THE EIGHTEENTH NATIONAL WORKSHOP ON FETAL AND NEONATAL PHYSIOLOGY

A WORKSHOP IN HONOUR OF PROFESSOR EUGENIE RUTH LUMBERS

Women's Health Institute
Royal Hospital for Women,
Barker St, Randwick, NSW
March 13-14, 2004

Organising Committee

Dr Megan Cock, Dr Karen Gibson,
Dr Leo Leader and Prof Richard Harding

Saturday 13th March

10.00-11.00 Registration / Morning tea

Session 1 Chair: Dr Leo Leader			
11.00	A1	Pitcher, Moore, Robertson, Cockington & Miles	Birthweight, gestational age and neuromotor outcomes in adult humans.
11.15	A2	Rodricks, Gibbs & Miller	The timing of prenatal insults determines memory impairment
11.30	A3	Kecskes, Kent, Dahlstrom & Hendry	Protein 14-3-3 in CSF following hypoxia-ischaemia – a study proposal
11.45	A4	Dalitz, Rees, Henschke ,Cock & Harding	Episodic alcohol consumption during pregnancy: mechanisms of fetal brain injury.
12.00	A5	Wong, Barfield, Wilkinson, McDermott & Walker	Use of cerebral intravascular oxygenation as parameter for cerebrovascular autoregulation
12.15-12.45 General discussion			

12.45-1.45 Lunch

Session 2 Chair: Prof Julie Owens			
1.45	A6	Yiallourou, Ramsden, Walker & Horne	Effects of sleep state and sleeping position on blood pressure in infants in the first 6 months of life
2.00	A7	Tuladhar, Harding, Adamson & Horne	Heart rate variability in sleeping infants during the first twelve months of life: effect of age and sleep state
2.15	A8	Sferruzzi-Perri, Robinson & Roberts	The effect of hypoxia on placental outgrowth during early pregnancy is mediated by insulin-like growth factor-II
2.30	A9	Supramaniam, Jenkin, Wallace & Miller	The site of production and release of activin A in the intra-uterine growth restricted ovine fetus
2.45	A10	Pringle, Kind & Roberts	Localisation of hypoxia, insulin-like growth factor-II (IGF-II) and hypoxia inducible factors (HIFs) in early murine implantation sites
3.00-3.30 General discussion			

3.30-4.00 Afternoon tea

Session 3 Prof E Lumbers Session Chair: Prof Richard Harding			
4.15	A11	Wintour-Coghlan	Eugenie Lumbers: her contribution to fetal physiology
4.30	A12	Boyce, Gibson, Wu, Burrell & Lumbers	The fetal cardiac renin-angiotensin system and effects of insulin-like growth factor I
4.45	A13	Gibson, Brown, Turner & Persson,	Measurement of net filtration pressure in the fetus and lamb
5.00	A14	O'Connell, Kumarasamy, Lumbers, Boyce & Gibson	The consequences of a midgestational asphyxial episode on the development of the fetus
5.15	A15	Lumbers, Kim, Boyce, Kumarasamy, Gibson & Burrell	Characteristics of cardiac myocytes following IGF-I infusion in late gestation fetal sheep
5.30-6.00 General discussion			

6.00 Pre dinner Drinks-

7.00 Workshop dinner – Seasalt Cafe

Sunday 14th March

10.00-10.30 Morning tea

Session 4 Chair: Assoc Prof Mary Wlodek			
10.30	A16	Jaquiery, Oliver, Bloomfield & Harding	Effects of periconceptional undernutrition on glucose and insulin metabolism in sheep.
10.45	A17	Dickinson, Moritz, Walker, Wintour & Dodic	Prenatal programming of hypertension in the spiny mouse
11.00	A18	Maduwegedera, Flower, Wintour & Denton	Chronic maternal hypertension and its effect on placental and kidney to body weight ratios in the rabbit
11.15	A19	Jaquiery, Oliver, Bloomfield & Harding	Effects of periconceptional undernutrition on pregnancy outcome and feto-placental growth in sheep.
11.30	A20	Grover, Coulter, Walker, Kind, Robinson & Owens	Effect of metyrapone on circulating cortisol and glucose homeostasis in the guinea pig.
11.45-12.15 General discussion			

12.15-1.30 Lunch

Session 5 Chair: Dr Tim Moss			
1.30	A21	Sozo, Filby, Hooper & Wallace	A technique to isolate genes that are critical for lung growth.
1.45	A22	Filby, Zahra, Sozo, Hooper & Wallace	VDUP1; a potential regulator of fetal lung growth and epithelial cell differentiation
2.00	A23	Yawno, Cock Hanna, Sozo & Harding	Altered airway epithelial structure following preterm birth in sheep.
2.15	A24	Suzuki, Hooper & Harding	Relation between lung size and pulmonary circulation, ventilation and gas exchange in neonatal sheep
2.30	A25	Allison, Crossley, Morley, Dargaville, Harding & Hooper	Negative extra-thoracic pressure (NEPT) ventilation in preterm lambs.
2.45-3.15 General discussion			

3.15-3.45 Afternoon tea

Session 6 Chair Prof Marelyn Wintour			
3.45	A26	Thiel, Probyn, Wallace, Hardinh, Morley & Hooper	Characterization of a novel maker of ventilator induced injury
4.00	A27	Moss, Shub, Nitsos, Ikegami, Jobe & Newnham.	Effects on fetal sheep of intra-amniotic injection of periodontal endotoxins.
4.15	A28	Wlodek	Placental restriction: a novel model system for study the relative roles of the prenatal and postnatal environments and the consequences of impaired lactation on growth and adult diseases.
4.30-4.50 General discussion			

4.50 Presentation of student prizes:

1. Best Oral Presentation
2. Best Discussant

A1

BIRTHWEIGHT, GESTATIONAL AGE AND NEUROMOTOR OUTCOMES IN ADULT HUMANS.

Julia Pitcher [1], Vivienne Moore [2], Alexandra Robertson [3], Richard Cockington [4] & Timothy Miles [3]. Departments of Obstetrics & Gynaecology [1], Public Health [2], and Discipline of Physiology [3], The University of Adelaide; Department of Child Development and Rehabilitation [4], The Women's & Children's Hospital, Adelaide, AUSTRALIA 5005.

Infants whose growth before birth has been restricted have increased rates of perinatal mortality and morbidity and increasingly, evidence of longer-term neurological and neuromotor problems into childhood. It is not known if the latter persist into adulthood, what their extent and impact on neuromotor function is for the individual and which underlying physiological determinants of that function have been impaired. Anecdotally, parents and teachers of these children often report "clumsiness" and poor motor skills. We have been examining if the individual born small for gestational age or of reduced birthweight into the normal range, exhibits impaired motor control in adult life, and if this is due to altered corticospinal function. We aim to determine if individuals who are small at birth for gestational age exhibit neuromotor functional deficits in early adulthood, to identify the physiological basis of any impaired neuromotor function in these individuals, and to determine which aspects of neuromotor development and function should be a focus for future investigations in younger contemporary cohorts and into the design and evaluation of interventions in infancy or childhood. The results being presented are preliminary data from 35 subjects studied to date. Our target sample is 120 young adults (now 27 years of age) who are members of the Adelaide Family Heart Study whose birth size was recorded in detail. This cohort comprises approximately 850 members and has been characterised in great detail in terms of blood pressure, glucose tolerance and other cardiovascular risk factors. Low birthweight has been linked with raised blood pressure (Moore et al., 1996) and insulin resistance, and hyperinsulinaemia (Flanagan et al., 2000) in this population. Selection for this study was made pseudo-randomly from the cohort on the basis of birth weight, so that comparisons of neuromotor control can be made across a spectrum of birth weights, from IUGR through to relatively high birth weights (eg. greater than 4000 grams) divided into birthweight quintiles, with quintile 1 being the lowest birthweight group. The assessments can be broadly grouped as motor skills tests and motor functions tests. Motor skills tested are handgrip (gross strength), finger tapping speed (fine), Purdue pegboard test (fine dexterity), and finger grip and lift (fine force scaling/sensorimotor integration). Motor functions tested are peripheral motor nerve conduction velocity, maximal compound muscle action potential (fibre density, current flow and temporal dispersion of the action potential), F waves (spinal motoneuron excitability), motor cortex stimulus-electromyogram response curves (corticospinal pathway function) and evoked potential silent periods (motor cortex inhibition). In addition, height, weight and handedness (Edinburgh laterality quotient) are recorded. Preliminary results indicate that low birthweight is associated with reduced handgrip strength in both hands in males. Similarly, a high body mass index is also associated with reduced handgrip strength. However, lower birthweight and shorter gestational age males have significantly faster finger tapping speeds than heavier birthweight males. This does not appear to be associated with any mechanical advantage related to smaller stature. There appear to be no similar relationships in females. In terms of corticospinal tract function, low birthweight is associated with a greater difference between the resting excitability thresholds of the left and right hemispheres of the motor cortex hand areas. This is evident in both males and females, but the relationship is much stronger in females. There is a weak trend for low birthweight to be associated with a steeper corticospinal stimulus-response curve. The finger grip and lift data have not yet been analysed. We intend to extend the study in the future to examine intracortical inhibition and facilitation, and transcallosal inhibition.

A2

THE TIMING OF PRENATAL INSULTS DETERMINES MEMORY IMPAIRMENT

Rodricks CL[ab], Gibbs ME[b] and Miller SL[a]
[a]Department of Physiology and [b]Pharmacology,
Monash University, Victoria, Australia, 3800
candice.rodricks@med.monash.edu.au

Background: Prenatal hypoxia is associated with neurological injury in newborn infants. Our previous study has shown that prenatal hypoxia impairs memory formation in the newly hatched chick. The mechanism by which this occurs is unclear but the timing of the insult influences the nature of the deficit. To cope with the stress of hypoxia it is possible that corticosterone levels are raised *in ovo* and may contribute to memory impairments at hatch. Alternatively, low oxygen availability may affect protein synthesis and the formation of neural pathways essential for memory formation.
Aims: This study aims to determine if hypoxic cognitive impairments are due to raised corticosterone levels or malnutrition *in ovo*. In addition, it will be determined if the duration of the insult (5 minutes to 4 days) affects memory formation.
Methods: To assess cognitive function, memory was tested using single trial (10 sec) discriminated avoidance learning. In this task, chicks are tested for their memory of an aversive red bead. Memory is measured by a discrimination ratio (DR), which is the proportion of pecks on red beads to blue beads. When the DR is close to 0.5, memory is poor and a DR approaching 1.0 is evidence of consolidated memory. Chicks were tested at 120 minutes which measures long term memory. Experiment #1 Hypoxia: low oxygen levels were imposed by reducing oxygen levels to 14% from E10-14 or E14-18. To determine if the onset of the insult was sufficient, 3 shorter periods of hypoxia were tested; 5 minutes of 0% oxygen (E14), 1 hour (E14) or 24 hours of 14% oxygen (E10 and E14). Experiment #2 Corticosterone administration: On E10-14 or E14-18 embryos were administered 60ng of corticosterone for 4 days onto the chorioallantoic membrane where it is rapidly taken up into circulation. Subsequent experiments administered one dose of corticosterone on E10 or E14. Experiment #3 Malnutrition: 5% and 7.5% of albumin was removed from the egg at the commencement of incubation.
Results: The discrimination ratios after prenatal hypoxia for 4 days and 24 hours were similar on E10 (0.59±0.08 and 0.58±0.02, respectively) and E14 (0.56±0.08, 0.54±0.01) and were significantly reduced compared to control chicks (0.93±0.01). On E14 no oxygen for 5 minutes (0.58±0.07) impaired memory formation but 14% oxygen for 1 hour did not severely impair (0.75±0.08). Administering corticosterone for 4 days from E10-14 did not impair memory (0.77±0.05) when compared to their control (0.87±0.08) and the hatch rate for administration from E14-18 was low (14%). A single administration of corticosterone on E10 (0.70±0.02) or E14 (0.52±0.03) impaired memory. Malnutrition had no effect on memory (0.83±0.05, 0.89±0.3).
Conclusion: It is the onset and not the duration of prenatal insults that are associated with postnatal cognitive deficits. This study has demonstrated that 24 hours of hypoxia produces the same cognitive deficits as 4 days of hypoxia. In addition, a single administration of corticosterone replicates the hypoxic insult and impairs memory on E14. Malnutrition was imposed from E0 and no memory deficits were evident because the embryo may have adapted to the insult during the developmental period. This study suggests that increased corticosterone levels *in ovo* after hypoxia potentially impairs cognitive development at hatch.

A3

PROTEIN 14-3-3 IN CSF FOLLOWING HYPOXIA-ISCHAEMIA – A STUDY PROPOSAL

Zsuzsoka Kecskes[1] Dr med, FRACP, Alison Kent[1] BMBS, FRACP, Jane Dahlstrom[2] MBBS (Hons) FPAC PhD FRCPA Grad Cert Ed St (higher educ), Ian Hendry[3] BSc DSc MBBS PhD

[1]Department of Neonatology, [2]Department of Anatomical Pathology, The Canberra Hospital, PO Box 11, Woden, 2606, ACT, Australia. [3]Division of Neuroscience John Curtin School of Medical Research, Box 334, Canberra City ACT 2601

Background: Severe perinatal asphyxia occurs in about 1 in every 500 births and is associated with significant morbidity and mortality. With clinical trials of neural protectant therapies on the horizon, it is essential to develop a method of quickly identifying those infants most likely to benefit from these treatments.

Protein 14-3-3 is an abundant protein comprising 1% of the total amount of soluble brain protein. Protein 14-3-3 is involved in signalling in cell division, cell cycle regulation and apoptosis. Recently, the detection of protein 14-3-3 in the cerebrospinal fluid has been shown to be a highly reliable test for the diagnosis of prion diseases such as Creutzfeldt-Jacob disease. Neuronal death caused by neurologic disease, seizures and ischaemia has been associated with an increase in protein 14-3-3. Protein 14-3-3 has never been measured following a hypoxic-ischaemic insult in a newborn human or animal experiment. We want to investigate whether protein 14-3-3 can be used as a marker of hypoxic-ischaemic damage in newborn animals.

Aims:

1. To measure protein 14-3-3 in the CSF of newborn rats following an hypoxic-ischaemic insult
2. To correlate protein 14-3-3 levels with histological evidence of brain ischaemia in newborn rats
3. To establish the earliest point at which protein 14-3-3 is predictive of outcome

Methods:

7-day old rat pups (males and females) will be subjected to ligation of the right carotid artery under general anaesthesia. One hour after the operation, hypoxia will be induced. A group of rats will be subjected to the same anaesthesia but no the surgery or hypoxia (control group) There will be 10 animals per group (5 controls, 5 study animals). Following the insult, the rats will be returned to and kept with their dams. Cerebrospinal fluid (CSF) will be obtained by puncture of the cisterna magna under general anaesthesia. Following this, the rats will be sacrificed.

CSF samples will be collected at the following time points in different animals: 0, 2, 4, 6, 12 hours and 72 hours after the insult. The presence of protein 14-3-3 will be assessed using Western Blot analysis. The primary antibody is commercially available. The brain will be removed from the skull and fixed. Tissue for light microscopic assessment will be processed using routine laboratories methods. Histology will be assessed using a standard neuronal loss score.

A4

EPISODIC ALCOHOL CONSUMPTION DURING PREGNANCY: MECHANISMS OF FETAL BRAIN INJURY.

Penelope Dalitz, BScHons [1], Sandra M Rees, PhD [2], Philip N Henschke[3], MBBS FRACP [3], Megan L Cock, PhD [1], Richard Harding, PhD, DSc [1].
[1] Physiology, Monash University, VIC 3800; [2] Anatomy & Cell Biology, University of Melbourne, Melbourne, VIC 3100 and [3] Womens Alcohol and Drug Service, Royal Women's Hospital, Melbourne, VIC 3053. Supported by the Pratt Foundation

BACKGROUND: Exposure to alcohol during fetal life has been associated with poor neurodevelopmental outcome; episodic (binge) exposure is considered to be particularly harmful. However, the mechanisms leading to altered neurological development are not fully understood. Potential pathways leading to altered brain development or fetal brain injury include oxidative stress, altered nitric oxide metabolism, increased rates of apoptosis and/or interference with neuronal migration.

OBJECTIVE: The aim of these on-going studies is to identify mechanisms of altered fetal brain development following repeated, "binge" exposure to ethanol (EtOH).

PRELIMINARY RESULTS: Our ovine model of binge drinking exposes pregnant sheep to EtOH for one hour on 3 consecutive days starting at 116 ± 1 days gestation. Blood alcohol concentrations in the mother and fetus reached maximal values of 0.11±0.01 g/dL (~20 mmol/L) ~1h after the start of EtOH infusions. During and after EtOH, fetuses were not hypoxemic or asphyxic, nor did their blood cortisol levels change. Histological analysis has revealed white matter gliosis in 3 of 8 EtOH treated fetuses, white matter damage in the cerebellum of 2 of 8 EtOH fetuses and no overt hippocampal damage in any fetus. Our results show that fetal cerebral hypoxia is not likely to be the cause of ethanol-induced alterations in fetal brain development.

FUTURE STUDIES: Potential pathways leading to altered brain development and/or injury include increased rates of apoptosis, interference with synaptogenesis and loss of cell mass and numbers, which may occur via increased oxidative stress and alterations in nitric oxide metabolism. These alterations to development will be examined histologically by glial fibrillary acidic protein (GFAP), lectin, Bielschowsky, caspase-3, TUNEL and 4-HNE staining. Increased oxidative stress will be assessed biochemicaly using a lipid hydroperoxidation (LPO) assay. Alterations to NO metabolism will be analysed through the quantification of cGMP (a measurable index of NO synthesis) via a radioimmunoassay. We will use ELISA-based DNA binding assay to examine activation of NF-κB and/or standard ELISA techniques to assess TNF-α and IL-6 levels in brain tissue.

A5

USE OF CEREBRAL INTRAVASCULAR OXYGENATION AS PARAMETER FOR CEREBROVASCULAR AUTOREGULATION

Wong F1,2, Barfield C1,2, Wilkinson MH1, McDermott EM1, Walker AM1
1 Ritchie Centre for Baby Health Research, Monash University, Clayton, Victoria, Australia; 2 Newborn Services, Monash Medical Centre , Clayton, Victoria, Australia

Background: Methods of assessing cerebrovascular autoregulation are being sought to monitor and reduce cerebral injury in preterm infants. Near Infrared Spectroscopy (NIRS) continuously measures changes in cerebral oxygenated (ΔHbO) and deoxygenated haemoglobin (ΔHb), enabling calculation of ΔHbD = (ΔHbO – ΔHb) as a measure of intravascular oxygenation. Assuming constancy of arterial oxygenation and cerebral oxygen metabolism, HbD would vary with cerebral blood flow (CBF). Moreover, CBF would fluctuate with mean arterial blood pressure (MAP) if cerebrovascular autoregulation was impaired. Thus, the degree of fluctuation of HbD with MAP offers a potential means to assess cerebrovascular autoregulation in preterm infants, but this is not yet established.

Aim: To quantify the degree of correlation between HbD and MAP in preterm infants using gain analysis and Fourier transforms, and to relate this parameter to the occurrence of intraventricular haemorrhage (IVH).

Method: NIRS (Hamamatsu NIRO-500) measurements (ΔHbO, ΔHb and ΔHbD) were recorded simultaneously with MAP measured via an indwelling arterial catheter, and arterial oxygen saturation (SpO2) measured by pulse oximetry. Data were separated into 21-minute blocks of uninterrupted recordings for gain analysis. Only periods in which SpO2 fluctuated <5% were included to minimize the influence of SpO2 on HbD. Correlation of HbD and MAP in each recording was quantified in a frequency-specific manner using Fourier Transform and transfer function analysis, and the result quantified as a gain (G), with a higher gain indicating a higher degree of correlation between the waveforms of HbD and MAP in a given frequency range (Fig 1 & 2),

Results: Preterm infants (n=17) with gestational age 26.3±1.7 (mean ± SE) weeks and birth weight 815±182 g were studied at postnatal age 21.6±17 hours. For infants with minimal (grade 1-2 IVH) or no cranial ultrasonographic abnormalities (n=13), the gain (median [IQR]) for the frequency range 0.04-0.07 Hz was 7.3 [4.6-8.1]. For infants with severe abnormalities (grade 3-4 IVH) on cranial ultrasonography (n=4) the gain was 4.4 [3.8-5.2]. There was no statistically significant difference between these groups (P=0.06), though there was a trend towards lower gain score in infants with severe lesions.

Fig 1. Correlating changes in HbD and MAP in a preterm infant of 26 weeks' gestation with gain of 8.0 at frequency range 0.04-0.07Hz

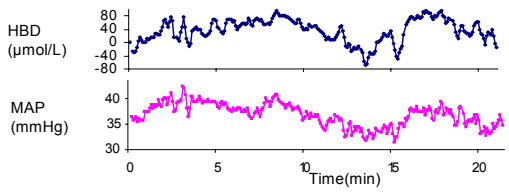
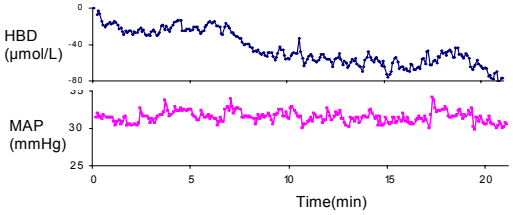


Fig 2. Poor correlattion of HbD and MAP in a preterm infant of 27 weeks' gestation with gain of 3.3 at frequency range 0.04-0.07Hz



Conclusion: High correlation between HbD and MAP theoretically indicate impaired cerebrovascular autoregulation, and this is expected to be associated with cerebral injury in preterm infants. However, our finding of lower gain scores in infants with severe IVH suggests a complex physiological relationship between HbD, autoregulation impairment and development of severe IVH.

A6

EFFECTS OF SLEEP STATE AND SLEEPING POSITION ON BLOOD PRESSURE IN INFANTS IN THE FIRST 6 MONTHS OF LIFE

Stephanie Yiallourou, Andrew Ramsden, Adrian Walker and Rosemary Horne
Ritchie Centre for Baby Health Research, Monash Institute for Reproduction and Development, Monash University, Melbourne

Background: Prone sleeping is the greatest risk factor for Sudden Infant Death Syndrome, and it has been shown that autonomic control of heart rate is impaired in this position, however the effects on other cardiovascular variables are unknown. Our group has recently validated a non-invasive device (Finometer ^{TM,} FMS, BV Arnhem, The Netherlands) for continuous monitoring of blood pressure (BP) in infants. In this study we aimed to determine the effects of sleep position and sleep state on BP control during the first six months of life.

Methods: Nine infants (6F/3M) born at term (38-42wks) with normal birth weights (3560 ± 90g) and Apgar scores averaging 9 and 9 at 1 and 5 minutes respectively were studied at 2-3 wks, 2-3 mo and 5-6mo postnatal age. Daytime polysomnography was performed following the infants normal sleep patterns at the Melbourne Children's Sleep Unit, Monash Medical Centre. Electroencephalogram (EEG), submental electromyogram (EMG), electro-oculargram (EOG), heart rate (HR), abdominal and thoracic respiratory movements, oxygen saturation, and behavioural patterns were recorded to determine sleep state. Measurements of BP were recorded continuously using a plethysmographic cuff placed around the infant's wrist (Finometer ^{TM}). Multiple measurements (n=4) were taken over two-minute epochs during both active sleep (AS) and quiet sleep (QS) in both the prone and supine positions.

Data Analysis: Movement artifacts were removed and data were averaged in 5s epochs for mean (MAP), systolic (SAP) and diastolic (DAP) arterial pressure. BP values were compared between sleep states, sleep positions, and across postnatal age using two way repeated measure ANOVA. Data are expressed as mean values ± sem, with $p<0.05$ considered as statistically significant.

Results: Data are summarised in Table 1. Sleep position had no effect on BP at any of the ages studied. BP was affected by sleep state, with MAP and DAP being higher in AS than in QS in both the prone ($p<0.05$) and supine ($p<0.01$) positions at 2-3 mo. SAP increased with postnatal age in the supine position (n=5), being significantly higher ($p<0.05$) at 5-6 mo than at 2-3 wks in AS.

	MAP(mmHg)			SAP(mmHg)			DAP(mmHg)		
	2-3 wks (n=5)	2-3 mo (n=9)	5-6 mo (n=8)	2-3 wks (n=5)	2-3 mo (n=9)	5-6 mo (n=8)	2-3 wks (n=5)	2-3 mo (n=9)	5-6 mo (n=8)
QS supine	61±2	64±3**	66±3	75±3	81±3	81±3	51±4	55±3**	58±3
AS supine	62±3	71±4**	75±4	77±3	87±4	91±4	55±3	62±3**	67±2
QS prone	65±3	66±3*	70±4	81±4	82±3	85±3	57±3	58±3*	62±2
AS prone	68±4	71±1*	74±4	85±4	88±1	89±4	60±3	63±4*	66±2

*****$p<0.05$, ** $p<0.01$ AS vs QS

Conclusion: Our study has found that sleep state and postnatal age have an effect on blood pressure. The finding that sleep position had no effect on blood pressure was unexpected, but further study is needed to confirm this.

This study was supported by the NHMRC (284357) and the Sudden Infant Death Research Foundation of South Australia.

A7

HEART RATE VARIABILITY IN SLEEPING INFANTS DURING THE FIRST TWELVE MONTHS OF LIFE: EFFECT OF AGE AND SLEEP STATE

Rita Tuladhar, * Richard Harding, T Michael Adamson, Rosemary SC Horne. Department of Paediatrics and Ritchie Centre for Baby Health Research, *Department of Physiology, Monash University, Melbourne, Victoria 3168. rita.tuladhar@med.monash.edu.au

AIM: The mechanisms responsible for the Sudden Infant Death Syndrome (SIDS) remain largely unexplained; however, it has been suggested that alterations in autonomic nervous system function may be involved. Heart rate variability (HRV) is a non-invasive index of sympathetic and parasympathetic control of heart rate[1]. HRV is a marker of clinical health and HRV has been found to be lower in SIDS victims and siblings of SIDS[2]. Autonomic control of the cardiovascular system undergoes functional maturation after birth and is altered by sleep state. Our aim was to characterise the maturation of autonomic control of the heart in term infants by examining HRV during sleep over the first year of life.

METHODS: Five term infants born at 38 to 41 weeks of gestational age with mean birth weights of 3756 ± 315g (mean ± SEM, range 3100 - 4600g) were each studied on 4 occasions: 3 mo, 6 mo, 9 mo and 12 mo of age. All infants were studied in the Melbourne Children's Sleep Unit, with overnight polysomnography. Heart rate (HR), EEG, submental EMG, EOG, respiratory movements, oxygen saturation, and visual observations of behaviour were recorded to determine sleep state.

DATA ANALYSIS: Sleep staging was performed according to standard criteria[3] into rapid eye movement (REM) sleep and into stages 1,2,3 & 4 of non-REM (NREM) sleep. Analysis was performed in 4 min epochs of each sleep stage using MacLab and HRV software (ADInstruments, Sydney). Data for mean R-R interval, low frequency (LF) power, high frequency (HF) power, the LF/HF ratio and the sum of the square of difference between adjacent R-R intervals (RMSSD) were collected for each sleep stage. All components of HRV were compared between sleep stages and between ages using 2-way ANOVA for repeated measures. All values are expressed as mean ± SEM and a value of $p < 0.05$ was considered significant.

RESULTS: The percentage of NREM was greater at 9 mo and 12 mo of age compared to 3 mo ($p<0.05$). In NREM sleep, time spent in stage 2 sleep was longer at 6 mo ($p<0.01$) and 9 mo and 12 mo ($p<0.05$) compared to 3 mo. Overall mean R-R interval was greater in NREM compared with REM sleep. Differences in R-R interval in the different sleep stages were: stage 2 (9 mo: $p<0.05$, 12 mo: $p<0.01$); stage 3 (6 mo: $p<0.05$, 9 mo: $p<0.05$, 12 mo: $p<0.01$) and stage 4 (6 mo: $p<0.01$, 9 mo: $p<0.001$; 12 mo $p<0.001$). However, there was no effect of sleep state on LF power, HF power, the LF/HF ratio or RMSSD. There was also no effect of age on the mean R-R interval although there was a tendency for the mean R-R interval to be greater in each sleep stage with increasing postnatal age. There was no effect of age on LF or HF power, on the LF/HF ratio or RMSSD although there was tendency for higher HF with age in each sleep stage.

CONCLUSION: Sleep architecture changed with age as NREM sleep increasing and REM decreasing with age. Heart rate was higher in REM than in NREM sleep. There was tendency for higher HRV with increasing postnatal age in the infants studied, suggesting a maturation of autonomic control of cardiovascular system during sleep.

References: 1. Akselrodet al 1981: *Science*, **213,** 220-3.; 2. Harper et al 1982: *Sleep*, **5,** 28-38. 3. Rechtschaffen et al 1968: *A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects*. UCLA Brain Information Service / Brain Res. Inst.

A8

THE EFFECT OF HYPOXIA ON PLACENTAL OUTGROWTH DURING EARLY PREGNANCY IS MEDIATED BY INSULIN-LIKE GROWTH FACTOR-II

Sferruzzi-Perri AN, Robinson JS and Roberts CT
Department of Obstetrics and Gynaecology, University of Adelaide, Adelaide, South Australia, Australia 5005

Background: Poor placental development has been associated with a variety of pregnancy disorders, including preeclampsia and intrauterine growth restriction. Early placentation is characterised by invasion and remodelling of the uterus and its vasculature by placental cytotrophoblasts (CTBs) to promote maternal blood flow to the placenta which is critical for fetal growth. Blood flow to the placenta is established at about the 11th week of pregnancy and therefore early placental development occurs in a relatively hypoxic environment. Insulin-like growth factor-II (IGF-II) is abundantly expressed by the invading CTBs and its gene expression is induced by hypoxia in other cell types. Transforming growth factor (TGF)-β1 is synthesised by decidual cells and to a lesser extent by CTB. TGFβ1 has opposing effects to IGF-II as it inhibits CTB invasion. TGFβ1 requires activation to have biological effects. Whilst the independent effects of oxygen, IGF-II and TGFβ1 have been investigated on CTB cell lines the interaction of these factors at the fetal-maternal interface have not. Therefore the aim of this investigation was to determine the effect and interaction of O_2 concentration and IGF-II on placental CTB outgrowth and activation of TGFβ1 during the first trimester *in vitro*.

Methods: First trimester (7-8 weeks gestation) human placental villous explants were cultured in serum-free media with 0 or 17nM IGF-II, in 1% or 20% O_2 (4 treatments). On day 6, villous explants were photographed for later assessment of CTB outgrowth and invasive behaviour. RNA was extracted and pooled (8 wells / treatment) for quantification of IGF-II, type 1 and 2 IGF receptors (IGF1R and IGF2R), urokinase plasminogen activator receptor (uPAR), TGFβ1 mRNAs and 18S rRNA using Real Time RT-PCR. Explant-conditioned media were assayed for IGF-II and total and active TGFβ1 protein using commercially available human ELISA kits.

Results: Placental explants formed 55% more CTB outgrowths when cultured in 1% O_2 than those cultured in 20% O_2 ($p<0.001$). Exogenous IGF-II enhanced CTB outgrowth by 35% in 20% O_2 explants ($p=0.001$), but not in those exposed to 1% O_2. IGF-II mRNA expression relative to 18S rRNA, was increased 3.5-fold in explants cultured in 1% O_2 compared with 20% O_2 ($p=0.04$), however there was no effect of treatment on IGF1R, IGF2R, TGFβ1 or uPAR transcription. Culture in 1% O_2 increased IGF-II protein secretion into the media by 10-fold ($p=0.02$). TGFβ1 activation was inhibited by 90% and 85% when placental explants were cultured in 1% O_2 ($p=0.001$) and by 65% in the presence IGF-II in 20% O_2 ($p= 0.05$). IGF-II mRNA and protein were positively correlated with CTB outgrowth and invasive behaviour, whereas activated TGFβ1 concentration was negatively correlated with these parameters.

Conclusion: Our data suggest that the low oxygen environment of the placenta during early pregnancy stimulates placental outgrowth and IGF-II expression, while it inhibits TGFβ1 activation. IGF-II appears to mediate the low oxygen response as addition of IGF-II to 20% O_2 explants promotes first trimester CTB outgrowth and inhibits TGFβ1 activation. This novel interaction of oxygen, IGF-II and TGFβ1 during pregnancy is likely to be an important determinant of CTB invasive behaviour and hence pregnancy outcome.

A9

THE SITE OF PRODUCTION AND RELEASE OF ACTIVIN A IN THE INTRA-UTERINE GROWTH RESTRICTED OVINE FETUS

Supramaniam VG[a], Jenkin G[a], Wallace EM[b] & Miller SL[a]
Departments Physiology[a] and Obstet & Gynaecol[b], Monash University, Clayton, Victoria
veena.supramaniam@med.monash.edu.au

Background: Placental insufficiency is thought to underlie a significant proportion of late pregnancy stillbirths and pregnancies complicated by intrauterine fetal growth restriction (IUGR). We have successfully established single umbilical artery ligation (SUAL) as a model of fetal growth restriction in mid-late pregnant sheep carrying singletons. In our previous study we saw an 8-fold increase in activin A in the amniotic fluid of the growth restricted fetus. Clinical studies have found activin A levels in maternal serum to be elevated in preeclamptic and IUGR pregnancies. Therefore, it has been suggested that activin A may be a useful marker of feto-placental compromise. Furthermore, in response to acute hypoxia in rats, there is also evidence that activin A may play a role in neuroprotection.

Aim: To use the model of SUAL in twin pregnant sheep to investigate the source of activin A in IUGR.

Methods: Surgery was performed on ewes carrying twin fetuses at gestational age 105-110 days (n=6). Catheters were inserted into the fetal femoral artery (FFA) and amniotic fluid (AF) of both fetuses. The two umbilical arteries were located in the cord of one of the fetuses, and a ligature was placed around one of the arteries. A catheter was also inserted into the maternal jugular vein. In the control group (n=4) the fetuses were catheterised but the umbilical cord was left intact. Samples were taken from the FFA and AF at the time of surgery, six hours post surgery and daily for 7 days. Post mortem was performed on day 7. Fetal and gestational tissues were collected for activin A measurement.

Results: SUAL resulted in fetal hypoxaemia, indicated by significantly decreased $\%SaO_2$ (43.53 $\pm$ 7.85%), in the ligated fetus, throughout the experimental period, compared to it's non-ligated twin (68.01 $\pm$ 3.83%; $p<0.05$) and the control group (mean = 66.07 ± 1.24%). AF activin A concentrations were significantly increased in the ligated fetus, within 48 hrs after surgery and remained elevated over the experimental period. On day 7, the activin A concentrations in the AF of the ligated fetuses (688.85 $\pm$ 279.55 ng/ml) was significantly elevated compared to the non-ligated fetus (27.40 $\pm$ 11.83 ng/ml; $p<0.05$) and the control group (22.40 ± 8.34 ng/ml). Preliminary results for activin A per gram tissue for twin SUAL (n=3) showed a 4-fold and 2-fold increase in activin A in the amnion and chorion, respectively, taken from the horn containing the SUAL fetus, compared to the control horn. There was also a 2–fold increase in activin A concentration in the lungs of the SUAL fetus compared to it's non-ligated twin. Interestingly, activin A appears to be elevated in the cerebellum, hippocampus and cortex regions of the brain of SUAL fetus compared to it's non-ligated twin.

Discussion: The high levels of activin A in the AF, chorion and amnion removed from the uterine horn containing the SUAL fetus confirm the human finding that the predominant source of activin A in pregnancy is the placenta and fetal membranes, and demonstrate that it is the chorion and amnion that increase production of activin A in response to chronic hypoxia. Interestingly, the brain regions that show an increase in activin A are regions that have been previously shown to be susceptible to hypoxic injury, which is at odds with endogenous activin A acting as a neuroprotectant.

A10

Localisation of hypoxia, Insulin-Like Growth Factor-II (IGF-II) and Hypoxia Inducible Factors (HIFs) in early murine implantation sites

Pringle KG, Kind KL, Roberts CT
Department of Obstetrics and Gynaecology, University of Adelaide, Adelaide 5005
Email: kirsty.pringle@adelaide.edu.au

In humans the oxygen tension in the intervillous space rises steeply from below 18 mmHg (2%) at 8 weeks to approximately 60 mmHg (8%) at 12 weeks of gestation [1]. This low oxygen tension in the first trimester is critical for successful pregnancy, as early onset of maternal blood flow can result in miscarriage. In mice, oxygen tension in the implantation site has not been measured and therefore it is unknown whether the early murine implantation site is exposed to hypoxia. Hypoxia Inducible Factors are transcription factors that are degraded under low oxygen conditions and stabilised under hypoxia, thereby mediating the effects of hypoxia. It was recently shown that Insulin-Like Growth Factor-II (IGF-II), a known target gene of HIF-1, is able to feedback and increase the levels of HIF-1α protein [2]. Interestingly both IGF-II and HIF-1α are essential for fetal and placental development. This study aimed to localise HIF-1α, HIF-2α and IGF-II, and identify cells exposed to hypoxia in early murine implantation sites.

C57/Bl6 males were mated with CBA F1 females and the day of the vaginal plug was designated as day 0.5 of pregnancy. An ontogenic study was performed in which mice from each day of days 5.5 - 9.5 of pregnancy were injected with pimonidazole (hypoxprobe-1) 2.5 hours prior to death. Pimonidazole is a novel marker of hypoxia that forms protein adducts in cells specifically exposed to oxygen concentrations of less than 10 mmHg. Uteri were then removed and sections processed by immunohistochemistry to localise IGF-II, HIF-1α, HIF-2α and the pimonidazole adducts.

IGF-II was present from days 5.5–9.5 gestation in the conceptus and mesometrial decidua. Decidual cells surrounding the apoptosed luminal epithelium from days 5.5-7.5, and the embryo on day 6.5, stained positively for pimonidazole adducts suggesting they were hypoxic. HIF-1α expression showed nuclear and cytoplasmic localisation throughout development and localised to similar regions as IGF-II, whereas HIF-2α protein was only localised to the cytoplasm in the decidua and trophoblast.

This study is the first to localise trophoblasts, hypoxia, HIF-1α and IGF-II in day 5.5-9.5 murine implantation sites. HIF-1α labelled nuclei throughout the mesometrial decidua and was co-localised with IGF-II, suggesting a role for HIF-1 in regulating gene expression in the early implantation site. Although regions of hypoxia were only detected up until day 7.5 of gestation the possibility that the oxygen levels may still be relatively low cannot be ruled out.

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A11

Eugenie Ruth Lumbers, MBBS, MD (Adel), D.SC (UNSW), FAA
Her contribution to fetal physiology
E.Marelyn Wintour, Dept of Physiology, Monash University, Clayton, Vic,3800

This is the story of an academic and scientist, who provides an excellent 'role model' for all scientists/academics. The story of Eugenie's life shows that you can achieve at an extremely high level, scientifically, whilst maintaining a well-rounded life, with interests in music, literature, art, and sailing, and producing and caring for a close and loving family.

Eugenie did Medicine at the University of Adelaide, 1959-1964, winning 5 prizes during the years. In 1964, one week before the final examination in Pediatrics, she married Bill Forbes, mathematician and teacher, and will celebrate her 40th wedding anniversary this year. Getting married didn't harm her exam results—she topped final year!! After a residency, she embarked on research for an MD degree, with Sandford Skinner, who was a little intimidated by attracting the top student. Nevertheless the research was extremely productive, and together they discovered the effects of oestrogen (in the contraceptive pill)on renin substrate. Eugenie went on to discover the prohormone form of renin—'inactive renin, aided by Brian Morris, who was one of Eugenie's first Honours students. These early papers have been well cited—(up to 254 times). In fact whereas the 'average' paper in Physiology is cited only 3 times, Eugenie's mean citation rate is 17.3. Eugenie's interests continued to be the Renin-Angiotensin System particularly in the pregnant mother and developing fetus. She was the first to show that renin was made in reproductive tissues, outside the kidney, and was essential to maintain normal renal function in the fetus. The cardiovascular system and it's regulation were also major topics of investigation. She graduated MD in 1970, being already the mother of one daughter, and about to have her second. Therefore establishing day-care facilities at the University of Adelaide was another of her 'little projects'. She was awarded a prestigious C.J.Martin scholarship from NHMRC in 1972-1974, and took it up at Oxford University, UK, where she worked with the renowned Joan Mott and Fiona Broughton-Pipkin. She subsequently returned to a Senior Lectureship at the University of New South Wales, in 1974 and was awarded a D.Sc in 1986. She became Professor in 1988, and Head of the School in of Physiology and Pharmacology, in 1991. In 1999 she was made Scientia Professor there, and in 2002 was made a Fellow of the Australian Academy. In 2003 Eugenie was awarded the Centenary Medal. Over her long career she earned $4.22 million in competitive research grants, and trained 26 Hons/B.Med Sci students and 15 postgraduate students. During all this time she was also bearing and raising three daughters (Nicole, Alexandra, and Heloise), two of whom are now mothers to her 5 grandchildren. They, and Bill, went on sabbatical with her (with Franz Ganong, 1980). Eugenie worked on innumerable NHMRC / NHF/ARC/ HRC -NZ committees, advised the government, established relations between the University of NSW and University of Harbin, China, and accredited medical courses at Flinders (x2), University of Queensland, and James Cook. Eugenie Ruth Lumbers has had a very distinguished career—throughout it all she has remained a 'really nice person', and made many good friends around the world. But her 'best friend' is her husband, Bill, who was unswervingly supportive during the last 40 years.

A12

THE FETAL CARDIAC RENIN-ANGIOTENSIN SYSTEM AND EFFECTS OF INSULIN-LIKE GROWTH FACTOR I

A.C. Boyce, K.J. Gibson, J. Wu, J.H. Burrell and E.R. Lumbers
Department of Physiology and Pharmacology, School of Medical Sciences, The University of NSW, Sydney 2052.

Insulin-like growth factor I (IGF-I) is thought to interact with the renin-angiotensin system (RAS) to promote growth of the developing kidney[1,2], and IGF-I infusions to fetal sheep in late gestation increase the activity of both the fetal circulating and intrarenal RASs[1]. These infusions also stimulate growth of the heart and left ventricle (LV)[1,2]. Since angiotensin II may be a cardiac growth factor, we wanted to find out whether the fetal cardiac RAS was upregulated by infusions of IGF-I that stimulated heart growth.

Hearts were obtained from 4 chronically catheterised fetal sheep that had been infused i.v. with 80 μg/h rhIGF-I for 6-10 days beginning at 120 days gestation (term=150), and from 6 vehicle infused fetuses (0.1% BSA in 0.15M saline). This IGF-I infusion had led to a marked, sustained increase in plasma renin levels and a ~75% increase in renal renin levels[1]. Cardiac tissue homogenates were incubated with excess sheep angiotensinogen (nephrectomised sheep plasma) at pH 7.5, 37°C to measure cardiac renin levels. The density and affinity of angiotensin type I and II receptors (AT_1R and AT_2R) were measured using competition and saturation binding assays, and mRNA levels for renin, angiotensinogen, AT_1R and AT_2R, relative to a calibrator, were measured by multiplex real time PCR using 18S rRNA as an endogenous control.

The LV free wall was more than 20% larger after IGF-I infusion ($P<0.05$), and 25% larger as a percentage of body weight ($P<0.05$). Total heart weights were not different between groups, but heart:body weight ratios tended to be greater ($P=0.085$). There was no difference between IGF-I and vehicle infused fetuses in any of the cardiac RAS components measured. In both groups AT_1R densities and affinities were similar between ventricles (Vehicle: LV AT_1R density, 57.6 ± 5.0, RV 63.4 ± 5.1 fmol/mg protein), as were AT_1R mRNA levels (Vehicle: LV 0.35 ± 0.05, RV 0.39 ± 0.05). The density and mRNA expression of the AT_2R, and angiotensinogen mRNA levels, were also similar between ventricles in each group. Renin mRNA was not detected in any sample. However, small amounts of renin protein were found in the left and right ventricle of fetuses from each group (Vehicle: 11.8 ± 1.3 and 12.3 ± 0.8 ng Ang I/mg protein, respectively; IGF-I: 13.8 ± 1.6 and 11.6 ± 2.9 ng Ang I/mg protein).

Thus infusions of IGF-I which stimulated fetal left ventricular growth and renal renin synthesis and secretion had no effect on the cardiac RAS. Moreover, the fetal heart at this stage in gestation does not express the renin gene. The renin protein that was found in the heart may result from uptake from the circulation. If so, the much higher levels of circulating renin in IGF-I infused fetuses did not lead to an increase in renin sequestration by the fetal heart. Interactions between IGF-I and the fetal RAS may be specific for renal renin.

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A13

MEASUREMENT OF NET FILTRATION PRESSURE IN THE FETUS AND LAMB

Karen J. Gibson*, Russell D. Brown[+], Anita J. Turner* and A. Erik G. Persson[+],
* Dept of Physiology & Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, 2052. [+]Dept of Physiology, Uppsala University, Uppsala, Sweden.

Aim: To determine normal values for the forces which determine single nephron glomerular filtration rate in the fetus and newborn.

Methods: Micropuncture studies were carried out in fetuses (133-143 days) and lambs (5-15 days after birth). For the fetal studies, pregnant ewes were anaesthetized by i.v. injection of thiopentone, intubated, ventilated and anesthesia was maintained with 1-3 % halothane in oxygen. The uterus was exposed and the fetus was catheterized (femoral artery, femoral vein and bladder). The fetus was then delivered into a shallow, saline-filled heated water bath, with care being taken to avoid stretching and drying of the umbilical cord. The fetal left kidney was exposed, all perirenal fat was removed, and the kidney was stabilized in a perspex cup in preparation for micropuncture. The kidney was viewed with a dissecting stereomicroscope and the outer layers of the renal capsule were carefully removed over a portion of the kidney cortex. Early proximal tubular segments were identified and punctured with a sharpened glass pipette (outer diameter ~ 5 μm) filled with 1M NaCl stained with lissamine green, using a micromanipulator. The pipette was connected to a servo-nulling pressure system so that intratubular pressure could be measured. Pressure was measured initially under free flow conditions (P_{ff}). Then the tubule distal to the pipette was blocked with oil, and additional readings were taken until a new stable pressure, the stop-flow pressure (P_{SF}) was reached. Similar measurements were made in anaesthetised (halothane 1-3%) paralysed lambs (vecuronium 0.1 mg/kg i.v.).

Results: Mean arterial pressures in the fetuses and the lambs were 45 $\pm$ 8 (s.e.m., *n*=12) and 54 $\pm$ 6 (*n*=4) mmHg respectively. In the fetus, P_{ff} was 8.1 $\pm$ 1.8 (*n*=tubules/animals, 32/12) mmHg and P_{SF} was 17.0 $\pm$ 5.7 (*n*=10/3) mmHg. In the lamb these values were 9.6 $\pm$ 1.5 (*n*=10/4) and 29.7 $\pm$ 4.7 (*n*=5/2). Thus net filtration pressure (P_{SF} - P_{ff}) was higher in the lamb (approximately 20 mmHg) than in the fetus (10 mmHg).

Conclusions: It is recognized that glomerular filtration rate is much higher in young lambs than in fetuses. For example Robillard *et al*. (1981) reported values of 0.42 $\pm$ 0.05 ml/min/g kidney weight in lambs 3-19 days old but only 0.11 $\pm$ 0.01 ml/min/g in fetuses >130 days. Our findings, although preliminary, suggest that an increase in net filtration pressure is partially responsible for this increase in glomerular filtration rate. We still need to measure the rate of filtration in single nephrons (SNGFR) in both groups and determine if there is any alteration in the ultrafiltration coefficient with development.

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A14

THE CONSEQUENCES OF A MIDGESTATIONAL ASPHYXIAL EPISODE ON THE DEVELOPMENT OF THE FETUS

O'Connell, AE, Kumarasamy, V, Lumbers, ER, Boyce, AC and Gibson, KJ.
Department of Physiology and Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, NSW, Australia, 2052.

Introduction: Previously we have shown that fetuses asphyxiated at midgestation retained relatively normal kidney structure and function, but they were unable to increase their urine flow rate enough to prevent the formation of hydrops fetalis[1]. We postulated that as the kidney matured they would be able to excrete the excess fluid, so hydrops would resolve. However, the overall development of the kidney, and other organs, may be disrupted leading to adverse consequences after birth.

Methods: Chronically catheterised fetal sheep were subjected to 30 min of complete cord occlusion at ~90 days gestation (term ~150 days). This asphyxial event was achieved by inflation of an occluder that was secured around the umbilical cord. A successful occlusion was demonstrated by an initial increase in fetal mean arterial pressure with a corresponding decrease in fetal heart rate, with hypoxaemia, hypercapnia and acidaemia at 5 min into occlusion[2]. A control group received no occlusion. A post mortem was carried out at ~130 days gestation. Body and organ weights were recorded, photos were taken of the kidneys to measure their dimensions, and the was heart processed for myocyte volume and nuclear number[3].

Results: At post mortem, there was no difference between the occlusion (n=8) and control (n=8) groups in terms of age, body weight or nose-rump length (NRL). Abdominal girth, however, was smaller in the occluded group (31.7 ± 0.9 v 35.0 ± 1.1cm, $p<0.05$). The occluded fetuses still seemed to be showing signs of hydrops, as their skin fold measurements at the shoulder and rump were increased when compared to control animals (4.4 ± 0.7 v 2.3 ± 0.2mm, $p<0.01$; 4.8 ± 0.4 v 2.9 ± 0.3mm, $p<0.01$ respectively). In the males, scrotal weight was higher (21.7 ± 1.8 (n=5) v 14.1 ± 1.7g (n=5), $p<0.01$), which is also indicative of subcutaneous oedema. All occluded fetuses were very stiff. The weights of the heart, lung and brain in the occluded group were significantly lower than that of the control group ($p<0.01$, $p<0.01$, $p<0.001$ respectively). There was a positive correlation between skin fold measurements at the shoulder and the weight of the liver in the occlusion group ($y=0.0015x+0.1333$, $r^2=0.681$, n=8). There was no significant difference between the groups in the percentage of either uni- or multinucleated myocytes in each ventricle. Uninucleated cells in the left ventricle were bigger in the occluded fetuses ($p<0.05$), as were mulitinucleated cells in the right ventricle ($p<0.01$).

Conclusions: Although body weights did not differ between groups, the occluded fetuses showed signs of residual hydrops. Because of this, and the fact that abdominal girth was decreased (i.e. thin body), it can be postulated that these fetuses are slightly growth restricted. The body stiffness and the reduced brain weight suggest that there was permanent damage to the brain. Fluid in the chest cavity, possibly combined with reduced fetal breathing movements, may have limited the growth of the lungs. All these changes, combined with the hypertrophy of cardiac myocytes suggests that although fetuses can survive a severe asphyxial episode at midgestation it will probably result in severe consequences after birth.

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A15

CHARACTERISTICS OF CARDIAC MYOCYTES FOLLOWING IGF-I INFUSION IN LATE GESTATION FETAL SHEEP

E.R. Lumbers, M-Y. Kim, A.C. Boyce, V. Kumarasamy, K.J. Gibson and J.H. Burrell
Department of Physiology and Pharmacology, School of Medical Sciences, The University of New South Wales, Sydney 2052.

Insulin-like growth factor I (IGF-I) stimulates hypertrophy and hyperplasia and may play a role in fetal cardiac growth and maturation. In late gestation fetal sheep, infusions of IGF-I stimulate growth of the heart and left ventricle, in the absence of hypertension[1,2,3]. We wanted to find out whether this growth was associated with hypertrophy and/or hyperplasia of cardiac myocytes, and if IGF-I altered rates of myocyte DNA synthesis and progression through the cell cycle.

Eight chronically catheterised fetal sheep aged 121 days gestation (term=150) were infused with rhIGF-I (80 μg/h) for 4 days. Control fetuses received vehicle (*n*=7; 0.1% BSA in 0.15M saline) or no infusion (*n*=3). On day 3, 250 mg of 5-bromo-2'-deoxyuridine (BrdU) were infused over 30 min. Sheep were sacrificed 3 days after the infusion stopped. Myocytes were isolated from the right (RV) and left ventricular free walls (LVFW) by collagenase digestion, stained with ethidium bromide and proportions of uni- and bi-nucleated myocytes, cell volume, and approximate number of myocytes were determined using confocal microscopy. BrdU incorporation and distribution of nuclei within stages of the cell cycle were assessed using fluorescent activated cell sorting (FACS).

BrdU incorporation tended to be increased in RV compared with LV during IGF-I infusion ($P<0.1$). At the end of infusion mean arterial pressure was not different between groups. Three days post infusion, heart weights were not different (control: 23.6 ± 1.1; IGF-I: 25.3 ± 1.8 g), nor were LV or RVFWs, although the RVFW:body weight ratio tended to be larger after IGF-I infusion (0.18 ± 0.01 vs 0.16 ± 0.01%, $P=0.07$). Kidney weights were increased by ~20% ($P<0.05$). Volumes of LVFW binucleated, and RVFW uni- and binucleated myocytes, were 8-10% larger in IGF-I infused fetuses ($P<0.05$). IGF-I did not change the proportions of each myocyte type within each ventricle (control, LV uninucleate 44.7 ± 3.1%, RV uninucleate 53.7 ± 3.1), or the approximate number of myocytes in each ventricle. Following IGF-I infusion, fewer RVFW nuclei were in the G0/G1 phases of the cell cycle, and more were in G2 and M phases ($P<0.05$), while in the LV, there tended to be fewer S phase nuclei ($P<0.1$).

IGF-I therefore failed to produce a lasting increase in cardiac mass. It did, however lead to hypertrophy of cardiac myocytes, and a stimulation of nuclear division in the RV that persisted for at least 3 days after infusion. These may have been direct effects as arterial pressure was not increased. The suppression of cell cycle activity in the LV suggests that there was a compensatory down regulation of cellular division after IGF-I infusion was withdrawn. This would account for the lack of LV hypertrophy in this study.

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A16

Effects of periconceptional undernutrition on glucose and insulin metabolism in sheep.

Jaquiery AL, Oliver MH, Bloomfield FH, Harding JE. Liggins Institute, Faculty of Medicine & Health Sciences, University of Auckland, Private Bag 92019, Auckland, NZ

Background Undernutrition during the periconceptional period in sheep alters duration of pregnancy, fetal growth patterns, and fetal and postnatal physiology. One hypothesis for the mechanism underlying these effects is that periconceptional undernutrition impairs maternal physiological adaptation to pregnancy.

Aims 1. To determine the effects of periconceptional undernutrition on maternal glucose and insulin metabolism 2. To determine the glucose and insulin responses to refeeding at 30 days gestation in previously well nourished and undernourished ewes

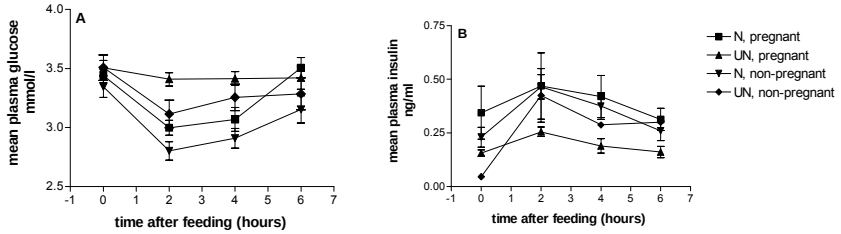
Methods 48 sheep were randomly assigned to receive either normal nutrition (N group, fed to 3-4% of body weight) or low nutrition (UN group, fed to 1-2% of body weight after an initial 2 day fast) from 61 days before mating to 30 days after mating. 10 of these sheep (5N, 5UN) were not mated. Maternal blood samples for analysis of plasma glucose and insulin were taken throughout the study period with additional samples on the day of refeeding (day 30). Mean glucose and insulin levels were compared using ANOVA.

Results 20 N and 21UN sheep completed the experiment.

1.Glucose and insulin metabolism Maternal plasma glucose levels initially decreased after fasting in the UN sheep, but then recovered and were not different from those in N ewes from just before mating to refeeding at 30d. Plasma insulin levels were consistently lower in UN sheep after day -57.

2.Refeeding Plasma glucose levels at 2 and 4 hours after refeeding were lower in N than in UN sheep. (p=.002 & .008 respectively). In all N sheep (pregnant and non-pregnant) and non-pregnant UN sheep glucose levels fell after feeding and returned to baseline levels by 6 hours. In contrast, in pregnant UN sheep glucose level did not change during the 6 hour refeeding period, despite a similar rate of food intake in all groups. Plasma insulin levels increased in both nutritional groups to a similar degree at 2 and 4 hours post-feed, but this increase was less in pregnant than non-pregnant sheep at 4 hours (p= 0.04). There was no difference in the insulin rise from baseline between pregnant N and UN sheep to account for the difference in glucose response in these 2 groups.

Figure. Changes in maternal plasma glucose (A) and insulin concentrations (B) over 6 hours after feeding (0 hr) at 30d gestation



Conclusions UN sheep were able to adapt to decreased nutritional intake and maintain glucose levels similar to N sheep after an initial adjustment period, apparently by decreasing plasma insulin levels. The observed fall in plasma glucose levels after feeding in all but the pregnant UN sheep suggests that once daily feeding in the other sheep may result in regular episodes of hypoglycaemia after feeds, associated with an inappropriately high or prolonged insulin response. The maintenance of stable plasma glucose levels in the pregnant UN sheep despite a similar rise in insulin to that seen in the pregnant N sheep raises the possibility of insulin resistance in the pregnant UN group.

A17

PRENATAL PROGRAMMING OF HYPERTENSION IN THE SPINY MOUSE

Dickinson H, Moritz K*, Walker DW, Wintour EM, Dodic M
Department of Physiology, Monash University, Clayton, Victoria, Australia
*Department of Anatomy, Monash University, Clayton, Victoria, Australia.
Hayley.Dickinson@med.monash.edu.au

Aim:

To investigate the effect of treating pregnant Spiny Mice at a 'preglomerular' stage in kidney development with dexamethasone for 48hrs on kidney structure, function and blood pressure in offspring.

Background:

In sheep & rat brief (2 days) exposure of the fetus to the synthetic glucocorticoid, dexamethasone, early in gestation when the kidney is at a preglomerular stage of development, results in a decrease of nephron number and hypertension in the adult offspring (1, 2). It is proposed to develop a third animal model in the Spiny Mouse (Acomys Cahirinus) to test the general application of the findings in sheep and rats. Spiny Mice are especially suited to perinatal research as they have a relatively long gestation (39-41 days), few offspring (1-3) and in many aspects of development (eg. placenta, brain, lung and kidney) are more similar to the human than other rodents.

Proposed Method:

Dexamethasone will be administered to pregnant Spiny Mice by implanting an Alzet® mini-pump subcutaneously on day 20 of gestation. Blood pressure will be measured in offspring at 8 weeks of age using an indwelling arterial catheter, and renal function will be determined by measurements of glomerular filtration rate (GFR), renal plasma flow (RPF), and urinary electrolyte and albumin concentrations. Animals will be killed at 8 weeks of age and kidneys collected. Nephron number will be counted using unbiased stereological methods and mRNA expression examined for genes known to be important in kidney development using real-time PCR.

Present Findings:

We have determined the time course of nephrogenesis in this species and found that it is completed during the fetal period. We have identified days 20-22 as the time when the ureteric bud enters the metanephric mesenchyme before the first glomeruli develop. We hypothesize that it is at this stage that the kidney is susceptible to the 'programming' effects of excess glucocorticoid.

Work in Progress:

Stereology is being used to determine total nephron number of adult spiny mice. Basal blood pressure measurements are being determined via surgically implanted arterial catheters in adult spiny mice. Animals are being mated in preparation for dexamethasone treatment.

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A18

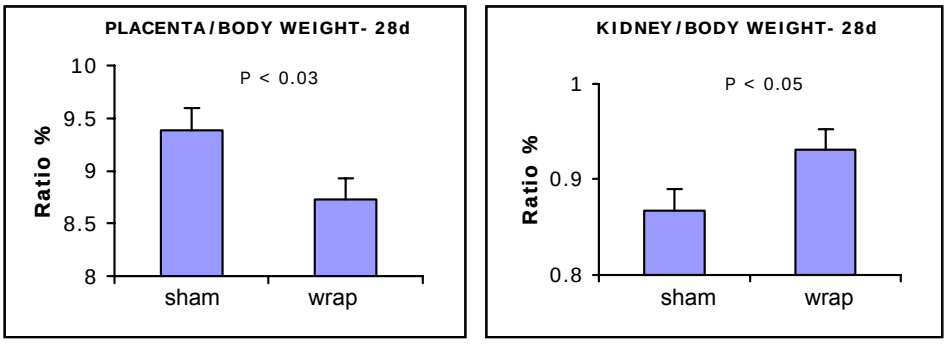
CHRONIC MATERNAL HYPERTENSION AND ITS EFFECT ON PLACENTAL AND KIDNEY TO BODY WEIGHT RATIOS IN THE RABBIT

Maduwegedera, D., Flower, R.L., Wintour, EM, Denton, K.M.
Department of Physiology, Monash University, Melbourne.

It is known that chronic hypertension complicates up to 5% of all pregnancies and this incidence is expected to rise following the demographic trend for women to have children in later life. Numerous studies have suggested a link between an adverse intrauterine environment and the development of diseases later in adult life. It has been suggested that permanent alterations in fetal development due to adverse conditions 'program' the fetus. We have previously shown that adult rabbit offspring of mothers with chronic renal hypertension have increased blood pressure (1). We have also shown that the offspring of hypertensive mothers are proportionately larger compared to offspring of normotensive mothers from as early on as gestational day 16. The aim of this study was to examine placental and fetal growth at various stages of gestation in order to better understand the developmental process in rabbits that may pre-dispose the offspring of chronically hypertensive mothers to develop hypertension in later life.

Three groups of rabbits were studied (1) two kidney, one wrap (2K-1W; renin dependent model, n = 18) hypertensive, (2) two kidney, two wrap (2K-2W; renin independent, n = 18) hypertensive and sham operated (n = 18) mothers. Four weeks following surgery rabbits were mated with normotensive males. Six rabbits from each group were overdosed with anaesthetic at gestational days 16, 21 and 28. Placental weight, fetal weight and dimensions were measured and amniotic fluid collected. Fetal kidneys and placenta were collected for determination of gene expression levels and histological analysis. Maternal mean arterial pressure (MAP), blood glucose levels and plasma renin activity (PRA) were measured throughout the study.

Preliminary results from 2K-1W hypertensive (n = 2, fetuses = 19) and normotensive (n = 4, fetuses = 35) mothers at day 28 of gestation are presented. Fetal body weight was significantly greater in the 2K-1W group (28.3±1.1 g (sham) vs. 32.1±1.3 g (2K-1W), P<0.03), but placental weights were not different in the sham (2.7±0.1 g) and 2K-1W (2.8±0.2 g) groups, respectively. Metanephric kidneys weighed 0.25±0.01 g and 0.30±0.01 g in the sham and 2K-1W groups, respectively (P<0.01). Thus the 28 day fetus in hypertensive mothers had a low placental (see figure) and a high kidney to body weight ratio.



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A19

Effects of periconceptional undernutrition on pregnancy outcome and feto-placental growth in sheep.

Jaquiery AL, Oliver MH, Bloomfield FH, Harding JE
Liggins Institute, Faculty of Medicine and Health Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand

Background Maternal undernutrition in pregnancy has been shown to affect fetal growth and physiology in late gestation, and to contribute to morbidity in later life. However there is less information on the effects of maternal undernutrition in the periconceptional period on pregnancy rate and fetal growth in early pregnancy.

Aims To determine the effects of periconceptional undernutrition on
1. pregnancy outcome
2. feto-placental growth in early pregnancy

Methods 38 sheep were randomly assigned to receive either normal nutrition (N group, fed to 3-4% of body weight) or low nutrition (UN group, fed to 1-2% of body weight after an initial 2 day fast) from 61 days before mating to 30 days after mating. Animals were killed at 50 days after mating and fetal and placental growth indices recorded. Pregnancy outcome in the 2 groups was compared using a $\chi 2$ test. Fetal and placental weights were compared using an unpaired t-test.

Results 31 sheep, 15N and 16UN, completed the experiment. Weights were similar in N and UN sheep at the beginning of the study (N 58.6±0.7 kg vs UN 59.4±0.90 kg). Mean weight gain in the N group from -61 to +50 days was 8.7kg (13%). Mean weight loss of the UN group to the time of refeeding (+30 days) was 9.6kg (17%) with a subsequent mean weight gain of 7.7kg (12%) between +30 and +50 days, so that at 50d UN sheep were still 11kg lighter than N sheep (N 67±0.66 kg vs UN 56±0.75 kg). Pregnancy outcomes were not significantly different in the two nutritional groups, with 8 singleton, 5 twin pregnancies and 2 dry ewes in the N group and 5 singleton, 6 twin, 1 triplet pregnancies and 4 dry ewes in the UN group. This was a total of 38 fetuses, 18N (12 male, 5 female, 1 not recorded) and 20UN (10 male, 10 female).
Fetal weight varied widely at 50 days with mean weight 17.7g, range 14.2-21.4g. Fetal weight was not affected by maternal nutritional group or pregnancy outcome (single vs multiple), but male fetuses tended to be heavier ($p=0.06$) in both nutritional groups. Placental weight also varied widely, with mean weight 110g, range 62 – 163g. Placental weight was not affected by nutritional group but was directly related to fetal weight.

Conclusions In this study, maternal undernutrition in the periconceptional period did not affect pregnancy rate or outcome. There was already a wide range of fetal and placental weights by 50 days of gestation. However this was not affected by maternal nutrition. Rather, the larger size of male fetuses at this early gestation suggests a genetic effect, while other reasons for the wide variation in fetal and placental size in early pregnancy remain obscure.

A20

Effect of metyrapone on circulating cortisol and glucose homeostasis in the guinea pig.

Grover S.[1, 2], Coulter C.L. [1], Walker M.R.[2], Kind K.L. [2], Robinson J.S. [2] and Owens J.A.[2]
School of Molecular and Biomedical Science, Discipline of Physiology[1], Department of Obstetrics and Gynaecology[2], The University of Adelaide, Adelaide, SA, 5005

Low birth weight in babies has been associated with an increased risk of developing the insulin resistance syndrome and coronary heart disease as an adult (1). Insulin resistance (IR) syndrome has many similarities with Cushing's syndrome and therefore it has been suggested that increased hypothalamic pituitary adrenal axis activity may underlie the development of IR following poor growth before birth. In support of this, low birth weight in humans is also associated with increased plasma cortisol concentrations in adult life. In the young adult guinea pig fetal growth restriction is associated with IR, glucose intolerance, and increased blood pressure (2) and with elevated salivary cortisol concentration. However, whether prenatally induced increases in circulating cortisol concentrations contribute to the IR, glucose intolerance and diabetes in adult life remains to be determined. Metyrapone decreases circulating cortisol in the guinea pig within two days of treatment through inhibition of 11β-hydroxylase, a key enzyme involved in cortisol biosynthesis (3). The aim of this study was to determine the impact of variable birth weight and circulating cortisol concentrations on fasting plasma glucose and glucose tolerance in the young adult guinea pig, before and after metyrapone treatment.

Vascular catheters were implanted at 100 days of age in young adult guinea pigs of known size at birth. An intravenous glucose tolerance test (IVGTT) was performed at 115 days of age after an overnight fast (0.5g/kg at 0 min; blood was sampled frequently between –10and 210 min). Animals were then either treated with vehicle or metyrapone for three days, and a second IVGTT was performed. Plasma cortisol was measured by specific radioimmunoassay and blood glucose by Glucometer. Glucose tolerance was calculated as the area under the glucose curve (GAUC).

Metyrapone treatment reduced the mean plasma cortisol concentrations during IVGTT (-10min to 210min), when compared to vehicle ($p=0.08$). Mean plasma cortisol throughout the IVGTT was 105.1nmol/L higher during the second IVGTT in vehicle treated animals, and 22.4nmol/L higher in metyrapone treated animals, when compared to their first IVGTT. This indicates that the first IVGTT presented a challenge in both treatment groups, which consequently increases circulating plasma cortisol concentration. However, a smaller increase in plasma cortisol was observed in metyrapone treated, compared to vehicle treated animals, indicating that metyrapone lowered the overall plasma cortisol concentration reducing the impact of the first IVGTT. Fasting blood glucose was higher prior to the second IVGTT, compared to the first ($p=0.001$), and was not altered by metyrapone treatment and GAUC was reduced by metyrapone treatment compared to vehicle ($p=0.06$). In females, GAUC during both IVGTTs correlated negatively with size at birth, regardless of treatment ($p<0.05$). However, GAUC at any given size at birth was higher at the second compared to first IVGTT after vehicle, but less so after metyrapone treatment. Finally, in males, GAUC correlated negatively with size at birth in the first IVGTT and after vehicle ($p<0.05$), but not after metyrapone treatment ($p<0.05$).

In summary, decreasing plasma cortisol concentration in the guinea pig improves glucose homeostasis in the challenged state, and may contribute to regulation of glycaemia as in humans and other species.

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A21

A TECHNIQUE TO ISOLATE GENES THAT ARE CRITICAL FOR LUNG GROWTH

Sozo F, Filby CE, Hooper SB and Wallace MJ.
Fetal and Neonatal Research Group, Department of Physiology, Monash University, Vic 3800, Australia

Background: The fetal lungs are filled with lung liquid, which maintains them in an expanded state. An adequate level of lung expansion is critical to normal fetal lung growth and development; if the level of lung expansion is reduced, lung growth ceases, whereas an increase in the degree of lung expansion accelerates lung growth. The mechanisms that mediate the effect of expansion on lung growth are not known, although they are likely to involve alterations in the expression of genes that have a role in fetal lung growth. Identification of the genes that lead to accelerated lung growth will aid in our understanding of how the lungs develop and may lead to the development of new therapeutic strategies to assist babies born with inadequately developed lungs.

Aims: To develop a technique to successfully isolate and identity genes that are differentially expressed following 36 hours of increased fetal lung expansion.

Methods: Surgery was performed on a sheep fetus to insert catheters into the trachea and left main bronchus. Liquid flow from the left lung was obstructed at 125d GA (term ~147d) for 36 hours to over-expand the left lung, whilst normal liquid flow from the right lung was maintained. Lung tissue from each side of the lung was collected, mRNA was extracted and then reverse transcribed into cDNA. Subtraction hybridization was used to isolate pools of genes up-regulated and down-regulated by increased lung expansion. The subtraction hybridization technique works on the principle that cDNA fragments that are expressed to a similar degree in the left and right lungs, bind to each other by homologous recombination and are eliminated; those that are differentially expressed are amplified and enriched for using PCR-based techniques. The potentially differentially expressed cDNA fragments were then sub-cloned and a colony array was produced. False positives are eliminated by colony hybridization and the remaining genes are sequenced and identified using the Genbank database.

Results: Subtraction hybridization followed by colony hybridization isolated both up-regulated and down-regulated genes. A preliminary analysis of these shows that we have isolated genes with known roles in cellular growth and differentiation, mitochondrial membrane proteins and ribosomal proteins; some genes remain unidentified. The differential expression of these genes is currently being investigated in separate groups of animals using northern blot analysis.

Conclusions: Subtraction hybridization was successful as a technique in isolating genes that are up-regulated and down-regulated following 36 hours of increased lung expansion; these are likely to be involved in fetal lung growth. The identification of these genes will provide a greater understanding of the mechanisms controlling fetal lung growth, which may assist in the development of improved therapeutic strategies for infants born either preterm or with lung growth deficits.

A22

VDUP1; A POTENTIAL REGULATOR OF FETAL LUNG GROWTH AND EPITHELIAL CELL DIFFERENTIATION

Filby CE, Sozo F, Zahra VA, Hooper SB & Wallace MJ
Fetal and Neonatal Research Group, Department of Physiology, Monash University, Victoria 3800, Australia
Email: Caitlin.Filby@med.monash.edu.au

Background: Inadequate lung development by the time of birth is the greatest cause of morbidity and mortality in the newborn. This can be caused by preterm birth when the lungs have had insufficient time to develop, or to inappropriate lung development in utero (lung hypoplasia). In order to improve the outcome of these infants, it is important that we understand the mechanisms that control lung growth and maturation. We have recently used a model of accelerated fetal lung growth (induced by increased lung expansion) to identify genes that are likely to control lung growth. Using the technique of subtraction hybridisation, one of the genes identified was VDUP1 (Vitamin D_3 Up-regulated Protein 1). VDUP1 is a known growth inhibitor and an inducer of cell differentiation. Consistent with its known roles, VDUP1 was down regulated at 2d of increased lung expansion when cell proliferation rates had increased by ~800%.

Aim 1: To investigate the expression of VDUP1 during normal lung development and in a model of accelerated fetal lung growth.
Aim 2: To compare VDUP1 mRNA levels with cell proliferation rates and with surfactant protein B (SP-B) expression as a marker of type-II alveolar epithelial cell differentiation.

Methods: Lung tissue was collected fetal sheep at 90, 105, 111, 128, 138 and 142 days GA (term ~147d) and from 2 week-old lambs (n=5 in each). Lung tissue was also collected from control fetal sheep and from fetal sheep with accelerated lung growth induced by increasing the degree of lung expansion (by obstruction of the trachea; TO) for 2, 4 or 10 days (n=5 each). Northern blots were generated from all tissues and used to examine the mRNA levels of VDUP1 and SP-B.

Results: VDUP1 mRNA levels were low during the canalicular phase of development (90-111d GA), increased 5-10 fold ($P<0.05$) during the saccular/alveolar phase of development and had a tendency to decrease after birth. SP-B mRNA levels followed a similar pattern and were positively correlated with the changes in VDUP1 ($r^2=0.8$, $p<0.001$). Cell proliferation rates have not yet been measured in those fetuses, however, in the rat, cell proliferation rates are high during the canalicular phase and substantially reduced during the saccular/alveolar stages. VDUP1 mRNA levels were down regulated by 63% at 2 days of increased lung expansion and remained below control levels at 4 and 10 days of increased lung expansion ($p<0.05$). SP-B mRNA levels followed a similar pattern as VDUP1 mRNA following increased lung expansion while cell proliferation rates were significantly increased at 2, 4 and 7 days of TO.

Conclusions: VDUP1 mRNA levels are positively correlated with SP-B expression (a marker of alveolar epithelial type-II cell differentiation) and negatively correlated with cell proliferation rates during normal lung development and in a model of accelerated fetal lung growth. We hypothesise that VDUP1 is a critical regulator of lung growth and type-II cell differentiation in the developing lung.

A23

ALTERED AIRWAY EPITHELIAL STRUCTURE FOLLOWING PRETERM BIRTH IN SHEEP

Tamara Yawno, Megan L. Cock, Mary Hanna, Foula Sozo, and Richard Harding. *Fetal and Neonatal Research Group, Department of Physiology, Monash University, Clayton, Victoria, Australia.*

Introduction: Recent epidemiological studies suggest that preterm birth can affect the development of the lungs and their postnatal function. Studies in infants, children and adolescents born preterm have shown an increased incidence of impaired airway function. However, there have been no studies that have investigated the structure of the airways following preterm birth, or the persistence of such alterations. Therefore, our aim was to examine the effects of preterm birth on postnatal airway development at both term-equivalent age and at 6 weeks post-term. We delivered the preterm lambs at an age when they could survive without ventilatory support, thereby avoiding the influence of confounding factors such as volutrauma and oxygen toxicity.

Methods: Premature birth was induced by administration of Epostane to pregnant ewes ~48 hours before delivery was required. Two groups of preterm lambs were used: ***Group 1*** were born at 133 days of gestation and examined at term (~147 d, ie 14 days after birth), and ***Group 2*** were born at 133 days and examined 6 weeks post term-equivalent (ie 8 wks after birth). These preterm groups were compared with control groups born at term and killed either 6-12 hours after birth (*Group 1*) or 6 wks after birth (*Group 2*). Pulmonary airways were dissected from generations 0 to 8 (trachea being generation 0) and processed for histological examination. We also examined the structure of bronchioles (mean diameter ~0.6 mm) in lung parenchyma. Sections were normally cut at 5μm and stained with hematoxylin and eosin; for more detailed analysis of the airway epithelium, sections were cut at 4 μm and stained with Periodic Acid-Schiff. Morphometric measurements of airway structure were made using Image-Pro analysis programme.

Results: Relative to the basement membrane, the areas of the airway lumen, smooth muscle and cartilage were not altered by preterm birth in the term-equivalent and post term animals; nor were the numbers of alveolar attachments altered. There was a significant reduction in the relative areas of airway wall components along the airway tree (generations 0 to 8) in all groups of lambs.

In 6 week old preterm lambs, the epithelium of large airways (generations 0 to 8) and bronchioles was, respectively, 50% (P=0.01) and 25% (P=0.01) thicker than in age-matched controls. At 8 weeks after birth, the epithelium of preterm lambs remained 28% larger (P=0.02) in large airways (generations 0 to 8) compared to age-matched controls. The increased thickness could be due to epithelial cell hypertophy or hyperplasia.

Conclusion: The increased thickness of the airway epithelium of preterm lambs may be due to environmental factors such as premature exposure to dry air or air-born pollutants. It remains to be determined if airway function is altered in these animals, and if these alterations persist.

A24

RELATION BETWEEN LUNG SIZE AND PULMONARY CIRCULATION, VENTILATION AND GAS EXCHANGE IN NEONATAL SHEEP

Keiji Suzuki, Stuart B Hooper, Richard Harding.
Department of Physiology, Monash University, Melbourne, Victoria, Australia.
E-mail: dks@med.monash.edu.au

Background and Objectives: Lung size is closely related to the mechanics of ventilation and the pulmonary circulation, as well as gas exchange. The aim is to study pulmonary haemodynamic properties, ventilatory mechanics and gas exchange in newborn lambs with various lung sizes.

Subjects and Methods: Subjects were 19 newborn lambs with various lung sizes. In 9 of them, lung hypoplasia was induced by tracheo-amniotic shunt and amniotic fluid drainage beginning at 98-112d gestation (term 147d). At 138-141d, fetuses were exteriorised under anesthesia to implant an ultrasonic flow probe around the left pulmonary artery before the lambs were delivered and ventilated for 2 hours. We recorded systemic and pulmonary arterial pressures, left pulmonary artery blood flow, airway pressure and air flow. We calculated oxygenation index (OI), alveolar-arterial difference of oxygen tension ($AaDO_2$) and ventilatory efficiency index (VEI). We also calculated total respiratory system compliance (Crs), total respiratory system resistance (Rrs), pulmonary vascular resistance (PVR) and pulmonary arterial compliance (PAC). We tested the correlations between data on (a) lung size (wet lung weight (LW), lung volume (LV), lung DNA) and (b) gas exchange indices (OI, $AaDO_2$, VEI) and (c) the data of ventilation and circulation mechanics (Crs, Rrs, PVR, PAC).

Results: Crs had a stronger correlation with VEI (r^2=0.67) and OI (r^2=0.34) than with lung size (LW, r^2=0.06; LV, r^2=0.13). Rrs had no significant correlation with any other variables. PVR was poorly correlated with gas exchange indices, but was negatively correlated with lung size (LW, r^2=0.27; LV, r^2=0.40). Similarly, PAC was poorly correlated with gas exchange indices, but was positively correlated with lung size (LW, r^2=0.36; LV, r^2=0.43). $AaDO_2$ was correlated with lung size (LW, r^2=0.38; LV, r^2=0.29). On the other hand, both OI and VEI were not significantly correlated with lung size.

Conclusions: Indices of pulmonary vascular mechanics (PVR, PAC) were better indicators of lung size than were indices of pulmonary ventilatory mechanics (Crs, Rrs). $AaDO_2$ was a better indicator of lung size than OI or VEI. We conclude that properties of the pulmonary circulation are more closely related to lung size than factors relating to pulmonary ventilation.

A25

NEGATIVE EXTRA-THORACIC PRESSURE (NETP) VENTILATION IN VERY PRETERM LAMBS.

Allison BJ[1], Crossley K[1], Morley, CJ[2], Dargaville, P[3], Harding, R[1] & Hooper SB[1].
Department of [1]Physiology, Monash University, [2]Neonatal Services, Royal Women's Hospital, [3]Neonatology, Royal Children's Hospital, Melbourne, Australia.

Infants that are born very preterm (<29 weeks gestation) have lungs that are small, often surfactant deficient, prone to collapse at end-expiration and are structurally immature. As a result these infants usually require artificial ventilation after birth but, although positive pressure ventilation can improve the oxygenation of these infants, it can also reduce the birth-related increase in pulmonary blood flow (PBF). Negative extra-thoracic pressure (NETP) has been used previously to ventilate adults and children, but the effect on the birth-related changes in PBF in preterm infants is unknown. We hypothesised that the application of NETP will increase pulmonary blood flow (PBF) and improve oxygenation in preterm lambs. Therefore, the aim of this study was to investigate the effects of NETP on PBF and oxygenation in preterm lambs.

Methods: Surgery was performed on five pregnant Merino-Border Leicester ewes at 120±2 days of gestation (term ~147 days) to implant catheters into a fetal carotid artery, pulmonary artery and jugular vein and a flow probe around the left main pulmonary artery. At 125±3 days, the fetus was delivered via caesarian section and intubated before it was ventilated using a tidal volume (V_T) of 5ml/kg and a positive end-expiratory pressure (PEEP) of 4 cmH_2O. After this stabilising period the lamb was ventilated with a NETP of either -2, -4, -6 and -8 cmH_2O for 10 mins each; to maintain transpulmonary pressures the peak inspiratory pressure (PIP) was reduced with each decrease in NETP. Following this study, the V_T was held constant by adjusting the PIP and the lamb was again ventilated at NETPs of -2 cmH_2O, -8 cmH_2O and -10 cmH_2O; between each NETP ventilation period, the lamb was ventilated for ten minutes at 4 cmH_2O PEEP with no NETP.

Results: The application of NETP while maintaining transpulmonary pressure, caused a reduction in PBF from 100±6 % to 62±3% which was greatest at the lower sub-atmospheric pressures (-8 cmH_2O). However, the lower NETP's were associated with an improvement in oxygenation as indicated by the decrease in alveolar-arterial difference in oxygen ($AaDO_2$) from 541±20 mmHg during ventilation at 4 cmH_20 to 436±43 mmHg when ventilated at -8 cmH_20, despite the lower PBF. When tidal volume (V_T) was not controlled, V_T decreased from 5.1±0.6 mL/kg to 3.1±0.3 mL/kg when NETP levels were decreased (more sub-atmospheric) despite an associated increase in transpulmonary pressure, indicating that NETP affects respiratory system mechanics.

When V_T was held constant, the application of NETP caused a reduction in PBF from 100±15 % to 76±8%, which was also greatest at the lower sub-atmospheric pressures (-10 cmH_2O). However, the lower NETP's were not associated with an improvement in oxygenation when V_T were held constant; 308±63 mmHg when ventilated at 4 cmH_2O to 279±64 mmHg when ventilated at -10 cmH_2O. Transpulmonary pressure significantly increased from 13±2.8 cmH_2O to 23±2.3 cmH_2O when NETP levels were decreased (more sub-atmospheric).

Conclusions: With the renewed interest in NETP as a ventilation strategy, there was a need to determine whether this form of ventilation would oppose the negative effects of PEEP on PBF. However, this study has shown that the application of NETP was found to produce similar effects on the pulmonary circulation as the application of PEEP. Future studies are needed to investigate the effects of NETP on preterm infant and the pulmonary circulation.

A26

CHARACTERIZATION OF A NOVEL MARKER FOR VENTILATOR-INDUCED LUNG INJURY?

Alison Thiel, Megan Probyn, Megan Wallace, Richard Harding, Colin Morley[1] & Stuart Hooper
Department of Physiology, Monash University and [1] Neonatal Services, Royal Women's Hospital, Melbourne, Australia.
alison.thiel@med.monash.edu.au

Introduction: Infants that are born very pre-term (<30 weeks) are at increased risk of developing respiratory distress syndrome (RDS) as their lungs are structurally immature, surfactant deficient and incompliant. RDS is closely associated with an acute inflammatory response within lung tissue, which may result from tissue injury caused by artificial ventilation, and may progress into chronic lung disease. To improve the treatment of, and outcome for, these infants, it is important to identify resuscitation and ventilation strategies that minimize lung injury as well as identify infants with injured lungs during the immediate inflammatory phase of the injury. Although we have been unable to show an increase in the inflammation markers NFkB, TNFα and TGFβ within 2 hours following the onset of artificial ventilation in preterm lambs, evidence of lung damage (i.e. the presence of neutrophils and RBC's in the airways) can be observed histologically. As such, the inflammatory pathways activated in response to ventilator-induced acute lung injury are largely unknown and there are no known markers that reliably predict the onset of lung injury. We have recently cloned a gene, interferon regulatory factor-2 binding protein-2 (IRF2-BP2) from the lung of fetal sheep and shown that its expression is increased within 2hs of lung aeration. Thus, our **aim** was to characterize the changes in expression of IRF2-BP2 in the lung of prematurely delivered lambs in response to different resuscitation and ventilation strategies. The protein encoded by this gene, and closely related proteins, play an important role in the activation and suppression of genes involved in cell cycle progression and inflammation. Thus, we **hypothesized** that it may play a role in the initiation of the inflammatory cascade associated with ventilator-induced lung injury.

Methods: At 126d (term~147d), fetal sheep were exteriorised by caesarian section and the fetal carotid artery and jugular vein were catheterised before the fetus was intubated and delivered.
Study 1: Lambs were resuscitated for 15 mins using either a neonatal resuscitation bag (BR; n=10), which administered 100% oxygen in the absence of PEEP, or the Drager Babylog 8000$^+$ mechanical ventilator (MV) which administered a tidal volume of either 5ml/kg (n=5) or 10ml/kg (n=5) with 8 cmH2O of PEEP. After 15 mins of manual resuscitation, BR lambs were transferred to MV with a V_T of 5ml/kg (n=5) or 10ml/kg (n=4). Ventilation was continued for 2 hrs.
Study 2: Lambs were mechanically ventilated for 15 mins at a V_T of 20ml/kg in the absence of PEEP; this was expected to cause lung injury. After 15 mins, the lambs were ventilated at a V_T of 5ml/kg and a PEEP of 4 cmH_2O for 15, 30, 60 and 120 mins (n=4 for each time point).
At the end of each experiment, lung tissue was collected and the expression level of IRF2-BP2 determined by Northern blot analysis.

Results: Three transcripts were detectable (7.25kb, 4.4kb and 3.25kbs). The 4.4kb transcript was the most abundant and its levels were expressed as a proportion of mRNA levels in 125d control fetal lung tissue.
Study 1: The mRNA levels of the 4.4kb transcript were 100.0 $\pm$ 5.0 % in 125d fetal lung tissue and were increased significantly following all forms of mechanical ventilation. The levels were 168.4 $\pm$ 3.8% in BR 5ml+MV, 152 $\pm$ 24% in BR 10ml+MV, 153.5 $\pm$ 12.5% in 5ml + MV and 142.1 $\pm$ 8.6% in 10ml + MV.
Study 2: The mRNA levels of the 4.4kb transcript were increased from 100.0 ± 14.7% in control fetal lung tissue to 214.3 ± 15.3% at 15mins, 247 ± 23.7% at 30mins, 231.6 ± 5.9% at 60 mins and 154.6 ± 29.5% at 120 mins after the injurious ventilation period.

Conclusions: The mRNA levels for IRF2-BP2 are significantly up-regulated with all forms of artificial ventilation, particularly in response to ventilation regimes designed to induce lung injury. However, further investigation is required to establish (1) whether the increased expression is lung injury specific, (2) if the expression is differentially regulated by the degree of lung injury and (3) which cells of the lung express IRF2-BP2. It is possible that IRF2-BP2 expression could serve as a useful early marker of the initial lung inflammation induced by mechanical ventilation.

A27

Effects on fetal sheep of intra-amniotic injection of periodontal endotoxins.

Tim Moss[1], Alexus Shub[2], Ilias Nitsos[1], Machiko Ikegami[3], Alan Jobe[3] & John Newnham[1].

[1]School of Women's and Infants' Health, The University of Western Australia, Crawley WA, [2]King Edward Memorial Hospital, Subiaco WA & [3]Division of Perinatal and Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati OH, USA.

Introduction
Intrauterine inflammation, manifest commonly as chorioamnionitis, poses great threat to successful pregnancy outcomes. Intrauterine inflammation often occurs without the presence of infectious organisms within the amniotic fluid, suggesting that the site of infection in such cases is distant from the uterus. It has recently become evident that periodontal disease is associated with preterm birth. We hypothesised that periodontal endotoxins would be potent activators of intrauterine inflammation and thus aimed to determine the intrauterine and fetal inflammatory responses to intra-amniotic injection of endotoxins from common periodontal bacteria.

Methods
Pregnant ewes bearing single fetuses received ultrasound-guided intra-amniotic injections of saline (n=13), *Escherichia coli* endotoxin (1mg, n=7; 10mg, n=6), *Porphyromonas gingivalis* endotoxin (0.2-10mg, n=22), or *Actinobacillus actinomycetumcomitans* endotoxin (1mg, n=6; 10mg, n=6) on day 118 of pregnancy. Fetuses were delivered by Caesarean section on day 124 of gestation. We measured the number of inflammatory cells in amniotic fluid, fetal umbilical arterial blood gases and white blood cell counts, and assessed inflammation and 'maturation' of the fetal lungs.

Results
Only 6 (out of 22) fetuses survived intra-amniotic injection of *P gingivalis* endotoxin. Intra-amniotic injection of 10mg *A actinomycetumcomitans* endotoxin killed 50% of fetuses. In fetuses that survived intra-amniotic injection of periodontal endotoxins blood gases were impaired compared to control. Lung inflammation was profound after intra-amniotic injection of periodontal endotoxins. Lung compliance was greater in fetuses exposed to intra-amniotic endotoxins from *E coli* or *A actinomycetumcomitans*, but not in those that survived intra-amniotic *P gingivalis* endotoxin.

Conclusion
Intra-amniotic injection of endotoxins from common periodontal bacteria causes more profound, and potentially lethal, intrauterine and fetal inflammation than intra-amniotic *E coli* endotoxin injection. Our findings demonstrate that the intrauterine and fetal inflammatory responses to endotoxins from different organisms are not equivalent. The mechanisms by which periodontal endotoxins elicit more severe effects than *E coli* endotoxin are unknown.

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PLACENTAL RESTRICTION: A NOVEL MODEL SYSTEM TO STUDY THE RELATIVE ROLES OF THE PRENATAL AND POSTNATAL ENVIRONMENTS AND THE CONSEQUENCES OF IMPAIRED LACTATION ON GROWTH AND ADULT DISEASES

Mary E Wlodek, Department of Physiology, University of Melbourne, Parkville, Victoria, 3010, Australia. m.wlodek@unimelb.edu.au

Impaired uteroplacental blood flow and subsequent reduced oxygen and nutrient delivery across the placenta to the fetus, is the major known feature of human pregnancies complicated by intrauterine growth restriction. Postnatally, maternal nutrient restriction and altered mammary function commonly limit nutrient delivery to the suckling neonate for whom mother's milk is the only source of nutrition. Thus the in utero and postnatal environments are critical periods in programming growth. Intrauterine growth restriction contributes significantly to perinatal morbidity and mortality. Low weight, thinness or shortness at birth are strongly associated with glucose intolerance, type 2 diabetes, obesity, hypertension and Syndrome X in adults. Insulin resistance clusters with a number of traits (hyperinsulinaemia, glucose intolerance, hypertension, adiposity, dyslipidaemia), to comprise metabolic Syndrome X which constitute major risk factors for diabetes and cardiovascular disease. Recent studies suggest that catch-up growth in infancy and accelerated growth later, particularly in those of low birth weight, independently increases the risk of adult diseases.

Growing recent evidence suggests that the neonatal lactational environment is of critical importance in programming growth restriction with subsequent effects on adult disease. Our novel finding is that placental restriction of prenatal growth in rats, which models human intrauterine growth restriction, also directly impairs mammary growth and development during pregnancy and the quantity and quality of milk supplied through breast-feeding during lactation. This has been achieved using a novel rat placental insufficiency model induced by bilateral uterine vessel ligation which causes perinatal growth restriction. Our preliminary data has demonstrated adult consequences following placental restriction including inducing postnatal catch-up growth and fasting hyperglycaemia and hypertension in the aged rat as well as glucose intolerance in males and increased adiposity in females. We have established innovative studies to directly test these hypotheses and establish how nutrition during fetal life, lactation and post-weaning separately and synergistically determine long-term growth and health. The life stages at which such changes emerge and their molecular basis will be established. Our recent cross fostering studies have clearly demonstrated that both prenatal growth restriction and postnatal mammary function separately influence postnatal growth. The Restricted pup grows better on a Control mother whereas the impaired mammary function of the Restricted mother slows the growth of the Control pup.

Using this model system we have the capacity to measure: body weight, body dimensions, growth rates, milk intake, tail vein blood samples and blood pressure and perform intravenous glucose tolerance test. Measurements and tissues from both males and females can be analysed from the following key developmental periods: fetal (20 days) and neonatal (6 days after birth) period, juvenile period around weaning (5 weeks) and at the time of catch-up growth (9 weeks) as well as in the mature and the aged rat (6 and 12 months). It is anticipated that this model system of placental and lactational insufficiency coupled with cross fostering studies will enable investigation of the consequences of perinatal growth restriction. Studies will identify markers of impaired lactation and developmental stages during which nutritional and other treatments have beneficial consequences for mammary function and the health of the infant and adult.

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Registrants

Ms Beth Allison
Dept of Physiology
Monash University
Melbourne Vic 3800
Beth.allison@med.monash.edu.au

Dr. Jane Black
Dept of Anatomy and Cell Biology
Monash University
Melbourne VIC 3800
jane.black@med.monash.edu.au

Dr Amanda Boyce
Dept of Physiology and Pharmacology
University of NSW
Sydney
a.boyce@unsw.edu.au

Ms Priscilla Cassaglia
Ritchie Centre for Baby Health Research
Monash Medical Centre
Melbourne VIC 3168
Priscila.Cassaglia@med.monash.eduau

Dr. Megan Cock
Dept of Physiology
Monash University
Melbourne Vic 3800
megan.cock@med.monash.edu.au

Dr. Coughtry Heather
Sydney
NSW 2773
coughth@wahs.nsw.gov.au

Dr. Kelly Crossley
Department of Physiology
Monash University
Melbourne Vic 3800
kelly.crossley@med.monash.edu.au

Ms. Penny Dalitz
Dept of Physiology
Monash University
Melbourne VIC 3800
penelope.dalitz@med.monash.edu.au

Ms. Nina De Boo
Liggins Institute
Dept of Medicine and Health Services
University of Auckland
Auckland, NZ
n.deboo@auckland.ac.nz

Dr. Kate Denton
Dept of Physiology
Monash University
Melbourne Vic 3800
kate.denton@med.monash.edu.au

Ms. Hayley Dickinson
Dept of Physiology
Monash University
Melbourne VIC 3800
hayley.dickinson@med.monash.edu.au

Dr. Simona Eremia
Liggins Institute
University of Auckland
Auckland, NZ
s.eremia@auckland.ac.nz

Dr. Susan Feng
Ritchie Centre for Baby Health Research
Monash Medical Centre
Melbourne VIC 3168
ying.feng@med.monash.edu.au

Ms. Caitlin Filby
Dept of Physiology
Monash University
Melbourne VIC 3800
Caitlin.Filby@med.monash.edu.au

Ms. Sharon Flecknoe
Dept of Physiology
Monash University
Melbourne VIC 3800
Sharon.flecknoe@med.monash.edu.au

Dr. Karen Gibson
Dept of Physiology
School of Medical Sciences
University of NSW Sydney 2052.
k.gibson@unsw.edu.au

Mrs. Sanita Grover
Dept of Obstetrics and Gynaecology
Lvel 6 Medical School North
Adelaide SA 5005
sanita.grover@adelaide.edu.au

Prof. Jane Harding
Liggins Institute
University of Auckland
Private Bag 92019
Auckland, NZ
j.harding@auckland.ac.nz

Prof. Richard Harding
Dept of Physiology
Monash University
Melbourne VIC 3800
Richard.Harding@med.monash.edu.au

Dr. Jon Hirst
Dept of Physiology
Monash University
Melbourne VIC 3800
jon.hirst@med.monash.edu.au

Dr. Rosemary Horne
Dept of Pediatrics
Monash Medical Centre
Monash University
Melbourne VIC 3800
rosemary.horne@med.monash.edu.au

Ms Lisa Hutton
Dept of Physiology
Monash University
Melbourne VIC 3800
l.hutton@med.monash.edu.au

Dr. Anne Jaquiery
Liggins Institute
Park Ave, Grafton Auckland NZ
a.jaquiery@auckland.ac.nz

Dr. Zsuzsoka Kecskes
Dept of Neonatology,
Canberra Hospital
Garran ACT 2605

Ms. Vasumathy Kumarasamy
Liggins Institute
Faculty of Medicine and Health Sciences
University of Auckland, New Zealand

Dr. Leo Leader
School of Obstet & Gynaecol
University of NSW
Sydney NSW 2052
l.leader@unsw.edu.au

Ms. Poonam Ligam
Dept of Physiology
Monash University
Melbourne VIC 3800
Poonam.ligan@med.monash.edu.au

Dr. Barbara Lingwood
Perinatal Research Centre
Royal Brisbane and Women's Hospital
Herston Qld 4029
b.lingwood@uq.edu.au

Professor. Eugenie Lumbers
Dept of Physiology and Pharmacology
University of NSW
Sydney 2052
e.lumbers@unsw.edu.au

Ms. Devaki Maduwegedera
Department of Physiology
Monash University
Melbourne VIC 3800
Dewaki.Maduwegedera@med.monash.ed.

Dr. Suzie Miller
Dept of Physiology
Monash University
Melbourne VIC 3800
suzie.miller@med.monash.edu.au

Dr. Karen Moritz
Dept Anatomy and Cell Biology
Monash University
Melbourne VIC 3800
Karen.moritz@med.monash.edu.au

Dr. Tim Moss
Dept of Obstet & Gynaecol
Uni. of Western Australia
Perth WA
tmoss@cyllene.uwa.edu.au

Dr. Makoto Nakamura
Newborn services
Monash Medical Centre
Clayton VIC 3168
JCB01257@nifty.com

Ms. Amanda O'Connell
Dept Physiology and Pharmacology
Wallace Worth Building
The University of New South Wales
Sydney NSW 2052
Canobolas@hotmail.com

Ms Rachel O'Dowd
Dept Physiology
University of Melbourne
Melbourne VIC
j.o'dowd@pgrad.umimelb.edu.au

Prof Julie Owens
The University of Adelaide
Adelaide SA 5005
Julie.owens@adelaide.com.au

Ms Hannah Palliser
Dept of Physiology
Monash University
Melbourne VIC 3800
Hannah.palliser@med.monash.edu.au

Dr Julia Pitcher
Dept Obstet & Gynaecol
The University of Adelaide
Adelaide SA 5005
Julia.pitcher@adelaide.edu.au

Mr Graeme Polglase
Dept of Physiology
Monash University
Melbourne VIC 3800
Graeme.polglase@med.monash.edu.au

Ms Kirsty Pringle
Dept Obstet & Gynaecol
The University of Adelaide
Adelaide SA 5005
Kirsty.pringle@adelaide.edu.au

Dr. Claire Roberts
Dept Obstet & Gynaecol
The University of Adelaide
Adelaide SA 5005
claire.roberts@adelaide.edu.au

Ms. Candice Rodricks
Department of Physiology
Monash University
Clayton VIC 3800
Candice.rodricks@med.monash.edu.au

Ms. Amanda Sferruzzi-Perri
Department of Obstetrics and
Gynaecology
University of Adelaide
Adelaide SA 5005
amanda.sferruzziperri@adelaide.edu.au

Ms Foula Sozo
Dept of Physiology
Monash University
Melbourne VIC 3800
foula.sozo@med.monash.edu.au

Ms. Janine Street
Liggins Institute
University of Auckland
Auckland New Zealand
j.street@auckland.ac.nz

Ms. Veena Supramaniam
Dept of Physiology
Monash University
Melbourne VIC 3800
Veena.supramaniam@med.monash.edu.au

Dr. Keiji Suzuki
Dept of Physiology
Monash University
Melbourne VIC 3800
dks@med.monash.edu.au

Prof. Barry Taylor
Dept of Women's & Children's Health
Dunedin School of Medicine
Dunedin New Zealand
barry.taylor@stonebow.otago.ac.nz

Ms Prema Thavaneswaran
Discipline of Physiology
School of Molecular and Biomedical
Sciences
The University of Adelaide
Adelaide SA 5005
prema.thavaneswaran@adelaide.edu.au

Ms. Alison Thiel
Dept of Physiology
Monash University
Melbourne VIC 3800
alison.thiel@med.monash.edu.au

Dr. David Todd
Fellow NICU
Westmead Hospital
Westmead NSW 2145
David.todd@wsahs.nsw.gov.au

Dr. Rita Tuladah
Dept of Pediatrics
Monash Medical Centre
Monash University
Melbourne VIC 3800
rita.tuladah@med.monash.edu.au

Mrs Anita Turner
Dept of Physiology and Pharmacology
University of NSW
Sydney NSW 2052
anita.turner@student.unsw.edu.au

Mr. Pierre van Zijl
Liggins Institute
University of Auckland
Auckland New Zealand
p.vanzijl@auckland.ac.nz

Prof. Adrian Walker
Ritchie Centre for Baby Health Research
Monash Medical Centre
Clayton VIC 3168
adrian.walker@med.monash.edu.au

Dr. Megan Wallace
Dept of Physiology
Monash University
Melbourne VIC 3800
megan.wallace@med.monash.edu.au

Dr Meredith Ward
Westmead Medical Centre
NSW

Ms Kerryn Westcott
Department of Physiology
University of Melbourne
Melbourne VIC
k.westcott@unimelb.edu.au

Prof Marelyn Wintour
Dept of Physiology
Monash University
Melbourne VIC 3800
Marelyn.Wintour-Coghlan@
med.monash.edu.au

Assoc Prof. Mary Wlodek
Department of Physiology
University of Melbourne
Melbourne VIC
m.wlodek@unimelb.edu.au

Dr. Ian Wright
Kaleidoscope Neonatal Intensive Care Unit
University of Newcastle
New South Wales 2310
iwright@mail.newcastle.edu.au

Dr. Flora Wong
Ritchie Centre for Baby Health Research
Monash Medical Centre
Clayton VIC 3168
Flora.Wong@med.monash.edu.au

Mr. Edwin Yan
Dept of Physiology
Monash University
Melbourne VIC 3800
edwin.yan@med.monash.edu.au

Ms Tamara Yawno
Dept of Physiology
Monash University
Melbourne VIC 3800

Ms. Stephanie Yiallourou
Ritchie Centre for Baby Health Research
Monash Medical Centre
Clayton VIC 3168
Stephanie.yiallourou@med.monash.edu.au