of Foetal and Neonatal Physiology and neurodevelopment. He focused his work on the central and peripheral mechanisms involved on the control fetal activity including breathing activity. The main questions were directed to explain the periodicity of fetal breathing activity, its conversion to continuous breathing at birth and the influence of hypoxia on the control mechanisms. Relevant questions which contributed to the understanding of apnea episodes during the neonatal period and SIDS. Besides he studied the influence of perinatal asphyxia and steroid (and neuroprotection) on developmental neurogenesis. During the last 10 years his interested shifted to the study of the mechanisms involved in the early origin of adult disease such us cardiovascular disease and obesity. He developed an original model for studying this question by using the chick embryo as a relatively independent embryo/fetus in development. Scenarios such as hypoxia, acute and chronic and malnutrion apply at different stages of development were created. This model allowed studying different organs, vessels and development after hatching providing insight onto the mechanisms involved for after birth diseases. Lately he became involved in developing projects (RCT) dealing with the prevention of obesity in teenagers. This group is targeted since they are the future parents and this is an ideal moment to empower them to make healthy life decisions. He is an active Neonatologist working as a Consultant at the National Maternity Hospital in Dublin, Ireland.

Abstract

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Developmental programming of mitochondrial function by hypoxia: A cold-blooded perspective

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Introduction: Due to the profound health implications of developmental programming, the impact of the prenatal environment on physiology has been extensively studied in mammals. Far less attention has been paid to ectothermic organisms which routinely develop in fluctuating environments. For example, crocodilians lay their eggs in subterranean nests where oxygen levels can range from 10-21%. Such severe levels of developmental hypoxia produce maladaptive phenotypes in mammals. In contrast, we hypothesised that hypoxia acts on developmental plasticity to produce novel, and possibly adaptive, phenotypes in embryonic organisms that regularly encounter hypoxia. To this end, we investigated the effects of developmental hypoxia on a fundamental aspect of alligator cellular function; mitochondrial oxidative phosphorylation.

Methods: Alligator eggs were incubated in 21% or 10% oxygen from 20-90% of development. Embryos were either harvested at 90% development or allowed to hatch and then reared in 21% oxygen for 3 years. Ventricular mitochondria were isolated from embryonic/juvenile alligator hearts and mitochondrial respiration was measured in an Oroboros microrespirometer.

Results: Developmental hypoxia had no effect on mitochondrial function in embryonic alligator hearts (Fig. 1A-C, black vs. white bars). However, at the juvenile life stage, animals from hypoxic incubations had lower levels of leak respiration and higher respiratory control ratios (Fig. 1B-C, dark grey vs. light grey bars), which is indicative of enhanced mitochondrial efficiency. These results are in contrast to the mammalian paradigm where prenatal hypoxia leads to mitochondrial dysfunction and the development of cardiovascular disease.

Conclusion: Here we show that mitochondrial function can be programmed in alligators by developmental hypoxia. The adaptive significance of the enhanced mitochondrial efficiency in the hypoxic phenotype is unknown; we speculate that it may provide an advantage when juveniles encounter hypoxic environments.

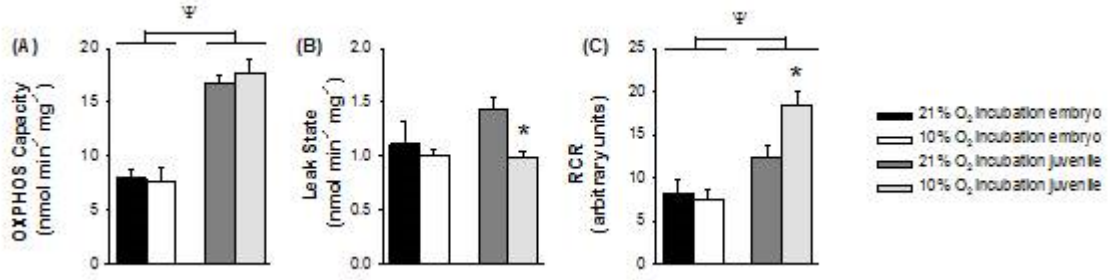

Figure 1. Effect of ontogeny and developmental hypoxia on mitochondrial respiration. Mitochondria were isolated from alligator embryos exposed to 21% and 10% oxygen during development (black and white bars, respectively; n= 5) and their juvenile counterparts (dark and light grey bars, respectively; n = 6-8) were subsequently raised in 21% oxygen. OXPHOS capacity (A), Leak state (B), and the respiratory control ratio (RCR, C) were measured with malate and pyruvate as substrates. Data is mean data ± SEM. * indicates a significant difference between 21% and 10% oxygen exposure groups, and indicates a significant difference between embryos and adults (2-way ANOVA). $P < 0.05$.

Abstract

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Gestational hypoxia induces NOX2-mediated oxidative stress and accelerated ageing in the developing ovary

Aiken, C.E.[1,2], Tarry-Adkins, J.L.[1], Spiroski, A.M.[3], Nuzzo, A.M.[3], Giussani, D.A.[3]*& Ozanne, S.E. [1*]

[1]University of Cambridge Metabolic Research Laboratories, Institute of Metabolic Science, Cambridge, United Kingdom, [2]Department of Obstetrics and Gynaecology, University of Cambridge, Cambridge, United Kingdom, 3 Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, CB2 3EG, UK
**These authors contributed equally.*

Introduction: Maternal smoking during pregnancy is known to be a key determinant of fetal ovarian reserve in human populations, however the underlying mechanism is unknown (Ruth et al., *Sci Rep* 6;24710, 2016). One possible candidate mechanism is chronic fetal hypoxia, which occurs with maternal smoking as a consequence of poor placentation (Kawasima et al., *PLoS One* 9(8);e106140, 2014). Chronic fetal hypoxia programmes cardiovascular dysfunction in the adult offspring (Giussani et al. *PLoS One* 7(2):e31017, 2012). However, whether chronic fetal hypoxia also programmes an impaired ovarian reserve in the adult female is unknown. The aim of this study was therefore to determine whether chronic fetal hypoxia has long-term effects on ovarian gene expression, oxidative stress and telomere length that could influence ovarian reserve.
Methods: Wistar rat dams were subjected to normoxia (N, 21%) or hypoxia (H, 13%) from days 6-21 of pregnancy. Offspring were raised under normoxic conditions. Ovaries were collected from 1 female per litter at 4 months of age, weighed and snap-frozen or fixed. Ovarian gene expression (q-rtPCR) and telomere length (Southern blotting) were determined..
Results: Chronic fetal hypoxia did not affect ovarian weight in female adult offspring (N: 0.08g±0.01 *vs.* H: 0.07g±0.01, p=0.17). Ovaries from adult offspring of hypoxic pregnancy showed up-regulation of components of NOX2-mediated oxidative stress (Gp91phox and P22phox, both $p<0.05$; Fig 1A and 1B), reduced telomere length compared to controls (Fig 1C and 1D), and an overall effect of hypoxia on telomere length ($p<0.05$).
Conclusions: We have demonstrated that exposure to chronic hypoxia during fetal development leads to oxidative stress and accelerated ovarian ageing in later life. These novel findings are in keeping with previous work suggesting that female reproductive physiology is exquisitely sensitive to the influence of the early-life environment. The major pathway affected by chronic fetal hypoxia in the ovary is NOX2-mediated oxidative stress, which could contribute to a decrease in ovarian reserve.
Supported by the British Heart Foundation

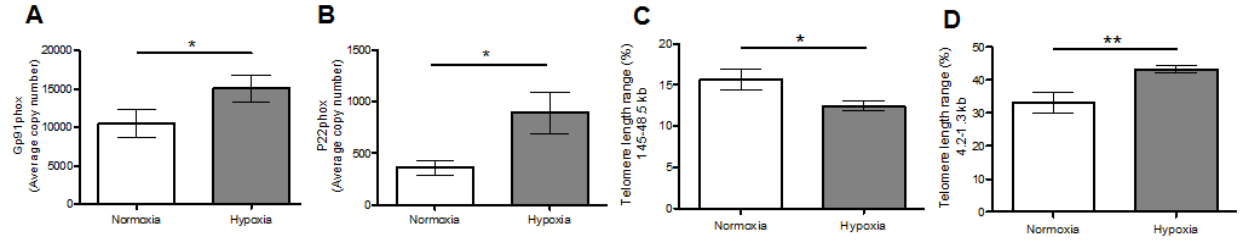

Figure 1. Gene expression in ovaries from adult offspring of normoxic (open bars) *vs*. hypoxic (grey bars) pregnancy for (A) Gp91phox, (B) P22phox, (C) percent of long telomeres, and (D) percent of short telomeres. Data are mean ± SEM, *$p<0.05$, **$p<0.01$.

Abstract

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Compensatory alterations in fetal fractional oxygen extraction are dependant upon both gestational age and birth weight centile

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Introduction: Fetal growth is linked to oxygen availability with fetal hypoxia associated with fetal growth restriction. In term infants fractional oxygen extraction (FOE) increases in response to hypoxia preventing anaerobic respiration. Whether this compensatory mechanism is consistent across gestation is not known. Therefore the aim of this study was to investigate the impact of fetal growth restriction on fetal FOE from 23-41 weeks gestation.

Methods: Umbilical cord arterial and venous blood samples were collected from neonates born at tertiary neonatal centre between November 2015 and February 2016 (n=906). The population was divided into three gestational groups: 23-29+6 weeks: 30-36+6 weeks: and ≥37 weeks) and customized birth weight centile calculated for each infant. Umbilical arterial and venous cord gas parameters were measured by co-oximetry and FOE calculated from venous and arterial pO_2.

Results: An inverse relationship between birth weight centile and FOE was seen for those infants < 30 weeks gestation (p=0.02). For these infants relationships between umbilical cord FOE and umbilical arterial lactate and pH were seen with arterial lactate increasing (p=0.03) and pH decreasing (p=0.02) with elevated FOE. These effects were not seen for the late preterm and term groups.

Conclusions: Whilst chronic fetal hypoxia secondary to placental insufficiency results in a reduction in fetal growth, irrespective of gestational age, this adverse intra-uterine environment is well tolerated in the more mature fetus. With greater immaturity the capacity for this compensatory adaptation to meet on-going systemic oxygen demands is limited, reaching a critical threshold. The relationship between FOE and arterial lactate in the very preterm growth restricted preterm neonate suggests a lowered oxygen margin of safety with FOE failing to meet on-going consumption. This could contribute to the higher incidence of morbidity and mortality in this high-risk population.

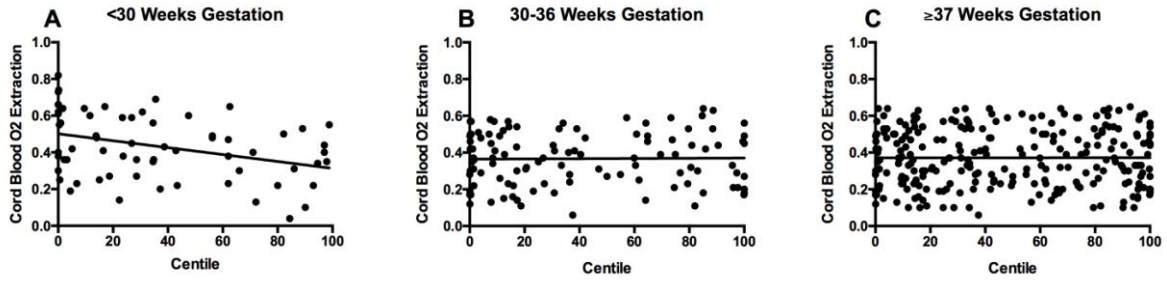

Figure 1. Relationship between Cord blood oxygen extraction and birth weight centile in neonates A) <30 weeks gestation (p=0.02), B) 30-36 weeks gestation and C) ≥37 weeks gestation.

Notes for Oral Session VII

Oral Session VIII

Utero-Placenta Studies A

Keynote Speaker: Paola Casanello
Chair: Abby Fowden

Keynote Speaker and Session Chair

Dr. Paola Casanello

Paola graduated as a Midwife in 1996 (Universidad de Concepción, Chile), specialized in Perinatology (1998) and trained in NICU. In 1998, as Instructor at the Department Obstetrics Obstetrics & Gynecology, lectured in obstetrics physiology to medical students. In 2000 started her MSc in Biological Sciences/Physiology where she studied mechanisms that lead lead to low NO production in IUGR-derived umbilical vessels, all of this at the Universidad de de Concepción. In 2002 started her PhD in Physiological Sciences at the P. Universidad Católica de Chile (PUC). She trained in placental and vascular physiology and adaptation to to hypoxia. In 2004 worked with Professor Anibal Llanos at the Universidad de Chile, in pulmonary hypertension of the newborn in sheep and llama. In 2005 as an Instructor and later as Assistant Professor (2007) at the Division of Obstetrics/Gynecology (PUC), studied negative regulators of NO synthesis, arginase-2 expression and regulation and placental dysfunction in IUGR-derived placentae. Presently, she is an Associate Professor at the Division of Obstetrics and Gynecology, and the Department of Neonatology, Division of of Pediatrics in the School of Medicine at PUC (since 2014), and the leader of the Programming and Perinatal Epigenetics Lab. This group is centred in studying epigenetic mechanisms leading to programming of vascular dysfunction in IUGR and macrosomic babies in placental vascular tissues, the effects on fetal growth, newborn and infant body composition, immune cell physiology and placental function of maternal supplementation with DHA in obese women. As Co-PI she studies vascular programming in offspring of IUGR-guinea pig and early markers of asthma in children from obese mothers. She develops interdisciplinary research where the antihypertensive effect of a a combination of drugs are being tested. She actively collaborates with groups in USA, Brazil, and UK. She has published over 50 full papers, has leaded 2 associative national grants (Fondef, Anillo, £450.000 each), 3 Fondecyt grants (£200.000 each) and co-investigator in 9 Fondecyt proposals. She is a Reviewing Editor for Current Vascular Pharmacology and Editor in Ars Medica (PUC, Chile). Dr. Casanello´s current research is focused on early origins of chronic disease at the clinical, basic science and whole animal level, studying the role of maternal nutrition and obesity obesity on the programming of vascular, immune and metabolic function in the newborn and the first years of postnatal postnatal life. Clinical nutritional interventions during pregnancy are also part of the interests and current projects in our our research team.

Professor Abigail Fowden

Abigail Fowden is Professor of Perinatal Physiology in the Department of Physiology, Development and Neuroscience and Head of the School of the Biological Sciences at the University of Cambridge. She was an undergraduate at Girton College and graduated with a first class degree in Physiology in 1975. She obtained her PhD from the University University of Cambridge in 1979 and immediately joined the staff of the Department of Physiology as a demonstrator. Since then, she has held positions as a University Lecturer Lecturer and Reader before being promoted to a personal chair in 2002. She obtained the the ScD degree in 2001 and was awarded the Joan Mott Prize of the Physiological Society Society for her research in 2008. Her research interests are in the factors controlling feto-placental growth and development during late pregnancy. The aims of her research are two-fold: first, to determine how hormones and other environmental cues regulate feto-placental development, and secondly, to establish how our experiences during early life alter the risk of degenerative diseases in adulthood. She is also a Professorial Fellow at Girton College, Cambridge.

Abstract

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Epigenetic programming of vascular function by fetal growth trajectory: the placenta as the black box

Casanello, P.[1,2], Hernandez, C.[1], Carrasco-Wong, I.[1] , Muñoz, E.[1], Uauy, R.[2], Krause, B.[2]

[1]Division of Obstetrics and Gynecology & [2]Department of Neonatology, Division of Pediatrics, School of Medicine, Pontificia Universidad Católica de Chile

Introduction: The association of low and excessive fetal weight with cardiovascular risk are in the central interest of DOHaD.

Aim: Due to the key role of endothelium in the umbilical vascular function we have studied markers of endothelial epigenetic heterogeneity in endothelium from umbilical arteries (HUAEC) and veins (HUVEC) from intrauterine growth restricted (IUGR), adequate for gestational age (AGA) and large for gestational age (LGA) fetuses.

Methods: In HUAEC from IUGR, AGA and LGA fetuses the expression of eNOS, Arg2, NOX4, GPX1, SOD1, Nrf2 and HO1 was determined under basal, hypoxia and oxidative stress conditions. The methylation status (by pyrosecuencing) of the promoter of these genes as well as histone modifications (H3K4 Me2/H3K4 Me3/H3K9 Me2/H3K9 Ac/H4K12 Ac) was determined by ChIP. Knockdown of DNMT1 was performed to study methylation-dependent changes.

Results: In IUGR and LGA-derived HUAEC there is an increase in the basal expression of eNOS. The mRNA of Arg2 and eNOS are induced by hypoxia in AGA-HUAEC but no change is observed in IUGR or LGA-HUAEC. Both eNOS and NOX1 showed significant changes in the methylation status at their promoter region in IUGR and LGA. ChIP showed an important amount of opened chromatin markers (H3K9 Ac, H4K12 Ac) in the *NOS3* promoter. Knockdown of DNMT1 modified the expression of eNOS in IUGR and LGA compared to AGA, and this was associated to changes in methylation in CpG -352 from TSS in *NOS3* gene.

Conclusion: In summary both extreme phenotypes (IUGR and LGA) show changes in some key endothelial genes. These changes are associated to epigenetic marks of vascular programming in fetuses in both extremes of the growth curve.

Funded: Fondecyt 1120928-1130801, CONICYT, Chile

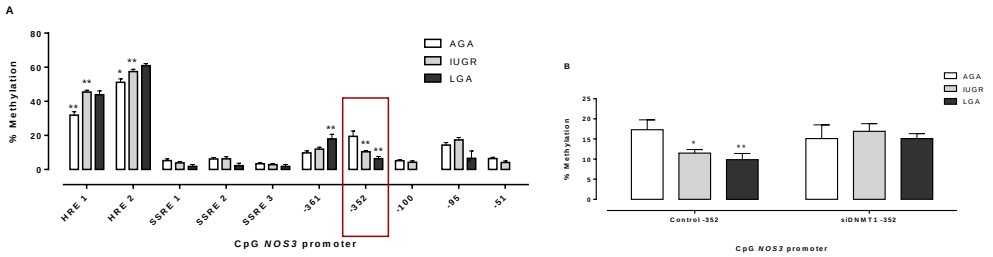

Figure 1. *NOS3* promoter methylation status in HUAEC from AEG, IUGR, LGA foetuses. A. The *NOS3* gene promoter is hypomethylated in -352 from the TSS in IUGR and LGA-derived HUAEC, compared to AGA. This correlates with the overexpression of eNOS mRNA and protein (not shown). **B.** This hypomethylation in -352 is reverted with DNMT1 knockdown.

Abstract

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Factors regulating the expression of the prorenin receptor-prorenin angiotensin pathway

Lumbers E.R.[1], Wang Y.[1], Delforce S.J.[1], Morris B.J.[2], Marques F.Z.[2], Broughton-Pipkin F.[3], Roberts C.T.[4], Pringle K.G.[1]

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Introduction: The human placenta expresses all the genes of the prorenin receptor (PRR)–renin-angiotensin system (PRR-RAS) required to produce angiotensin II (Ang II), as well as both Ang II receptor subtypes. Ang II, acting via the AT_1 receptor, stimulates cytotrophoblast proliferation, migration, invasion and angiogenesis. Expression of the PRR-RAS is greatest in early gestation placenta, but the mechanisms controlling its expression are unknown. We are investigating the role of cAMP, oxygen and miRNAs in regulating the placental PRR-RAS.

Methods: The effects of 0.3 mM 8-bromo-cAMP, or culture in differing oxygen tensions (1, 5, and 20%) on expression of the PRR-RAS in HTR-8/SVneo cells were studied. HTR-8/SVneo cells are a transformed first trimester extravillous trophoblast cell line.

Human placentae were collected between 8–10 weeks, 12–16 weeks, and at term (n=4 placentae per group) and screened for 2006 miRNAs using an Agilent microRNA array and analysed using the Partek Genomic suite to detect significant changes. miRNAs that were predicted to target the PRR-RAS were studied further. miRNA expression was validated in 8 placentae at each gestation. Vectors containing miRNAs were transfected into HTR-8/SVneo cells to determine their effects on prorenin gene and protein expression.

Results: In HTR-8/SVneo cells, 0.3 mM 8-bromo-cAMP stimulated expression of prorenin, PRR, and AT_1R, while incubation in 1% oxygen stimulated expression of AT_1R and VEGF genes and enhanced ACE protein levels. Renin gene and protein expression were greatest in early gestation placentae ($P<0.01$). The miRNA hsa-miR-181a (shown to regulate human renal renin gene and protein expression) was, however, most highly expressed in term placentae ($P<0.01$). There were also strong inverse correlations between hsa-miR-181a expression and both renin gene expression ($P<0.001$, $rho=-0.64$, n=23) and prorenin protein levels ($P<0.001$, $rho=0.69$, n=21).

HTR-8/SVneo cells that overexpressed hsa-miR-181a expressed 40% less renin mRNA and 60% less prorenin protein (both $P<0.001$) than scrambled controls.

Conclusions: Regulation of the placental PRR-RAS pathway in early gestation is complex involving the interaction of a low oxygen environment, miRNAs and cAMP. Abnormalities in its regulation could lead to poor placentation and abnormal pregnancy outcomes as well as a predisposition to disease in adult life.

Abstract

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IGF2 is essential for the expansion of the fetoplacental vasculature during late gestation development

Constancia, M., Fowden, A., Burton, G., Georgopoulou, A., Sferruzzi-Perri, A. & Sandovici, I.

Centre for Trophoblast Research, University of Cambridge

Introduction:
In eutherian mammals the fetoplacental vascular tree grows and expands to meet nutrient demands of the fetus in late gestation. How genes specifically contribute to this expansion remains largely unknown. Also unknown is the extent to which endocrine signals emanating from the fetus might control this process. In this study, we investigated the contribution of paracrine and endocrine Igf2 in the growth of the placental vascular tree using genetically engineered mice.
Methods: Mice with a global reduction of Igf2 levels in embryonic-derived cell types, but normal levels in extra-embryonic cells (Igf2 Meox2Cre k.o), and mice with a specific deletion in endothelial cells (Igf2 TekCre k.o) were generated. We measured placental vascularization by stereology, the number of placental endothelial cells and their proliferation by flow cytometry and immunohistochemistry, and performed RNA transcriptome studies.
Results: Igf2 deletion from fetal organs leads to reduced fetal growth (57% of normal (N) by E19) and circulating Igf2 levels (32% N), and a disproportionately smaller labyrinthine layer of the placenta (57% N of labyrinthine volume) with reductions in fetal capillary volume, surface area, length (~50% N) and endothelial cell number (29% N) at E19. Molecular signatures of abnormal angiogenesis were found by array and RNA Seq (e.g. reduced expression of Angpt1 and Tie2, Vegfb, Hey2). A selective loss of placental endothelial cells within the labyrinthine compartment was observed, associated with reduced endothelial cell proliferation and increased expression of the p21 cyclin-dependent kinase inhibitor. RNA Seq revealed that Igf2 is the top expressed gene in placental endothelial cells. Igf2 specific deletion from endothelial cells leads to a much less pronounced growth and angiogenesis phenotypes compared to Igf2 Meox2 k.o. (i.e. 84%N reduction in fetal growth, 83%N in circulating Igf2 levels and 60%N in number of endothelial cells, at E19).
Conclusions: Loss of Igf2 leads to severe impairment of placental endothelial cell expansion during late gestation through de-regulation of pro-angiogenic and anti-angiogenic factors, leading to defects in cell proliferation and number. The angiogenesis defects were more pronounced in mice that lack Igf2 in circulation, thus suggesting that endocrine Igf2 is required to stimulate placental angiogenesis/vascular tree expansion in late gestation.

Abstract

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Periconceptional alcohol exposure reduces trophoblast giant cell differentiation and outgrowth capacity in the rat

Kalisch-Smith, J.I., Simmons, D.G., Pantaleon, M., Moritz, K.M.

The University of Queensland, Australia

Introduction: Maternal periconceptional alcohol (PC-EtOH) exposure in the rat causes fetal growth restriction and sex-specific changes to placental morphology in late gestation. This may be the result of perturbations to pre-implantation embryo development and/or its capacity to interact with the uterine cells and form a placenta. This study aimed to examine cell allocation in the pre-implantation embryo and behaviour of trophectodermal (TE) derivatives after PC-EtOH exposure.

Methods: Sprague Dawley dams were administered 12.5% v/v EtOH or a control diet from 4 days prior (E-4) to 4 days after conception (E4) in a liquid diet. Blastocysts were collected at E5 and assessed for total cell count and allocation to either the TE (CDX2 positive) or the inner cell mass (ICM). A subset was also used to assess nuclear CDX2 expression in TE cells when stained control and PC-EtOH embryos were stained in tandem. To determine whether EtOH affects the differentiation capacity of *in vivo* derived embryos, a subset was flushed at E5 and cultured *in vitro* for 6 days to obtain trophoblast outgrowths. Outgrowths were immunolocalised for Pan-Cytokeratin to positively label trophoblasts, and counted for those forming parietal trophoblast giant cells (nuclear area >1000um^2).

Results: No differences were found between control and PC-EtOH treatments in forming a competent blastocyst at E5, when assessed for total cell number and cell allocation to the TE or the ICM. However, PC-EtOH embryos showed significant reductions in nuclear TE CDX2 fluorescence intensity ($P<0.0001$). Embryos exposed to *in vivo* PC-EtOH prior to culture *in vitro* showed reduced trophoblast outgrowth area ($P<0.05$), and formation of the parietal trophoblast giant cells ($P=0.01$).

Conclusions: This study shows *in vivo* PC-EtOH can affect differentiation and behaviour of the TE lineage, which may contribute to altered placental development, fetal growth restriction and the programming of adult disease.

Abstract

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Therapeutic effect of human placental-derived mesenchymal stromal cells on a lipopolysaccharide Induced mouse model of preeclampsia

Nuzzo, A.M.[1], Barrile, R.[1], Mele, P. [2,3], Eva, C. E.[2,3], Todros, T.[1] Rolfo, A.[1]

[1]Department of Surgical Sciences, University of Turin, Italy
[2] Neuroscience Institute of the Cavalieri-Ottolenghi Foundation Orbassano (Turin), Italy
[3]Department of Neuroscience, University of Turin, Italy

Introduction: Preeclampsia (PE), the most severe human pregnancy-related syndrome, is a leading cause of fetal-maternal mortality and morbidity and lacks of an effective therapy. Main hallmarks of PE are severe maternal hypertension and proteinuria, expression of a generalized endothelial damage and inflammation that could lead to Fetal Growth Restriction (FGR). Human Placenta-Derived Mesenchymal Stromal Cells (hPDMSCs) are well renowned for their pro-angiogenic and anti-inflammatory effects exerted via paracrine interactions. Herein, we tested the effects of hPDMSCs-derived soluble molecules on a mouse model of preeclampsia.
Methods: An injectable bioactive formulation (CB-ChMF-11) containing pure hPDMSCs trophic mediators was kindly provided by Corion Biotech S.r.l. (Italy). Preeclampsia was induced in pregnant C57BL/6NCrl mice by endovenous bacterial Lipopolysaccharide (LPS) injection. Starting from d9, maternal blood pressure and proteinuria were monitored until d19. At d11 of pregnancy, dams were injected with E.Coli LPS (1µg/Kg). At d12, mice were randomly divided into two groups (n=7 each) and treated endovenous as follow: plain vehicle (300µl, placebo) and CB-ChMF-11 (300µl, treated). At d19, mice were sacrificed. Number of fetuses, FGR, fetal reabsorption and placental weight were evaluated. Next, placentae were processed for mRNA isolation and cDNA preparation. sFlt-1, IL-6 and TNF-α gene expression were evaluated by Real Time PCR.
Results: Injection of CB-ChMF-11 on d12 significantly decreased maternal systolic blood pressure ($p<0.05$) and proteinuria ($p<0.05$) by day 13 until term relative to placebo group. No FGR and/or reabsorbed fetuses were delivered by CB-ChMF-11 treated PE mice, while 5 FGR fetuses were found in the placebo group ($p=0.02$). No differences were found in placental weight between groups. CB-ChMF-11 treatment significantly decreased sFlt-1, IL-6 and TNF-α mRNA levels ($p<0.05$) in PE mice relative to placebo group.
Conclusions: Our data indicate that hPDMSCs-derived trophic mediators can reverse PE-like features during pregnancy, suggesting a therapeutic role for hPDMSCs for the treatment of preeclampsia.

Abstract

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Placental uptake and metabolism of vitamin D

Simner, C.L., Lewis, R.M., Cooper, C., Harvey, N.C., and **Cleal, J.K.**

Institute of Developmental Sciences, Faculty of Medicine, University of Southampton
MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK

Introduction: Low vitamin D levels are common in pregnancy and are linked to suboptimal fetal growth. Maternal supplementation with inactive-25-hydroxyvitamin D (25(OH)D) is recommended. It is unclear whether both 25(OH)D and active-1,25-dihydroxyvitamin D (1,25(OH)2D) are taken up by placenta. Vitamin D circulates bound to carrier proteins, vitamin D binding protein (DBP) and albumin, but their role in placental vitamin D uptake is unclear. We investigated whether both 25(OH)D and 1,25(OH)2D are taken up by human placenta, and whether carrier proteins impact upon uptake.
Methods: Term human placentas were collected within 30 min of delivery. Placental villous fragments were cultured for 8 h in Tyrodes buffer containing 20 μM 25(OH)D (n=6), 25(OH)D + 0.7 mM albumin (n=6), 50 nM 1,25(OH)2D (n=11) or 1,25(OH)2D + 0.7 mM albumin (n=11). Endocytic mechanisms of 1,25(OH)2D uptake were investigated by adding 5 mM amiloride (n=5) or 80 μM dynasore (n=5). mRNA expression of CYP24A1, a vitamin D responsive gene, was measured by qRT-PCR. Villous fragments were incubated with 150 nM FITC-albumin (n=5) with or without endocytic blockers (n=3) for ≤1 h at 4°C and 37°C. Samples were fixed, stained with lectins, and viewed using confocal microscopy. Data were analysed by one- and two-way ANOVA.
Results: CYP24A1 mRNA expression increased with 25(OH)D ($p<0.001$) compared to controls; this increased further with the addition of albumin ($p=0.01$). 1,25(OH)2D increased CYP24A1 mRNA expression ($p<0.001$) compared to controls, which did not increase further with albumin ($p=0.16$). FITC-albumin uptake increased with time ($p=0.08$) at 37°C but not at 4°C ($p=0.004$). Dynasore did not alter CYP24A1 expression compared to 1,25(OH)2D with or without albumin. Amiloride reduced CYP24A1 mRNA expression compared to 1,25(OH)2D with ($p<0.001$) and without albumin ($p=0.006$) and also reduced FITC-albumin uptake ($p=0.03$).
Conclusion: These data suggest that both 25(OH)D and 1,25(OH)2D are taken up into the placenta and can induce vitamin D dependent gene expression, implying the placenta converts 25(OH)D to 1,25(OH)2D. Furthermore uptake of 25(OH)D may be enhanced by albumin, while amiloride inhibited albumin uptake and 1,25(OH)2D stimulated CYP24A1 expression, suggesting endocytic uptake.

This work was supported by the Gerald Kerkut Charitable Trust

Abstract

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Maternal and fetal genomes interplay through phosphoinositol 3-kinase (pi3k)-p110α signalling to modify placental resource allocation to fetal growth

Sferruzzi-Perri, A.N., López-Tello, J., Fowden, A.L., & Constancia, M*.

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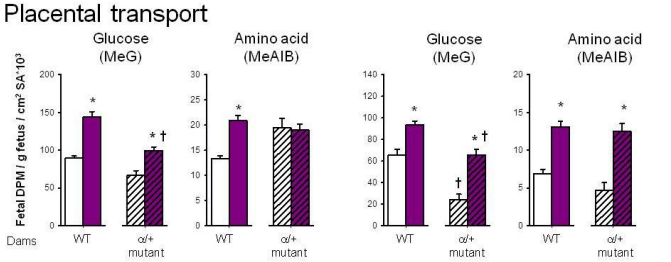
Introduction: The successful outcome of mammalian pregnancy depends on balancing resource allocation between the genetically-determined fetal drive for growth and the maternal nutrient requirements to support pregnancy and lactation. Failure to achieve the right balance can lead to pregnancy complications and abnormal fetal development with long-term consequences for both maternal and offspring health. As the site of materno-fetal substrate transfer, the placenta is central this tug-of-war over nutrient allocation. Previous work indicates that the placenta can adapt dynamically to both fetal signals of nutrient demand and maternal signals of nutrient availability to ensure appropriate allocation of available resources (reviewed by Sferruzzi-Perri and Camm 2016). The fetus and mother therefore, have to cooperate to optimise both offspring and maternal fitness but, little is known about the relative importance of the fetal versus the maternal genome, in balancing resource allocation at the placental level.
Methods: We used genetic inactivation of the growth and metabolism regulatory protein PI3K-p110α (α/+) in mice as a tool to examine the interplay between the maternal and fetal genomes on placental phenotype. This was achieved by studying feto-placental growth, placental morphology and transport in litters of mixed genotype that were generated through reciprocal crosses of wildtype and α/+ mice. Data were collected on days 16 and 19 (term=20 days).

Results:Placental growth and structure were impaired and associated with reduced growth of α/+ fetuses at both days of pregnancy(Figure). Despite its defective development, the α/+ placenta adapted functionally to increase the supply of maternal glucose and amino acid to the fetus, particularly near term(Figure). The specific nature of these changes however, depended on whether the mother was α/+ or wildtype and were related to alterations in endocrine and metabolic profile induced by maternal PI3K-p110α deficiency.
Conclusions: Our findings show that the maternal genotype and environment programmes placental growth and function and identify the placenta as critical in integrating both intrinsic and extrinsic signals governing materno-fetal resource allocation. Furthermore, our study highlights that the α/+ mouse is a useful tool for investigating the mechanisms and role of maternal-fetal genome interactions in determining how health of offspring may be programmed by the maternal environment.

Conceptus weights

	Day 16				Day 19			
Dam	WT		α/+ mutant		WT		α/+ mutant	
Conceptus	WT	α/+ mutant	WT	α/+ mutant	WT	α/+ mutant	WT	α/+ mutant
Fetus (mg)	416±5	325±8*	409±5	343±5*	1137±16	978±15*	1131±22	1024±25*
Placenta (mg)	116±2	107±3*	124±2	113±2*	93±2	84±2*	108±2†	95±3*†

Placental transport



2 way ANOVA linear mixed model. *P<0.05, effect fetal genotype, †P<0.05, effect maternal genotype both by pair-wise comparison. SA= placental surface area

Notes for Oral Session VIII

Oral Session IX

Utero-Placenta/ Partuirtional Physiology B

Chair: Donald Peebles

Session Chair

Professor Donald Peebles

Professor Donald Peebles obtained a BA in the History of Art from Cambridge University in 1983 and his MBBS from the University of London in 1986. During his early career he worked with Professor Mark Hanson at UCL, establishing his continuing interest in fetal physiology. He was appointed as Professor of Maternal Fetal Medicine at UCL in 2008 and Head of the Research Dept MFM in the UCL Institute for Women's Health until 2016. He has a number of research interests that focus on improving the outcomes for women and their babies following complicated pregnancies. Particular research areas include: 1) maternal innate immunity, infection, inflammation and preterm labour 2) the role of hypoxia and inflammation in causation of perinatal brain injury 3) fetal physiology (especially fetal responses to acute and chronic substrate deprivation) and 4) the development of novel molecular and cellular methods for treatment of fetal disease. His main grant funding relates to developing novel methods of fetal therapy including gene therapy for fetal growth restriction. He has published over 100 peer reviewed papers and is a faculty member of the Infection, inflammation and Immunity Theme of the NIHR UCLH Biomedical Research Centre In parallel with his research activities he is also a sub-specialty accredited Consultant in Maternal Fetal medicine at UCLH with a particular interest in the management of pregnancy complicated by maternal disease or poor obstetric history and fetal medicine, including fetal therapy, prenatal diagnosis and management of fetal growth restriction. In 2013 he was appointed as co clinical Director for the NHS England London Maternity Strategic Clinical Network, since when he has worked with his co-Director to reduce stillbirth and maternal mortality rates in London, as well as to improve women's experience of maternity services. Since Jan 2015 he has also been the Clinical Director for Women's Health at University College London Hospitals Trust. He is President of the Blair Bell Research Society and a member of the RCOG Academic Board.

Abstract

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Significance of the first hour of the fetal heart rate monitoring during labour: nonreactive vs. reactive initial trace

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Nuffield Department of Obstetrics & Gynaecology, University of Oxford

Introduction: Fetal heart rate (FHR) 'cyclicity' is defined as alternating episodes of quiet sleep and reactivity (active sleep). Antepartum, a 'normal' trace require at least one episode of FHR reactivity in an hour. During early labour, identification of the nonreactive FHR may also be informative owing to its association with pre-existing fetal compromise, such as neurological injury, infection or inflammation (Phelan & Kim, Seminar Perinatol 24;2000).
We used computerised methods to detect nonreactive FHR patterns at the onset of FHR monitoring in clinical practice and report their incidence and association with perinatal outcome.
Methods: All singleton births at Oxford in Jan'93-Dec'11, of gestation >35 weeks with intrapartum FHR monitoring of ≥1 hour were included; excluded were breech presentations and congenital abnormalities. The first hour of the FHR was analysed.
Nonreactive FHR was defined with computer analysis as Decelerative Capacity <1bpm (Georgieva et al, BJOG 128;2014) and no accelerations during the first hour. Severe compromise was a composite outcome of stillbirth, neonatal death (<28 days), neonatal encephalopathy, seizures; or resuscitation at birth followed by ≥48hrs of intensive care.
Results: In total 52,632 cases were included; 184 (0.34%) were nonreactive (34% before established labour, 57% in the first stage of labour). Their characteristics were compared with the remaining 52,448 births (Table 1). Those with nonreactive patterns were three times more likely to have severe compromise.

Table 1. Comparison of the cases with nonreactive initial FHR and the remaining deliveries.

	Nonreactive (n_1 = 184)	Remaining births (n_2 = 52,448)	Odds Ratio (95% Confid. Interval)
Nulliparous	116 (63.04%)	30,536 (58.22%)	1.22 (0.91; 1.65)
Pre-eclampsia	24 (13.04%)	5,919 (11.29%)	1.18 (0.77; 1.81)
Emergency Caesarean	47 (25.54%)	6,763 (12.89%)	**2.32 (1.66; 3.23)**
Thick meconium	24 (13.04%)	4,160 (7.93%)	**1.74 (1.13; 2.68)**
Oxytocin	78 (42.39%)	21,582 (41.15%)	1.05 (0.79; 1.41)
Low Apgar	10 (5.43%)	1,338 (2.55%)	**2.20 (1.16; 4.16)**
Resuscitation	7 (3.80%)	564 (1.08%)	**3.64 (1.70; 7.78)**
Convulsions	0 (0%)	101 (0.19%)	-
SCBU ≥48hrs	16 (8.70%)	1,778 (3.39%)	**2.71 (1.62; 4.54)**
Severe compromise	6 (3.26%)	425 (0.81%)	**4.12 (1.82; 9.36)**
Meconium aspiration syndrome	2 (1.09%)	255 (0.50%)	2.17 (0.54; 8.78)
Baby below the 3rd Yudkin centile	7 (3.80%)	853 (1.63%)	**2.39 (1.12; 5.11)**
Baby above the 97th Yudkin centile	12 (6.52%)	2609 (4.97%)	1.33 (0.74; 2.40)
Stillbirth or Neonatal Death	0 (0%)	27 (0.05%)	-

The sensitivity of the test was only 1.39%, but because a nonreactive FHR was so rare (0.34%), the false positive rate was also very low (about 3 in 1,000).
The risk for severe compromise was 1 in 124 for the entire dataset, but increased to 1 in 31 in the nonreactive cases; and to 1 in 5 in the nonreactive cases with thick meconium.
Conclusions: Persistently nonreactive FHR in the first hour of intrapartum monitoring is rare. Computerised FHR monitoring can reliably alert clinicians if such a pattern is present. Nonreactive initial FHR in the presence of thick meconium carries a 1 in 5 risk for severe compromise, indicating the need for prompt intervention.

Abstract

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A heretical view: Placental 11β-HSD2 may not perform a placental protective function but rather act to provide substrate for fetal peripheral cortisol synthesis

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Center for the Study of Fetal Programming, Department of Animal Science, University of Wyoming

Introduction: Obese (OB) pregnant ewes and their fetuses display elevated blood cortisol from mid to late gestation. Cortisol levels higher than appropriate for the current stage of fetal development are associated with adverse development and postnatal offspring health. Elevated fetal cortisol in maternal OB occurs without increased fetal ACTH suggesting a nonadrenal source. This study was conducted to determine the source of this fetal cortisol rise, - fetal and/or maternal origin.

Methods: Multiparous ewes ate either 100% National Research Council recommendations (NRC) (CTR, n=7) or 150% NRC (OB, n=7) starting 60 days before conception until necropsy day 135, 0.9 gestation. At necropsy, maternal jugular and umbilical venous blood were collected. Fetal liver, perirenal fat and cotyledonary tissues were snap frozen. Cortisol concentrations in maternal plasma and cortisol and cortisone in fetal plasma were determined by radioimmunoassay. 11β hydroxysteriod dehydrogenase 1 (11β-HSD1), hexose-6-phosphate dehydrogenase (H6PD), and 11β-HSD2 protein were determined by Western blot in fetal liver, perirenal fat and cotyledonary tissue. Group differences were determined via proc MIXED procedure of SAS. Data M ± SEM.

Results: At 0.9G, maternal plasma cortisol (79.1 ± 8.57 vs. 19.9 ± 6.78 ng/ml, $P<0.001$), fetal plasma cortisol (45.5 ±5.07 vs. 23.7 ± 5.48 ng/ml) and cortisone (37.6 ± 4.08 vs. 21.9 ± 3.92,) were higher in OB vs CTR, $P<0.01$. Expression of 11β-HSD2 (which converts cortisol to cortisone) was higher ($P<0.05$) in cotyledonary tissue of OB versus CTR fetuses (0.5 ± 0.05 vs. 0.4 ± 0.04 arbitrary units [au]). Expression of 11β-HSD1(which converts cortisone to cortisol in the presence of the cofactor, H6PD) was increased ($P<0.05$) in OB versus CTR in fetal liver (0.7 ±0.13 vs.0.3 ± 0.13 au), and perirenal fat (1.7 ± 0.17 vs 1.19 ± 0.17 au). Fetal H6PD increased ($P<0.05$) in OB vs. CTR perirenal fat (0.4 ± 0.03 vs 0.3 ± 0.03 au) and tended to increase in liver (0.7 ± 0.06 vs. 0.5 ± 0.05 au, $P<0.10$).

Conclusions: **Novel, heretical hypothesis:** In maternal OB, maternal cortisol is converted to cortisone by the placenta. Cortisone can then act as a substrate for peripheral fetal cortisol production.

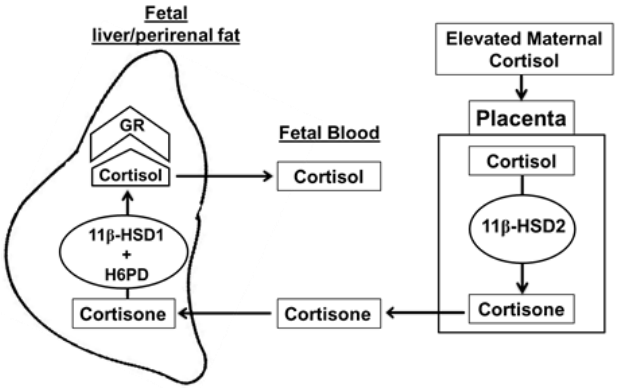

Figure 1. Maternal-placental and fetal glucocorticoid metabolism in OB. 11β-HSD2 up regulation within OB placental cotyledons converts elevated maternal cortisol to fetal cortisone. Elevated fetal hepatic and peripheral adipose tissue11β-HSD1 and H6PD then use circulating fetal cortisone as a substrate to synthesize cortisol which may may act locally and/or systemically in the circulation.

Abstract

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Fetal growth and the risk of spontaneous preterm birth (sPTB) in a prospective cohort study of Nulliparous women

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Background Previous studies have suggested an association between fetal growth restriction and the risk of sPTB. However, there are significant methodological hurdles to addressing this association.
Methods We conducted a prospective cohort study of nulliparous women with a viable singleton pregnancy (Lancet 2015;386:2089-97). Ultrasonic fetal biometry was performed at 20 and 28 weeks of gestational age (wkGA). All biometric measurements were expressed as gestational age-adjusted z-scores. Fetal growth velocity was quantified by the change in z-score between 20 and 28wkGA. The outcome was sPTB, defined as delivery ≥28 and <37 weeks, associated with labour in the absence of a method of induction of labour. The risk of sPTB was analysed using Cox regression.
Results There were 3,892 women eligible for the current analysis and 98 (2.5%) had a sPTB. There was a highly significant association between the growth velocity of the fetal femur between 20 and 28 weeks and the risk of sPTB (Figure): the lowest decile was associated with a hazard ratio of 2.37 (95% CI: 1.43-3.93, P<0.001). Adjustment for maternal characteristics was without material effect (adjusted HR: 2.50, 95% CI: 1.50-4.14, P<0.001). There were no significant associations between other fetal measurements and the risk of sPTB.
Conclusions We conclude that fetal growth restriction is associated with an increased risk of sPTB and speculate that serial assessment of fetal growth may enhance prediction of the condition.

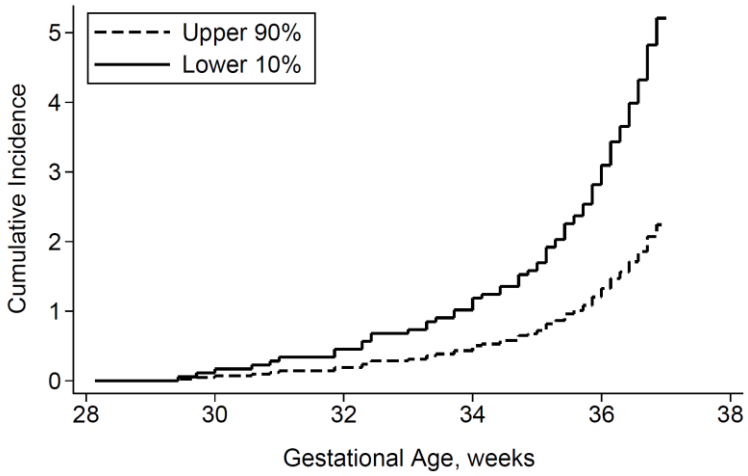

Figure. Cumulative incidence (%) of sPTB between 28+0/7 and 36+6/7 wkGA comparing fetuses with the lowest decile of femur length growth velocity between 20 and 28 weeks (solid line) and all other fetuses (dashed line) (P=0.001).

Abstract

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Sexual dimorphism in feto-placental circulation during the second half of pregnancy: a longitudinal study

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Introduction: Sexual dimorphism in placental size and function has been described, but longitudinal studies evaluating serial changes in feto-placental blood flow based on sex are lacking. Sex differences are not taken into account when evaluating feto-placental hemodynamics. We hypothesized that placental blood flow is different between male and female fetuses. Our objective was to investigate gestational age-specific serial changes in umbilical vein blood flow (Q_{uv}) during the second half of normal pregnancy and establish sex-specific reference ranges."
Methods: This was a prospective longitudinal study of singleton low risk pregnancies. UV diameter and blood flow velocity were serially measured using ultrasonography at the intra-abdominal portion of the UV during 19-40 weeks. Q_{uv} was calculated as the product of mean velocity ($0.5xV_{max}$) and cross-sectional area of the vessel, and normalized for estimated fetal weight.
Results: Of 179 women (87 male and 92 female fetuses) included in the final analysis,746 observations were used to construct sex-specific reference ranges. We found no statistically significant differences in UV diameter, $V_{max,}$ and absolute or normalized Q_{uv} between male and female fetuses. However, they appeared to have some differences in the temporal development pattern of normalized Q_{uv} during the second half of pregnancy with cross-overs at 24 and 32 weeks of gestation.
Conclusions: Feto-placental blood flow is similar among male and female fetuses in quantitative terms, but the pattern of gestational age-dependent temporal changes may be different with important physiological implications in regards to *in utero* development and maturation of feto-placental unit.

Abstract

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Changes in myometrial potassium channel function and expression in dysfunctional human labour

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Introduction: Strong labour contractions require calcium influx through voltage-gated calcium channels. Thus, myometrial smooth muscle membrane potential is critical for strong labour progress. Dysfunctional labour (DL) is a significant problem in the labour ward, and is the most common indication for caesarean delivery. We recently discovered a marked excessive negative membrane potential in myometrium of both lean and obese DL women. We hypothesized that abnormally high expression and/or activity of potassium channels leads to a large negative resting membrane potential which suppresses opening of voltage-gated calcium channels resulting in weak contractions and DL necessitating caesarean delivery.
Methods: Electrophysiology was used to record membrane potential and K^+ channel activity simultaneously with contraction in myometrial strips and isolated cells from women at term not-in-labour (NIL) and in labour (IL). K^+ channel protein expression was determined using western blotting.
Results: Myometrial strips from women progressing well IL had spontaneous contractions (n=7). DL strips did not contract spontaneously (n=9), although contraction could be achieved using experimental depolarizations. Resting membrane potential in myometrium from normally progressing women NIL was -58±1mV (n=17) and IL was -58±1mV (n=7). DL myometrium was significantly more negative IL (-73±2mV, n=9). Blockade of K_V7 channels, using XE-991, returned resting potential to normal levels (-61±2mV) in high negative DL myometrium. Expression of $K_V7.1$ protein did not change in myometrium from normally progressing women before versus in labour, but was significantly increased in DL (21.2±2.4, n=5) versus normal progress IL (11.9±1.6, n=5, p=0.02). Levels of $K_V7.4$ protein were also significantly increased in myometrium from DL IL women (1.18±0.14, n=5) versus normally progressing labour (0.26±0.04, n=5, p=0.0008). In acutely isolated myometrial cells the K_V7 current (at 20mV) was enhanced in IL DL (6.1±1.1pA/pF) versus normal progress (2.5±0.5pA/pF, p=0.02). In DL myometrium, depolarization evoked by oxytocin (10nM) was 8±2mV, insufficient to reach threshold for the opening of voltage-gated calcium channels and so did not cause contraction.
Conclusions: K_V7 channels have a major input into determining the level of membrane potential in human myometrium at term and increased expression of these channels induces excessive negativity resulting in the weak contractions of dysfunctional labour, necessitating caesarean delivery.

Notes for Oral Session IX

Oral Session X

Developmental Metabolism

Keynote Speaker: Susan Ozanne
Chair: Julie Owens

Keynote Speaker and Session Chair

Professor Susan E Ozanne

Professor Ozanne is Professor of Developmental Endocrinology in the Institute of Metabolic Science Metabolic Research Laboratories and the MRC Metabolic Diseases Unit at the University of Cambridge. She is also a Fellow of Churchill College, Cambridge, U.K. Prof. Ozanne obtained a first class honours degree in Biochemistry from the University of Edinburgh, Scotland in 1990. She then went to Christ's College at the University of Cambridge where she obtained her PhD in 1994 under the mentorship of Professor Nick Hales. Before being appointed to her current post, she was a Diabetes U.K. R.D. Lawrence Fellow, a Wellcome Trust Career Development Fellow, a British Heart Foundation Lecturer and a British Heart Foundation Senior Fellow. Her research interests are focused on understanding the mechanistic basis of the relationship between suboptimal early nutrition and growth and risk of diseases such as type 2 diabetes, obesity and cardiovascular disease in later life. Initially her work was directed towards understanding how under-nutrition during fetal life influenced long-term health but her research has now expanded to include studying the link between maternal over-nutrition and obesity on the long-term health of her offspring. Her research group works on animal models of early dietary manipulation as well as on biopsy material from low birth weight humans. She is the author of over 180 peer-reviewed papers on the early origins of health and disease and is a member of the council of the Society for the Developmental Origins of Health and Disease.

Professor Julie Owens

Prof Owens is Pro-Vice Chancellor Research Strategy at the University of Adelaide. She was previously the inaugural Head of the School of Paediatrics and Reproductive Health and Associate Dean Research in the Faculty of Health Sciences and earlier, Head of Discipline of Physiology and Associate Dean Research in the Faculty of Sciences. She joined the University as an ARC QEII Fellow and subsequently an NHMRC Research Fellow, engaged in research into pregnancy and fetal and placental functional development. This continues, extending to the early life programming of obesity and cardio-metabolic health of offspring by paternal and maternal obesity and diabetes and other exposures, funded by NIH, NHMRC, ARC and other bodies. Prof Owens is Deputy President of the scientific society. Development Origins of Health and Disease ANZ, which she helped establish as a chapter of the international society. She also helped establish links with and currently participates in various international research collaborations, including the EU Early Nutrition FP7-Funded Research Program, the MRC University of Bristol Integrative Epidemiology Unit and the International Weight Management in Pregnancy Consortia.

Abstract

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Eating for two during pregnancy-Programming by maternal diet-induced obesity

Ozanne, S.E.

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Obesity prevalence is increasing at an alarming rate in both the developed and developing world. This is observed in all groups within the population, including women of childbearing age. Indeed recent statistics demonstrate that over half of pregnant women in the UK are now either overweight or obese during pregnancy. Therefore for the first time being of healthy weight during pregnancy in the UK now places a woman in the minority. This is of particular concern as obesity during pregnancy not only has immediate detrimental consequences for both mother and baby, there is growing evidence to suggest that developing *in utero* in an obesogenic environment has a long term impact on the metabolic and cardiovascular health of the child. These effects are not simply mediated by transmission of obesity genes from mother to child but occur through epigenetic mechanisms. This has been termed the developmental origins of health and disease and has been supported by studies in humans and in animal models. The strongest evidence from humans to suggest that development in an obesogenic *in utero* environment "programmes" increased risk of obesity and cardio-metabolic disease has come from the study of siblings, living in the same household but born either before or after maternal bariatric surgery. These studies revealed that the sibling born post surgery when the mother was leaner had reduced adiposity, lower blood pressure and increased insulin sensitivity compared to their sibling born prior to maternal weight reducing surgery. We have used a mouse model of diet-induced obesity to define the mechanisms by which obesity during pregnancy impacts on the long-term cardio-metabolic health of the offspring. These studies have demonstrated that the offspring of obese dams develop insulin resistance, cardiac dysfunction and fatty liver even when weaned onto a healthy low fat diet. They are also more susceptible to diet-induced obesity that further exaggerates the detrimental health consequences of maternal obesity. Our studies have identified maternal insulin as a key 'programming" factor and therefore highlight it as an important target of interventions studies.

Abstract

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Maternal obesity (MO) programs dysfunction of the oxidative phosphorylation pathway producing oxidative stress (OS) and leads to hepatic steatosis in offspring (F1) male rats exacerbated by aging

Lomas-Soria, C.[1,2], Bautista, C.J.[1], Reyes-Castro, L.A.[1], Vega, C.[1], Rodríguez-González, G.L.[1], Cox, L.[2], Nathanielsz, P.W.[3] and Zambrano, E[1].

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Introduction: MO predisposes F1 to later life metabolic disorders through several mechanisms. Non-alcoholic fatty liver disease (NAFLD) is the commonest liver disorder. With aging, the liver undergoes substantial changes in structure and function that are associated with significant impairment of hepatic metabolic activities. However, the mechanism (s) whereby MO and/or aging affect NAFLD development are poorly understood. A decline in oxidative phosphorylation is proposed as a mechanism central to limitation of life expectancy. We hypothesize that MO causes F1 liver electron transfer chain (ETC) gene down regulation, increasing ROS, leading to NAFLD. **Methods:** F0 female rats ate control (C) or obesogenic diet (MO) from weaning through lactation. After weaning F1 males ate C diet. At PND 110 and 650 serum and liver were collected for histology, liver triglycerides (TG) and OS parameters, and immunohistochemistry (IHC) for sirtuin 2 (SIRT-2). We performed F1 liver transcriptomics (RNA-seq) for differentially expressed genes (DEG) by age and diet. Data M ± SE, one-way ANOVA for all four groups for RNAseq, M±SE Log2. **Results**: MO F1 at 110d show impaired hepatic lipid function, increased NAFLD markers OS and serum lipid, exacerbated in MO at 650d vs. C 650d. MO liver lipoperoxidation and ROS increased while antioxidant enzymes activity and gene expression decreased earlier than in C F1 (data not shown). We found DEG in natural aging (C 110 vs C 650) with 97% down regulated as well as DEG between F1 male C and MO livers also 95% down-regulated. We used KEGG to identify candidate pathways related to premature aging in F1 from MO. All oxidative phosphorylation complexes showed gene down regulation in F1 from MO at 110d (Fig 1A-1D). Sirtuins are anti-aging proteins. In MO 110d SIRT-2 mRNA and protein was decreased to levels seen in F1 at C650d indicating premature aging. **Conclusions:** MO programs F1 metabolic dysfunction and aging trajectory predisposing F1 to premature aging. MO increases F1 liver fat accumulation and increased ROS and decreased antioxidant enzymes, potential mechanisms for programming MO F1 life course metabolic dysfunction.

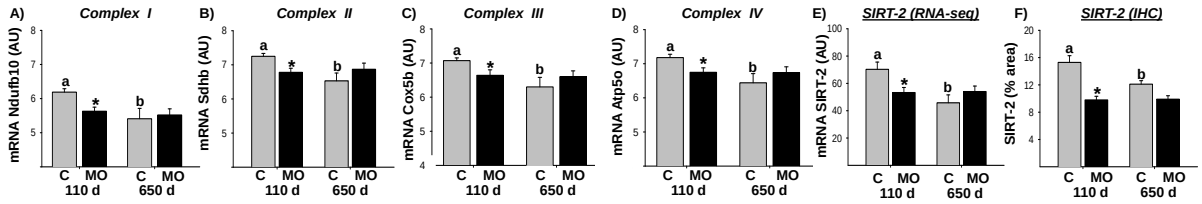

Figure 1. Genes down-regulated in premature aging from oxidative phosphorylation pathway, **A)** Ndufb10, **B)** Sdhb, **C)** Cox5b and **D)** Atp5o, **E)** SIRT-2. **F)** Protein SIRT-2 by IHC. P < 0.05 for data not sharing a letter on the same maternal diet (different age), * p <0.05 obese vs C in the same age. n=5-7 non-littermate rats. Genes are considered differentially expressed if p <0.05 vs C110d.

Abstract

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Creatine metabolism in human pregnancy

Dickinson, H.[1], Ellery S.J.[1], Della Gatta P.[2], Lappas, M.[3], Murthi, P.[1], Snow R.J.[2] & Walker, D.W.[1]

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Introduction: Creatine is an amino acid derivative, and when phosphorylated replenishes ATP preserving cellular energy levels. Creatine is obtained from a diet rich in fish, meat or dairy, or by endogenous synthesis from the amino acids arginine, glycine and methionine. When supplemented in pregnant animals, creatine prevents birth asphyxia induced damage to the fetus. Here we present the first evidence for *de novo* placental creatine synthesis, and for altered creatine synthesis and transport in pregnancies with fetal growth restriction (FGR).
Methods: These data were generated from 2 cohorts of placenta samples: (1) control term caesarean section deliveries (n=11) at Monash Medical Centre; (2) age-matched idiopathic FGR (n=13) and control (n=20) deliveries at 27-40 weeks gestation from The Royal Women's Hospital, Melbourne. In Cohort 1, creatine synthesis was determined by *ex vivo* biosynthesis assay with creatine concentrations resolved with HPLC. Tissue localisation of the creatine synthesising enzyme AGAT, and the creatine transporter, slc6a8, determined by immunohistochemistry. GAMT protein was identified by western blot. For Cohort 2, expression of AGAT, GAMT and slc6a8 were assessed using Fluidigm BioMark HD system TaqMan chemistry.
Results: The term placenta synthesis creatine *ex vivo* and expresses the creatine transporter and AGAT enzyme on the syncytiotrophoblast layer on fetal villi. mRNA expression of slc6a8 (R^2=0.6420, P<0.01) and AGAT (R^2=0.5554; P<0.01) declined with advancing gestation in control placenta. In FGR placenta, expression of AGAT and slc6a8 remained steady with advancing gestation and were 2-fold higher at any given gestational age than age matched controls.
Conclusions: The human placenta is not only a site of creatine transport, but also of synthesis. We hypothesize that pregnancy results in displacement of creatine synthesis from the maternal renal-hepatic axis to the placenta, thus ensuring creatine supply to the fetus. An adaptation to FGR may include up-regulation of creatine synthesis and transport.

Abstract

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Hypothyroidism induces hyperplasia of unilocular adipocytes in perirenal adipose tissue of the ovine fetus

Harris, S.E.[1], De Blasio, M.J.[2], Wooding, F.B.P.[2], Blache, D.[3], Meredith, D.[1], Fowden, A.L.[2] & Forhead,A.J.[1,2]

[1]*Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK*
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Introduction: Thyroid hormones are important regulators of fetal growth, although their mechanism of action remains unclear. In the sheep fetus, thyroid hormone deficiency increases plasma insulin and leptin concentrations. This study investigated the effects of hypothyroidism on perirenal adipose tissue (PAT) development and adipose insulin signalling pathways in fetal sheep.
Methods: All procedures were performed under the UK Animals (Scientific Procedures) Act 1986. In 10 twin-bearing pregnant ewes at 105-110 days of gestation (d; term~145d) and under general anaesthesia, one fetus was thyroidectomised (TX), while the other was sham-operated. After maternal and fetal euthanasia, PAT was collected from the fetuses at 143d, weighed, and frozen or processed for histology and stereological assessment. Protein and mRNA content was determined by Western blotting and qRT-PCR. Data (mean±SEM) were assessed by Student's t-test.
Results: Absolute and relative PAT mass was increased in TX fetuses compared to sham fetuses (absolute: sham 10.9±1.1g, TX 14.7±1.1g, $P<0.05$; relative: sham 3.1±0.3g/kg, TX 4.8±0.4g/kg $P<0.05$). This was due to a 2-fold increase in absolute and relative mass of unilocular (white) adipocytes (absolute: sham 3.3±0.6g, TX 7.2±0.7g, $P<0.001$; relative: sham 1.1±0.2g/kg, TX 2.3±0.3g/kg, $P<0.05$), with no change in the mass of multilocular (brown) adipocytes. Relative unilocular adipocyte mass correlated positively with plasma insulin ($r=0.76$, $P<0.001$) and leptin ($r=0.64$, $P<0.002$). Unilocular adipocyte perimeter was unaffected by TX which indicated that thyroid hormone deficiency in utero induced hyperplasia rather than hypertrophy of unilocular adipocytes. In PAT from TX fetuses, increases were observed in protein levels of proliferating cell nuclear antigen, the insulin-sensitive glucose transporter-4 and phosphorylated S6-kinase, and in mRNA and protein levels of the differentiation marker, peroxisome proliferator-activated receptor-γ ($P<0.05$).
Conclusions: In the ovine fetus, development of unilocular adipocyte mass in PAT is sensitive to changes in thyroid hormones, which may be related, in part, to altered insulin concentrations in utero. These findings have implications for the control of adipose function and leptin secretion before and after birth.

Abstract

51

Decreased substrate oxidation and increased lactate parallel hepatic glucose production in fetal sheep with intrauterine growth restriction

Wesolowski, S.R., Brown, L.D., Rozance, P.J., Wilkening, R.B. & Hay, W.W.

Department of Pediatrics, University of Colorado School of Medicine, Anschutz Medical Campus, Aurora, Colorado, USA

Introduction: Intrauterine growth restricted (IUGR) fetal sheep have increased hepatic glucose production (HGP). IUGR fetal livers also have increased *LDHA* and *PDK4* mRNA expression. Thus, we hypothesized that IUGR fetal livers would have increased lactate production and utilization, increased amino acid uptake, and decreased substrate oxidation, which would support HGP.

Methods: Placental insufficiency and IUGR fetal sheep were produced by exposure to elevated temperatures. Chronic hepatic catheterization was used to measure fetal hepatic blood flow and net flux of glucose, lactate, pyruvate, amino acids, and oxygen across the left liver lobe (CON, n=7; IUGR, n=4). Fetal liver tissue metabolites were measured with NMR (CON, n=6; IUGR, n=7). Primary fetal hepatocytes were isolated (CON, n=7; IUGR, n=12) and glucose production rate (GPR) was measured after 24h in basal media (minus glucose) or with substrates: lactate (Lac), pyruvate (Pyr), mixed amino acids (AA), or Lac+Pyr+AA.

Results: IUGR fetuses weighed 40% less and had lower blood oxygen contents and plasma glucose and insulin concentrations, and higher plasma lactate concentrations ($P<0.05$). Hepatic blood flow, adjusted for liver weight, was similar, yet hepatic oxygen uptake was 40% lower in IUGR fetuses (Fig.1). CON fetuses had hepatic glucose uptake, which was negligible in IUGR fetuses (Fig.1). Hepatic lactate uptake and pyruvate release were similar between CON and IUGR fetuses, yet uptake of Gly, Ala, Met, Leu, Phe, and Lys were reduced in IUGR fetuses ($P<0.05$). IUGR livers had decreased metabolite concentrations of total nucleotides, ATP, and cholesterol, and increased concentrations of Ala, beta-hydroyxbutyrate, and N-acetylglutamate ($P<0.05$). In IUGR hepatocytes, basal GPR was increased 80% compared to CON. GPR was increased with Lac, Lac+Pyr, AA, and Lac+Pyr+AA in IUGR hepatocytes ($P<0.05$), whereas only Lac+Pyr+AA increased GPR in CON hepatocytes.

Conclusions: HGP in IUGR fetuses is associated with decreased hepatic oxygen uptake, amino acid uptake, and nucleotide and ATP concentrations, indicating decreased substrate oxidation. Increased hepatic Ala concentrations and increased hepatocyte GPR with Lac and AA suggest use of these carbon substrates for HGP. We speculate that these glucogenic substrates are produced or alternatively metabolized, spared from oxidation, and thus used to fuel HGP in the IUGR fetus.

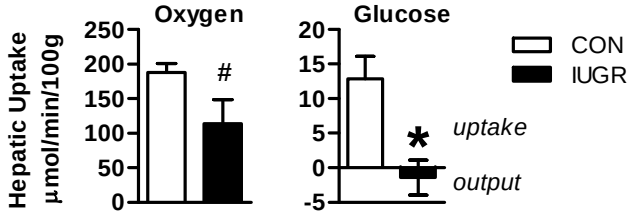

Figure 1. Fetal hepatic oxygen and glucose metabolism. Hepatic oxygen uptake and net hepatic glucose uptake measured with chronic hepatic catheterization in CON and IUGR fetal sheep. #P=0.09, *P<0.05.

Notes for Oral Session X

Dawes Lecture

Keynote Speaker: Andrew Murray

Chair: Dino Giussani

Keynote Speaker and Session Chair

This year the Geoffrey Dawes Lecture will be given by Dr Andrew Murray from University of Cambridge.

Dr Murray studied for a Masters in Biochemistry at the University of Oxford, before continuing in the Department of Physiology, Anatomy and Genetics as a British Heart Foundation- supported DPhil student in the laboratory of Professor Kieran Clarke, studying the control of cardiac energy metabolism in heart failure and diabetes.
Andrew continued in Oxford as a postdoctoral researcher, before moving to the University of Cambridge as a Research Councils UK Academic Fellow in 2007.

Andrew is currently a University Senior Lecturer in the Department of Physiology, Development and Neuroscience, and the WYNG Fellow in Natural Sciences at Trinity Hall, where he is also the Admissions Tutor for Sciences. Andrew is a Principal Investigator at the Centre for Altitude, Space and Extreme Environment Medicine, and has taken part in several large-scale high altitude research expeditions including Caudwell Xtreme Everest in 2007 and Xtreme Everest 2 in 2013. As part of these projects, Andrew and his team have measured cardiac and skeletal muscle energetics in climbers returning from the summit of Everest and muscle mitochondrial function in Lowlanders and Sherpas at Mt Everest Base Camp. Andrew has published over 50 research papers to date, including articles in the *Lancet*, *Diabetes*, *Cell Metabolism* and *Nature Cell Biology*. In 2010, he was invited by the Royal Society to represent the UK at the UK-Brazil Frontiers of Science symposium.

Professor Dino A. Giussani

Professor Giussani graduated with a first class BSc (Hons) Physiology at Royal Holloway of the University of London and Doctor of Philosophy at University College London under the mentorship of Professor Mark Hanson. He was a Post-Doctoral Fellow at the Universidad de Chile with Professor Anibal Llanos and at Cornell University with Professor Peter Nathanielsz, before taking up a tenured Lectureship (Assistant Professor) at the University of Cambridge in 1996, where he has been since.
He was promoted to Reader (Associate Professor) in 2004 and to full Professor in 2011. He also holds a Professorial Fellowship at Gonville & Caius College in Cambridge, where he is Director of Studies in Medicine. In 2016 he received a ScD from the University of Cambridge.
Professor Giussani is the current President of The Fetal & Neonatal Physiological Society. He has secured over £11 M in grant funding, published over 170 full papers and his research has won 15 international prizes including: The Lister Institute Prize, The Royal Society Wolfson Research Merit Award, The Netherlands Wim Schellekens 2007 Prize, the 2009, 2012, 2013 Lang-Pardi Foundation Awards, The 2015 David Barker Memorial Lecture at OHSU and The 2015 Sir Peter Tizard Lecture from The Neonatal Society. He is Reviewing Editor for The Journal of Physiology, Section Editor for Pediatric Research and for the American Journal of Physiology.
Professor Giussani's current programmes of research use an integrative approach at the whole animal, isolated organ, cellular and molecular levels to determine the role of fetal oxygenation and reactive oxygen species in cardiovascular development, and in setting an increased risk of cardiovascular disease in later life.

Abstract

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Life at the limit-studies of human energy metabolism at extreme high altitude*

Murray, A.J.

Department of Physiology, Development and Neuroscience, University of Cambridge, UK

** Dawes Lecture*

At high altitude, barometric pressure falls and with it inspired P_{O2}, compromising oxygen delivery to the tissues. With sufficient acclimatisation, the erythropoietic response increases red blood cell mass such that arterial O_2 content is restored; however arterial O_2 pressure remains low, and the diffusion of O_2 from capillary to mitochondrion is impaired. Mitochondrial respiration and aerobic capacity are thus limited, whilst reactive oxygen species (ROS) production increases. Use of supplementary O_2 does not fully recover aerobic capacity, hinting at a lasting peripheral impairment. In 2007, as part of the Caudwell Xtreme Everest expedition we studied Lowlander mountaineers returning from extreme high altitude at the summit of Mt Everest (8848 m), and found that cardiac energy levels fell and altered skeletal muscle energetics were seen. This was accompanied by a loss of skeletal muscle mitochondrial density, which may explain the lasting peripheral impairment. In contrast with Lowlanders, the Himalayan Sherpas, a human population of Tibetan descent, are highly adapted to life in the hypobaric hypoxia of high altitude. Physiological features and genetic signals of Tibetan groups have hinted at adaptations in oxygen supply to the tissues, however little was known about oxygen utilisation. In 2013, we therefore investigated Sherpa and Lowlander subjects before and during exposure to hypobaric hypoxia during an ascent to Mount Everest Base Camp (5300 m; Figure 1). Here we found that Sherpas have a metabolic adaptation to life at high altitude that is associated with enhanced efficiency of mitochondrial oxygen utilisation, improved muscle energetics and protection against oxidative stress compared with Lowlanders, in part associated with a putatively advantageous allele for the *PPARA* gene. Our work therefore suggests that metabolic adaptations underpin human evolution to life at high altitude, and could impact upon our understanding of human diseases where hypoxia is a feature.

Figure 1. Mitochondrial function at Mt Everest Base Camp. Dr James Horscroft and Dr Aleksandra Kotwica carry out High Resolution Respirometry to measure mitochondrial function in biopsies of their own skeletal muscle at Mt Everest Base Camp, Nepal (5300 m above sea level), as part of Xtreme Everest 2 in 2013.

Notes for Dawes Lecture

Posters

Developmental Neuroscience (1)

Poster

1

A novel non-invasive MRI biomarker revealing premature brain aging in the young adult female baboon resulting from developmental programming

Franke, K., Clarke, G.D., Dahnke, R., Gaser, Ch., Li, C., Schwab, M., Nathanielsz, P.W.

Structural Brain Mapping Group, Dept. of Neurology, University Hospital Jena, Germany and Southwest National Primate Research Center, Texas Biomedical Research Institute, San Antonio, TX, United States

Introduction: Decreased fetal nutrient delivery is widespread worldwide, resulting in disturbances of early organizational processes in cerebral development, major impairment of fetal brain structure, and life-long cognitive alterations. This study applies our innovative, non-invasive *in vivo* MRI biomarker to evaluate effects of fetal undernutrition on individual brain aging in the closest available species for translation to human brain development, eliminating confounds occurring in epidemiological studies. We hypothesized that subjects exposed to fetal undernutrition would show premature brain aging during adulthood.
Methods: First, MRI data from 29 control subjects (*Papio hamadryas*; aged 4–22y; human equivalent 16–88y) were used to construct a reference curve for brain aging in baboons, applying our novel baboon-specific MRI preprocessing pipeline and pattern recognition algorithms. Second, individual deviations from normal brain aging were analyzed in 5 young adult female baboons (aged 4–7y) that were exposed to fetal undernutrition, resulting in intra-uterine growth restriction (IUGR), and 5 same-aged female controls.
Results: Premature brain aging by +2.7 years (p<0.01) was observed in the sample of young adult female IUGR offspring.
Conclusions: This study is the first to reveal premature brain aging during young adulthood resulting from developmental programming due to fetal undernutrition. Our innovative *in-vivo* biomarker will enable tracking longitudinal effects of (preventive) treatments, developmental and environmental influences on individual brain aging.

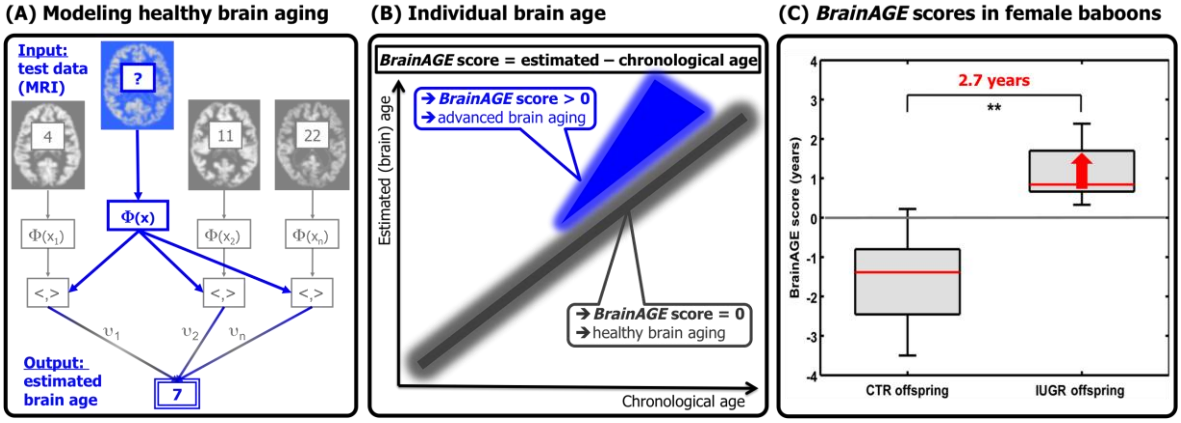

Figure 1. Depiction of the *BrainAGE* concept and study results. (A) The model of healthy brain aging is trained with chronological age and segmented structural MRI data of a training sample (gray). Subsequently, the individual brain age of a new test subject is estimated (blue). **(B)** The difference between estimated and chronological age results in the individual *BrainAGE* score. **(C)** *BrainAGE* scores were significantly increased in young adult female IUGR offspring (**p<0.01). [Funding: NIH 1 R24 RR021367-01 A1]

Poster

2

Antenatal maternal glucocorticoid treatment produces premature brain aging in the male middle-aged baboon offspring as revealed by *in-vivo* MRI

Franke, K., Clarke, G.D., Dahnke, R., Gaser, Ch., Li, C., Schwab, M., Nathanielsz, P.W.

Structural Brain Mapping Group, Dept. of Neurology, University Hospital Jena, Germany &
Southwest National Primate Research Center, Texas Biomedical Research Institute, San Antonio, TX, United States

Introduction: Synthetic glucocorticoids (sGC) are administered to women threatening premature delivery to accelerate fetal lung maturation, a treatment with clear benefits on offspring morbidity and mortality. However, exposure to inappropriately high levels of sGC for the current stage of brain development has been shown to result in adverse outcomes of brain development in several species. In our baboon model of fetal sGC exposure we have already shown sex-specific behavioral effects in the juvenile offspring. This study examines brain aging of the sGC-exposed animals during mid- adulthood.
Methods: First, *in-vivo* MRI data from 29 control baboons (aged 4–22y; human equivalent 16–88y) were used to construct a reference curve for brain aging, applying our novel baboon-specific MRI preprocessing pipeline and pattern recognition algorithms. Second, individual deviations from normal brain aging were analyzed in 4 mid-adulthood male sGC baboons (10y; human equiv. 40y) with maternal treatment of 3 courses of betamethasone (175 µg/kg/day on two consecutive days) at 0.6, 0.64, and 0.68 gestation (human equiv. 24, 26, 28 weeks pregnancy) and 4 age-matched saline exposed male controls.
Results: Premature brain aging by +1.3 years (p<0.05) was observed in the male mid-adult offspring of mothers who received sGC treatment during pregnancy.
Conclusions: This study is the first to reveal premature brain aging during adulthood resulting from developmental programming by sGC treatment during gestation. There is a need for further studies on the brain tracking longitudinal effects of sGC exposure in the baboon model and human samples to enable early diagnosis and preventive treatment.

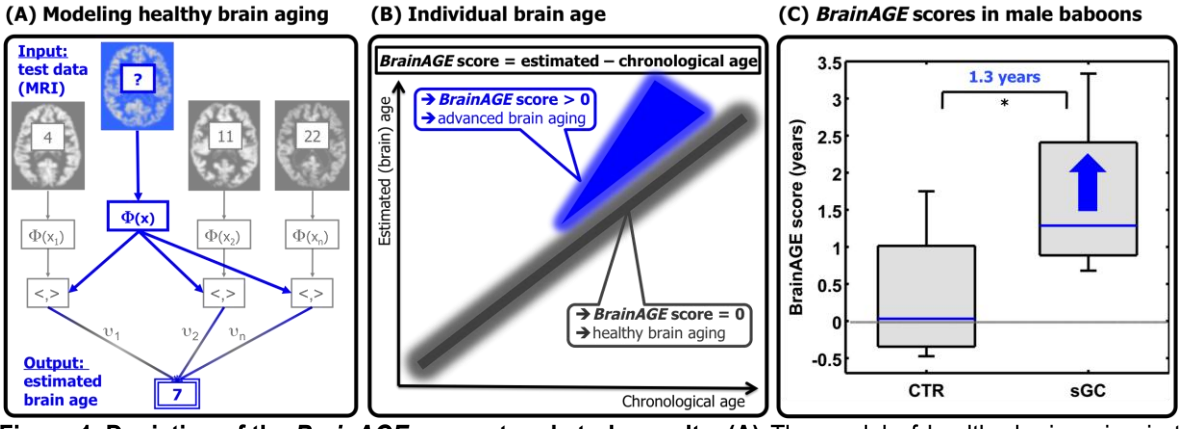

Figure 1. Depiction of the *BrainAGE* concept and study results. (A) The model of healthy brain aging is trained with chronological age and segmented structural MRI data of a training sample (gray). Subsequently, the individual brain age of a new test subject is estimated (blue). **(B)** The difference between estimated and chronological age results in the individual *BrainAGE* score. **(C)** *BrainAGE* scores were significantly increased in the adult male sGC offspring by 1.3 years (*p<0.05). [Funding: NIH 1 R24 RR021367-01 A1]

Poster

3

Sensorimotor gating deficits following pre and postnatal stress in guinea pig offspring

McInerney, K.M., Palliser, H.K., Shaw, J.C. and **Hirst, J.J.**

Mothers and Babies Research Centre and School of Biomedical Sciences and Pharmacy, University of Newcastle, NSW, Australia

Introduction: Infants born following compromised pregnancies are often exposed to further stress, such as maternal separation, during their time in the NICU. Perinatal stress increases vulnerability to anxiety and other behavioural/attention disorders in childhood and beyond. Previous studies have shown that the long-term effects of prenatal stress on offspring behaviour are enhanced by a range of postnatal maternal stressorsEmack and Matthews, 2011). Anxiety spectrum disorders and cognitive impairments are associated with sensorimotor gating (prepulse inhibition, PPI) deficits. Neurosteroids have been recently found to play an important role in the regulation of PPI and neurosteroid deficiencies have also been linked to perinatal stress and the development of these disorders. Our aim was to develop a model of prenatal maternal stress followed by postnatal separation stress in the guinea pigs that results in sensorimotor gating deficits for the future assessment of the neurosteroid based anxiolytics and their potential use as therapies for reducing behavioural disorders.

Methods: Pregnant guinea pigs were exposed to psychosomatic stress (strobe light exposure, 2hrs/day every 5 days, GA50 to term) or were subjected a control exposure (no light) before delivering spontaneously. Neonates from these psychosomatic stress, and a subset of control pregnancies underwent maternal and peer separation from PND2-8 (postnatal stress, 2hrs/day; pre + postnatal stress). Offspring underwent acoustic startle (SR-LABs) testing at 28 days of age. Acoustic startle testing involved measuring the startle response to a 120dB stimulus with and without presentation of a low intensity, non-startling pulse (3-15dB above background) immediately prior to the stimulus to assess prepulse inhibition (PPI).

Results: Baseline startle response was inhibited in offspring from both the post and pre+post stress groups. PPI was disrupted in pre+post stressed offspring compared to controls (Figure).

Conclusions: Dual (pre and postnatal) stress decreases sensorimotor gating in offspring that is consistent with an anxious phenotype. These findings indicate postnatal stress markedly potentiates the adverse effects of maternal stresses during late pregnancy and suggests this is a suitable model for the evaluation of therapeutic interventions. Further assessment of longer term behavioural and neurodevelopmental outcomes is warranted.

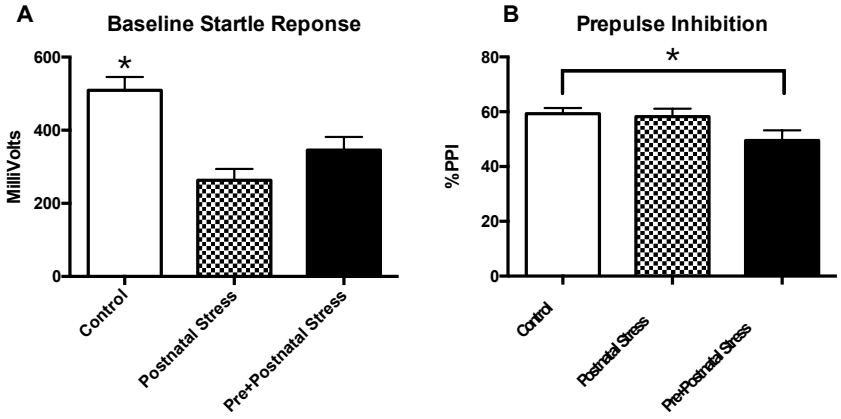

Figure 1. Baseline acoustic startle response (A) and prepulse inhibition (B) in 28 day old guinea pig offspring following postnatal alone and pre+postnatal stress.

Poster

4

Differential Expression of Neurogenesis Mediators by Physiological and Preeclamptic Placenta-derived Mesenchymal Stromal Cells

Barrile, R.[1], Nuzzo, A.M.[1], Leonardi, R.[1], Mele, P.[2], Eva, C.[2], Todros, T.[1], Rolfo, A[1].

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[2]*Department of Neuroscience, University of Turin, Turin*

Introduction: The placenta is involved in the modulation of fetal neurodevelopment. In particular, the syncithiotrophoblast metabolizes fetal serotonin from maternal circulating tryptophan until the second trimester of pregnancy. We recently reported that, within the chorionic villi, Placenta-derived Mesenchymal Stromal Cells (PDMSCs) express Brain-derived Neurotrophic Factor and Neurotrophins 3/4, key modulators of neurogenesis. Importantly, we demonstrated that these molecules are over-expressed in PDMSCs isolated from pregnancies complicated by PE-FGR, severe placenta-related disorders associated with neurological disorders and intellectual disability for the newborn. PDMSCs contribution to fetal neurodevelopment has never been investigated.
Our aim is to characterize the expression of neurogenesis mediators Doublecortin and NCAM, of indolamine-2,3-dioxygenase (IDO), responsible for the production of neurotoxic tryptophan metabolites, and of miRNA-124, -134 and -181a, intracellular regulators of neurogenesis, in normal and PE-PDMSCs in order to understand their role in physiopathological fetal neurodevelopment.
Methods: PDMSCs were isolated from healthy (n=7) and PE-FGR (n=7) placentae. Doublecortin, NCAM and IDO mRNA and miRNA-124, -134 and -181a levels were determined by Real-Time PCR. Western blot was used to evaluate NCAM "polysialic acid-modified" (PSA-NCAM) protein levels. PSA-NCAM expression is inversely correlated to that of NCAM, acting as a negative modulator of neurodevelopment. Spatial expression of IDO, PSA-NCAM and Nestin, a neurofilament typically found in neuroblast, was detected using immunocytofluorescence (ICF).
Results: Doublecortin and NCAM mRNA levels were over-expressed ($p<0.05$), while IDO mRNA expression was decreased ($p<0.05$) in PE-FGR vs normal PDMSCs. In contrast, PSA-NCAM protein levels were down-regulated ($p<0.05$) in PE-FGR vs normal PDMSCs. miRNA-124, -134 and -181a expression was higher in normal vs PE-FGR PDMSCs. PSA-NCAM, IDO and Nestin expression was confirmed by ICF.
Conclusions: We characterized, for the first time to our knowledge, the expression of neurogenesis mediators in normal and PE-FGR PDMSCs. Doublecortin and NCAM mRNA increase together with PSA-NCAM, IDO and miRNAs down-regulation suggest that PE-PDMSCs try to counteract impaired fetal neurodevelopment by promoting the expression of pro-neurogenic molecules and avoiding placental accumulation of neurotoxic metabolites. Further investigations are required.

Poster

5

The Role of Neonatal Dexamethasone Exposure on Adult Psychiatric Phenotypes in a Rodent Model

Yates, N. J., Martin-Iverson, M. T., Robertson, D., and Rodger, J.

The University of Western Australia, School of Animal Biology

Introduction: The effects of early life stress *in utero* or as neonates has long-term consequences on hypothalamic-pituitary-adrenal (HPA) stress axis function and neurodevelopment. These effects extend into adulthood and may underpin a variety of mental illnesses such as schizophrenia. Additionally early life difficulties associated with glucocorticoid treatment in infants are associated with adult mental health issues. Yet no study has comprehensively attempted to mechanistically link early life HPA axis activation with schizophrenia phenotypes. We examined the potential developmental programming effects on adult behaviour and brain function of neonatal glucocorticoid exposure using the synthetic glucocorticoid dexamethasone in rat pups.

Methods: Rat pups were injected with 1.5 mg/kg dexamethasone solution or saline vehicle from postnatal day 5 to 10 inclusive. We utilized a comprehensive battery of assessments for adult behaviour (i.e. startle reactivity, dopamine sensitivity, anxiety), brain function (i.e. EEG, auditory evoked potentials) and gene expression (i.e. dopamine and glucocorticoid genes) to determine if elevated early life HPA activation is associated with adult-onset schizophrenia-like traits.

Results: Neonatal dexamethasone exposure in rats increased startle reactivity under all conditions tested, yet it decreased sensitivity of sensorimotor gating to dopaminergic disruption – opposite of what is observed in schizophrenia and other psychiatric illnesses. There also appeared to be mild long-term reductions in stress and anxiety-related behaviours with neonatal dexamethasone exposure. Electrophysiology revealed that there were no schizophrenia-like abnormalities in auditory processing or resting state brain function with dexamethasone exposure. However, neonatal dexamethasone altered auditory cortex glucocorticoid activation, and auditory cortex synchronization.

Conclusions: Neonatal glucocorticoid exposure in has subtle yet long lasting effects on neurodevelopmental programming. Our results indicate that neonatal HPA axis activation by dexamethasone alters several aspects of adult brain function and behaviour, but is not specifically related to any particular neuropsychiatric disease such as schizophrenia. Changes in dopamine sensitivity and startle reactivity indicate that excessive neonatal glucocorticoids may alter the ability to filter sensory stimuli as adults, which has broad implications on learning and attention. In addition the phenotype indicates that neonatal dexamethasone exposure may induce long-term changes in emotional stress-reactivity.

Poster

6

Creatine protects the fetal brain from the effects of severe *In Utero* hypoxia

Dickinson,H.[1,2], Rajakaruna, S.[1], Ellery, S.[1], Muccini, A.[1], Hale, N.[1], Snow, R.[3], Walker, D.[1,2]

[1]The Ritchie Centre, Hudson Institute of Medical Research,[2] Department of Obstetrics & Gynaecology, Monash University; [3]Institute for Physical Activity & Nutrition, Deakin University Melbourne, Australia

Introduction: Protecting the brain from injury through the perinatal period is imperative to preventing brain injury and neurodevelopmental disabilities acquired *in utero* or at birth. Supplementation of creatine in late pregnancy has been shown to be an effective neuroprotective agent in the context of birth asphyxia in spiny mice (Ireland et al, 2011). In this study we examined the effect of increasing creatine in fetal sheep on the extent of cell death and brain injury caused by *in utero* cerebral hypoxia/ischaemia induced by umbilical cord occlusion (UCO).

Methods: Singleton fetuses of 11 pregnant ewes were surgically prepared at 118 days gestation with an inflatable cuff placed around the umbilical cord, and catheters for intravenous infusion of either creatine (6 mg/kg/h, n=6) or the equivalent volume of saline (6 ml/kg/h, n=5) from 122-135 days gestation. Fetal arterial blood was sampled daily, and frequently before and after 10 mins UCO at 132 days gestation. The ewe and fetus were killed humanely at 135 days to obtain the fetal brain for analysis of cell death (TUNEL), and extravasation of serum protein into the brain.

Results: Quantitative analysis revealed that creatine treatment reduced the number of TUNEL and sheep serum +ve cells in the subcortical white matter and deep white matter, and in the striatum (**Figure).**

Conclusions: Increasing the creatine supply to the fetus in late gestation protects the fetal brain from the effects of transient *in utero* hypoxia, and may therefore be an effective prophylactic treatment for all pregnant women, especially where the level of obstetric surveillance is low or restricted.

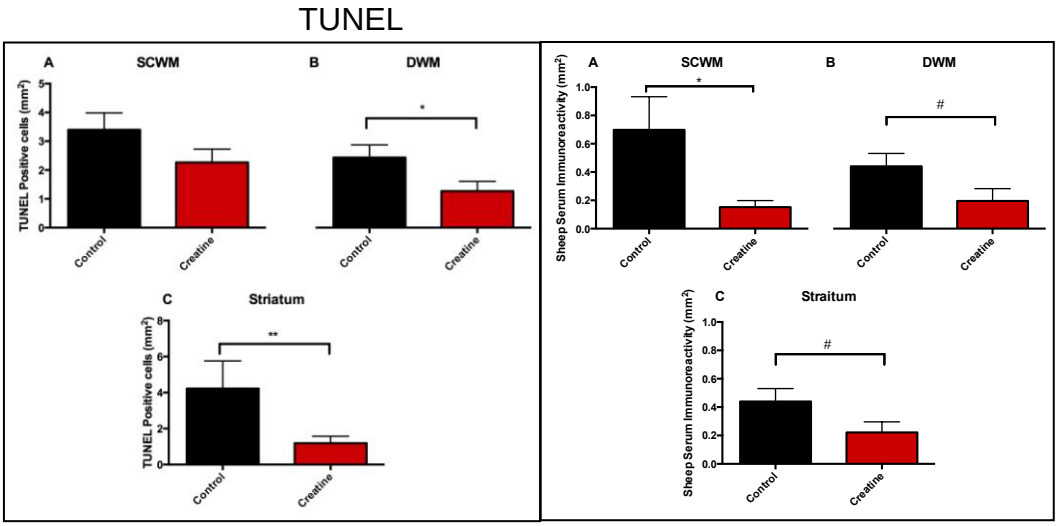

Figure 1. TUNEL and Serum protein +ve cells (no. cells/mm^2) in sub-cortical white matter (SCWM), deep white matter (DWM) and striatum at 72 h after UCO in fetal sheep infused with saline (black bars) or creatine (red bars) for10 days prior to UCO at 132 days gestation.

Posters

Developmental Programming

Poster

7

The effect of maternal obesity in mice on pup anxiety and mother-pup interactions in the first week of life

Rasool, A.[1], Green, L.R.[1], Teeling, J.L.[2], Cagampang, F.R.[1], Poore, K.R.[1]

[1] Institute of Developmental Sciences, University of Southampton, Southampton, UK; [2] Biological Sciences, University of Southampton, UK

Introduction: The obesity epidemic is leading to an increase in obesity during pregnancy, which has implications for the health and wellbeing of offspring. Maternal obesity influences offspring behaviour such as anxiety/stress in adult life, potentially via the influence of low-grade inflammation on the development of neural pathways such as the hypothalamo-pituitary adrenal (HPA) axis [1]. This study used ultrasonic vocalisations to investigate pup anxiety, a marker of HPA function, in the first week of life following exposure to maternal obesity induced by high fat (HF) feeding. Since maternal behavior towards her pups also has long lasting consequences for offspring HPA stress responsiveness [2], we also assessed early markers of maternal care in HF-fed mothers.

Methods: Female C57BL/6 mice were fed either a high fat diet (HF; 45% kcal fat) or control diet (C; 7% kcal fat) 6 weeks before mating, throughout pregnancy and lactation. In 7 day-old male and female pups (C, n=20; HF, n=19) anxiety was assessed by measuring ultrasonic vocalisations (USVs) using a bat detector (70 KHz) during 4 min isolation from the mother. Maternal behaviour (C, n=5; HF, n=6) was assessed by recording times of first dam-pup interaction and pup retrieval to the nest in the 2 min following pup return (averaged across all littermates). Data were analysed by *t* test.

Results: There was a significant reduction ($P<0.05$) in pup USVs in female but not male pups from HF mothers. The incidence of mothers failing to return pups to the nest was greater for HF-fed mothers, however there was no significant difference in the times taken to interact with pups or return pups between C and HF mothers.

Conclusions: The effect of maternal HF diet on pup anxiety was sex-specific, with pup USVs reduced only in females. Although mother-to-pup interactions were not different between C and HF mothers at day 7, further studies of behaviour of these offspring at multiple time points in their lifespan will determine whether maternal, as well as postnatal HF feeding, will affect adult offspring behaviour, including anxiety and memory and learning.

Refs:
1. Sullivan *et al.* (2012) Physiology and Behaviour 123: 236-242.
2. Liu *et al.* (1997) Science 277: 1659-62.

Poster

8

Prenatal high fat diet exposure in mice primes offspring for increased neuroinflammation and altered hypothalamic stress markers

Rasool,A.[1], Green, L.R.[1], Teeling, J.L.[2], Cagampang, F.R.[1], **Poore, K.R.**[1]

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Introduction: Obesity during pregnancy is a rapidly increasing global issue with 5% of pregnant women classed as obese in the UK [1]. Maternal obesity influences offspring behaviour and stress responses, however the mechanisms remain unclear. One possibility is that the hypothalamo-pituitary adrenal (HPA) axis, which regulates the stress response, is influenced by an inflammatory response to a high fat (HF) obesogenic diet. This study investigated markers of HPA function and of neuroinflammation in offspring from HF-fed obese mothers, with or without subsequent postnatal HF-feeding.
Methods: Female C57BL/6 mice were fed either a high fat diet (HF; 45% kcal fat) or control diet (C; 7% kcal fat) 6 weeks before mating and throughout pregnancy and lactation. From weaning onwards, offspring were fed C or HF diet resulting in 4 offspring groups: C/C (n=5), C/HF (n=4), HF/C (n=6), HF/HF (n=4) (pre/postnatal diet). In 15 week-old male offspring brain, gene markers of inflammation (IL-1β, TNF-α, FCγR1) and HPA function (corticotropin releasing hormone [CRH], glucocorticoid receptor [GR]) were measured by RT-PCR in the hypothalamic paraventricular nucleus (PVN). Data were analysed by 2-way ANOVA.
Results: IL-1β but not TNF-α was significantly increased (P<0.01) and GR was significantly decreased (P<0.05) in HF-fed male offspring PVN, but only when their mothers were HF-fed (HF/HF). Maternal HF diet also increased CRH and decreased FCγR1 in offspring PVN, regardless of postnatal diet (P<0.05).
Conclusions: Maternal high fat diet influences markers of both inflammation and stress in the PVN of 15 week-old male mice offspring. The effects on IL-1β and GR specifically in HF/HF offspring suggest that a maternal high fat diet may prime offspring to the adverse effects of subsequent high fat diets in postnatal life. Further studies of offspring from this dietary model later in their lifespan will determine whether these changes persist into old age and have implications for stress responsiveness.

Refs:
1. Centre for Maternal and Child Enquiries (2010). Maternal obesity in the UK: findings from a national project. London: CMACE

Poster

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Maternal protein restriction (MPR) during pregnancy accelerates aging of sperm quality in male rat offspring (OFF)

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Introduction: We have shown that MPR during pregnancy leads to premature aging of male reproductive capacity. It has been suggested that early life nutritional adversity is associated with increased oxidative stress (OS). OS is implicated in male infertility and aging. We hypothesized that MPR during pregnancy accelerates the aging process in sperm quality and that OS plays a role.

Methods: We studied male rat OFF whose mothers ate either control (C) (20% casein) or a restricted (R) (10% casein) isocaloric diet during pregnancy. After birth all rats were fed C diet. At postnatal day (PND) 110, 450 and 850 male OFF were euthanized and sperm from the epidiymal tail and vas deferens were obtained to measure: 1) reactive oxygen species ROS – by fluorescence), 2) sperm quality (concentration, viability and motility). Data are M ± SEM; two-way ANOVA analysis; n=6, $p<0.05$.

Results: At all ages ROS concentrations were higher in the R group vs C. Sperm quality was reduced at all ages in the R group. ROS levels, sperm quality and fertility were analyzed at different ages within groups. In both groups, ROS levels did not change with age and sperm concentration decreased at PND 450 with no further changes. For both groups sperm viability decreased at PND 450. In the R group sperm viability declined further by PND 850. In both groups, sperm motility decline by PND 850, however in R group the motility was already reduced at PND 450. (Fig. 1).

Conclusions: MPR increases sperm ROS which may play an important role in the trajectory of aging in the sperm quality. The increase of ROS in the sperm of young adults may lead to the programming of diseases in the next generation via the male germ line, currently a little studied area that requires more investigation.

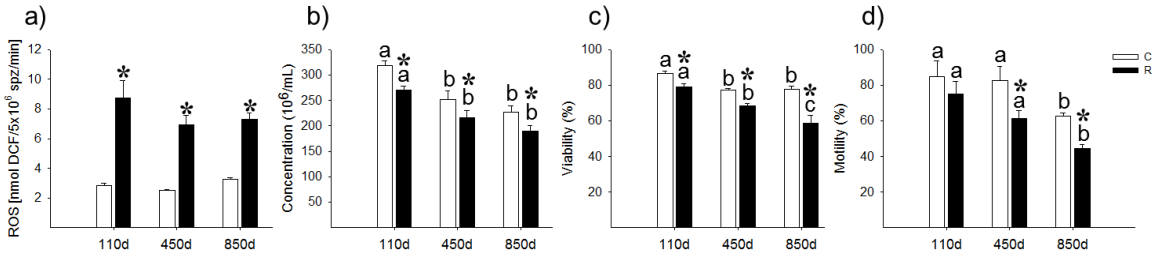

Figure 1. Sperm characteristics across the life course: a) ROS, b) sperm concentration, c) sperm viability; d) sperm motility. M ± SEM, n=6, $p<0.05$ for data not sharing at least one letter on same maternal diet. * indicates $p<0.05$ vs C.

Poster

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Maternal obesity (MO) during pregnancy and lactation increases oxidative stress is the neonatal rat testis

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Introduction: Growing evidence shows that MO during pregnancy and lactation has long-lasting consequences for offspring health (chronic metabolic, endocrine and reproductive disorders). We have shown that MO during pregnancy and lactation increases testicular and sperm oxidative stress and impacts male fertility in adult life. Nrf2 serves as a master regulator to control the basal and inducible expression of an array of antioxidant and detoxification enzymes. We hypothesized that the alterations observed in adult life such as the increase in testicular reactive oxygen species and the decrease in male offspring reproductive capacity are already programmed since early stages of development.

Methods: Control maternal rats (C) ate normal chow (5%-fat) while MO mothers ate a high fat diet (20% animal lard and 5% fat) from weaning through pregnancy and lactation. After weaning all rats ate C diet. We determined Nrf2 (qPCR), malonaldialdehyde (MDA – by spectrophotometry), reactive oxygen species (ROS – by fluorescence) and superoxide dismutase (SOD – by spectrophotometry) activity in neonatal testis of 36 postnatal days.

Results: Nrf2, the master gen of the antioxidant response was increased in the neonatal testis from the MO group. MDA and ROS levels, as well as SOD activity was also higher in the testes homogenate at postnatal day 36 (Fig.1).

Conclusions: MO produces signs of oxidative stress in offspring testes at the time of puberty. Thus oxidative stress may be one mechanism by which MO reduces male offspring reproductive capacity in adult life (Int J Obes 2016;39(4):549-56).

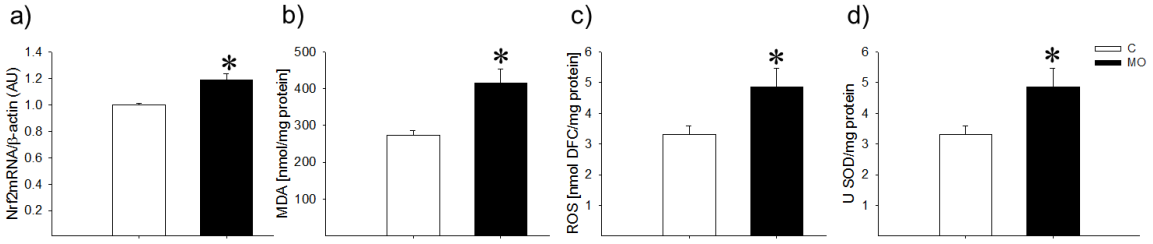

Figure 1. Testis at 36 postnatal days: a) Nrf2, b) MDA, c) ROS; d) SOD. M ± SEM, n=6,* p<0.05 vs C.

Poster

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Maternal obesity (MO) up-regulates the protein associated with stress in the late gestation baboon fetal frontal cortex

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Introduction: Maternal obesity (MO) in pregnancy results in poor fetal development predisposing to adult neurological dysfunction. The frontal cortex (FC) of the brain plays an important role in memory, learning, and complex cognition A study of obesity in pregnancy in Japanese macaque mothers showed greater anxiety in offspring (Sullivan EL et al J Neurosci. 2010; 30:382), **Hypothesis:** MO affects the fetal FC stress signalling pathways by up-regulating glucocorticoid receptors (GR) and increasing protein expression of the serotonin transporter (5-HTT).
Methods: Animals ate Purina Monkey Diet. Female baboons, similar age and weight were randomly assigned to control (CTR, n = 25) or MO diet (n = 19; 45% energy fat, 4.62% glucose, 5.64% fructose, and 2.32% sucrose, energy 4.03 kcal/g and free access to high-fructose sodas). CTR diet contained 12% energy fat, 0.29% glucose, 0.32% fructose energy 3.07 kcal/g. Diets were maintained through pregnancy. Fetal FC was collected at Caesarean section under general anaesthesia at 90% gestation. GR, phosphor-GR (p-GR) and 5-HTT were measured by immunohistochemistry (IHC) with Image J quantification of stained fraction. Analysis was by Student's t-test: $P < 0.05$.
Results: % area stained for GR and 5-HTT were greater ($P < 0.05$) in gray and white matter of MO fetal FC, and p-GR protein tended to increase ($0.05 < P < 0.1$) [Fig. 1].
Conclusions: Increased GR and p-GR will tend to accelerate FC cellular differentiation at the expense of proliferation resulting in postnatal neurological dysfunction of stress signalling pathways. Up-regulation of 5-HTT would increase serotonin uptake by the presynaptic neuron resulting in local serotonin deficiency in the synaptic cleft. **Conclusions:** MO results in changes in fetal FC associated with the stress signalling pathways by late gestation that may explain the programming of offspring of MO mothers to anxiety and depressive mood.

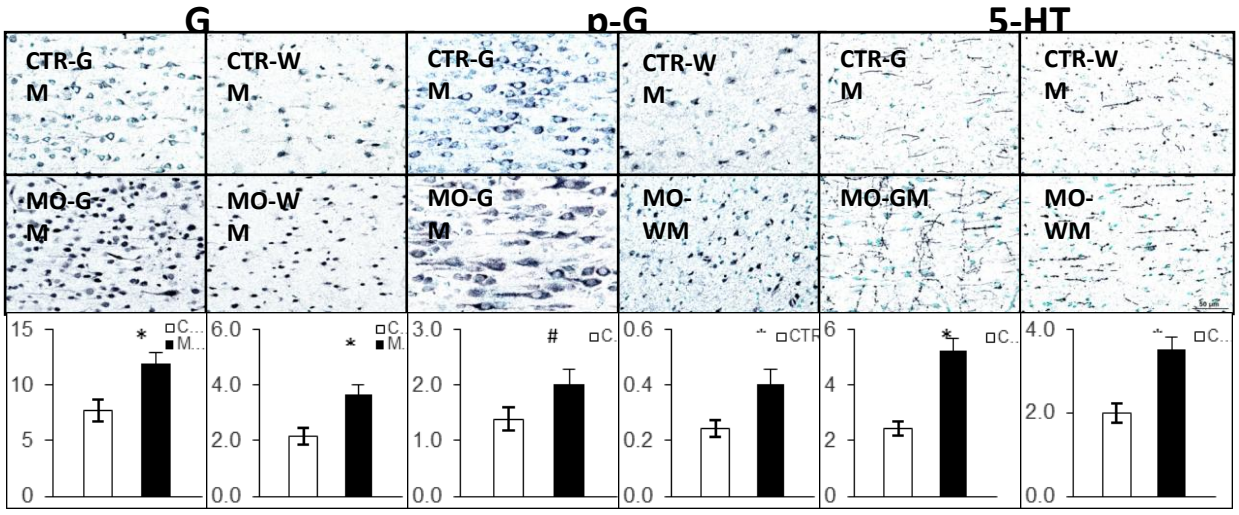

Figure 1. * $P < 0.05$, GR and 5-HTT immunoreactivity were increased in both grey matter and white matter of the frontal cortex of baboon foetuses of MO mothers. p-GR was up regulated in white matter. # $P = 0.069$.

Poster

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Intrauterine growth restriction produces accelerated cardiac aging in male and female adult baboons

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Introduction: Extensive rodent studies show that poor perinatal nutrition programs later life cardiovascular disease. To enable translation to humans, we developed cohorts of baboon offspring of mothers fed *ad lib,* control or 70% control diet in pregnancy and lactation. Offspring were IUGR at birth. Since we have shown premature brain aging in these IUGR offspring, we hypothesized that IUGR offspring show impaired heart function and accelerated cardiac aging. **Methods:** We studied IUGR baboons (8 male, 8 female) age 5.7 years and control (CTL) offspring: (8 male, 7 female), age 5.6 years and elderly baboons (OLD), (6 male, 6 female), age 15.9 years Human equivalent 64 years, using cardiac MRI to evaluate left ventricular (LV) and right ventricular (RV) functional parameters, normalized to body surface area. Analysis two-way ANOVA by group and sex with $p < 0.05$. **Results:** Ejection fraction (EF), three-dimensional sphericity index (3DSI), cardiac index, diastolic volume and normalized LV wall thickness normalized for body surface area differed by group. Group and sex differences were found for normalized LV wall thickening and myocardial mass, without interactions. Decreased LV EF was noted in IUGR and OLD (58±3%, 45±2%, 50±3%, CTL, IUGR, OLD, mean ± SEM, ANOVA $p<0.01$). Similarly, increased LV globular morphology (0.32±0.01, 0.39±0.02, 0.41±0.02, $p=0.0005$) and decreased filling rate (88.8±7.1mL/sm2, 63.5±7.0mL/sm2, 62.0±7.3mL/sm2, $p<0.05$) were present. RV EF was depressed in IUGR and OLD (49±2%, 32±3%, 39±3%, $p<0.0001$), accompanied by decreased stroke volume (26.5±1.8mL/m2, 20.0±1.8mL/m2, 17.5±2.2mL/m2, $p<0.01$). RV and LV ejection fractions were correlated ($r=0.80$, $p<0.001$). (Figure). LV filling rate and diastolic 3DSI normalized for body surface area were not correlated in CTL but strongly correlated in OLD ($r=0.69$, $p<0.01$) and IUGR baboons ($r=0.81$, $p<0.01$). **Conclusions:** IUGR programs reduced systolic and diastolic function, producing myocardial remodelling resulting in emergence of premature cardiac aging. To our knowledge this is the first demonstration of the specific characteristics of cardiac programming and functional decline with aging in any species. Further studies across the life span will determine progression of cardiac dysfunction. Non-invasive imaging identifies early cardiac biomarkers of IUGR in this susceptible population, improving recognition of clinical presentations and management of treatment strategies.

FIGURE

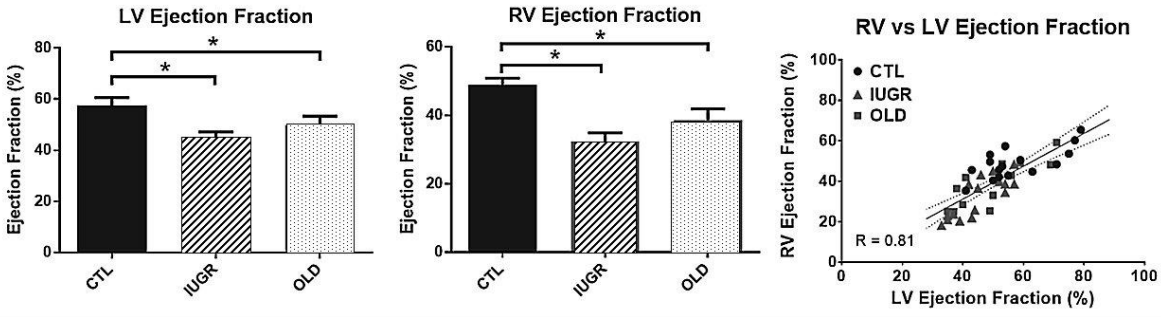

Figure: LV and RV Ejection fractions normalized to body surface area and their correlation in control (CTL) baboon offspring, (8 male, 7 female; age 5.6 years), IUGR baboon offspring (8 male, 8 female; age 5.7 years) and normal elderly baboons (OLD), (6 male, 6 female, age 15.9 years). Analysis was by two-way ANOVA by group and sex with * $p < 0.05$.

Posters

Clinical Fetal and Neonatal Physiology

Poster

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Retinal microvascular plasticity in a premature neonate

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Introduction: Dilation and abnormal tortuosity of retinal vessels are the hallmarks of severe retinopathy of prematurity (ROP) in premature infants and, if left untreated, may lead to haemorrhage, retinal detachment and blindness. Deregulated signalling pathways involving hypoxia-inducible factors such as vascular endothelial growth factor (VEGF) are involved in the pathogenesis of ROP. VEGF-antagonists are increasingly being used as "off-label medication" to treat this condition, with some success.

Methods: We present Baby SM (female), who was born prematurely at 24 weeks gestation, birth weight 640g, in a tertiary neonatal intensive care unit. The baby developed severe hyaline membrane disease complicated by pulmonary haemorrhage. In total, she required 375 hours mechanical ventilation followed by 789 hours continuous positive pressure support. On screening at 35 weeks postmenstrual age (PMA), she was noted to have ROP, which became severe (Grade 3) by 37 weeks PMA. She received 1 dose of intravitreal VEGF antagonist (Bevacizumab) She was discharged home at 127 days of age, and she will regularly be reviewed until the age of 3 months.

Results: The retina before and after receiving VEGF antagonist are shown in Figure 1. After receiving intravitreal VEGF-antagonist, the vessels tortuosity and dilation decreased markedly. Circulating VEGF- antagonist levels have been shown to last 4 to 6 weeks. Repeat imaging at 4 weeks shows re-emergence of vessel tortuosity.

Conclusions: We believe the observed changes demonstrate the inherent retinal microvascular plasticity in premature neonates. With improved survival of extremely premature neonates and the availability of retinal imaging technology, we are now able to monitor this plasticity, allowing closer assessment of both new treatments and the underlying retinal microvascular physiology in the premature neonate and, presumably, the fetus.

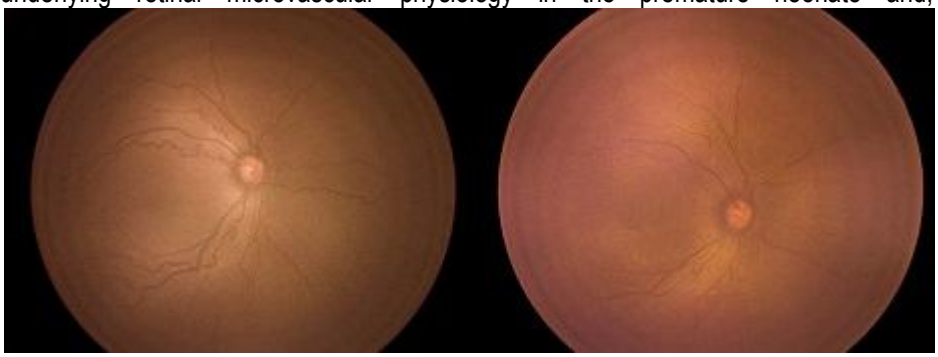

Figure 1. Image of the retina before (Left) and 2 weeks after (Right) intravitreal injection of VEGF-antagonist. The arterial and venous tortuosity had reduced dramatically.
(Retinal images were acquired by using RetCam III, Clarity Medical System, CA, USA).

Poster

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Perinatal prognosis using of the 5-tier system of assessing fetal heart rate tracing

Murabayashi, N., Kubo, M., Nii, M., Maeda, Y., Shimura, M., Magawa, S., Kawamura, T., Tanaka, K., Tanaka, H., Osato, K., Umekawa, T., Kamimoto, Y., Parer, J., and Ikeda, T.

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Introduction: To determine whether the 5-tier system of assessing fetal heart rate tracing can result in reduced metabolic acidemia without increasing obstetrical intervention.

Methods: Rates of umbilical artery pH and base excess values, and operative intervention delivery were determined in 14 hospitals in Japan, all of which accept patients in all risk categories. Results were compared for 2 years before and 2 years after a 6-month training period in the Japan Society of Obstetrics and Gynecology 5-tier system of assessing fetal heart rate tracings. All of the perinatologists and nursing staff were educated by lecture or DVD in the 5-tier system, and had weekly discussions in every hospital.

Results: Deliveries were about 10,000 every year, which is equivalent to 1% of all the Japanese deliveries. Although the percentage of low base excess cases were reduced 27% in the post training period, the pre- and post-training rates of umbilical arterial pH<7.10 (1.4% vs. 1.3%, p=0.86) and base excess <-12mEq/L(2.2% vs. 1.6%, p=0.35) did not differ significantly. The rates of unscheduled Cesarean deliveries (6.5% vs. 7.6%, p=0.54) and vacuum deliveries (7.8% vs. 7.4%, p=0.83) did not differ significantly.

Conclusions: The 7-fold reduction in metabolic academia seen in a low risk population (Katsuragi et al, 2015) was not seen in this high risk population, possibly because of the higher intrinsic risk of metabolic academia, and fore-knowledge by practitioners over the past 5 years of the Japanese 5-tier system.The 5-tier system may be useful for reduction of newborn metabolic acidemia without increasing operative intervention.

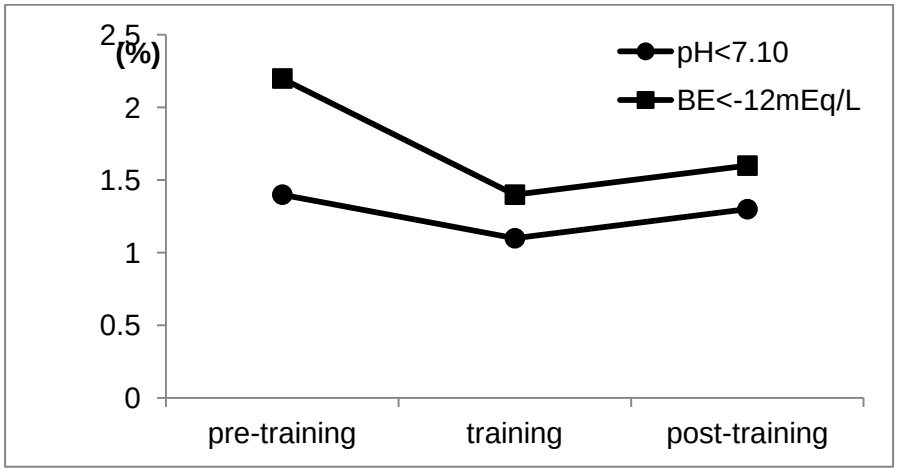

Figure 1. Rates of acidosis. Percentage incidences of umbilical cord blood at birth with a mean umbilical arterial pH <7.10 and umbilical base excess <-12 mEq/L in every period. Low base excess cased were reduced after training.

Poster

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Magnesium sulfate reduced fetal ventricular tachycardia and Torsa de Pointes in congenital long QT syndrome

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Introduction: Congenital long QT syndrome occurs in one out of 5000 people. In some cases, they may develop ventricular fibrillation, ventricular tachycardia, and Torsa de Pointes.

Case: A 30 years old Japanese woman at 33 weeks of gestation was referred to our hospital. Her fetus had a bradycardia (60bpm). We intravenously administered ritodrine hydrochloride against threatened preterm labor. We found that the fetus had 2:1 AV block, intermittent VT, and TdP. We administered magnesium sulfate instead of ritodrine hydrochloride, as we considered that the fetus might be long QT syndrome. Fetal heart rate became sinus rhythm in a few days. At 34 weeks and 5 days of gestation, the mother vaginally delivered the baby. We diagnosed the baby as long QT syndrome because the corrected QT time was prolonged (600 msec). The baby developed VT and TdP, and they were treated with magnesium sulfate and beta blockade. She discharged 26 days after birth with the medication of carvedilol and mexiletine

Discussion: Ritodrine hydrochloride is recommended as a first choice for treating threatened preterm labors, and magnesium sulfate as a second choice in Japan. In this case, ritodrine hydrochloride may have triggered VT/TdP. When we encounter fetal bradycardia, we have to keep in mind the fetus might be long QT syndrome. However, it is difficult to detect long QT syndrome prenatally using ultrasonography. Magnetocardiography is the only available method for detection of long QT syndrome.On the other hand, magnesium sulfate is an antiarrhythmic drug for adults. It is known to reduce the early afterdepolarization and stabilize the membrane of the myocardium. There are a few reports about a transplacental treatment for fetus arrythmia using magnesium sulfate. Lidocaine and beta-blocker are also known as transplacental drugs for VT/TdP. However, we are used to administering magnesium sulfate to pregnant women. In addition, it is easy to control its blood concentration.

Conclusion: Transplacental magnesium sulfate is one of the options for fetal VT/TdP in cases of congenital long QT syndrome in terms of its effectiveness, safety and controllability.

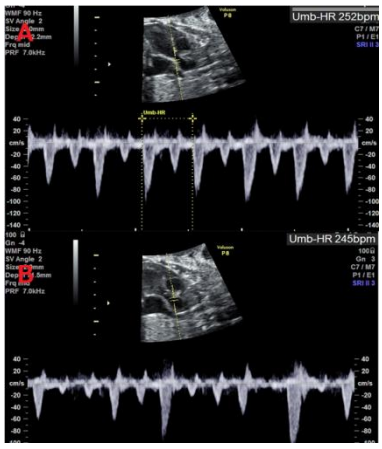

Figure 1. Doppler ultrasonography(SVC-Ao) Gestational age 33 weeks (after administration of ritodrine hydrochloride).**A**: Regular waves of ventricular tachycardia, heart rate is 250bpm;**B**: Polymorphic wave pattern of ventricular tachycardia, Torsa de Pointes.

Poster

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A data-driven system for continuous fetal monitoring in labour: the Oxford prototype

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Introduction: Intrapartum cardiotocography (CTG) is widely used. Maternal and fetal risk factors confound the relation of CTG patterns to fetal health; visual interpretation is unscientific, without reliable estimates of the risk for the fetus. Unnecessary operative deliveries are performed whilst some babies at risk are not delivered in a timely fashion. Commercial computerised systems aid visual assessment and standardise expert opinion, but do not quantify the associated fetal risks or incorporate other risk factors.
We report the performance of a prototype data-driven system (OxSys) that objectively quantifies the intrapartum CTG. It takes into account clinical risk factors and relates to the perinatal outcome in a large cohort.
Methods: Considered were all normally formed babies, born Mar'00-Dec'11 at Oxford, who had intrapartum CTG, paired cord gas analyses, and gestation >35 weeks (23,903 births). We studied four exclusive groups:
Compromise:
- Severe (composite of stillbirth, neonatal death, neonatal encephalopathy, seizures; and resuscitation at birth followed by intensive care for ≥48hrs);
- Moderate (umbilical cord arterial blood pH at birth <7.05);
- Mild (7.05≤pH<7.15);
Normal: all remaining.
The OxSys prototype analysed each CTG with a moving 15min window and alerted if the first hour of the trace was nonreactive or, at any time, the Decelerative Capacity (Georgieva et al, BJOG 128;2014) exceeded a threshold, adjusted for preeclampsia or thick meconium.
Results: Table 1 shows that, when compared to the rate of emergency deliveries due to 'fetal distress' as recorded in the patients' notes at birth, OxSys had significantly lower alert rates in Normal (i.e. lower false positive rate, 14.46% vs. 16.40%, p<0.001) and slightly higher alert rates in the compromise groups (i.e. higher sensitivity). We demonstrate that the false positive rate was high and the sensitivity was low for both clinical practice and OxSys.

Table 1. Relation of clinical practice and OxSys to perinatal outcomes

Outcome groups (exclusive)	**Compromise** *(sensitivity)*			**Normal** *(false positive rate)*
	Severe	**Moderate**	**Mild**	
Number of births	190	629	3,233	19,851
Detected in clinical practice	71 (37.37%)	198 (31.48%)	733 (22.67%)	3,255 (16.40%)*
Detected by OxSys 1.5	83 (43.68%)**	225 (35.77%)***	795 (24.59%)†	2,871 (14.46%)‡

*972 (29.9%) were Caesarean sections.
Chi squared test, OxSys 1.5 vs. Clinicians: ** p = 0.21; *** p = 0.11; †p = 0.07; ‡ p<0.001.

Conclusions: The Oxford prototype intrapartum system OxSys compares favourably to assessment in clinical practice. Further work is needed to achieve better performance. Our approach to evaluating diagnostic accuracy allows methods for CTG interpretation to be compared and improved. We can include any new knowledge in the system and test its contribution by measuring changes in sensitivity and/or the false positive rate.

Poster

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The three cases of urinary tract obstruction with bladder rapture

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Introduction: The lower urinary tract obstruction (LUTO) are the most common cause of mechanical infravesical obstruction, and causes distention of the bladder, hydronephrosis. In case of severe obstruction, hypoplastic lung due to oligohydramnios and renal failure due to obstruction are often developed. We report the three cases of LUTO to take irregular courses by bladder rupture.

Case: Case1: MM-twin. Enlarged bladder was diagnosed by ultrasonography at 11weeks of gestation. We found that the dilated urinary bladder enlarged with "key-hole sign", the sign of bladder outlet obstruction. The enlarged bladder shrunk suddenly at 23 weeks of gestation. At 29 weeks and 2days of gestation, spontaneous labor occurred and she delivered her babies. Infant 1 had cloacal anomaly, vertebral defect (L4-5, S1-2) and atrial septal defect (ASD).

Case2: Enlarged bladder was diagnosed by ultrasonography at 27 weeks of gestation. There was no sign of oligohydramnios at diagnosis. At 36 weeks of gestation, the enlarged bladder shrunk and oligohydramnios appeared, we decided her delivery mode Caesarean section. After that, we operated the bladder of the baby.

Case3: Ascites, bladder rupture and oligohydramnios was diagnosed by ultrasonography at 27 weeks of gestation. We injected amniotic fluid and then followed up. Then, the sign of oligohydramnios was disappeared and bladder enlarged gradually again. Oligohydramnios and hydronephrosis caused at 34 weeks of gestation, so caesarean section was performed.

Conclusions: This three cases took irregular courses by bladder rupture. We should be closely monitored by ultrasonography.

Poster

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Fetal behavioural state in late gestation is affected by maternal sleep position

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Introduction:
Maternal sleep position has been associated with the risk of late stillbirth. In the developed world a large number of stillbirths are unexplained. In our physiological studies of maternal and fetal responses, we hypothesised that maternal sleep position would affect fetal activity states overnight. The aim of this study was to describe the effects of maternal sleep positions on fetal behavioural states (FBS) in healthy fetuses.
Methods: Healthy pregnant women (35-38 weeks gestation, n=30) underwent overnight studies with polysomnography during which continuous transabdominal fetal ECG (Monica AN24 ™) recordings were made. Two independent observers blinded to maternal position assessed FBS by cardiotocography (inter-observer Kappa = 0.8). Repeated measures analyses were undertaken to compare 1F and 2F to allow for the differences in proportions of the women in each sleep position and length of sleep.
Results: Over 7 hours of fetal data per participant were available for analysis. Fetuses spent most time in state quiet sleep state (2F, 83.2%) followed by (active sleep state (1F, 12.5%), very little time being in active awake state (4F, 4.3%). State 4F occurred almost exclusively when the mother was in a left or right lateral position. State 4F was seen mostly in the early part of the night. In repeated measures analyses comparing state 1F and 2F we found that state 1F was less common in the right position and more common in the supine position compared to the left position. The effect of maternal sleep position on fetal state also appeared to be restricted to the earlier part of the night when women tended to remain in one position for the longest periods of time.
Conclusions: Maternal sleep position affects the behavioural state a fetus is most likely to be in. Maternal supine position is associated with more time in 1F and less time in 4F than other positions. This would be consistent with the fetus taking up a lower oxygen consuming state as a response to adverse effects of the supine position.

Poster

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Renal function in the first month of life in Australian Indigenous and non-Indigenous preterm neonates

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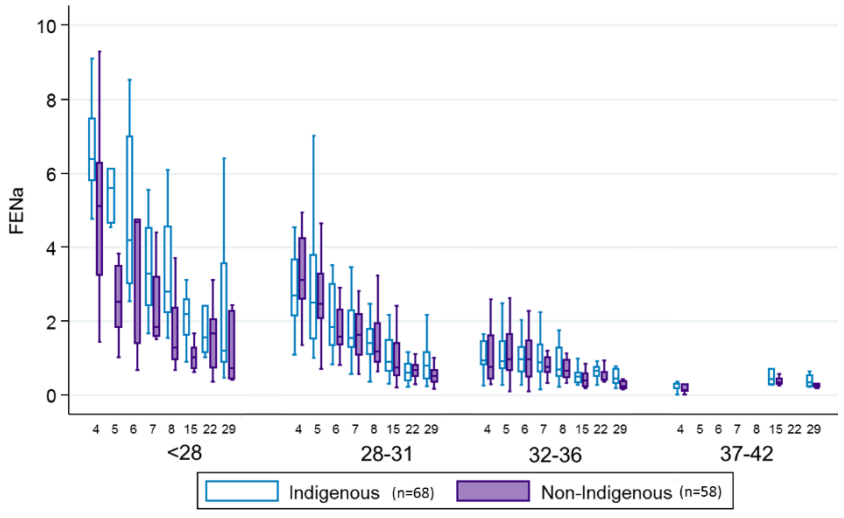
Introduction: Preterm birth (birth < 37 weeks' gestation) occurs in ~10% of births world-wide. In Australia the incidence is very high in the Indigenous population; ~14% in Indigenous Australians compared to ~9% in non-Indigenous Australians. Preterm birth occurs at a time when the kidneys are structurally and functionally immature and nephrogenesis is still ongoing in many preterm infants. Hence, it is likely that renal function in the preterm neonate will be adversely affected by renal immaturity and/or postnatal injury. The aim of this study was to compare renal function in the first month of life in Australian preterm and term Indigenous and non-Indigenous neonates.

Methods: Indigenous and non-Indigenous infants recruited at the Royal Darwin Hospital, were grouped by gestational age at birth: ≤ 28, 29–32, 33-36 and >36 weeks. Twenty-four hour pooled urine samples were obtained at days 3-7, 14, 21 and 28; creatinine clearance (CrCl; measure of glomerular filtration rate), fractional excretion of sodium (FENa; measure of tubular function) and urine output (ml/kg/hr) were calculated.

Results: During the first week of life, CrCl increased with gestational age at birth and postnatal age, while FENa decreased with increasing gestational age at birth and postnatal age. These trends continued until Day 28 of life. Notably, the FENa during the first month of life was greater in the Indigenous infants born < 28 weeks than age-matched non-Indigenous infants, indicating a significant difference in tubular function. Urine output was markedly lower in term infants of both groups at Day 4, increasing thereafter.

Conclusions: This study demonstrates that renal function in preterm neonates is dependent on gestational and postnatal age, with kidneys of extremely preterm Indigenous Australians particularly vulnerable. The low urine output of term babies on Day 4 reflects low oral intake, compared to all preterm babies, where specified fluid intakes drive urine output. This brings into question the amount of fluid currently used in fluid management of preterm babies.



Posters

Developmental Cardiovascular Physiology

Poster

20

The effect of moderate preterm birth on the structure and growth of cardiomyocytes in the right ventricle of adult sheep

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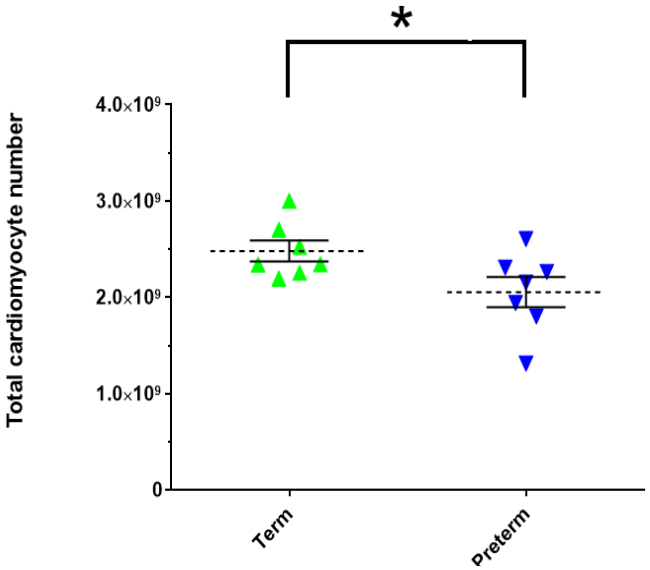
Introduction: Preterm birth (< 37 weeks of gestation) affects approximately 10% of births worldwide, with the majority of infants born moderately preterm. Recent studies show that there is remodelling of the myocardium in response to preterm birth and that there is altered growth and function of the myocardium in adulthood; these effects are particularly evident in the right ventricle (RV). The aim of this study was to examine the effect of moderate preterm birth on the growth of cardiomyocytes in the RV of the adult heart using a clinically relevant sheep model.

Methods: Singleton male lambs were delivered moderately preterm (132±1days; n=7) or at term (147±1days; n=7). Ewes assigned to deliver preterm were administered a clinical dose of betamethasone antenatally (11.4 mg at 48 hours and 24 hours prior to birth). The preterm and term born sheep were grown to adulthood and their hearts examined at 14.5 months of age.

Results: Preterm sheep remained smaller in weight and stature throughout life. At 14.5 months of age, there were significantly fewer cardiomyocytes in the RV of preterm sheep compared to terms, with cardiomyocyte number proportional to both body weight and heart weight. There were no differences in the size of cardiomyocytes or in myocardial interstitial collagen deposition in the RV of the adult preterm and term born sheep.

Conclusions: The reduced number of cardiomyocytes in the RV of the adult heart following preterm birth may be a direct consequence of preterm birth or to the exposure to antenatal corticosteroids; alternatively, it may relate to the attenuated postnatal growth. A reduced number of cardiomyocytes is likely to impact on the adaptive and functional capabilities of the RV if challenged during adulthood.



Cardiomyocyte Number in the RV: Total cardiomyocyte number in the RV of term (n = 7) and preterm (n = 7) 14.5 month old male sheep. There was a significant reduction (P=0.046) in the number of cardiomyocytes in the RV of adult sheep that were born preterm compared to those born at term.

Poster

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miR-133a and miR-15 family target gene expression in the fetus and 6 month old sheep heart in response to myocardial infarction

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Introduction: The adult mammalian heart has very little capacity to repair after damage because adult cardiomyocytes lack the ability to proliferate. This lack of response to injury is in stark contrast to the adult zebrafish, newborn mouse and fetal sheep whose cardiomyocytes have the capacity to repair through the proliferation of existing cardiomyocytes. The expression of microRNAs (miRNAs) such as miR-133a and the miR-15 family increase at the time that proliferation ceases in rodents and sheep and have been implicated in regulating cell cycle entry and cardiomyocyte proliferation. We therefore aimed to investigate the expression of these miRNAs and their target genes after myocardial infarction in sheep hearts before (105 d gestation) and after (6 months) birth when the response to injury is different.

Methods: We used sheep, which have a similar pattern of cardiomyocyte development relative to birth compared to humans, as a model to investigate the effect of age on cardiac damage, by ligating the second diagonal of the left anterior descending (LAD) coronary artery. Surgery was performed on fetuses (MI, n=5; SHAM, n=5; 102 days gestation when all cardiomyocytes are proliferative) and postnatal sheep (MI, n=4; SHAM, n=4; 6 months of age when all cardiomyocytes contribute to heart growth by hypertrophy). Three days later, infarct size was visualized using 2,3,5-triphenyltetrazolium chloride (TTC) staining of heart sections. Total RNA was extracted and qRT-PCR was used to quantify expression of miRNA and their target genes.

Results: Mean normalized expression (MNE) of target genes of miR-133a (e.g. SRF and IGF1R) and the miR-15 family (e.g. CCND2 (Figure 1)) were increased in myocardial tissue from the infarcted area compared with the remote zone in the fetus. In contrast, the opposite expression pattern was observed in the 6 month old sheep.

Conclusions: These results indicate that miR-133a and the miR-15 family have significant roles in regulating cardiomyocyte proliferation in sheep. Low expression in the fetus allows cardiac repair after damage with high expression after birth resulting in limited capacity for repair.

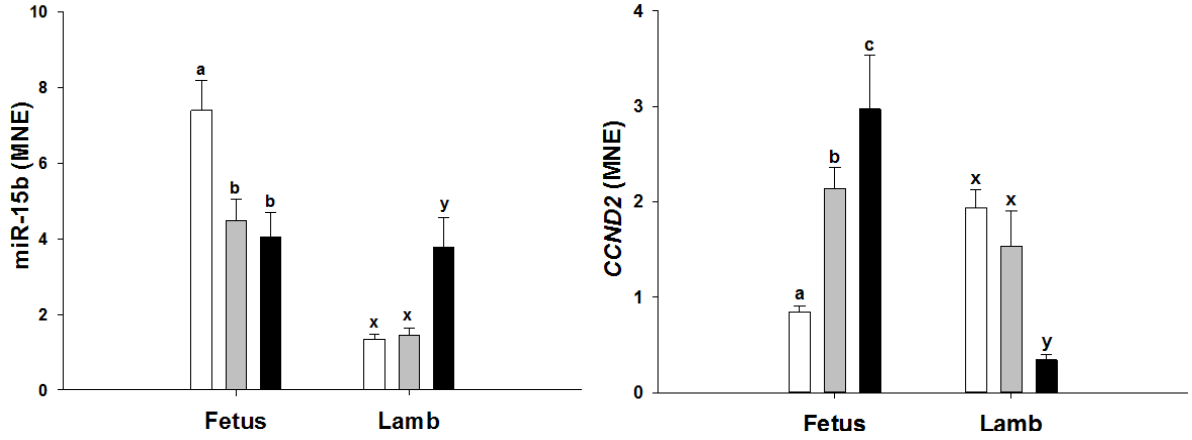

Figure 1. Normalised miR-15b and *CCND2* mRNA expression in the infarct, border zone and remote zone in the fetal (105 d gestation) and adult (6 month old) sheep heart in response to ligation of the LAD.

Poster

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The redistribution of cardiac output by vasopressin infusion in the premature fetal sheep

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Introduction: The pioneering works using fetal sheep have clarified their cardiovascular responses to hypoxia. The responses serve to reduce oxygen consumption of the whole body and to redistribute the cardiac output away from the periphery towards hypoxia-sensitive organs, such as adrenal glands, heart and brain. A low dose of vasopressin infusion [1.37 ± 0.11 mU/kg/min (mean ± SEM)] has been reported to induce the redistribution in late preterm fetus. But, it is unknown whether it can induce the same responses in more premature fetus. Our purpose was to determine whether the low dose infusion of vasopressin could induce the redistribution of the organ blood flow in premature fetal sheep.
Methods: The experiment was performed between October 2012 and March 2014 with the approval of the Animal Care and Use Committee (2013MdA-008/009). Ten sheep fetuses between 99 and 121 days of gestation were chronically instrumented and infused vasopressin intravenously; 0.93 ± 0.10 mU/kg/min. Fetal actual organ blood flows were measured using the colored-microsphere technique and statistically compared between before and after the vasopressin infusion.
Results: After the vasopressin infusion, the fetal arterial pressure raised from 40.0 ± 1.6 to 51.3 ± 2.0 mm Hg and the heart rate fell from 192.6 ± 5.9 to 159.3 ± 2.0 beats/min. The fetal blood flows of cerebral cortex and medulla oblongata increased, whereas myocardial and adrenal blood flows decreased.
Conclusions: The cardiovascular response to the vasopressin infusion in premature fetus might be different from that in late preterm fetus because the vasopressin infusion reduced the blood flows of myocardium and adrenal glands while it could maintain the cerebral blood flow.

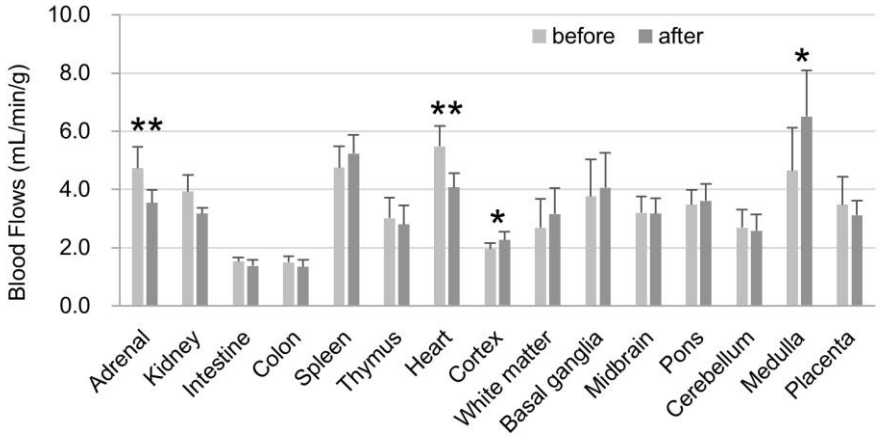

Figure. Comparison of organ blood flows before and after the vasopressin infusion.
All data were expressed as mean ± SEM. *, p <0.05, the blood flows of brain cortex and Medulla oblongata significantly increased. **, p <0.05, the myocardial and adrenal blood flows significantly decreased (Wilcoxon signed-rank test, respectively).

Poster

23

The effect of cinaciguat (bay-582667) on the cardiopulmonary circulation in hypoxic neonatal lambs at high-altitude

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Introduction: High altitude hypoxia induces an increased production of reactive oxygen species (ROS), which reduces the function of soluble guanilyl cyclase (sGC). In fact, lambs gestated and born at 3600 m, show a reduced sGC. We hypothesised that cinaciguat, a drug that activates oxidized sGC, will enhance vasodilatation in the pulmonary hypertensive neonatal lamb.
Methods: Twelve newborn lambs, born and raised at Putre Research Station, INCAS (3,600 m), were catheterized in the pulmonary artery (Swan Ganz) at 3-4 days of age. Six lambs received a daily infusion of cinaciguat (35 $\mu g.kg^{-1}.d^{-1}$) into the pulmonary circulation and six controls received vehicle. On day 8, lambs were subjected to an acute episode of superimposed hypoxia (30 min of basal, 30 min of hypoxemia and 30 min of recovery). We determined pulmonary arterial pressure (mPAP), heart rate (HR), cardiac output (CO) and pulmonary vascular resistance (PVR). At 15 days of age, the lambs were euthanized, heart chambers were dissected and weighed, and pulmonary samples were collected for molecular biology, vascular function (myography) and histology (small pulmonary arteries) studies. All procedures were approved by the Local Bioethical Committee (0643 FMUCH CBA).
Results: Cinaciguat changed the PVR and CO in the last 2 days of treatment, and resulted in a decreased response in mPAP, PVR and CO during the episode of acute hypoxia ($p<0.05$). Cinaciguat treatment reduced the RV/LV + septum ratio ($p<0.05$) and decreased the muscle layer in small pulmonary arteries ($p<0.05$). Further, small pulmonary arteries from cinaciguat group showed a 30% greater vasodilatation when exposed to SNP and when exposed to sildenafil showed more sensitivity than the control group. Additionally, protein expression of cyclase and PD5 (phosphodiesterase 5) was similar between groups.
Conclusion: Cinaciguat decreased pulmonary artery responsiveness in daily treatment and during acute superimposed hypoxia, further improving cardiopulmonary remodelling and vasodilatation.

Funding: FONDECYT 1140647, 1120605, 1130424 & 1151119.

Poster

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Antenatal melatonin modulates cellular pro-oxidant sources in newborn lambs with pulmonary hypertension

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Introduction: Chronic hypobaric hypoxia during gestation cause intrauterine growth restriction and neonatal hypertension (1). One of the mechanisms involved in the pathophysiology of pulmonary hypertension is oxidative stress, which implies an increased generation of reactive oxygen species (ROS) and/or a decreased antioxidant capacity (2). Melatonin is a neurohormone with antioxidant properties that improves pulmonary vascular function in the neonatal lamb (3). Therefore, we hypothesised that antenatal treatment with melatonin improves the antioxidant/prooxidant ratio and reduces oxidative stress in neonatal lungs.
Methods: Twelve singled pregnant ewes were maintained under chronic hypobaric hypoxia (Putre, 3,600 masl). Six of them conform a control group (CN, 5ml vehicle) and 6 the melatonin treated group (MM, 10 mg.d^{-1} melatonin in 5ml ethanol 1.4%). Treatments were given to the pregnant ewes, daily at 18h (oral) in the last third of gestation (100-150 d). All lambs were born and left with their mothers in a common pen. At 12 d old, lambs were euthanized and lung tissue and plasma were sampled. We performed protein analyses (Western blot) for the antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). Further, oxidative stress was measured by 8-isoprostanes, 4HNE and nitrotyrosine levels. The pro-oxidant sources were assessed by NADPH oxidase, xanthine oxidase and mitochondrial ROS-generation in lung tissue. Finally, we determined the plasmatic antioxidant capacity (FRAP).
Results: Antenatal administration of melatonin decreased the expression of antioxidant SOD, CAT and GPx. In addition, the generation of superoxide anion by the main pro-oxidant sources decreased in the melatonin treated group (Fig. 1), associated with lower oxidative stress markers in lung tissue as: 4HNE, 8-isoprostane and nitrotyrosine, and decreased FRAP.
Conclusion: Antenatal melatonin diminished oxidative stress by programming a decreased ROS generation in the postnatal pulmonary sources.

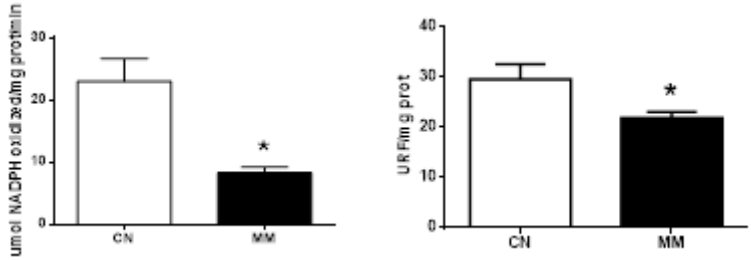

Figure 1. Pulmonary cellular pro-oxidants sources. A) NADPH oxidase, B. Mitochondria. Average ± SEM for control (CN), and antenatal melatonin treated neonates (MM). Significant differences (t-test, $p<0.05$): * *vs* CN.
Funding: FONDECYT 1130424, 11130232, 1140647 & 1151119.
References.
1. Herrera et al. *Am J Physiol.* 299:R1676-R1684, 2007.
2. Gao & Raj. *Compr Physiol.* 1(1):61-79, 2011.
3. Torres et al. *J Pineal Res.* 58(3):362-73, 2015.

Poster

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Can fetal heart rate variability identify the phases of injury after asphyxia in preterm fetal sheep?

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Introduction: Exposure to hypoxia-ischaemia (HI) before birth is a major mediator of brain injury. Neuroprotection treatments including hypothermia are most effective when begun early (latent phase) after the insult. However, many babies do not benefit from treatment and this may be the injury has already evolved beyond the optimal window for treatment (secondary phase). Currently we lack reliable biomarkers to identify fetuses at risk of brain injury after HI, and phases of injury. The aim of this study was to identify whether fetal heart rate variability (FHRV) could distinguish between injurious and non-injurious HI insults and whether patterns over time predicted phases of injury.

Methods: Singleton fetal sheep at 0.7ga were randomly assigned to one of two groups: 25min of complete umbilical cord occlusion (UCO) (n=29) or 15min (n=18). Fetal heart rate (FHR) and four measures of FHRV (STV, LTV, SDNN, RMSSD) were assessed until 72 h after HI in two phases: latent and secondary phases.

Results: During the latent phase (first 6h post UCO) FHRV was biphasic with initial suppression followed by elevation in both groups. The 25min group was tachycardiac, whereas the 15 group were mildly bradycardic. In the secondary phase (6-72h) FHRV was suppressed in the 25min group, while FHR was normal or lower. FHRV remained elevated in the 15 min group while FHR normalised.

Conclusions: A pattern of increased FHRV may identify the late latent phase of injury after HI, accompanying tachycardia in the early latent phase suggests that the insult was injurious. Suppression of FHRV was observed during the secondary phase only after an injurious duration of asphyxia. These findings suggest that analysis of FHR and FHRV after HI may be able to help determine the timing and severity of fetal HI and therefore provide information to better direct neonatal care.

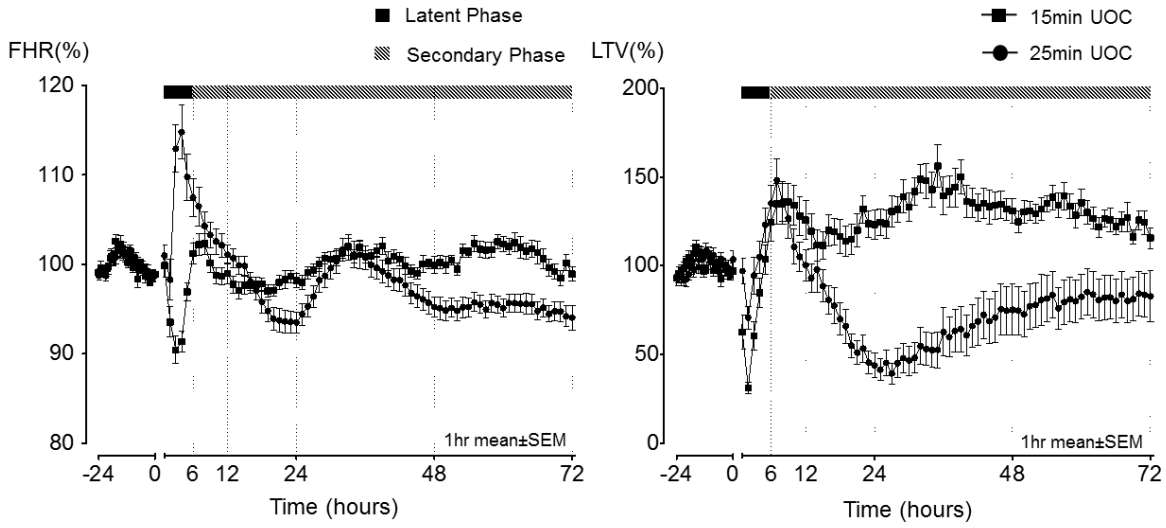

Figure 1 shows the changes in the latent and secondary phases in fetal heart rate (FHR) and long-term variability (LTV) after 15 or 25 min UCO in preterm fetal sheep.

Developmental Neuroscience (2)

Poster

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Umbilical cord blood derived mesenchymal stem/stromal cells protect against preterm white matter brain injury following hypoxia-ischemia

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Introduction: Preterm infants, particularly those born <32 weeks gestational age, are at high risk for cerebral palsy and neurological deficits. We have reported that, following acute hypoxia-ischaemia (HI), the decrease in oligodendrocytes observed in the preterm fetal sheep brain is correlated to the number of activated microglia present **(Figure)** and that early administration of allogeneic umbilical cord blood (UCB) cells reduces white matter brain injury following ischemia and following term neonatal asphyxia. Mesenchymal stem/stromal cells (MSCs) are receiving strong interest for neural repair, in adolescents and adults, in both preclinical and clinical studies due to their strong anti-inflammatory actions. Although the number of MSCs in human term UCB is very low, preterm UCB contains a relatively higher concentration of MSCs. This study examined the neuroprotective effects of allogeneic MSCs derived from preterm ovine UCB in a fetal sheep HI model of white matter brain injury induced by acute umbilical cord occlusion.

Methods: UCB was collected from a cohort of preterm sheep at ~0.75-gestation. UCB-MSCs were isolated by plastic adherence and morphology, and expanded until passage 3. Quad-lineage differentiation potential, clonogenicity and self-renewal ability of the cells was confirmed. A different cohort of chronically instrumented fetal sheep (0.7 gestation) received either 25min total umbilical cord occlusion or sham occlusion. Ten million preterm UCB derived MSCs, or saline (control), were administered iv to fetuses at 12h after HI. Fetal brains were collected 10d after occlusion or sham occlusion for histopathology.

Results: HI reduced the density of myelin fiber tracts (CNPase+) in the periventricular white matter (PVWM) and internal capsule (IC; P<0.05) and induced microglial activation in the white matter and systemic inflammation. UCB-MSC administration preserved myelination following HI and significantly promoted cell proliferation (Ki-67+) in all white matter regions examined (subventricular zone, PVWM, and IC) compared to control and HI alone fetuses (P<0.05). UCB-MSC administration reduced plasma inflammatory TNFα concentrations at 3d post-HI (P<0.05) and moderately suppressed microglial activation within the brain.

Conclusions: Administration of UCB-MSC, derived from preterm UCB, preserved white matter structure in preterm fetuses, following HI, by promoting cell proliferation in the preterm brain and by decreasing systemic inflammation.

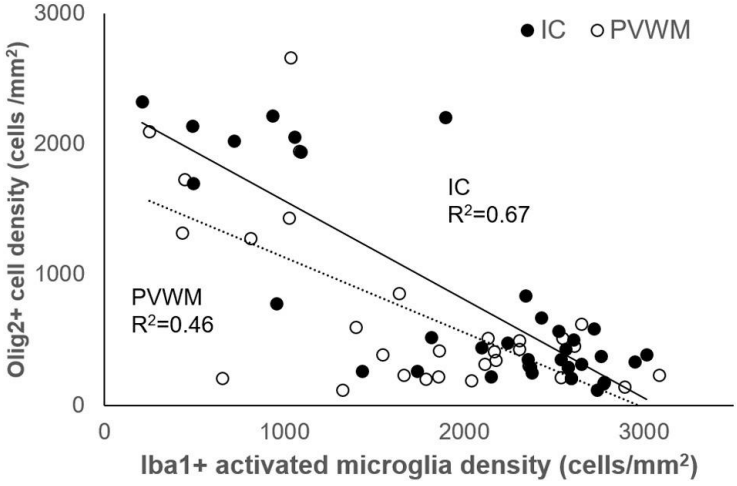

Figure. Correlation analysis between the number ofoligodendrocyte (Olig2+) and activated microglia (Iba-1+) cells revealed a significant

negative correlation in the PVWM (R2 = 0.46, P b 0.001) and IC (R2 = 0.67, P b 0.001).

PVWM: periventricular white matter; IC: internal capsule.Li et al. (2016) http://dx.doi.org/10.1016/j.expneurol.2016.06.017

Poster

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[redacted]

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Institute for Women's Health, University College London

Poster

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Effects of maternal obesity on cognitive function in the adult offspring in the rat

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Introduction: Obesity is a low-grade inflammatory state, which constitutes an inhospitable intrauterine environment. After birth, children of obese mothers are at increased risk of neurodevelopmental abnormalities, e.g. reduced cognitive capacity (~ 3 IQ points lower at 10 years), developmental delay, attention deficit hyperactivity disorder, and autism spectrum disorders. In adults, high BMI and metabolic syndrome are associated with lower cognitive performance, and poor executive function and memory. The hippocampus and prefrontal cortex are major centres for memory, learning and decision making, and emotional regulation. The aim of our study was to determine the effects of obesity in early life, *in utero* and lactation, on cognitive function in rats.

Methods: Long Evans rat dams were placed on control chow (CC) or a high fat/ cafeteria (HF) diet for 6 weeks and then mated with lean males. Offspring were retained on the diet of their dam or, at weaning, were switched to the other diet. At 14 -16 weeks of age, cognitive function was assessed using the 8-arm radial maze. When 17-19 weeks old, the brains were studied *in vitro* using multi-electrode arrays to record electrical activity in hippocampal and cortical brain slices in 0-Mg^{2+} artificial aCSF.

Results: Cognitive function in HF male and female offspring was impaired in terms of both working memory and reference memory. Switching HF offspring to CC at weaning rescued working memory but reference memory remained impaired. Similarly, placing CC offspring onto HF diet at weaning resulted in impaired working, but not reference memory. When brain slice electrical activity was studied, there was a dramatically greater tendency for oscillatory network (epileptiform) activity in the CA3/dentate gyrus of the hippocampus in offspring from HF dams versus CC counterparts. Similar bursts also occurred in the motor cortex and prefrontal cortex (PFC). Pro-inflammatory markers were increased in HF hippocampus.

Conclusions: Whereas some cognitive function can be rescued by diet, the offspring of obese mothers may experience some cognitive impairment throughout life. Electrical hyperactivity in the hippocampus and prefrontal cortex is likely to be an important contributor to this impairment of cognitive function.

Poster

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Maternal protein restriction around conception alters the foetal mouse brain by increasing neuronal differentiation during gestation, and is associated with adult memory deficits

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Introduction: Maternal malnutrition during pregnancy is detrimental to foetal development and increases the risk of many chronic diseases in later life i.e. neurological consequences such as increased risk of schizophrenia. Previous studies have shown maternal protein malnutrition during pregnancy and lactation compromises brain development in late gestation and after birth, affecting structural, biochemical and pathway dynamics with lasting consequences for motor and cognitive function. However, the importance of nutrition during embryogenesis for early brain development is unknown. We have previously shown maternal low protein diet confined to the preimplantation period (Emb-LPD) in mice is sufficient to induce cardiometabolic and behavioural abnormalities in adult offspring.

Methods: Using a diet model, female mice were fed different diets from conception to the end of pregnancy: normal protein diet (NPD), low protein diet (LPD) or embryonic LPD (Emb-LPD: LPD for 3.5 days, NPD thereafter). Foetal brains were analysed at three time points in gestation (E12.5, E14.5 & E17.5), with in vivo analysis using FACS and immunofluorescence for neural stem cell and neuron markers, and in vitro techniques using the neurosphere culture assay. We have also carried out a number of follow up behavioural tests at multiple time points in the adult offspring, including the short-term memory novel object recognition.

Results: We have shown that Emb-LPD and sustained LPD reduce neural stem cell (NSC) and progenitor cell numbers through suppressed proliferation rates in both ganglionic eminences and cortex of the foetal brain at E12.5, E14.5 & E17.5 (p=0.01). Moreover, Emb-LPD causes remaining NSCs to upregulate the neuronal differentiation rate in compensation beyond control levels during gestation independently of sex. In the short-term memory test the Emb-LPD adult offspring show a highly significant deficit in memory Fig 1 (p=0.00001).

Conclusions: This data are the first to clearly demonstrate that poor maternal nutrition around conception has adverse effects on early brain development and is associated with adult memory deficits.

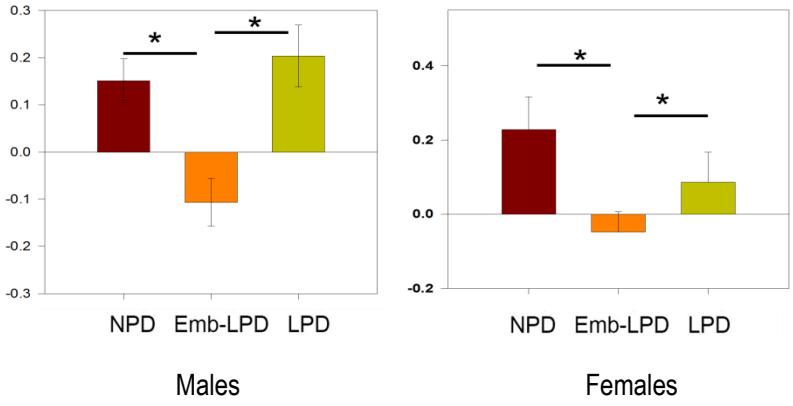

Figure 1. Novel object recognition test analysis. The 'Discrimination Index' (DI) is represented by standard error of the mean. Novel object is assessed between the adult offspring at PND 64 between three died groups NPD, Emb-LPD & LPD. (P=0.00001 mothers =11 n=28).

Poster

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Impact of hypercapnia on neurovascular coupling in the fetal sheep and newborn lamb

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Introduction: Neurovascular coupling (NVC) leads to increased cerebral blood flow (CBF) and cerebral oxygenation upon increased neural activity. Hypercapnia (HC) increases baseline CBF and attenuates NVC-induced CBF response in the adult brain. HC is common in preterm infants undergoing intensive care, but effects of HC on NVC in the immature brain remains unknown. We examined the impact of HC on NVC in the fetal sheep and neonatal lamb using cerebral oxy- and deoxy-haemoglobin (ΔoxyHb and ΔdeoxyHb) measured by near infrared spectroscopy (NIRS), after median nerve stimulation.

Methods: Under isoflurane anaesthesia EEG and NIRS electrodes were positioned bilaterally over the somatosensory cortex of fetal sheep (128-132 days gestation; n=8) and 4-8 day old neonatal lambs (n=7). Median nerve electrical stimuli of 8s duration was used to evoke a somatosensory evoked potential, and ΔoxyHb and ΔdeoxyHb were recorded before, during and after HC induced by ventilatory adjustments.

Results: $PaCO_2$ [mean (SD)] before, during and after HC were 48.2(5.6), 69.6(5.5) and 52.7(6.9)mmHg respectively in fetal sheep, and 39.5(3.2), 64.6(6.3) and 41.6(4.4)mmHg respectively in newborn lambs. Three patterns of cerebral functional responses were observed after the median nerve stimulation: the positive response denotes an increase in ΔoxyHb in the contralateral cortex, the negative response denotes a reduction in contralateral ΔoxyHb, whereas the biphasic response consists of an initial reduction followed by a later rise in contralateral ΔoxyHb. Changes in response patterns before, during and after HC are shown in table 1. During HC, 7 of the 8 fetal sheep showed higher ΔoxyHb in the contralateral cortex after median nerve stimulation compared to before HC [mean (SEM) ΔoxyHb: -1.4 (8.6) vs 14.9 (2.7) μM.cm before and after HC respectively, n=8, p=0.07]. For newborn lambs, 4 of the 7 animals showed higher ΔoxyHb in contralateral cortex during HC [0.7 (4.9) vs 9.5 (3.0) μM.cm before and after HC respectively, n=7, p=NS].

Conclusions: The rise in cerebral oxygenation after somatosensory stimulation tends to increase with HC in fetal and newborn lambs, in contrast to findings in adult studies. This may be related to different cerebral vasoreactivity in the developing brain and recruitment of cerebral vessels during HC.

Fetal sheep				Newborn lamb			
Animal no.	Before HC	During HC	After HC	Animal no.	Before HC	During HC	After HC
F1	Neg	Pos	Neg	NB1	Neg	Pos	Pos
F2	Pos	Pos	Pos	NB2	Pos	Pos	Pos
F3	Pos	Pos	Pos	NB3	Pos	Pos	Pos
F4	Neg	Pos	Pos	NB4	Neg	Neg	Neg
F5	Biphasic	Biphasic	Biphasic	NB5	Pos	Pos	Pos
F6	Pos	Pos	Pos	NB6	Pos	Biphasic	Pos
F7	Neg	Pos	Neg	NB7	Neg	Pos	Pos
F8	Biphasic	Biphasic	Biphasic				

Table 1. Cerebral functional response patterns in the fetal sheep and newborn lambs before, during and after HC. Pos: positive response, Neg: negative response.

Poster

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Development of mitochondrial function in the cortex and cerebellum of the ovine fetus: role of thyroid hormones

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Introduction: Mitochondria of immature brains are involved in regulating inflammation, synaptic development and connectivity in addition to ATP generation (*Lancet Neurol* **13**:217, 2014). Adult brains have a high rate of oxygen consumption but little is known about mitochondrial function in fetal brains. The aim of this study was to determine the ontogenic changes in mitochondrial respiratory function in the fetal sheep brain, and their dependence on thyroid hormones known to regulate fetal oxygen consumption.

Methods: All experiments were carried out under the Animals (Scientific Procedures) Act 1986. Twin-bearing ewes (n=6) were anaesthetised at 102-105 days of gestation (d; term ~145d) for fetal thyroidectomy (Tx) or sham operation. Ewes and fetuses were euthanised at 140-145d, together with 4 additional un-operated twin-bearing ewes at 102-105d. Samples of cortex and cerebellum were placed in biopsy medium before homogenisation in respirometry medium, for assessment of mitochondrial function using an Oroboros oxygraph respirometer and a substrate-uncoupler-inhibitor titration protocol (sequential addition of pyruvate: 5mM; ADP: 10mM; glutamate: 10mM; succinate: 10mM and rotenone: 0.5µM). Respiration rates were corrected for protein concentration, measured by the bicinchoninic acid assay.

Results: Pyruvate-supported leak respiration (not shown) and oxidative phosphorylation (following ADP addition; Fig.1A,E) did not alter significantly with age in either brain region (all $p>0.05$). In cortex, complex II-supported and maximal oxidative phosphorylation rates (complex I+II) increased during gestation (Figs.1C,D, $p<0.05$). In cerebellum, complex I and II-supported, and maximal oxidative phosphorylation (complex I+II) rates, increased significantly with gestational age (Figs.1F-H, $p<0.05$). Oxidative phosphorylation was greater in cerebellum than cortex at both gestational ages (all $p<0.05$). Tx did not alter mitochondrial respiratory function in either brain region (Fig.1A-H, all $p>0.05$).

Conclusions: Mitochondrial respiratory function increases up to 2-fold towards term in both brain regions, with a greater respiratory capacity in cerebellum than cortex. Thyroid hormones do not appear to regulate mitochondrial respiration in the fetal brain near term.

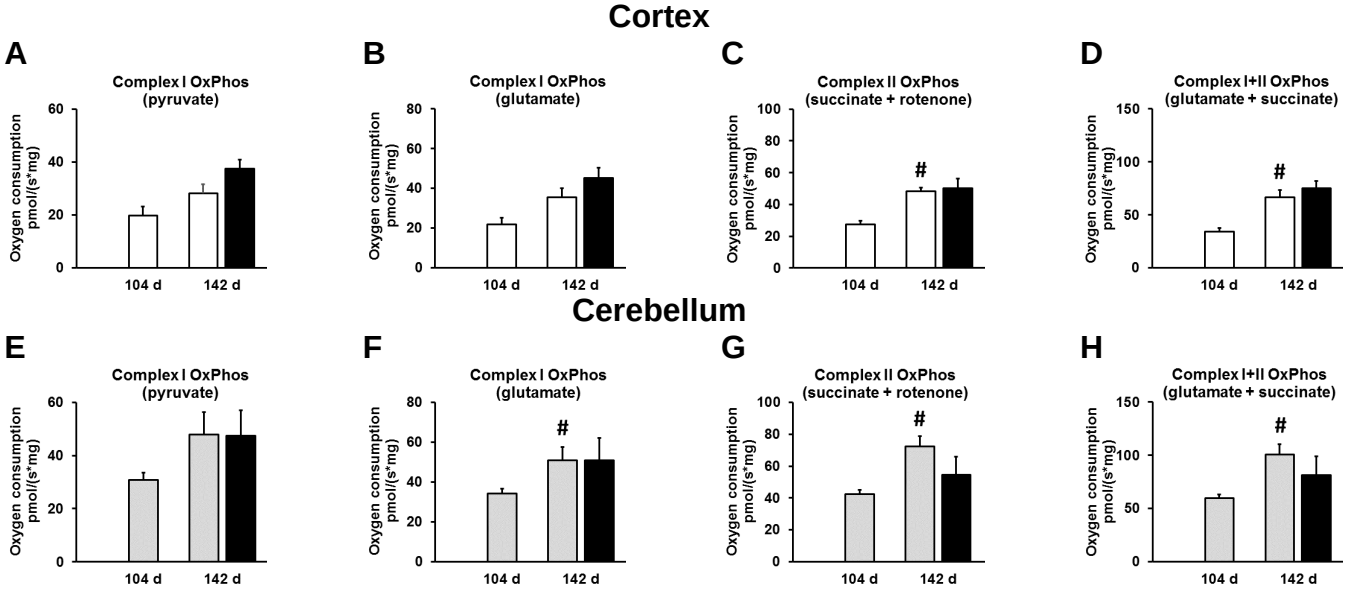

Figure 1. Mitochondrial oxygen consumption in fetal cortex and cerebellum. Values are mean ± S.E.M. in ontogeny/sham (white and grey bars) and Tx (black bars) fetuses. (A,E) Pyruvate-supported respiration. (B,F) Complex I-supported respiration with glutamate. (C,G) Complex II-supported respiration with succinate and rotenone. (D,H) Maximal (Complex I+II) oxidative phosphorylation with glutamate and succinate. Two-way ANOVA with Tukey test, # $p<0.05$ 104d vs. 142d.

Posters

Developmental Hypoxia

Poster

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Chronic fetal hypoxia and programming of cardiovascular dysfunction: the role of xanthine oxidase

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Introduction: The chick embryo is the only established animal model to isolate the direct effects of environmental changes during fetal development in programming the physiology of the adult offspring independent of effects on the mother and/or placenta. We used an integrative and longitudinal approach to determine the role of xanthine oxidase (XO) during hypoxic incubation in the programming of cardiovascular dysfunction in chick embryo and in the adult bird. We combined studies of in vivo physiology with those at the isolated organ and cellular levels.

Methods: Fertilized chicken eggs were incubated under normoxia or hypoxia (14% O_2) ± the XO inhibitor allopurinol from d13 of incubation (term is 21 days). Allopurinol (5 $mg.kg^{-1}$), or vehicle (H_2O) was administered daily into the air cell for 6 consecutive days. At d19, fetal femoral arteries were isolated for myography and tested with acetylcholine (ACh) and fetal cardiac function was determined under Langendorff. Some animals were allowed to hatch and raised until 6 months of adulthood. At adulthood, birds were chronically instrumented under general isofluorane anaesthesia with vascular catheters. Five days later, in vivo echocardiography and basal and stimulated cardiovascular function with and without L-NAME treatment was established to determine the contribution of NO bioavailability to any changes. Stereology was carried out on the adult hearts.

Results: Chronic in ovo hypoxia resulted in endothelial and cardiac dysfunction in the chick embryo (Fig. 1 A,B). At adulthood, offspring of hypoxic incubation had higher basal arterial blood pressure, a significant reduction in NO bioavailability, signs of compensatory cardiac hypertrophy and enhanced cardiac contractility (Fig. 1 C-F). In ovo treatment with allopurinol during hypoxic incubation protected against the endothelial and cardiac dysfunction in the chick embryo and normalised in vivo NO bioavailability in the adult bird. However, these effects of allopurinol were insufficient to restore hypertension and the enhanced cardiac contractility in the adult bird.

Conclusions: XO-derived oxidative stress has a direct role in programming cardiovascular dysfunction during hypoxic development.

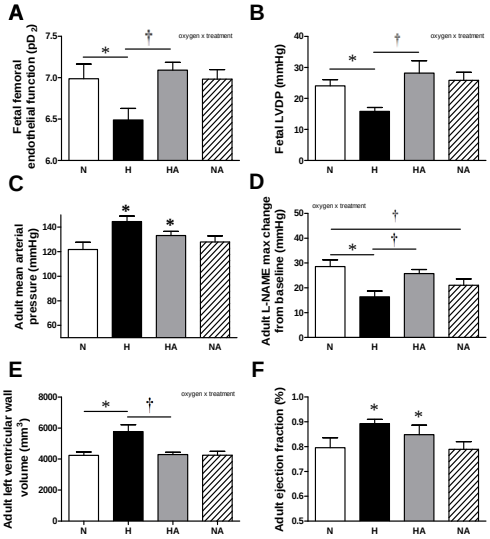

Figure 1. Values are mean ± S.E.M. for the fetal femoral relaxant sensitivity to ACh (pD2; A), fetal left ventricular developed pressure (LVDP; B), adult mean arterial blood pressure (MAP, C), adult MAP max change from baseline to L-NAME (D), adult left ventricular wall volume (E) and adult cardiac ejection fraction (%). Chickens were incubated in normoxia (N), hypoxia (H), hypoxia with allopurinol (HA) and normoxia with allopurinol (NA), n=6-12 per group. Two-way ANOVA for the effect of exposure to hypoxia (* $p<0.05$) or treatment with allopurinol (†$p<0.05$). Where a significant interaction is observed between factors (denoted as 'oxygen x treatment'), the outcome of Student-Newman-Keuls post hoc comparison between each group is displayed.

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Poster

33

Intergenerational transmission of protection against heart disease via the maternal mitochondria

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Introduction: Many studies have reported the intergenerational transmission of increased risk of disease in humans and animals. Conversely, the transmission of overt protective or advantageous traits between generations has been mostly restricted to reports in plants, such as in rice or studies with invertebrate species, such as in *C elegans* (Agrawal et al. *Nature* 401(6748):60–63, 1999; Rechavi et al. *Cell* 147(6): 1248-56, 2011). Here, we show in a mammalian model of intergenerational programming by developmental hypoxia that mothers can transfer onto their offspring cardio-protective traits via mitochondrial mechanisms.

Methods: Pregnant rats (n=24, F0) underwent normoxic (N) or hypoxic (H: 14% O2) pregnancy from days 6-20 of gestation. At 12 weeks, F1 offspring were mated with partners from outside the colony to produce an F2 generation which did not experience hypoxia from both parental lineages. In F1 and F2 male offspring, cardiac recovery from ischaemia/reperfusion (I/R, Langendorff) at 4 months was assessed. Protein kinase C epsilon (PKC-ε) is known to have a cardio-protective role against I/R via established mitochondrial mechanisms, such that a fall in its expression increases cardiac susceptibility to I/R damage (Patterson et al. *Circ Res*. 107(3): 365-73, 2010). Therefore, the expression of PKC-ε was determined in hearts of 4 month old F1 and F2 offspring of normoxic and hypoxic pregnancies with and without the I/R challenge in adulthood.

Results: F1 and F2 paternal lineage offspring of hypoxic pregnancy showed impaired cardiac recovery from I/R (Fig. 1a and 1b). In contrast, F2 maternal lineage offspring of hypoxic pregnancy showed normal recovery to I/R (Fig. 1c). Hearts of offspring from hypoxic pregnancy showed a reduced fall in PKC-ε protein expression following I/R compared to those of offspring of normoxic pregnancy (Fig. 1d). This cardio-protective trait was present in F2 male offspring via the maternal but not via the paternal line (Fig. 1e and f).

Conclusions: We show the heritability of an overt advantageous trait transmitted from mother to offspring via the maternal mitochondria in a major organ system such as the heart and circulation in a mammal for the first time.(*Supported by the British Heart Foundation*)

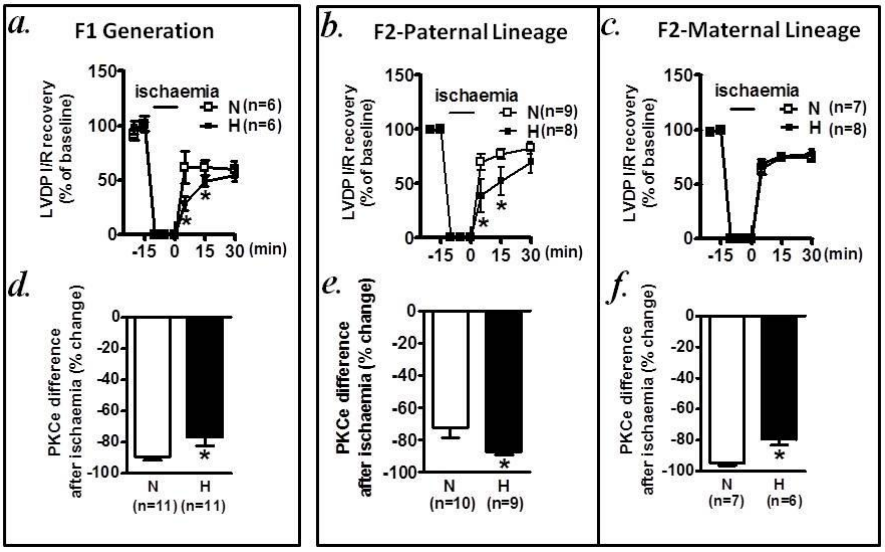

Figure 1. Recovery of left ventricular developed pressure (LVDP) to a 10 minute episode of ischemia and reperfusion (I/R) challenge (top row) and the cardiac protein expression of PKCe difference before and after I/R in F1 and F2 adult offspring hearts of normoxic and hypoxic pregnancy via paternal or maternal lineages. A reduced cardiac PKCe expression difference before and after I/R is cardio-protective. *P<0.05, N *vs*. H (Two-Way ANOVA plus Tukey test).

Poster

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Mitochondrial-derived oxidative stress and the developmental programming of cardiac dysfunction – studies in the chicken

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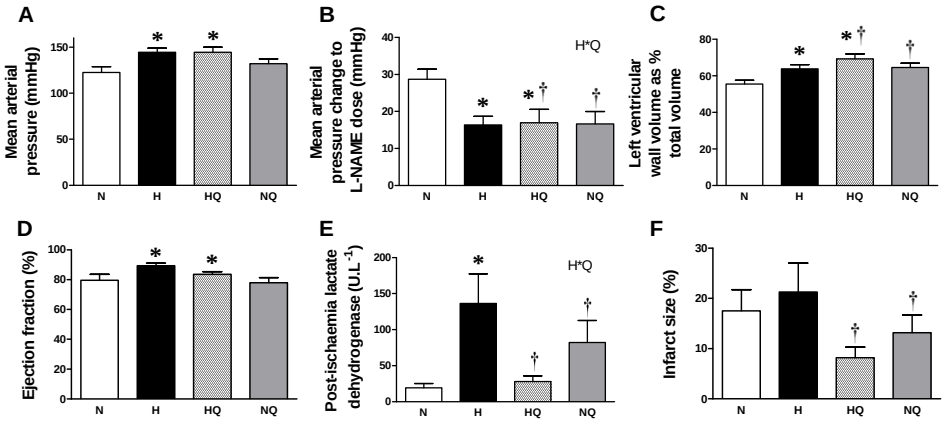
Introduction: Development under chronic hypoxia increases the risk of cardiovascular dysfunction in adult life. It has been demonstrated that this occurs secondary to an increase in oxidative stress as treatment with the antioxidant vitamin C is protective (Giussani et al. *PLoS ONE* **7(2)**:e31017, 2012). However, Vitamin C treatment is only effective at high doses which are not clinically viable. Hence, there is a need to find alternative antioxidant strategies. Mitochondria are a major source of cellular oxidative stress, hence we investigated the effects of treatment of hypoxic development with the mitochondrial targeted antioxidant MitoQ.

Methods: Fertilised chicken eggs were incubated in normoxia (21% oxygen) or hypoxia (14% oxygen) from day 1 of incubation (term=21 days). From days 13-18, embryos were treated with vehicle (water) or MitoQ (0.2 $mg.kg^{-1}.day^{-1}$). On day 19, all eggs were moved to normoxia, allowed to hatch and chickens raised to adulthood (6 months). At 6 months, chickens were surgically instrumented with femoral vascular catheters allowing measurement of blood pressure and responsiveness to drug infusion. Cardiac function and structure was assessed using echocardiography, the Langendorff preparation and stereological techniques.

Results: Adult chickens from hypoxic incubations showed hypertension (Fig.1A), low basal nitric oxide (NO) levels (Fig. 1B), hypertrophic hearts (Fig.1C) which worked hard to overcome the increased afterload (Fig. 1D) and an increase release of lactate dehydrogenase following ischaemia (Fig. 1E). Hypoxic MitoQ treated hearts showed no improvement in blood pressure, NO availability or cardiac hypertrophy (Fig. 1 A-D) but hadve reduced levels of LDH following ischaemia (Fig. 1E). MitoQ in both normoxic and hypoxic animals also reduced infarct size.

Conclusions: Developmental hypoxia programmes cardiovascular dysfunction in adult chickens. MitoQ treatment during hypoxic development offers some protection against ischaemic reperfusion challenges later in life.

Supported by the *British Heart Foundation*



Cardiovascular function in the adult chicken. Values are mean ± SEM for mean arterial pressure (A), pressure response to an I.V. dose of L-NAME (B), left ventricular wall volume as a % of total ventricular volume (C), cardiac ejection fraction (D), post-ischemia levels of lactate dehydrogenase (E) and post-ischaemia infarct size (F). Chickens were incubated in normoxia (N; white bar), hypoxia (H; black bar), hypoxia with MitoQ treatment (HQ; white striped bar) or normoxia with MitoQ treatment (NQ; grey bar). Two-way ANOVA for the effect of exposure to hypoxia (* $p<0.05$) or treatment with MitoQ († $p<0.05$). Where a significant interaction between factors is observed, the outcome of the Tukey *post hoc* comparison between each group is displayed

Poster

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Developmental programming of pulmonary hypertension by chronic prenatal hypoxia

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Introduction: Late gestation hypoxia-induced intrauterine growth restriction (IUGR) is associated with left heart dysfunction in young adult rats and ageing-associated pulmonary hypertension (PHT; Rueda Clausen et al. *Card Res.* **81**:713-22, 2009). However, whether chronic prenatal hypoxia programmes PHT in later life in the absence of IUGR is unknown. We hypothesised that chronic prenatal hypoxia would programme PHT in adulthood independent of IUGR and postnatal normoxia.

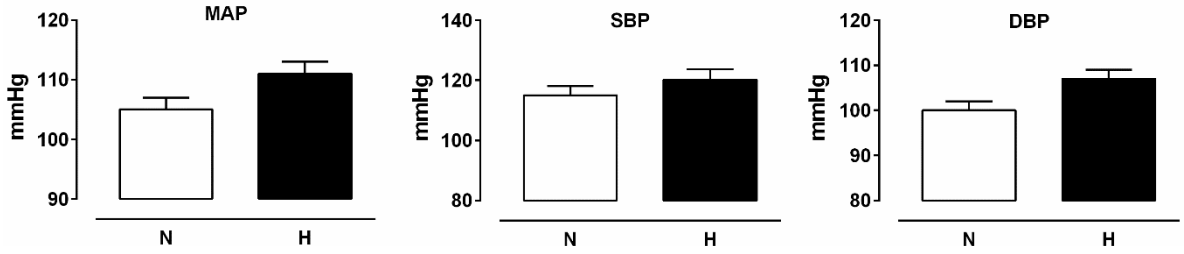
Methods: All experiments were carried out under the Animals (Scientific Procedures) Act, 1986. Pregnant Wistar dams were exposed to hypoxia (H; 13% O_2) or normoxia (N; 21% O_2) from 6-21 days gestation (term ~22 days). Offspring were maintained in normoxia to 4 months of age. One male per litter was selected for either echocardiography, performed under 10 mg▪kg^{-1} xylazine and 100 mg▪kg^{-1} ketamine (N: n=11, H: n=8), or conscious cardiovascular assessment. Femoral catheters were inserted under isoflurane anaesthesia and cardiovascular function was determined 4-5 days post-operatively (N: n=10, H: n=8).

Results: Pulmonary valve velocity time interval (PV VTI) peak pressure (mmHg) and velocity (mm▪s^{-1}) were elevated and tricuspid valve E/A ratio (TV E/A) was greater in H vs. N offspring (Fig. 1A). The ratio of diastolic right ventricle to left ventricle internal diameter (RVIDd/LVIDd) relative to bodyweight was greater in H compared with N offspring (0.42±0.01 *vs.* 0.36±0.03, $p<0.05$). M-Mode measurement of ejection fraction and pulse wave Doppler mitral E/A ratios were not different. Similarly, mean arterial (MAP), systolic and diastolic blood pressures (SBP and DBP, respectively) were not different (Fig. 1B).

Conclusions: These data suggest that chronic prenatal hypoxia programmes PHT independent of IUGR and postnatal normoxia, prior to the development of significant left heart and systemic dysfunction.

Supported by funding from the British Heart Foundation

A. Echocardiography



B. Arterial blood pressure

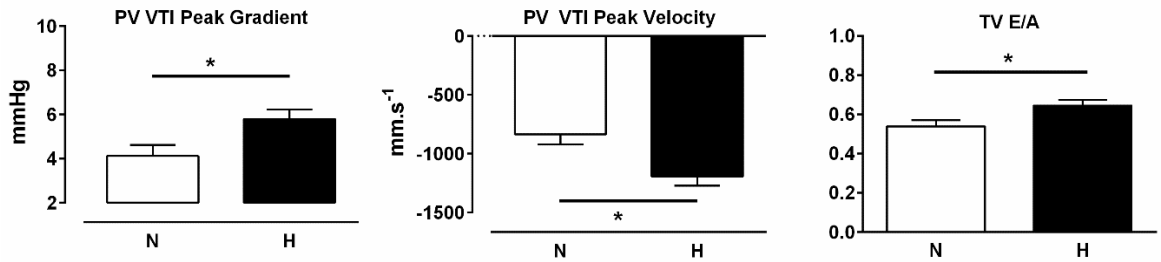


Figure 1. Cardiovascular Function. Cardiovascular measures were analysed in normoxic (N, white) and hypoxic (H, black) offspring by *t*-test. Values are mean ± SEM; *$p<0.05$.

Posters

Utero-Placental Studies

Poster

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Treatment administering tadalafil for severe preeclampsia with fetal growth restriction

Tanaka, H., Kubo, M., Nii, M., Maeda, Y., Shimura, M., Magawa, S., Kawamura, T., Tanaka, K., Murabayashi, N., Osato, K., Umekawa, T., Kamimoto, Y., and Ikeda, T.

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Background: For severe preeclampsia (PE) with fetal growth restriction (FGR), the only treatment is early delivery of the placenta. Clinicians are often forced to end the pregnancy for maternal indications. We report the case of severe PE with FGR whose PE temporary was improved, and pregnancy was successfully prolonged with Tadalafil as hosphodiesterase 5 inhibitor (PDE 5 inhibitor).
Case: A 35-year-old primigravid woman presented at 27 3/7 weeks of gestation with severe PE and FGR and started to administer Tadalafil. Biochemical markers temporarily improved after administering Tadalafil, hypertension and proteinuria temporality improved, and importantly a prolongation of the pregnancy by 14 days after initiation of Tadalafil.
Conclusion: Tadalafil may be a novel treatment for severe PE with FGR to prolong the period of pregnancy.

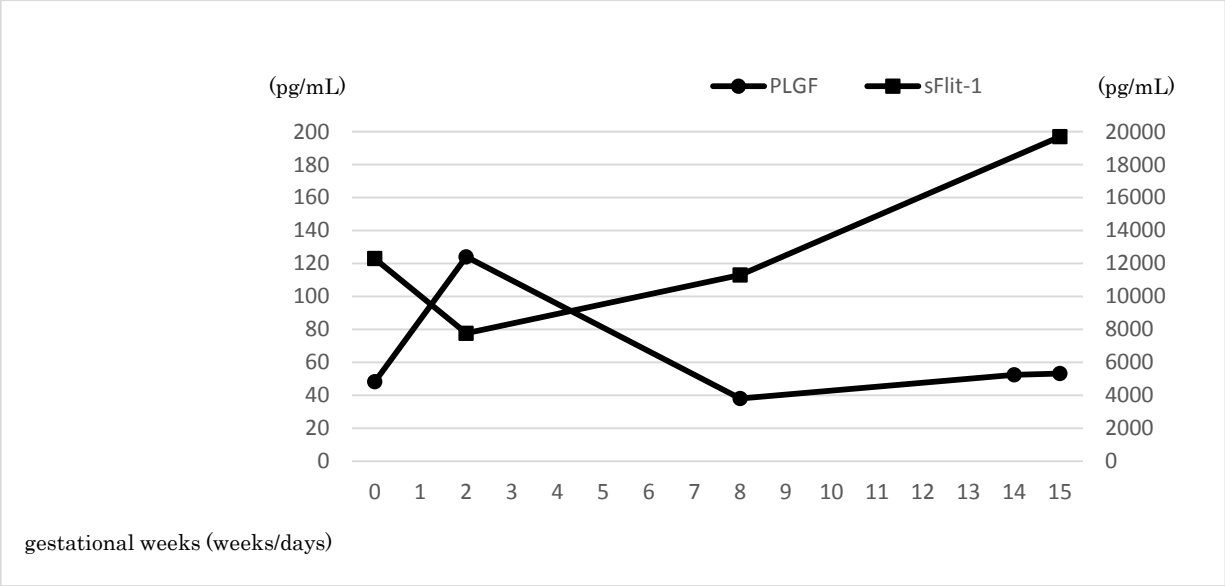

Figure 1. Angiogenic biomarkers of preeclampsia after start of administering Tadalafil. ; Soluble fms-like tyrosine kinase 1 (sFlt-1) fell with Tadalafil. and placental growth factor (PlGF) rose.

Poster

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The mouse dam fails to metabolically adapt to the pregnant state in response to a defieiency of Igf2 inplacental endocrine cells

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Introduction: Insulin-like growth factor (IGF)-2 is a growth regulatory imprinted gene highly expressed in feto-placental tissues. We and others have demonstrated that IGF2 regulates fetal growth and the morphogenesis and nutrient transport capability of the placenta during physiological and environmentally-manipulated pregnancies. However, less is known about the specific role of IGF2 in placental endocrine function and putative adaptation of maternal metabolism to favour fetal nutrient delivery. This is important as failure to adapt maternal physiology can lead to pregnancy complications and abnormal fetal development with long-term consequences for maternal and offspring health.
Aim: To investigate the significance of IGF2 in placental endocrine regulation of maternal metabolism and fetal growth during pregnancy.
Methods: Transgenic mice were crossed to produce entire litters with a deficiency in *Igf2* in the endocrine zone of the placenta (junctional zone, Jz) using the placental endocrine cell specific *Cre* line, *TpbpaCre* and *Igf2*-floxed mice (Figure A-B). On day (D) 16 of pregnancy, dams underwent a glucose tolerance test or were anesthetised, blood collected and maternal organs and fetuses weighed. Data were compared to age-matched non-pregnant (NP) females and pregnant dams on D16 that carried litters with unaltered *Igf2*.
Results: Deletion of *Igf2* in the placental Jz impaired the ability of the mother to expand her liver and kidney masses, increase plasma cholesterol and triglyceride concentrations and increase glucose availability in the circulation (AUC) in the pregnant state (Figure C-D). Moreover, circulating non-esterified free fatty acids were diminished and fetal growth marginally reduced on D16 of pregnancy in response to Jz-*Igf2* deficiency (Figure D-E).
Conclusions: *Igf2* expression in placental endocrine cells is important for driving maternal metabolic changes that promote fetal nutrient acquisition and growth during pregnancy. Work is underway to further assess changes on D19 of pregnancy. Supported by the Royal Society and COST Epiconcept STSM.

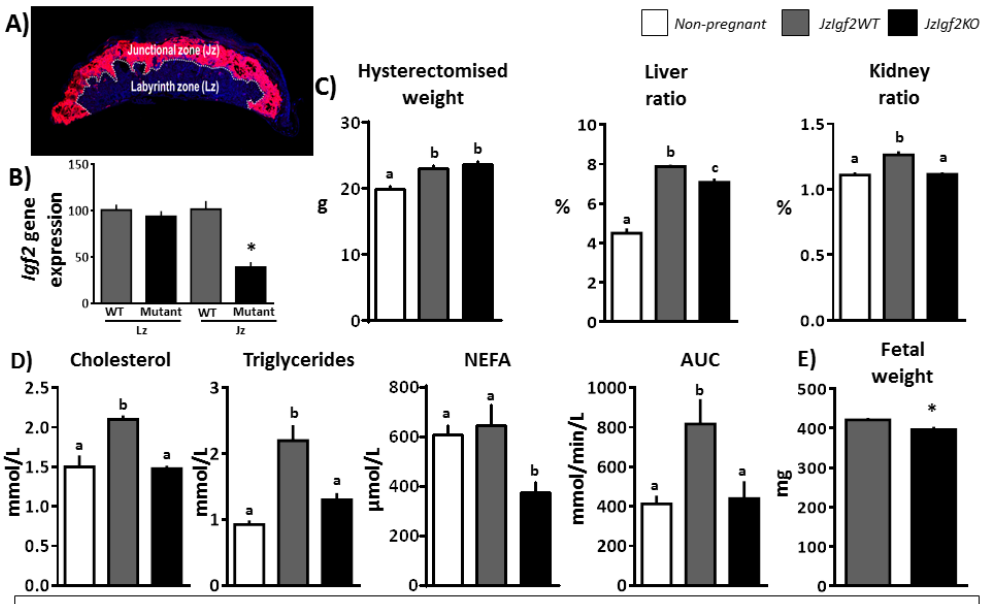

Figure A) *Use of TdTomato fluorescent Cre reporter line to show that TpbpaCre is exclusively active in the endocrine (Jz), but not in the transport zone (Lz) of the mouse placenta. B) qPCR to show* Igf2 *gene expression is reduced in the Jz but not in the Lz of mutant (Igf2/Tpbpa) placenta compared to wildtype (JzIgf2WT). Determined in litters of mixed genotype.* ***C)*** *Maternal organ weights as a percentage of hysterectomised weight.* ***D)*** *Maternal circulating lipids (NEFA, non-esterified fatty acids) and glucose clearance (AUC, area under the curve following glucose injection; 1 g/kg, i.p.).* ***E)*** *Fetal weights. Data from 4-15 dams and analysed by ANOVA and T test. Different superscripts indicate significant differences among groups (P<0.05). * compared to JzIgf2WT (P<0.05).*

Poster

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Labour-associated proinflammatory genes in the amnion are marked by bivalent epigenetic histone modifications

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Introduction: The co-occurrence of activating (H3K4me3) and repressive (H3K27me3) histone-3 (tri)methylations on nucleosomes near the promoter is a characteristic of developmentally regulated genes poising them for activation at the correct stage of development. Here we have explored whether this "bivalent" epigenetic modification pattern is involved in the activation of proinflammatory genes that may trigger term or preterm labour.
Methods: Amnion was collected in early pregnancy (12-16 wks) and after term elective caesarean section and spontaneous labour. Chromatin immunoprecipitation (ChIP) and sequential ChIP (ChIP-reChIP) was used to determine the level and co-occurrence, respectively, of the H3K4me3 and H3K27me3 modifications at the promoters of the labour-associated inflammatory genes PTGS2, BMP2 and NAMPT. Histone methyltransferase and demethylase expression was measured by quantitative RT-PCR.
Results: Both modifications were present at the proximal promoters of the 3 genes. There was a significant rise in the H3K4me3:H3K27me3 ratio at term prior to labour, compared to early gestation, due to increased H3K4me3 levels. H3K27me3 levels did not change between early pregnancy and term. The H3K4me3:H3K27me3 ratio decreased at the PTGS2 promoter with labour. ChIP-reChIP demonstrated co-occurrence of the H3K4me3 and the H3K27me3 marks on nucleosomes at the PTGS2 and BMP2 promoters throughout gestation. The H3K4 methyl-transferases KMT2A, -B, -C, -D, -F and G were expressed in the amnion with KMT2F (SET1A) and KMT2G (SET1B) mRNA levels increasing prior to term labour. Both H3K27me3 demethylases were expressed and KDM6B, but not KDM6A (UTX), mRNA abundance increased during pregnancy and at term labour. KDM6A mRNA level increased at preterm labour, and H3K27 methyltransferase (EZH2) expression decreased sharply after early pregnancy.
Conclusions: Our study of prototypical labour-associated genes in the amnion suggests that this group of genes is regulated epigenetically by bivalent histone modifications at the promoter. Activating H3K4me3 levels increase at term potentially by multiple H3K4 methyltransferase complexes, while suppressive H3K27me3 levels are established in early pregnancy by the EZH2-containing methyltransferase complex (PRC2) and remain stable until labour. We conjecture that the resulting bivalency controls the extent and the trajectory of labour-promoting gene expression as pregnancy advances ultimately determining the time of labour and providing defence against preterm birth.

Poster

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Enlarged uterus in amniotic fluid embolism and C1 esterase inhibitor

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[3] Mie University, Department of Obstetrics and Gynecology

Introduction: Amniotic fluid embolism (AFE) is one of the main causes of maternal death. The reported incidences of amniotic fluid embolism varied prominently from 1.7 to 7.7 per 100,000 deliveries or pregnancies. And the mortality rate is 20-60%. Typical symptoms of AFE are acute dyspnea, hypotension, DIC, and atonic bleeding.
Case: We experienced two cases of clinical amniotic fluid embolism. In both cases in addition to DIC, severe uterine edema was observed. Although improvement of DIC was admitted by the FFP, severe edema of the uterus persisted. We gave C1 esterase inhibitor (C1INH) in both cases and the uterus remarkably contracted in a short time.
Discussion: In amniotic fluid embolism, uterus becomes edematous due to anaphylactoid reaction accompanied by increased level of bradykinin.
C1INH improves the edematous change of the uterus by inhibiting the kallikrein-kinin system, which leads to decrease the production of bradykinin which causes atony of the uterus. In both cases we proved the decreased levels of C1INH before we had given C1INH. C1INH may possibly be used as a therapeutic medication for amniotic fluid embolism in three points: 1) it inhibits the production of bradykinin and contracts the uterus, 2) it controls the conhealing fibrinolysis system, 3) it inhibits the activation of the complements. There is no specific medicine for AFE. Tamura et al reported mean C1 esterase inhibitor(C1INH) activity level in clinical AFE cases was 30.1% , which was significantly lower than those of normal postpartum women 62.0%. C1INH , major inhibitor of complements, Factor XIIa and kallikrein could be the therapeutic medicine in AFE in which anaphylactoid reaction, coagulopathy, and activation of elements are the reported mechanisms.
Conculusion:C1INH may help treat AFE with an edematous uterus.

Poster

40

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Posters

Developmental Metabolism

Poster

41

Maternal fructose-sweetened beverage intake renders offspring physiologically-sensitive to further fructose intake

Gray, C[1,4]., Coppi, A.A[3,5]., Lobo-Ladd, A.A.B[5]., Lobo-Ladd, F.V[5]., Gardiner, S.M[2]. and **Gardner, D.S[1].**

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Introduction: The Western diet is typically high in fructose, which has myriad direct effects on cardiovascular, renal and metabolic function. Each physiological system may also be influenced by maternal diet. Here, we outline effects of moderate fructose-sweetened beverage intake on the dam, her placenta and offspring cardiovascular, renal and hepatic function and function.

Methods: Pregnant rats were fed water (+/-10% fructose) before and during gestation and through lactation. Male and female offspring were weaned onto standard laboratory chow. From 9-14 weeks of age, cardiovascular parameters (basal, circadian, stimulated) were assessed continuously by radiotelemetry, renal parameters (Na, K, free water clearance) via metabolic cage and hepatic structure and composition by serum enzymes and lipidomics, respectively.

Results: Maternal fructose intake led to significant repartitioning of maternal fat from peripheral to central deposits and significantly reduced placental volume (0.14 vs. 0.40 ± 0.04 cm^3). Offspring BP was unaffected by prenatal fructose but sensitive to postnatal dietary intake (i.e. increased ~5 mm Hg within hours), an effect exacerbated in female offspring from fructose-fed dams (i.e. increased ~ 10 mm Hg). Moreover, circadian analyses revealed non-dipping of nocturnal pressure in fructose-exposed offspring, which also had higher urine flow (34±3 vs. 25±2 ml/day kg BW^{-1}). Despite similar liver mass, triacylglycerol and glycogen content, fructose-exposed offspring had small, but significant shifts in hepatic fatty acid composition, even after no direct exposure to fructose (see Figure).

Conclusions: Moderately increased maternal fructose-sweetened beverage intake has myriad direct effects on the dam and her placenta and multiple persistent effects on the offspring, which would render them more susceptible to metabolic syndrome.

Figure 1. Fatty Acid composition of Offspring Liver: Fatty acids were measured by GC-MS after methylation extraction and are expressed as nmoles % of total hepatic tryglyceride. Data are; Control (n=7) and Fructose-exposed (n=6) offspring.

Poster

42

Maternal low protein intake early in gestation specifically impacts hepatic glucose metabolism in adult offspring of sheep

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Introduction: Differences in maternal diet can account, in part, for variation in metabolic competence of the subsequent individual as an adult. Developmental programming may affect growth of organs to limit functional capacity in adulthood, when environment may assume a greater role e.g. the rate of functional decline being exacerbated by obesity. Here, we have tested an interaction between prenatal nutritional 'thrift' and postnatal nutritional excess on gluco-regulatory function in an ovine model.

Methods: Ewes received either a standard diet (18% protein; CP, n=20) or a diet low in protein only (9% protein) fed during early gestation (0-65 days, term ~147 days; LPE, n=37) or late gestation (65-147 days; LPL, n=17). A proportion of ewes were culled at 65 days for fetal sampling. Remaining ewes lambed naturally, were weaned at 10 weeks and offspring handled accordingly to maintain body weight as 'lean' (at 1.5 years of age) or 'obese' (i.e. after 6 months exposed to an 'obesogenic environment'). In lean and obese states, body composition was determined by DXA in parallel with GTT and ITTs and organ tissue sampling after euthanasia.

Results: Low protein diet had little obvious effect on the dam or fetus, but term weight was reduced by ~500g in LPL. Homeostasis model assessment indicated relative insulin resistance in male LPE (Fig, left), an effect observed during adolescence. iAUC confirmed this observation (Fig, centre) and molecular analyses coupled with physiological assessment suggested insulin-signalling pathways in the liver, rather than muscle, as the target organ affected by low protein diet during early gestation (Fig, right).

Conclusions: Marked variation in maternal protein intake has 'silent' effects on fetal organ growth that are only revealed later in life when environmentally challenged by nutritional excess and physical inactivity. Livers of these offspring appear more susceptible to wider excursions of plasma insulin during gluco-regulatory challenges, an effect that will compound age-related, functional metabolic decline.

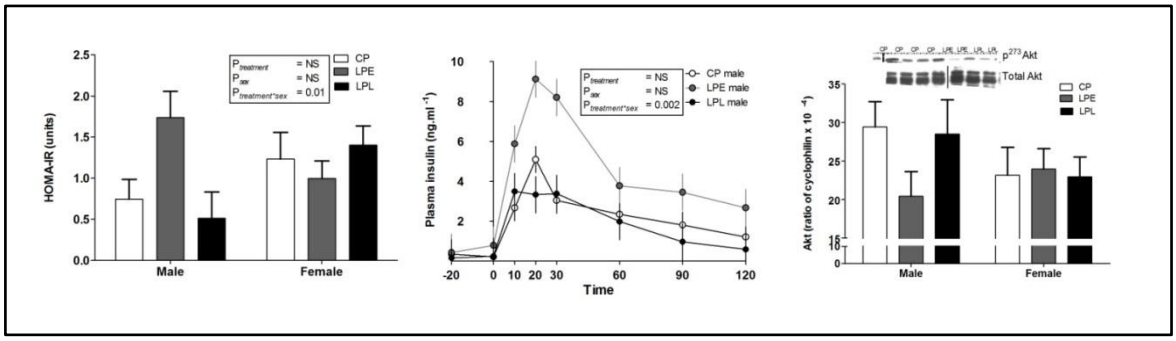

Figure 1. Gluco-regulatory functions of low protein exposed offspring.

Glucose and Insulin were measured in plasma obtained by venepuncture at timed intervals after an *i.v.* bolus of glucose (time zero; 0.5g/kg BW^{-1}). Data for males only are; Control (CP, n=8), Low Protein Early (LPE, n=8) or Low Protein Late (LPL, n=8). HOMA, homeostasis model assessment.

Poster

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Perinatal changes in mitochondrial function in ovine fetal skeletal muscle

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Introduction: The energy requirement of skeletal muscle increases at birth as it acquires the roles of locomotion and thermoregulation. Mitochondria are the primary source of energy through ATP production by oxidative phosphorylation. Additionally, mitochondria regulate oxidative stress and the generation of reactive oxygen species (ROS). Little is known, however, about the perinatal maturation of skeletal muscle mitochondrial density and function in preparation for the changes in energy demand and oxygen availability at birth.

Methods: Under the Animals (Scientific Procedures) Act 1986, twin-bearing ewes and their fetuses were euthanised (200mg/kg sodium pentobarbitone) at 102-105 days (d) of gestation (n=6 ewes; term ~d145), d126-129 (n=8 ewes) or d140-144 (n=8 ewes). Additionally, six 1-2 day old lambs from twin pregnancies were euthanised (one lamb per ewe). Samples of *biceps femoris* muscle were collected immediately into preservation medium, or frozen in liquid nitrogen. Muscle fibres were dissected and permeabilised to measure fat (palmitoylcarnitine; 40μM)-, carbohydrate (pyruvate; 5mM)-supported, and total (10mM glutamate and 10mM succinate) ADP (5mM)-coupled oxygen consumption. Citrate synthase (CS) activity, a marker of mitochondrial density, was measured spectrophotometrically. Data were analysed by one-way ANOVA with Tukey's *post-hoc* test.

Results: Muscle CS increased progressively with offspring age (fig.a; $P<0.0001$). Similarly, the rate of oxygen consumption with all substrates was higher in the neonates than prenatally ($P<0.001$ all cases; total shown in fig.b). When normalised to CS activity, pyruvate-supported and total oxygen consumption per muscle mitochondria decreased with increasing offspring age, with the highest values in the 102-105d fetuses and lowest values in the newborns ($P<0.01$ for both; total shown in fig.c).

Conclusions: Mitochondrial density increases towards term and continues to rise after birth, which would allow the rise in energy production expected for the increased requirement after birth. However, the functional capacity per mitochondrial unit appears to decrease over this period. This may play a protective role in reducing ROS production during labour and upon exposure to higher postnatal oxygen tension.

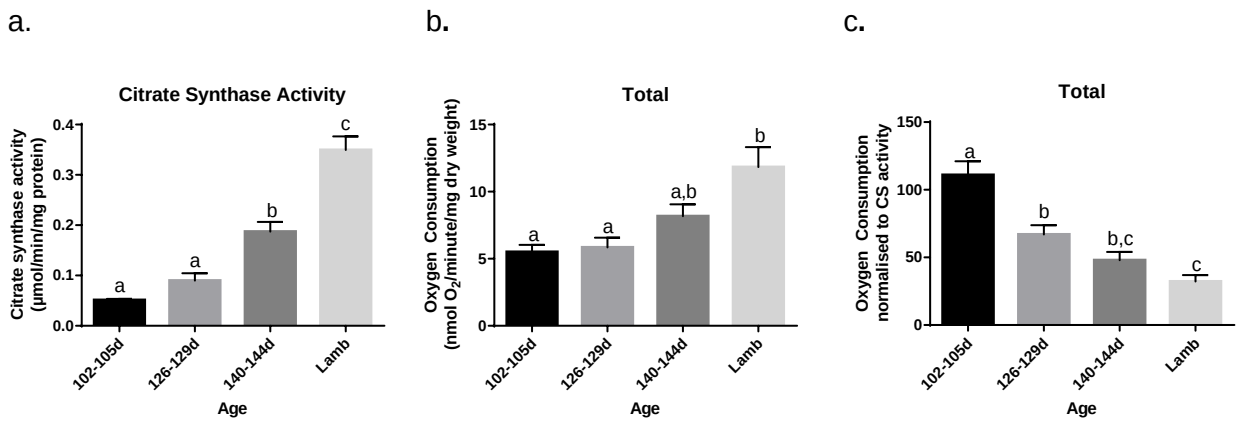

Figure. Mean±SEM a) CS activity and b&c) total ADP-coupled mitochondrial oxygen consumption rate with age in ovine skeletal muscle. Means with different letters are significantly different.

Poster

44

Lipid accumulation in the primate fetal liver with maternal obesity may be regulated by novel epigenetic mechanisms

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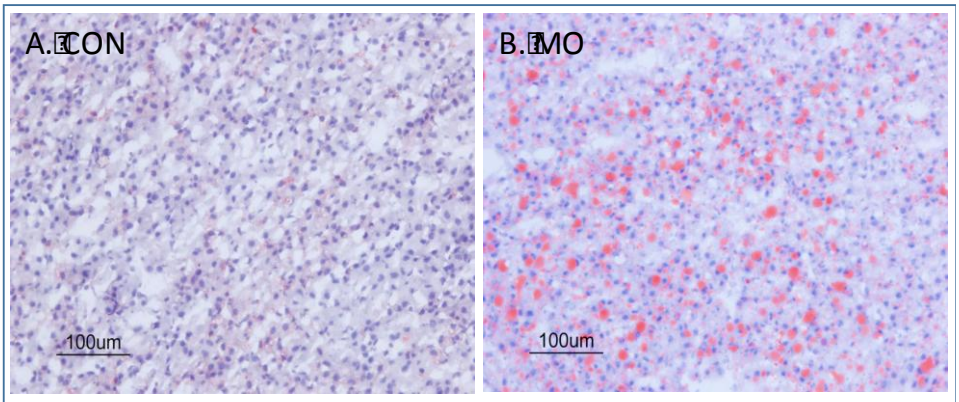
Introduction: The liver is a central organ regulating lipogenesis, gluconeogenesis and cholesterol metabolism. It is also considered the main detoxifying organ of the body playing a central role in metabolic homeostasis and is a major site for synthesis, storage and redistribution of carbohydrates, proteins and lipids. It is well-established that maternal obesity (MO) increases risk of offspring cardiovascular disease (CVD), diabetes and obesity. Mechanisms by which the MO intrauterine environment predisposes offspring to CVD and metabolic dysregulation are unknown. The goal of this study was to assess the impact of MO on primate fetal liver and identify underlying molecular mechanisms.
Methods: Unbiased hepatic gene (arrays) and microRNA (small RNA Seq) abundance were quantified in near-term (0.9G) baboon fetal livers (control (CON) = 6, MO = 5) and subjected to network analysis (GeneSifter, Ingenuity Pathways) to determine whether fetal responses to MO represent a coordinated molecular response. Lipid content (CON = 16; MO = 16) was quantified by Oil Red O staining and Computer Assisted Stereology Toolbox (CAST).
Results: We identified 875 differentially expressed genes between MO and CON, with 350 genes up-regulated and 583 down-regulated. Network analysis revealed Wnt/β catenin signaling central to the MO response suggesting that MO impacts lipid metabolism in fetal liver. In addition, 53 differentially expressed miRNAs that target genes in the Wnt/β catenin signaling pathway are inversely expressed with their target genes. We found hepatic lipid content was 3-fold greater in MO than CON fetal livers (p=0.02).
Conclusion: Fetal livers in the MO intrauterine environment display early indications of metabolic dysregulation with marked lipid accumulation. Molecular genetic analyses suggest that this dysregulation is mediated by Wnt/β catenin signaling, a central pathway in fatty acid metabolism and lipid storage. These findings are supported by inverse expression of miRNAs that target Wnt/β catenin signaling genes. The miRNA results also suggest an epigenetic mechanism regulating the Wnt/β catenin pathway with MO. Our findings show that MO has an impact on the fetal liver and predicts the impact on offspring impaired metabolic health.



Quantification of lipid content in CON and MO fetal baboon livers. Sections were stained with Oil Red O and quantified with stereomicroscopy. Panel A shows a representative control fetal liver and B MO fetal liver. Red indicates lipid.

Poster

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Similarities and differences in outcomes in the term fetal baboon pancreas to challenges of maternal under nutrition and obesity

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Introduction: Many outcomes in response to developmental challenges have U-shaped programming outcomes. For example the Harvard Nurses Study, one of the very first programming studies, showed that poor adult cardiac outcomes were related to birth weight in a J-or U-shaped fashion. We evaluated the fetal pancreas for insulin, key growth and differentiation factors as well as indices of oxidative stress and glucocorticoid receptors with immunohistochemical (IHC) techniques at term in pregnancies in which the mother was 1) on a moderate maternal restriction diet or 2) in obese mothers on a high fat high energy diet.

Methods: Control baboons ate Purina monkey diet *ad libitum* (CTR) while mothers experiencing moderate nutrition reduction ate 70% of control throughout pregnancy (IUGR). OB mothers were obese at the time of conception due to eating a high fat, high-energy diet (45% energy from fat with 4.62% glucose and 5.64% fructose) and unlimited access to fructose drinks from at least nine months before and throughout pregnancy. Fetuses were retrieved at C-section at Term. IHC for area stained with image J quantification was analyzed by Student's T-test.

Results: Fig. 1 shows that IUGR fetuses had reduced pancreatic insulin while OB fetuses had increased pancreatic insulin due to presumably to the increase in circulating secretagogues. Similarly IGF1 was down in IUGR and up in OB which could account for differences in pancreatic growth. PDX1, an important molecule in pancreatic differentiation was decreased in both IUGR and OB but proportionately more in IUGR. HNF4α was decreased approximately by the same percentage in both IUGR and OB. Reactive oxygen stress (ROS) is considered by many to be a basic mechanism in developmental programming outcomes. It is, thus, of interest that nitrotyrosine was increased similarly in both IUGR and OB as was NDUFV2 and citrate synthase. Phospho-GR was decreased in both situations.

Conclusions: Similarities and differences exist in fetal pancreatic outcomes in response to fetal nutrient deficiency and excess which may reflect the fact similarities and differences in predisposition to diabetes in later life that result from these two challenges.

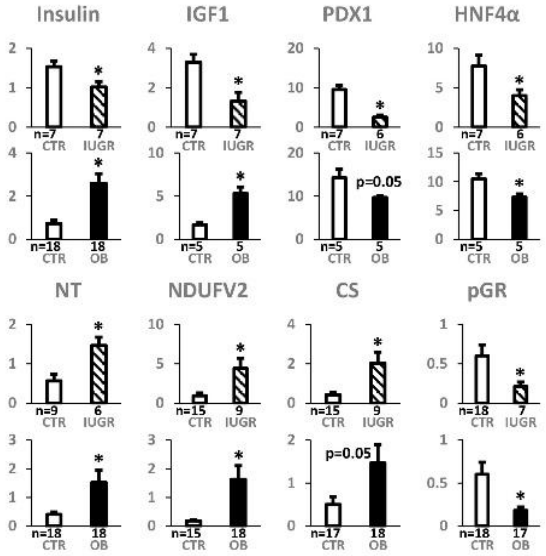

Figure 1. Immunohistochemistry in pancreas of foetuses of IUGR and OB mothers. NT – Nitrotyrosine, CS – Citrate synthase, pGR – Phospho-Glucocorticoid receptor. Data: percentage fraction of section stained. M + SEM, *p< 0.05.

Poster

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Maternal nutrient restriction (MNR) or excess (MNE) increases oxidative stress in multiple fetal baboon tissues at 0.9G: all roads lead through Rome?

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Introduction: Maternal nutrition during pregnancy is crucial to development of many fetal neuroendocrine, cardiovascular and metabolic systems. We and others have shown increased oxidative stress in several tissues of offspring of rodent mothers exposed to either nutrient reduction (MNR) or maternal nutrition excess (MNE). There is a need to determine if oxidative stress plays a role in responses to maternal dietary challenges in nonhuman primates.
Hypothesis: Both MNR and MNE increase oxidative stress in multiple fetal tissues.
Methods: Healthy female baboons of similar body weight (10-15 kg) were fed normal chow (CTR, n=26; 12% energy from fat with 0.29% glucose and 0.32% fructose), or 70% CTR global diet from 0.16 - 0.9 gestation (MNR n = 11) or a high energy diet (MNE, n=19; 45% energy from fat with 4.62% glucose and 5.64% fructose) plus *ad lib* fructose sodas prior to pregnancy. Fetuses were removed by C-Section at 0.9G under general anesthesia, frontal cortex (FC), heart left ventricle (LV), liver, pancreas, kidney and skeletal muscle vastus lateralis (SM) were fixed and stained for Nitrotyrosine (NT), superoxide dismutase 1 and 2 (SOD1 and SOD2) (Santa Cruz, 32757, 11407, 133254). Data M + SEM, Student's t-test, *p< 0.05.
Results: Fig. 1 shows NT protein expression increased in MNR fetal FC, heart LV, liver and pancreas as well as in MNE fetal FC, heart LV and pancreas. SOD1 decreased in MNE fetal FC, SOD2 decreased in MNR fetal FC, heart LV and MNE fetal FC. However SOD2 increased in both MNR and MNE fetal pancreas. No differences were observed in kidney and muscle (not shown).
Conclusions: Altered fetal nutrient supply both an increase and decrease in nutrient availability stimulate oxidative stress. If these changes persist into adult life they may constitute a mechanism whereby poor or excess fetal intrauterine nutrition predisposes to widespread later life organ dysfunction.

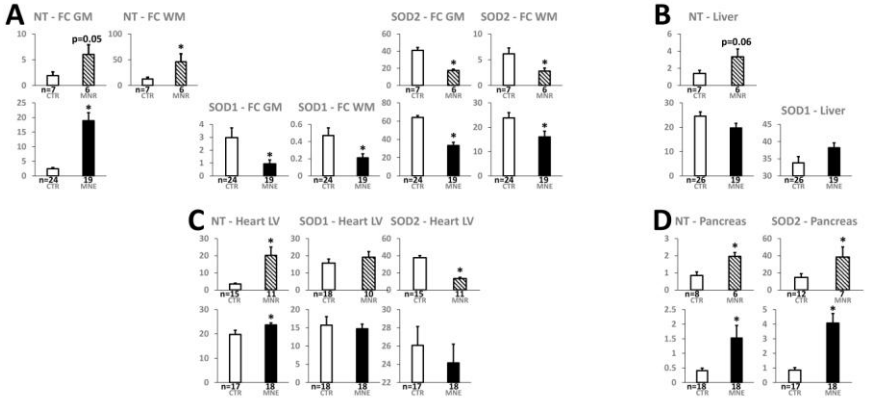

Figure 1. Immunohistochemistry. (A) FC – Frontal cortex, GM – Gray matter, WM – White matter, (B) Liver, (C) Heart LV – Heart left ventricle, (D) Pancreas. Data: percentage fraction of section stained. M + SEM, *p< 0.05.

Poster

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Offspring (F1) sex differences in postnatal growth characteristics in a Nonhuman Primate Model of Programming by Maternal Obesity (MO)

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Introduction: MO in pregnancy predisposes F1 to a variety of chronic health problems. We recently completed an NIH project to establish well-documented cohorts of F1 baboons of both sexes derived from MO mothers eating a high fat, high energy diet (HF-HED) plus unlimited fructose sodas to compare outcomes of F1 of same age, normal weight control mothers eating standard primate center Purina Monkey diet *ad libitium*. NIH requires that resources developed on R24s be advertised as available for collaborations. Therefore we summarize here their phenotype to describe this cohort for investigators interested in studying F1. F1 fetal and postnatal tissues are available for collaborative studies on challenges, exposures, mechanisms and outcomes responsible for programming due to MO and HF-HED combined in well-characterized nonhuman primate model. It is also possible to design *in vivo* studies.

Methods: Female baboons housed in group cages were fed *ad lib* Purina Monkey diet [CTR] or *ad lib* CTR diet with *ad lib* HF-HED diet [MO] for at least 9 months prior to breeding and through pregnancy and lactation. MO group had continuous access to high fructose beverage (Kool-Aid).

Results: MO raised maternal BMI (20%) and increased maternal body fat (12%) above CTR at conception. By 0.9 of gestation, maternal glucose in MO increased above CTR 79%, insulin 181% and triglycerides 31%. Fetal glucose was unchanged but fetal insulin increased 170% and fetal triglycerides 28%. At postnatal age 0.75 – 1.0 years percentage body fat in MO F1 was increased above CTR (6.6% females and 2.5% males). Female F1 of MO mothers were heavier than CTR until 10 months of age, males were of similar weight (Fig 1). Over the first year of life body weight, head, chest and abdominal circumference were all increased at some stages while not in males.

Conclusions: Strengths of baboon programming studies are 1) ability to control challenges and remove confounds present in epidemiological studies, 2) genetic similarity to humans, 3) precocial stage at birth. Tissues and F1 are available for study.

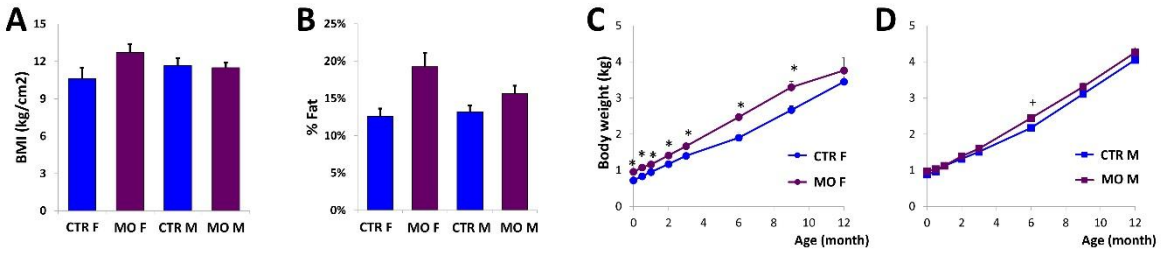

Figure 1. Morphometry. (A) Body mass index (BMI) and (B) Body fat percentage (%Fat) at 0.75 – 1.00 years of age; Postnatal body weight of female(C) and male (D). Purple – offspring of maternal obese baboon (MO) vs Blue – F1 control, Data: M+ SEM, *p< 0.05, 0.05<+p< 0.10.

Poster

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Liver transcriptome outcomes differ in adult male and female rat offspring exposed to maternal obesity (MO) in pregnancy and lactation

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Introduction: MO predisposes offspring (F1) to chronic diseases, including non-alcoholic fatty liver disease (NAFLD). NAFLD is the hepatic manifestation of the metabolic syndrome. Prevalence of NAFLD is higher in males. However, mechanisms underlying NAFLD in F1 of MO mothers are poorly understood. We conducting RNA-seq to evaluate the transcriptome of F1 male and female from MO and Control (C) mothers to evaluate mechanism involved in development of NAFLD. **Methods:** F0 female Wistar rats ate control or obesogenic diet from weaning through lactation. After weaning all F1 males and females ate control diet. At PND 110 male and female serum and tissues were collected to analyze biochemical parameters, H&E histology, liver triglycerides (TG), immunohistochemistry (IHC) for nitrotyrosine (NT) and oxidative stress (OS) parameters, as well as F1 liver transcriptome with RNA-seq to identify differentially expressed genes (DEG). M ± SE, analysis one-way ANOVA. **Results**: Male and female F1 of MO show increased total fat TG, and HOMA without body weight changes vs. C. Liver: weight, fat content (Fig 1A), TG (Fig 1B), NT (Fig 1C) and glutathione peroxidase (GPX) (Fig 1D), increased in males whereas only liver TG and NT increased in females MO vs. C. Expression of 3,054 and 170 genes was changed by MO in males and females, respectively ($P < 0.05$, Fig 1E). For males 108 genes (3.5%) were up-regulated and 2,946 (96.5%) were down-regulated. From the list of DEG down-regulated, KEGG pathway database identified genes related to metabolic pathways (314 genes), oxidative phosphorylation (39 genes), insuling signalling (37 genes), cell cycle (25 genes). In contrast, in female, only 122 genes were differentially expressed up (72%) and 48 genes down (28%) (Fig 1E), with no relevant KEGG pathways enrichment. **Conclusions:** MO programs metabolic dysfunction and down regulation of key cell cycle genes and oxidative phosphorylation, especially complex 1 the major site of ROS production. Changes are sex-specific and can be associated with liver dysfunction observed in F1. The adult liver of male MO adult F1 shows global down regulation of genes that are required for normal function of the transcription process.

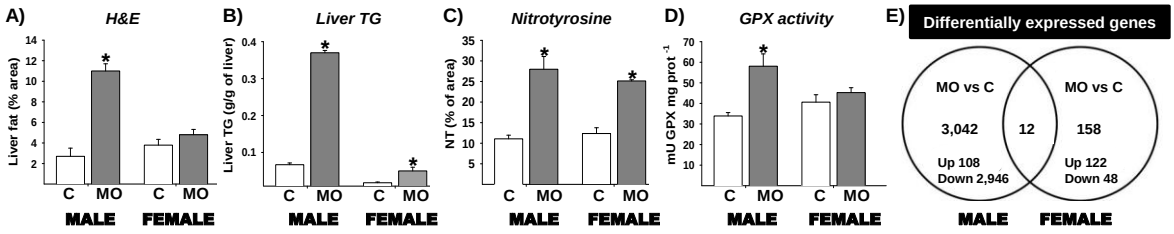

Figure 1. A) Liver % fat by histology, **B)** Liver TG, **C)** NT IHC, **D)** GPx activity and **E)** RNA-seq analysis of differential expressed genes. Mean ± SE, n = 5-7 male and female F1 from different litters. * p <0.05 obese vs C in the same sex.

Poster

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Impaired post-partum weight loss associated with inflammation and lipid peroxidation in rats exposed to low-protein diet *in utero*

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Introduction: Maternal obesity is increasingly prevalent globally. It is recognized that progressive inter-pregnancy weight-gain is a common route to obesity in reproductive age women. However mechanisms influencing post-partum weight loss are not well understood. We investigated whether exposure to a suboptimal early-life environment influenced the metabolic response to pregnancy and subsequent post-partum weight-loss.

Methods: Wistar rat dams were fed an 8% protein diet during pregnancy, and offspring suckled by control-fed mothers (recuperated). All first-generation (F1) offspring were weaned onto standard chow. First-generation offspring were bred at 3 months and their weight-gain serially monitored during pregnancy and lactation, alongside a non-pregnant paired-sibling control. At the end of lactation, tissues were collected and intra-abdominal fat mass measured. Gene expression was measured via q-rtPCR.

Results: There was no difference in pregnancy weight-gain between control and recuperated F1 mothers. By the end of lactation, the weight of the control mothers was not significantly different to their non-pregnant sibling-controls, however recuperated mothers remained significantly heavier than either their control counterparts or their non-pregnant sibling-controls ($p<0.01$). Ovarian fat pads were heavier in postpartum recuperated mothers than in controls ($p<0.05$). Gene expression in intra-abdominal adipose tissue showed up-regulation of inflammation and lipoxygenase pathways in recuperated mothers compared to both control mothers and non-pregnant sibling-controls (Alox12 $p<0.05$; IL6 $p<0.05$).

Conclusions: Exposure to suboptimal intrauterine nutrition impairs recovery from the metabolic challenge of pregnancy and leads to retention of adipose mass post-partum. This study suggests that the early-life environment may have an important role in influencing inter-gestational weight gain, hence contributing to maternal obesity.

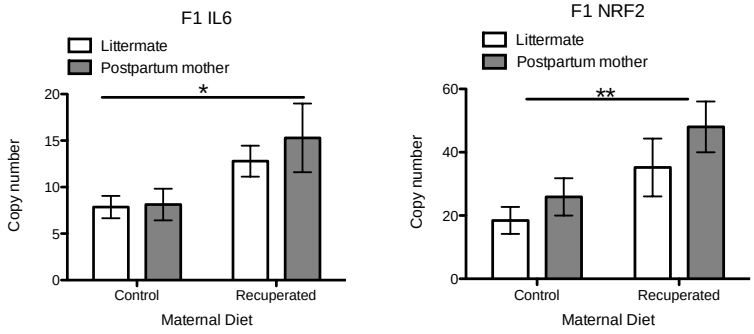

Figure 1. Gene expression in the adipose tissue of F1 mothers and non-pregnant littermates (A) IL6 is elevated in the recuperated animals compared to controls ($p<0.05$), but no significant difference by pregnancy status was observed (B) NRF2 expression is highly up-regulated in recuperated animals ($p<0.01$), and there is a significant interactive effect of pregnancy status and maternal diet ($p<0.05$).

Poster

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Differential effect of lower vitamin D status on fetal hind limb skeletal muscle and bone structure in sheep

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Introduction: Vitamin D deficiency affects a substantial proportion of the human population, including during pregnancy. Lower maternal vitamin D status has been associated with altered human fetal bone growth, reduced handgrip strength in 4 year olds and altered muscle structure in young rodents. The fetus is reliant on maternal vitamin D status, and therefore we looked directly at fetal vitamin D status in relation to bone and skeletal muscle structure during fetal life.

Methods: Welsh mountain ewes were fed 100% nutrient requirements (C, 2000 IU/kg vitamin D_3, n= 9), or a diet lacking the vitamin D_3 supplement (VDD, 0 IU/kg vitamin D, n=10) from 17 days prior to conception until post-mortem at 126-130 days of gestation. Maternal and fetal serum total 25-hydroxyvitamin-D concentrations ([25(OH)-D]) were measured by HPLC tandem mass spectroscopy. 3-dimensional images of fetal femur were captured using microcomputer tomography (SkyScan). Type-I and –II myofibre density and cross-sectional area (CSA) were assessed in 10µm sections of frozen vastus muscle by immunohistochemistry (MY-32 anti-fast myosin). Data (age-adjusted) were analysed by Student's t- or Mann-Whitney U-test and linear regression with [25(OH)-D].

Results: Bone length and structure, and vastus type-I and –II myofibre density and type-II CSA were not significantly different between C and VDD groups. In the fetal femur, lower fetal serum [25(OH)-D] was associated with thinner trabeculae ($p<0.01$, $R^2=0.36$), and with greater trabecular surface-to-volume ratio ($p<0.01$, $R^2=0.39$), lower mid-shaft wall thickness-to-diameter ratio ($p<0.05$, $R^2=0.21$), and greater length ($p<0.05$, $R^2=0.30$). Fetal serum [25(OH)-D] was not associated with vastus type-I/II myofibre density or CSA. Longer fetal femur length was associated with lower total ($p<0.05$, $R^2=0.30$) and type-II myofibre density ($p<0.05$, $R^2=0.30$), and with greater type-II myofibre CSA ($p<0.01$, $R^2=0.46$).

Conclusions: The association of lower fetal [25(OH)-D] with altered bone, but not skeletal muscle, structure shows a tissue-specific hind limb adaptation. Our data highlight an association between longer femur length and reduced vastus myofibre density. Thus the preservation of femur length at the expense of strength with lower fetal [25(OH)-D], may alter skeletal muscle structure and influence hind limb strength in later life.

Poster

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Effect of preterm birth on mRNA expression of drug transporters in guinea pig liver

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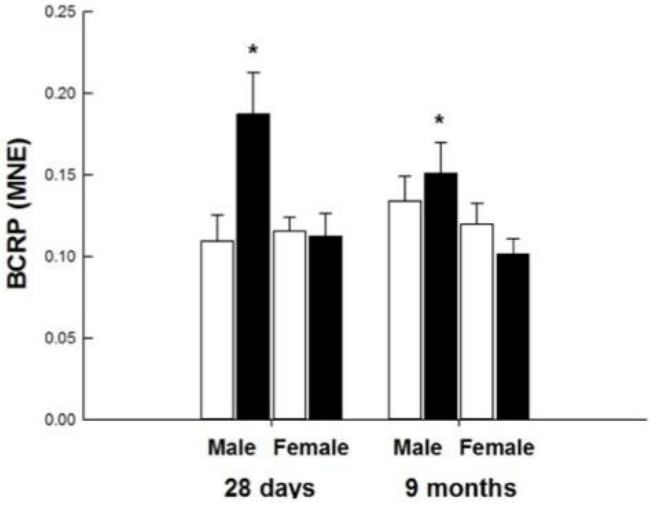
**denotes equal contribution*

Introduction: Preterm birth affects approximately 10% of live births every year and these babies are likely to be treated with drugs during their lifetime. There is some evidence that preterm birth may alter neonatal drug disposition. Due to advancements in perinatal medicine, more preterm babies are surviving into adulthood; however, little is known regarding the ex-preterm's ability to clear medication from its system in later life.

Methods: Guinea pigs were mated and randomised to term and preterm groups. Standard clinical care for women at risk of preterm delivery was replicated by administration of two doses of betamethasone 48 and 24 hours before induction of preterm delivery at day 62 (Term=69d) in the preterm group. Liver tissue was collected at 28d (Term males, n=5; Term females, n=8, Preterm males, n=6, Preterm females, n=6), representing adolescence, and 9 months (Term males, n=9; Term females, n=8, Preterm males, n=8, Preterm females, n=6), representing adulthood. qRT-PCR was used to determine the mRNA expression of drug transporters (Breast cancer resistant protein (BCRP) and P-glycoprotein (P-gp)) as well as factors that can regulate the expression or function of drug transporters (Glucocorticoid pathway (Glucocorticoid receptor (GR), 11-Beta hydroxysteroid dehydrogenase-1 (11βHSD1) and -2 (11βHSD2)), Pregnane X Receptor (PXR) and the most common active dimer complex of NF-κB (p50 and p65).

Results: We found an increase in BCRP mRNA expression in the liver of preterm males at 28 days and this effect persisted into adulthood. Interestingly, preterm birth had no effect on BCRP mRNA expression in females. There was no effect of preterm birth on P-gp mRNA expression. Although, preterm birth had no effect on GR mRNA expression, males had higher GR mRNA expression compared to females. Interestingly, at both ages preterm birth resulted in an increase in 11βHSD1 mRNA expression in males but a decrease in females. There was no effect of preterm birth on mRNA expression of Pregnane X Receptor, and the most common active dimer complex of NF-κB, p50 and p65.



Conclusions: This preliminary data shows that preterm birth may affect drug disposition in later life. In addition there appears to be differential effects of sex.

Figure 1: Preterm birth resulted in increased mRNA expression of BCRP in male guinea pigs at 28d and 9 months, but not in female guinea pigs. **P*<0.05, effect of preterm; white bars, term; black bars, preterm.

Poster

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Earthquake and perinatal medicine in Japan-disaster liaison in paediatrics and perinatal medicine (DLPPM)

Unno, N., Tsuruwa, M., Itoh, T., Tsuda, N., Nishigaya, Y., Hattori, K., Sugawara, J., Suzuki, M., Nakai, A., Wada, K., Nakamura, T. & Masuzaki, H.

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Introduction: In Japan, 47% of all the deliveries are cared in private clinics while 52% in private or public hospitals. After the experience of the Great East Japan Earthquake in 2011, we learned that, during the acute phase of large scale earthquake, most private obstetric clinics and small hospitals are unable to continue their practice until recovery of lifelines. Deliveries in the remaining hospitals increase drastically. For these hospitals, immediate support by teams of medical professionals and provision of sterilized medical supplies for delivery and surgery are essential to continue necessary service to the pregnant women and neonates suffered by the earthquake. For this purpose, an efficient communication system between headquarters for disaster control in the local government and delivery units of local hospitals in the affected area must be established.

Methods: 1) Following the discussion in disaster control committees of academic societies in paediatric and perinatal field, by the end of 2015, we decided to launch a new training system for obstetricians and paediatricians to become a "disaster liaison" who collects the support needs for maternal-fetal-neonatal medicine in the affected area, and plays a role in logistic arrangements such as patient transportation to prenatal centres in the unaffected areas. 2) In April 16, 2016, a huge earthquake (Magnitude 7.3) struck Kumamoto Prefecture in Kyushu district of Japan. Due to serious damage, the largest perinatal centre in Kumamoto with 18 NICU beds, Kumamoto City Hospital lost its function completely, and an immediate evacuation and a transfer of all the patients to other centres in the unaffected areas were conducted. In response to the request of disaster control headquarters in Kumamoto, a team consisted of 3 paediatricians and 3 obstetricians experienced with disaster medicine dashed into Kumamoto to serve as DLPPM.

Results: After 10 days' vigorous activities in the Kumamoto headquarters and evacuation shelters, the DLPPM team withdrew and took over their mission to local physicians. In Kumamoto area, no serious damage was observed in pregnant women or in the patients of perinatal centres obliged to be transported after the earthquake.

Conclusions: 1) Although not well-prepared, an instant team of DLPPM played a significant role during the acute phase of Kumamoto Earthquake in 2016. 2) The experience in Kumamoto Earthquake suggests that a training system of DLPPM may enhance our ability of disaster control in the field of paediatrics and perinatal medicine.

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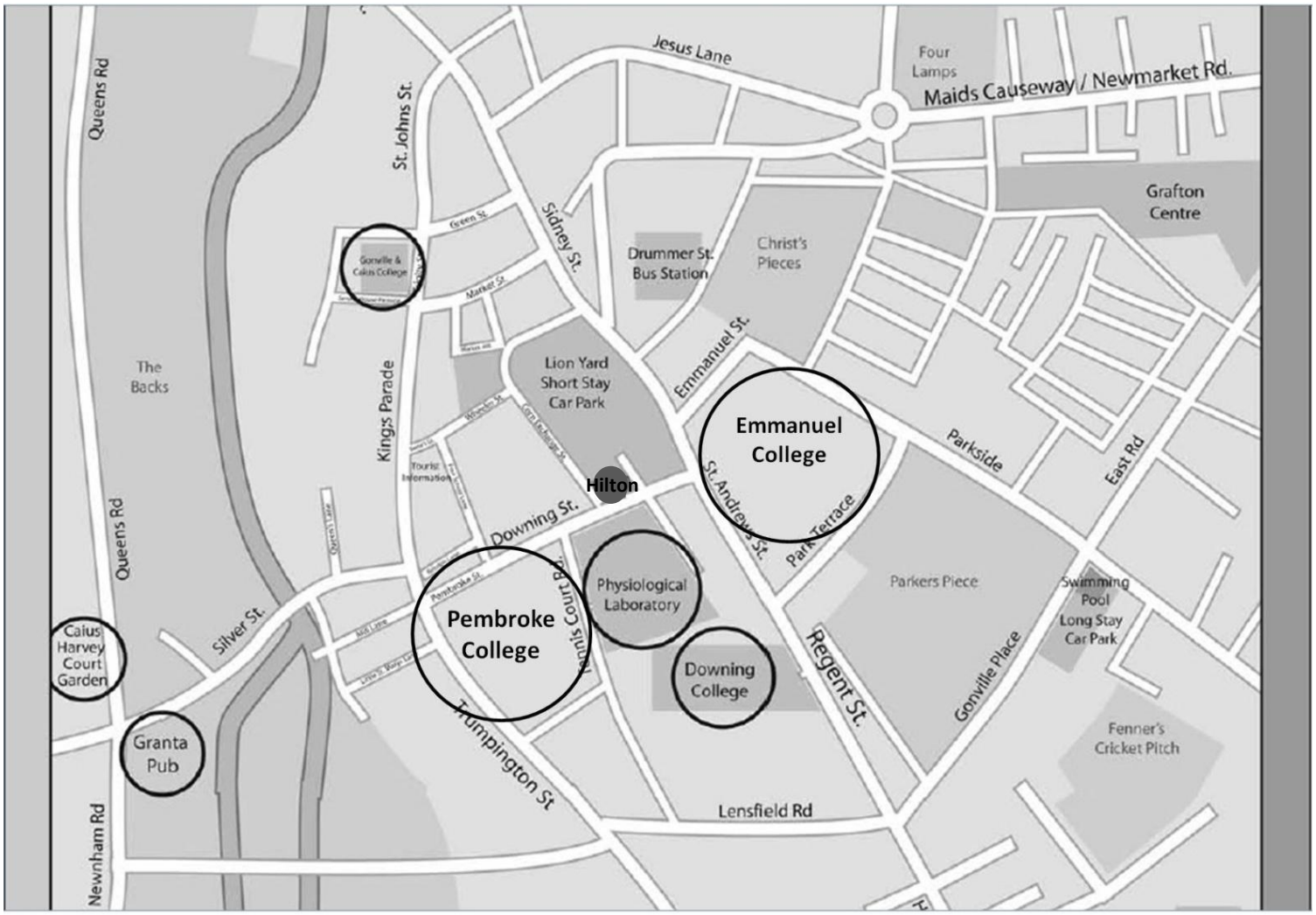

Notes:

- Entrance to the Main Physiology Lecture Theatre via Downing Site on Downing Street;
- Entrance to Emmanuel College via Porter's Lodge on St Andrew's Street;
- Entrance to Pembroke College via Porter's Lodge on Trumpington Street;
- Entrance to Gonville & Caius College via Porter's Lodge on Trinity Street;
- Entrance to Granta Pub located on Newnham Road;
- Entrance to Harvey Court and the Stephen Hawking building via Porter's Lodge on West Road;
- Caius Fellows' garden is located just behind Harvey Court, on West Road.

Fetal and Neonatal Physiological Society

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