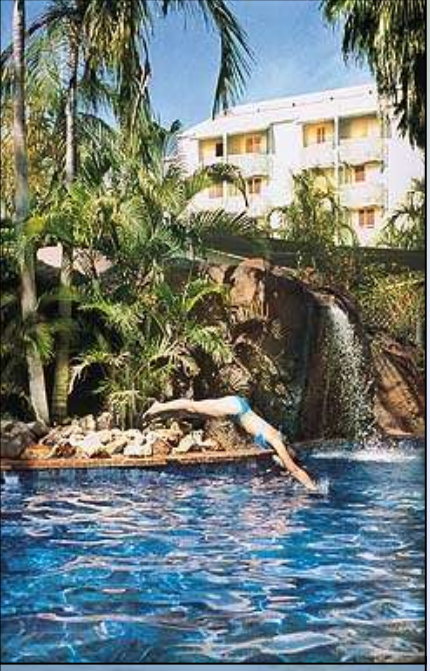




The Fetal and Neonatal Workshop of Australia and New Zealand

23rd Annual Meeting

Travelodge Mirambeena Resort, Darwin

17-18th April, 2009

2009 Organising Committee:

Rob DeMatteo	Monash University
Richard Harding	Monash University
Vicki Stokes	Monash University
Gurmeet Singh	Charles Darwin University

NGED

ARC/NHMRC Research Network in Genes and Environment in Development

Program Outline

FRIDAY 17TH APRIL

8.30am-9.00am
Registration

9.00am-10.30am
Session 1

10.30am-11.00am
Morning Tea

11.00am-12.30pm
Session 2

12.30pm-1.15pm
Lunch

1.15pm-2.30pm
Session 3 Part 1

2.30pm-3.00pm
Afternoon Tea

3.00pm-4.15pm
Session 3 Part 2

5.45pm
Meet at the end of Stokes Hill Wharf on the right hand side to commence boarding at 5.45pm for our sunset dinner cruise departing at 6pm and returning at 8.45pm.

SATURDAY 18TH APRIL

9.30am-11.00am
Session 4

11.00am-11.30am
Morning Tea

11.30am-1.00pm
Session 5

1.00pm-2.00pm
Lunch

2.00pm-3.15pm
Session 6

3.15pm-3.45pm
Afternoon Tea

3.45pm-5.15pm
Session 7

SCIENTIFIC PROGRAM

DAY 1- FRIDAY 17th APRIL

Registration: 8.30am-9.00am

Session 1: Chair – Rosemary Horne			
9.00am	**A1**	Jonathan Hirst	Sex specific differences in the effects of betamethasone treatment on steroidogenic enzyme expression in normal and growth restricted fetal guinea pigs
9.15am	**A2**	Nicolette Hodyl	Placental cytokine responses following in-vitro lipopolysaccharide stimulation and capacity for inhibition by cortisol: effects of maternal asthma and fetal sex
9.30am	**A3**	Annette Osei-Kumah	Sex specific differences in human placental micro RNA expression
9.45am	**A4**	Heidi Richardson	Effects of gender on arousal from sleep responses in sleeping infants
10.00am	**A5**	Noreen Ishak	Gender effects on lung development in preterm lambs
10.15am			**General discussion**

Morning tea: 10.30am-11.00am

Session 2: Chair – Ian Wright			
11.00am	**A6**	Megan O'Reilly	Does exposure of the newborn mouse lung to hyperoxia affect development of the pulmonary airways?
11.15am	**A7**	Melissa Siew	The relationship between lung aeration and lung liquid removal at birth
11.30am	**A8**	Beth Allison	Morphological changes within the lung following ventilation with 100% oxygen, room air or nitrogen in immature sheep.
11.45am	**A9**	Sharon Flecknoe	Pulmonary vasculature development regulates alveolar epithelial cell differentiation
12.00pm	**A10**	Nadine Brew	Effects of brief ventilation on small airway structure in the very immature lung
12.15pm			**General discussion**

Lunch: 12.30pm-1.15pm

Session 3: Chair – Alison Kent			
1.15pm	**A11**	Joanna Goh	Effects of alcohol exposure during late gestation on the fetal heart
1.30pm	**A12**	Robert DeMatteo	Does altered nutrition and growth following preterm birth affect cardiovascular, respiratory and metabolic function and structure in later life?
1.45pm	**A13**	Leo Leader	Prenatal anxiety & maternal sensitivity … Associations with infant cortisol & reactivity
2.00pm	**A14**	Kathryn Gatford	Interventions to prevent diabetes after IUGR – preliminary outcomes in the twin IUGR lamb
2.15pm			**General discussion**

Afternoon tea: 2.30pm-3.00pm

Session 4: Chair – Leo Leader			
3.00pm	**A15**	Jonathan Bensley	Characterising an ovine model of chronic chorioamnionitis: effects on offspring from birth to 2 months of age. A preliminary report
3.15pm	**A16**	Karen Gibson	The renal response to water restriction is unimpaired in the offspring of ewes with renal insufficiency
3.30pm	**A17**	Robert Galinksy	The effect of chorioamnionitis on the developing human kidney
3.45pm	**A18**	Kimberley Ong	The effects of hyperoxia on kidney development
4.00pm			**General discussion**

Conference dinner: Sunset dinner cruise- boarding at 5.45pm, departing at 6pm

SCIENTIFIC PROGRAM CONT.

DAY 2- SATURDAY 18th APRIL

Session 5: Chair - Rob De Matteo			
9.30am	**A19**	Richard Dalton	Effects of uterine development on pulmonary vascular development and function – the origin of postnatal cardiopulmonary disease?
9.45am	**A20**	Oksan Gezmish	Effect of life-long vitamin D deficiency on cardiac function and susceptibility to ischemia/reperfusion injury in the adult rat heart
10.00am	**A21**	Keiji Suzuki	Contractile properties of excised murine pulmonary arteries to various vasoactive agents commonly used in neonatal units
10.15am	**A22**	Mary Wlodek	Being born small reduces the number of cardiomyocytes which can be increased by improving postnatal nutrition
10.30am	**A23**	Stephanie Yiallourou	Assessment of baroreflex sensitivity in the frequency and time domains during sleep in term infants
10.45am			**General discussion**

Morning tea: 11.00am-11.30am

Session 6: Chair – Anne Jaquiery			
11.30am	**A24**	Mary Berry	Cardiovascular autonomic function is altered in pre-pubertal lambs born preterm
11.45am	**A25**	Kitty Bach	Effect of ventilator gas flow rate and mode of ventilation on early lung injury markers in the preterm lung
12.00pm	**A26**	Julia Pitcher	How might motor cortex development influence specific learning difficulties in children born preterm?
12.15pm	**A27**	Graeme Polglase	The effect of volume loading on cardiopulmonary haemodynamics in ventilated preterm lambs
12.30pm	**A28**	Nicole Witcombe	Pre-eclampsia: Effects on the preterm infant cardiovascular system
12.45pm			**General discussion**

Lunch: 1.00pm-2.00pm

Session 7: Chair – Julie Owens			
2.00pm	**A29**	Michael Stark	Determinants of inter-uterine growth in patho-physiological pregnancy
2.15pm	**A30**	Anne Jaquiery	Insulin response to glucose tolerance test is altered by preterm birth, twinning, sex and postnatal nutrition in lambs
2.30pm	**A31**	Long Nguyen	The effect of maternal undernutrition on gene expression in visceral fat of the late gestation guinea pig
2.45pm	**A32**	Sandra Orgeig	Intrauterine growth restriction delays maturation of the pulmonary surfactant system in the sheep fetus
3.00pm			**General discussion**

Afternoon tea: 3.15pm-3.45pm

Session 8: Chair – Mary Wlodek			
3.45pm	**A33**	Hayley Dickinson	Who determines the length of gestation – mother, fetus or placenta? (a study in progress)
4.00pm	**A34**	Bree O'Connell	The maintenance of pregnancy in the spiny mouse (a study in progress)
4.15pm	**A35**	Scott Sands	A new method for quantifying stability during respiratory instabilities and determining the efficacy of intervention
4.30pm	**A36**	Stuart Hooper	Imaging lung motion using phase contrast X-ray imaging
4.45pm	**A37**	Julie Owens	Maternal folic acid supplementation and altered metabolic health of offspring: molecular basis
5.00pm			**General discussion**

End of Workshop

SEX SPECIFIC DIFFERENCES IN THE EFFECTS BETAMETHASONE TREATMENT ON STEROIDOGENIC ENZYME EXPRESSION IN NORMAL AND GROWTH RESTRICTED FETAL GUINEA PIGS

Amy A. McKendry[1], Hannah K. Palliser[1], David W. Walker[2] & Jonathan J. Hirst[1]
[1] Mothers and Babies Research Centre, University of Newcastle, Newcastle, Australia
[2] Department of Physiology, Monash University, Melbourne, Australia

Background: Exposure to elevated levels of glucocorticoids in late gestation has been shown have adverse effects on the brain including delayed myelination. Intrauterine growth restriction (IUGR) may chronically raise glucocorticoid levels and this elevated exposure may be further increased by synthetic glucocorticoid treatment with betamethasone. 5α-reduced neuroactive steroids such as allopregnanolone have been found to promote myelination following tissue damage and may be important in normal myelination. Betamethasone and IUGR may suppress the synthesis of these steroids in the fetal brain and/or in peripheral tissues. The consequent reduction in the production may mediate some of the effects of glucocorticoids brain cell death and myelination. 5α-reductase type 2 has a key rate-limiting role in allopregnanolone production and the expression of this enzyme differs with fetal sex. Thus, there may be sex differences in the sensitivity of this enzyme to suppression by glucocorticoids.

Aims: Our aim was to examine the effect betamethasone treatment and IUGR on 5α-reductase type 2 expression in the brain and adrenal glands of male and female fetuses and in the placenta.

Methods: Placental insufficiency was induced in guinea pigs by the ablation of uterine artery branches at mid gestation (term 71d). This resulted in fetal IUGR characterised by brain sparing. Sham and growth restricted fetuses were treated with vehicle or betamethasone (1mg/kg/day) for 4 days prior to sacrifice (65d). Real time RT-PCR was used to determine 5α-reductase-2 mRNA expression.

Results: Brain 5α-reductase-2 mRNA expression was reduced in males but not females of all treatment groups compared to controls. Betamethasone treatment reduced placental 5α-reductase-2 mRNA expression, but expression was not affected by either growth restriction alone or with betamethasone. 5α-reductase-2 expression in the adrenal glands was higher in males than females and was reduced by growth restriction.

Conclusions: In males betamethasone and IUGR reduced brain 5α-reductase-2 enzyme expression, whereas females retained normal levels. Betamethasone in IUGR fetuses overcame glucocorticoid suppression of expression in the placenta. These alterations in neurosteroidogenic enzymes potentially reduces brain neurosteroid levels, this may be reduced even further in males. These changes could adversely affect brain development, particularly myelination, and cell death as well as leaving these fetuses vulnerable to asphyxic insults.

PLACENTAL CYTOKINE RESPONSES FOLLOWING IN-VITRO LIPOPOLY-SACCHARIDE STIMULATION AND CAPACITY FOR INHIBITION BY CORTISOL: EFFECTS OF MATERNAL ASTHMA AND FETAL SEX

Nicolette Hodyl, Naomi Scott, Annette Osei-Kumah, Michael Stark & Vicki Clifton.
Robinson Institute, School of Paediatrics and Reproductive Health, University of Adelaide, SA, 5005, Australia.

Background: Asthma affects 12% of pregnant women in Australia, and results in reduced female fetal growth when left untreated. Reduced fetal growth in the presence of other inflammatory conditions is associated with increased placental pro-inflammatory cytokine production. Whether this also occurs in the presence of asthma is currently unknown. Given that foetal growth is reduced in females, but not males, we propose that activation of pro-inflammatory pathways in the placenta are dependent on foetal sex. We have previously demonstrated sexually dimorphic placental metabolism of cortisol in the presence of asthma, which, given its immuno-suppressive function, may also contribute to differential degrees of placental inflammation between the sexes.

Aims/Hypothesis: We aimed to identify whether asthma potentiates placental cytokine production and sensitivity to inhibition by cortisol *in-vitro*, in a sex-specific manner. We hypothesise that increased pro-inflammatory cytokine production will be evident in placentae from females compared to males, and will be further increased in the presence of asthma. Additionally, inhibition of cytokine responses by cortisol will exhibit sexual dimorphism, with females more responsive than males.

Methods: Placentae were collected following spontaneous delivery from control, non-asthmatic women (n=10) and women with asthma (n=12). Placental explants were incubated for 24 hours and then stimulated with lipopolysaccharide (LPS; 1ng/ml). Supernatant was collected after 2 and 24 hours, and cytokine concentrations (tumour necrosis factor (TNF) α, IL (interleukin) 1β, IL6, IL8 and IL10) were measured using Luminex multiplex system.

Results: In control placentae, no sex-specific differences were observed in any measured cytokine at the 2 hours time-point. At 24 hours, females exhibited increased concentrations of IL8 relative to males ($p<0.05$). In the asthmatic group at 2 hours, increased concentrations of IL-1β were observed in females relative to males, while at 24 hours increased levels of IL-10 were also observed. Placental cytokine production was equivalent between the asthma and control groups in the presence of a male foetus. However, in the presence of a female fetus TNFα, IL-1β and IL6 were increased in the asthma group at 2 hours ($p<0.05$), yet decreased at 24 hours relative to controls ($p<0.05$). Cortisol inhibited the TNFα and IL-1β response at 2 and 24 hours in both sexes, but inhibited TNFα and IL-1β in the asthmatic group at 2 hours, and in both the control and asthmatic groups at 24 hours.

Conclusions: We observed increased pro-inflammatory cytokine production in placentae collected from women with asthma, only in the presence of a female fetus. Increased sensitivity to inhibition by cortisol was observed in the asthmatic group, regardless of fetal sex. These findings in the presence of asthma are consistent with the observations of reduced fetal growth in other inflammatory conditions. Previously, we have identified sex-specific differences in regulatory pathways that may influence pro-inflammatory cytokine production. Further investigation of these differences in the context of maternal inflammation is the current focus of our research.

SEX SPECIFIC DIFFERENCES IN HUMAN PLACENTAL MICRO RNA EXPRESSION

Annette Osei-Kumah, Nicolette Hodyl, Wee-Ching Kong, Julie Owens & Vicki Clifton
Robinson Institute, University of Adelaide, Frome Rd, SA 5005

Background: We have previously identified sex specific differences in placental gene expression associated with strategies for optimal growth and fetal survival. Specifically, we have shown differences in placental cytokine mRNA and glucocorticoid receptor expression as well as 11beta-hydroxysteroid dehydrogenase type 2 activity. We have also identified sex specific differences in global placental gene expression using microarray. Micro RNAs (miRs) are non-coding small RNAs and act as important post-transcriptional regulators of gene expression by altering the abundance or translational efficiency of mRNAs. The current study sought to examine miR expression in the placenta, to determine if they are involved in the regulation of the differential gene expression observed in male and female placenta.

Aims/Hypothesis: We hypothesise that post-transcriptional regulation of genes by miRs may play an important role in conferring sexual dimorphism in placental gene expression during development. The aim of the present study was to determine if there are any differences in placental miRs between male and female placentae.

Methods: RNA was extracted from male (n=4) and female (n=4) placenta from normal pregnancies and miR analysis was conducted. miRGEN target prediction algorithm database (http://www.diana.pcbi.upenn.edu/cgibin/miRGen/v3/Targets.cgi) was used to identify predicted targets for identified miRs. Target genes were then imported into Ingenuity Pathways Analysis (IPA) software to identify functional networks of differentially expressed miRs. Since clustered miRs are thought to be regulated together, pathway analysis using IPA was performed on common predicted targets of clustered miRs.

Results: One hundred and six miRs were differentially expressed between male and female placentae based on a *P*-value of less than 0.05. Sixty miRs were up-regulated and 46 were down-regulated in female placentae compared with males. Most of the differentially expressed miRS were clustered on chromosomes 13, 14, 16, 17, 19 and 20. Some of the miRs were also clustered on the X chromosome. Pathway analysis identified networks involved in innate immune activation (toll like receptor), cytokine signalling (IL-4, 1L-8, 1L-13, STAT6, IFNγ and MAPK), glucocorticoid receptor signalling, cellular growth and proliferation.

Conclusions: There are differentially expressed miRs in male and female placenta which target genes involved in cytokine gene expression and other immune pathways in the placenta. This may be related to the differential gene regulatory mechanisms initiated by males and females in-utero for growth and survival.

EFFECTS OF GENDER ON AROUSAL FROM SLEEP RESPONSES IN SLEEPING INFANTS

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Ritchie Centre for Baby Health Research, Monash Institute of Medical Research, Monash University, Melbourne, Australia

Background: Sudden Infant Death Syndrome (SIDS) is thought to involve an impaired ability to arouse from sleep in a life threatening situation. There is also evidence to suggest that future SIDS victims may have pre-existing arousal abnormality which inhibits the progression of sub-cortical activation to full cortical arousal. Approximately 60% of SIDS victims are male and it has been suggested that males have delayed cortical maturation when compared with females. We therefore hypothesised that the proportion of induced cortical arousals would be depressed in male infants compared with female infants.

Aim: To evaluate induced arousal processes in male and female infants from both active (AS) and quiet (QS) sleep, whilst accounting for the progression of sub-cortical activations and cortical arousals.

Methods: 37 healthy term infants (14 male, 23 female) were studied with daytime polysomnography at both 2-4 wk and 2-3 mo after birth. Whilst infants slept supine, arousal from sleep was induced using a pulsatile jet of air to the nostrils at increasing pressures. Arousal responses were scored as sub-cortical activations (SCA) and cortical arousals (CA) according to standard criteria and were expressed as proportions of total arousal responses. Two-way ANOVA was used to assess effects of gender and age on the frequencies of SCAs and CAs.

Results: During AS, male and female infants exhibited similar CA proportions at both 2-4 weeks (males 41 ± 6%, females 35 ± 5%) and 2-3 months (males 33 ± 9%, females 29 ± 5%) postnatal age. During QS, at 2-4 weeks, CA tended to occur less frequently in males (15 ± 5%) when compared with females (23 ± 6%), though failed to reach statistical significance. Furthermore, there were no gender effects on arousal processes observed at 2-3 months (males 21 ± 7%, females 22 ± 4%).

Conclusions: This study has demonstrated that there were no consistent effects of gender on induced arousal processes, which supports our previous finding that total arousability is also unaffected by gender. Therefore, although males are at increased risk of SIDS when compared with females, this risk is not associated with differences in arousal progression.

GENDER EFFECTS ON LUNG DEVELOPMENT IN PRETERM LAMBS

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Introduction: Preterm birth exposes newborn infants to a number of complications due to the immaturity of their organ systems. It is now well established that the incidence of respiratory distress syndrome (RDS) and respiratory illness is higher in male preterm infants than in female preterm infants. To date, the exact mechanisms responsible for the gender differences in respiratory outcomes are unclear but they are postulated to include developmental differences in the lungs and/or the effects of sex hormones. In the last 5 years, our laboratory has been using a model of moderate preterm birth in sheep, in which lambs are born at 132-3 days of gestation; this age is the earliest that most lambs survive without requiring respiratory support. At this age, we have found that a majority of male preterm lambs die within 24 hours from respiratory insufficiency whereas the majority of females survive.

Aim: To determine whether there are structural and biochemical differences between lungs of male and female fetal sheep at a stage of lung development that is comparable to moderate preterm birth.

Methods: We used the lungs from male (n=8) and female (n=9) fetal sheep at ~131d gestational age (GA; term ~147d). The right lungs had been fixed via the trachea at 20 cmH_2O, and fresh, frozen tissue was collected from the left lungs. Lung weight and volume were determined and the lungs were then analysed morphometrically. We measured: the percentages of tissue and air space in the lung parenchyma, collagen and elastin deposition, and indices of lung cell proliferation and apoptosis. In addition, real-time PCR was performed to determine mRNA levels of surfactant proteins (SP)-A, -B, -C and -D and tropoelastin.

Results: No significant differences were observed between the male and female fetuses in body weight, lung tissue weight and volume, lung morphometric parameters (percentages of tissue and air space, collagen and elastin contents, cell proliferation and apoptosis) and biochemical parameters (SP-A, -B, -C and -D and tropoelastin gene expression).

Conclusions: Despite the increased incidence of respiratory distress syndrome in males born preterm, compared to females born preterm, this study has demonstrated that the lungs of male and female ovine fetuses are structurally and biochemically similar. Therefore, this finding suggests that the gender differences in the incidence of RDS may be mediated by other factors. Further research into this topic is needed.

NOTES

DOES EXPOSURE OF THE NEWBORN MOUSE LUNG TO HYPEROXIA AFFECT DEVELOPMENT OF THE PULMONARY AIRWAYS?

Megan O'Reilly, Richard Harding & Foula Sozo
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Background: Very preterm infants are born with immature lungs and usually require respiratory support, including supplemental oxygen therapy. However, exposing the immature lung to physiologically high concentrations of oxygen can lead to hyperoxia-induced lung injury, which is characterised by alterations in lung structure that are similar to those observed in bronchopulmonary dysplasia (BPD). The long-term respiratory effects of BPD include reduced lung function, which may be attributed to airway dysfunction. Despite this, little is known about the effects of hyperoxia exposure on the structure of the airways in the immature lung and its persistence into adulthood.

Aim: To determine the immediate and long-term effects of moderate hyperoxia exposure on the structure of the developing lung in newborn mice.

Methods: Neonatal mice (C57Bl/6J) born at term were continuously exposed to hyperoxia (65% oxygen) from birth until 7 days postnatal age (7d PNA). Following 7 days of hyperoxia, mice were either (a) culled immediately (n=33), or (b) allowed to live in room air (normoxia; 21% oxygen) for a further 7 weeks until they were culled at 56d PNA (n=26). Controls for each group were age-matched mice exposed to normoxia (7d PNA n=35; 56d PNA n=31). Newborn mice were marked for identification and then weighed on a weekly basis from the day of birth. At necropsy, the lungs were fixed via the trachea at 25cmH_2O with 4% paraformaldehyde and processed for morphometric analysis. In the lung parenchyma we measured (1) the percentage of tissue and airspace, and (2) the diameter of the saccules/alveoli using the mean linear intercept method. Components of the walls of small conducting airways were morphometrically analysed.

Results: Mice exposed to 65% oxygen for 7 days were significantly lighter (p<0.001) than age-matched controls. The significant reduction in body weight persisted up to 3 weeks following hyperoxia (p<0.05), after which there was no treatment effect. Immediately following hyperoxia exposure at 7d PNA, the saccules/alveoli in hyperoxic mice were significantly larger (p<0.001) compared to controls; this persisted into adulthood at 56d PNA (p<0.05). Hyperoxic mice had a significantly lower percentage of parenchymal tissue at 7d PNA (p<0.001) compared to controls; this was also observed at 56d PNA (p<0.05). In the conducting airways, there was no significant difference in epithelial thickness between hyperoxic and control mice when assessed at 7d PNA. However, mice exposed to hyperoxia for 7d had a thicker airway epithelium compared to control mice when assessed at PNA 56d (p<0.001).

Conclusions: Exposure to a moderate level of hyperoxia for the first 7 days after birth can alter body growth and induce structural alterations in the lung parenchyma and conducting airways. The persistence of these changes into adulthood, even in the absence of continued hyperoxia exposure, could contribute to reduced lung function.

THE RELATIONSHIP BETWEEN LUNG AERATION AND LUNG LIQUID REMOVAL AT BIRTH

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Background: At birth, the lungs must immediately clear the airways of liquid to allow the entry of air and the onset of gaseous respiration. However, very preterm newborns often fail to adequately remove liquid from the airways which impairs effective gas exchange and may lead to respiratory failure. We have observed the transition of the lung from a fluid-filled to an air-filled organ at birth in mechanically ventilated very preterm rabbit pups using phase contrast X-ray imaging (PCXI). Although it is logical to assume that the volume of air entering the lung after birth must be proportional to the volume of liquid leaving the airways, plethysmography and PCXI are only able to measure the volume of *air entering the lungs*, which may or may not equal the volume of liquid cleared. This study aims to determine if lung aeration corresponds to airway liquid removal or whether liquid remains within the airways and is cleared more slowly.

Hypothesis: We hypothesise that lung aeration corresponds to lung liquid clearance.

Methods: Pregnant New Zealand White rabbits (28dGA) were anaesthetised and the pups delivered by cesarean section. Each pup was surgically intubated with an ET tube preloaded with 0.1ml of FITC-albumin. The instillate was slowly flushed in and out ~20 times before the pup was ventilated for 7 minutes with a tidal volume of 7ml/kg, a positive end expiratory pressure of 5cmH_2O and a rate of 24 breaths/minute. Following ventilation, the pup was killed, the lungs removed and re-inflated before being frozen in OCT. The frozen lung was sectioned at 15 µm in a cryostat and observed under a fluorescent microscope, taking note of the exact co-ordinates of each field of view. The sections were then fixed in acetone, stained with haemotoxylin and re-aligned using the co-ordinates noted previously. The fluorescent and haemotoxylin images were merged together using ImagePro plus. Liquid-filled fetal lungs also received FITC-albumin, but pups were killed and the lungs collected without ventilation.

Results: In the ventilated lungs, areas of strong fluorescence were observed in close association with the airway epithelium although in some cases it penetrated just beyond the epithelial layer into the interstitial tissue. Sub-epithelial penetration of the dye is likely due ventilation-induced damage of the airway epithelium. Although fluorescence most commonly lined a portion of an airway a thin band of strong fluorescence was found to line the entire circumference of numerous small airways. In contrast, the control lungs did not demonstrate any concentrated areas of fluorescence, showing a relatively consistent concentration across the airways.

Conclusion: The concentrated areas of fluorescence that line the airways in the aerated lung suggest that the majority of the pre-existing lung liquid has left the airspace. A significant volume of lung liquid does not exist in aerated alveoli and therefore, the volume of aeration seen in the phase contrast X-ray images must closely correspond to the volume of lung liquid cleared.

MORPHOLOGICAL CHANGES WITHIN THE LUNG FOLLOWING VENTILATION WITH 100% OXYGEN, ROOM AIR OR NITROGEN IN IMMATURE SHEEP

Beth J Allison[1], Sharon J Flecknoe[1], Carmen Williams[1], Kelly J Crossley[1], Colin J Morley[2] & Stuart B Hooper[1]
[1]Dept Physiology, Monash University, Melbourne, and [2]Neonatal Services, Royal Women's Hospital, Melbourne.

Background: Many premature infants develop ventilation-induced lung injury (VILI) resulting from artificial ventilation needed to support their life. It is known that long term ventilation can lead to bronchopulmonary dysplasia a disease characterised by changes in lung structure including simplified alveoli, hypercellularity and alveolar fibrosis. Bronchopulmonary dysplasia is strongly associated with high inspired oxygen. We have recently established a model where fetal sheep are ventilated *in utero* to reduce the number of confounding factors often associated with ventilating preterm infants. As the fetus can be ventilated much earlier using this model, the injurious effects of ventilation can be determined at a stage of development that closely reflects the morphological and cellular development of the lung in very preterm infants. Furthermore, fetal lung liquid can be replaced at the end of the ventilation period, allowing time for pathological changes to manifest, thereby avoiding the need to ventilate for prolonged periods of time. This model is therefore highly suitable to investigate the changes in lung morphology which result from exposure to oxygen.

Aim: The aim of this study was to investigate the effects of high (100%), low (21%) or no (100% nitrogen) oxygen (O_2) on lung morphology. We hypothesized that 100% oxygen would be more injurious to the lung and more likely to cause developmental arrest than 21% oxygen or 100% nitrogen.

Methods: A tracheotomy was performed in fetal sheep at 105d GA (n=8; term ~147d) to insert an endotracheal tube which was connected to a neonatal ventilation circuit. At 110d GA, lung liquid was drained and fetuses were ventilated for 6 hrs using 21% or 100% O_2 or 100% nitrogen with a PEEP of 6 cmH_2O and a PIP of 35 cmH_2O. After this lung liquid was replaced and normal tracheal liquid flow restored. At 117d, ewes and fetuses were humanely killed and lung tissue collected for histological analysis of alveolar development. Unventilated age-matched controls (at 111d and 118d GA) were used for comparison. Data are presented as mean ± SEM.

Results: Ventilation with oxygen (21% and 100%), but not the nitrogen group caused a significant ($p<0.05$) delay in the thinning of inter-alveolar septa compared to age-matched controls. However, all ventilation groups had at least ~35% reduction in secondary crests compared to controls ($p<0.01$). Furthermore, the pattern of elastin deposition appeared to be altered following ventilation in all ventilation groups.

Conclusions: These results indicate that changes in lung structure can occur in response to only 6 hours of ventilation. These results also demonstrate a high FiO_2 may be causal in some aspects of abnormal lung development after very premature delivery, but cannot account for all changes.

PULMONARY VASCULATURE DEVELOPMENT REGULATES ALVEOLAR EPITHELIAL CELL DIFFERENTIATION

Sharon J. Flecknoe[1], Shannon J. Simpson[2], Caitlin L. Filby[1] & Stuart B. Hooper[1]
[1]Dept. of Physiology, Monash University, Vic 3800, [2]Dept. of Zoology, La Trobe University, Vic 3086

Background: Type-I and type-II alveolar epithelial cells (AECs) line the internal surface of the gas exchange region of the lung (alveoli). Type-I AECs provide a large surface area and narrow barrier for gas exchange whereas type-II AECs produce pulmonary surfactant. Given their critical roles in lung function after birth, it is important to understand the factors that regulate type-I and –II AEC development from their undifferentiated precursor cells. From our previous observations of lung development in developing dunnarts, we believe that type-I AEC differentiation is dependent on signalling with capillary endothelial cells. This presentation will discuss some of our previous observations in dunnarts as well as our recent findings following pulmonary vasculature disruption in fetal sheep.

Aims: To determine the relationship between the developing pulmonary vasculature and type-I AEC differentiation.

Hypothesis: We hypothesize that type-I AEC differentiation is dependent on signalling with capillary endothelial cells and that targeted disruption of pulmonary capillaries will inhibit type-I cell differentiation.

Methods:
Dunnarts: Lung tissue was collected from postnatal dunnarts aged between 0 and 100d and processed for electron microscopy. Emphasis was placed on the ultrastructural appearance of the epithelium and developing pulmonary vasculature.

Fetal sheep: Pregnant ewes underwent surgery at ~90 days gestation (GA) to implant left pulmonary artery catheters. From 95 to 104d GA, fetal lungs were embolised via daily injections of microspheres into the pulmonary artery. Age matched controls were used for comparison. Fetal lung tissue was collected at 104d GA and the proportions of AECs analysed using electron microscopy.

Results: Type-I AECs were present at the time of birth in dunnarts, despite their otherwise immature lung structure. However, type-I AEC differentiation coincided with the development of nearby capillaries, with many type-I AECs exhibiting unilateral cytoplasmic projections in the direction of the underlying capillary.

Following embolisation of fetal sheep lungs, the proportion of undifferentiated cells was increased (control: 41.2±6.0%; embolised: 69.3±2.5%) whereas the proportion of type-I AECs was decreased (control: 47.6±7.2%; embolised: 25.9±3.0%) compared to controls.

Conclusion: Partial pulmonary embolisation inhibits type-I AEC differentiation from undifferentiated cells. In combination with our observations of type-I AEC development in dunnarts, this research demonstrates that the pulmonary vasculature plays an important role in the initiation of type-I AEC differentiation.

EFFECTS OF BRIEF VENTILATION ON SMALL AIRWAY STRUCTURE IN THE VERY IMMATURE LUNG

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Background: *In utero* ventilation (IUV) of fetal sheep has been used to study the mechanisms underlying the development of bronchopulmonary dysplasia (BPD) in very immature lungs[1]. Many BPD survivors display impaired lung function in later life[2], suggesting altered development of the small pulmonary airways. In a previous study using IUV, it was shown that 7 days after 12 hours of IUV of very immature ovine lungs, the small airways display thickened epithelium, increased smooth muscle and collagen deposition and increased rates of proliferation and apoptosis[3].

Aim: The present study aimed to characterise small airway structure close to term two weeks after a short period of IUV.

Method: Seven fetal sheep at 125 days of gestational age (DGA; term is ~147 DGA) underwent aseptic surgery during which the trachea was intubated and the lungs were mechanically ventilated for 2 hours using a positive pressure ventilator (Dräger Babylog 8000, positive end expiratory pressure=0mmHg, positive inspiratory pressure=~40mmHg, tidal volume=5ml/kg). Five unventilated age-matched fetuses were controls. Ewes and fetuses recovered well from surgery and remained healthy, with normal blood gases and pH, until necropsy near term (140 DGA), when the fetal lungs were collected for structural analysis.

Results: Small bronchioles, with a basement membrane perimeter (P_{bm}) of 300-1,000µm, which are often implicated in airway hyperresponsiveness, were examined for this study. At 140 DGA there was a 9% reduction in epithelial area, relative to P_{bm}, compared to controls ($p<0.01$) and a 63% reduction in the proportion of epithelial cells undergoing proliferation ($p<0.01$) compared to controls. The area of smooth muscle, relative to P_{bm}, in the outer airway wall was not different between ventilated and control lungs.

Conclusions: This study shows that brief mechanical ventilation can induce airway epithelial remodelling in the very immature lung which persists for two weeks in the absence of continued ventilation. Future studies will examine other aspects of the airway walls.

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NOTES

EFFECTS OF ALCOHOL EXPOSURE DURING LATE GESTATION ON THE FETAL HEART

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Introduction: Numerous studies have demonstrated adverse effects of high levels of fetal alcohol exposure on the developing heart. However, the effects of more moderate levels of alcohol on the fetus are poorly understood. Depending on the timing of the insult, the effects on organ development are likely to be different; a late gestational insult is likely to affect the heart since cardiomyocytes undergo a process of maturation late in gestation.

Hypothesis and Aim: We hypothesized that repeated exposure to moderate levels of alcohol during late gestation would affect the coronary arteries, impair the growth and/or maturation of cardiomyocytes and increase the collagen deposition in the fetal heart. Our aim was to determine the effects of daily fetal exposure to a moderate dose of alcohol on the media to lumen ratio of coronary arteries, the percentage of collagen deposition in the myocardium, and the number of cardiomyocyte nuclei.

Methods: Pregnant ewes were infused with either ethanol or saline from gestational days 95 to 134 of the 147 day pregnancy. The fetuses were killed and heart tissue was collected from 8 alcohol exposed fetuses and 7 control fetuses. Hearts were weighed, dissected and the volumes of the walls of the ventricles were measured using the Cavalieri Principle. The hearts were then examined for collagen deposition and the media to lumen ratio of coronary arteries determined by staining with Picrosirius red and analysing the sections using image analysis. Cardiomyocyte nuclei number was stereologically determined.

Results: Our results show that there are no significant differences in cardiomyocyte nuclei number, myocardial collagen deposition or media to lumen ratio of the coronary arteries in the hearts of fetuses exposed to alcohol compared to control fetuses. However, when heart weight to body weight ratio and ventricular tissue volume to body weight ratio were compared between alcohol exposed fetuses and controls, there was a significant increase in both ratios (relative heart weight: 9.04±0.65 vs 7.22±0.51g/kg, respectively; $p=0.05$ and relative ventricular tissue volume: 5.66±0.12 vs 5.23±0.13mm^3/g, respectively; $p=0.03$).

Conclusion: The results showed that maternal administration of moderate levels of alcohol during late gestation leads to hypertrophy of the fetal heart. This does not appear to be due to an increase in the number of cardiomyocytes or increased deposition of extracellular matrix. However, cardiomyocyte size is yet to be examined.

DOES ALTERED NUTRITION AND GROWTH FOLLOWING PRETERM BIRTH AFFECT CARDIOVASCULAR, RESPIRATORY AND METABOLIC FUNCTION AND STRUCTURE IN LATER LIFE?

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Background: In developed countries, such as Australia, 6-12% of all live-born babies are born before term. Not only does preterm birth pose a major physiological challenge for the infant, due to incomplete development or injury to important organs, appropriate nutrition of the preterm infant remains a significant challenge; most preterm infants are growth-restricted by term-equivalent age. In addition, recent studies have found significant associations between postnatal growth patterns and an increased risk of adult onset disease. Accelerated postnatal growth has been shown to increase the risk of developing obesity and type ll diabetes in adults whereas a slow growth rate has been associated with an increased risk of developing adult cardiovascular diseases, such as stroke. Studies in sheep have shown that slow postnatal growth alters the structure of the lung parenchyma and airways in the adult[1, 2].

Nutrition and growth during early life can have important life-long consequences for health outcomes. Although growth trajectory from birth to maturity is known to be of importance, there is very little data on how organ development and function are affected by differing growth rates following premature birth, *per se*, at a time when many organs are still maturing.

Aims/Hypothesis: To determine how the structure and function of major organs are altered by reduced or increased postnatal nutrition in lambs born prematurely.

Methods: We will use an established model of preterm birth, *per se*, in sheep which avoids the potentially confounding effects of other medical interventions, such as corticosteroid administration and mechanical ventilation. In this model, ewes are induced to deliver vaginally at 133 days of gestation (term ~ 145 days GA) which is the earliest gestational age at which lambs do not require ventilatory support; this age is analogous to 32–34 weeks gestation in humans. Lambs will be randomly assigned to either an under-nutrition, over-nutrition or a control group. *Under-nutrition*: From previous growth data records in our lab, we are aiming to achieve a growth rate below the 10th percentile, or approximately 125g/day. *Over-nutrition*: From previous growth data records in our lab, we are aiming to achieve a growth rate above the 90th percentile, or approximately 270g/day. Morphometric measurements and blood samples (for lipid profiles) will be taken weekly. At 8 weeks post-term equivalent age (PTEA) we will assess cardiovascular function (basal arterial pressure, baroreflex test), metabolic function (glucose tolerance test, hyperinsulinaemic euglycaemic clamp) and lung function (carbachol challenge, multiple breath nitrogen washout test). At 10weeks PTEA lambs will be killed and major organs taken for structural analysis.

Outcomes: Preliminary studies have shown the feasibility of altering growth rates in preterm lambs. We anticipate that an altered postnatal nutrition, either accelerated or delayed, following moderate preterm birth, will modify the structure and function of key organs at 2 months of age.

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PRENATAL ANXIETY & MATERNAL SENSITIVITY … ASSOCIATIONS WITH INFANT CORTISOL & REACTIVITY

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Background: There is increasing evidence that maternal stress and anxiety during the perinatal period may impact on the fetus and the infant after birth. This study explored associations between maternal pre- & postnatal anxiety, maternal sensitivity, & infants' behavioural & physiological responses to social challenge.

Aims/Hypothesis: The rationale underlying this study is the fetal programming hypothesis. This states that maternal stress or anxiety during sensitive periods of fetal development can permanently alter or "program" stress regulatory systems in offspring.

Methods: Participants were 84 mothers & their 7 month-old infants. Women were originally recruited during pregnancy using a stratified sampling approach to ensure that anxious pregnant women were adequately represented in the study. Stratification was based on scores on the Antenatal Risk Questionnaire, a questionnaire routinely administered during booking-in at the hospital's antenatal clinics. The Still Face Test (SFT) was administered during the seventh postnatal month to assess i) maternal sensitivity and ii) infant behavioural and cortisol reactivity. Observed maternal behaviours were rated using Murray's Global ratings of Mother-Infant Interaction.

Results: The SFT was effective in eliciting a stress response in 7 month-old infants. Although maternal anxiety was marginally associated with parenting sensitivity, the interaction between prenatal anxiety and maternal sensitivity had a significant effect on infant outcomes. Prenatal anxiety was related to heightened infant reactivity but only when the quality of observed maternal care was also rated as poor. Infants' patterns of cortisol response following the SFT were also found to differ as a function of maternal prenatal anxiety.

Conclusions: This evidence suggests that individual differences in infant stress reactivity may originate before birth. This may have clinical implications for the development of psychopathology later in life.

INTERVENTIONS TO PREVENT DIABETES AFTER IUGR – PRELIMINARY OUTCOMES IN THE TWIN IUGR LAMB.

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Background: Intrauterine growth restriction (IUGR) increases the risk of later diabetes, in part via inadequate increases in insulin secretion to compensate for insulin resistance, implying impaired capacity for β-cell plasticity. In the rat, placental-restriction of fetal growth similarly leads to diabetes and loss of β-cell mass and function after birth, partly via epigenetic modifications. In this model of IUGR in the rat, exendin-4 treatment in the neonate, but not after weaning, can reverse epigenetic changes to the Pdx-1 promoter and prevent later diabetes. Reduced methyl nutrient availability and impaired use for DNA occurs in placental restriction in the rat and is implicated in a range of epigenetic changes in various tissues. We therefore tested whether exendin-4 or maternal methyl nutrient supplementation could prevent or reverse impairment of insulin action during or after IUGR in sheep. We chose this species because, as in humans, the pancreas is more mature at birth than in the rat.

Aims/hypotheses: We hypothesised that maternal nutrient supplementation to increase circulating methyl donors and cofactors in late gestation, or neonatal exendin-4 treatment, would improve postnatal insulin action after restricted fetal growth due to twinning in the sheep.

Methods: Glucose tolerance and insulin secretion (IVGTT), insulin sensitivity (hyperinsulinaemic euglycaemic clamp), and body composition (PM) were measured in lambs at 12-16 d of age:

- Control lambs from singleton-bearing ewes (CON)
- IUGR lambs (IUGR), one twin of non-supplemented twin-bearing ewes
- IUGR + exendin-4 lambs (IUGR + ex-4), other twin of non-supplemented twin-bearing ewes), injected s.c. daily from 1 d old with 1 nmol exendin-4 per kg
- IUGR + gestational methyl donor plus cofactor supplement (IUGR + methyl), twins from ewes supplemented with rumen-protected methionine (rate), folate (rate), sulphur (rate, to increase ruminal sulphur amino acid production), and cobalt (rate, to increase ruminal Vitamin B12 production)
-

Results: Exendin-4 increased glucose-stimulated insulin secretion by 155% and decreased fat accumulation by 60% in IUGR twin lambs, and methyl donor and cofactor supplementation increased insulin sensitivity by 40% in IUGR twin lambs, compared to untreated IUGR twins.

Conclusions: Neonatal exendin-4 and maternal late gestation methyl plus cofactor supplementation improve neonatal insulin secretion and sensitivity respectively in the twin IUGR lamb. We now need to determine whether this persists to improve insulin action, secretion and plasticity in the adult.

NOTES

CHARACTERISING AN OVINE MODEL OF CHRONIC CHORIOAMNIONITIS: EFFECTS ON OFFSPRING FROM BIRTH TO 2 MONTHS OF AGE. A PRELIMINARY REPORT

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Background: Chorioamnionitis, an infection of the chorion/amnion, is one of the primary risk factors for preterm delivery in humans. Chorioamnionitis has been shown to have a deleterious effect on the human fetus, including cerebral damage (particularly in regards to neurodevelopmental outcome[1] and visual cortex performance. However, some studies have shown that a strong fetal immune response to chorioamnionitis is associated with improved survival after preterm birth in humans[2]. In the sheep, chorioamnionitis has been induced via LPS injected into the amniotic fluid but it does not result in preterm birth[3]; however there have been no studies investigating the effect of chronic *in utero* exposure to LPS on postnatal growth and organ development (focusing particularly on the heart) in offspring born before term.

Aim/Hypothesis: To examine the effect of chronic intra-amniotic LPS exposure on body growth, survival and organ development in lambs delivered preterm. We hypothesise that survival will be improved, and heart development will be altered in preterm lambs exposed to chorioamnionitis.

Methods: LPS was infused continuously into the amniotic fluid using surgically implanted osmotic mini-pumps from day 110 to 133 days, when preterm birth was induced. Four ewes carrying singleton fetuses were given LPS. Eight preterm lambs (unexposed to LPS) were used as controls. We measured body growth, heart weight (including left ventricle, right ventricle, and atria), arterial pressure and heart rate.

Preliminary Outcomes: LPS infused lambs demonstrated superior survival compared to preterm controls. The survival rate for male LPS treated preterm lambs was 66% (2 of 3); in our previous studies with no LPS administration, the majority of male preterm lambs die (male untreated preterm survival is 14.3% (1 of 7), female untreated preterm survival is 78% (7 of 9), with an overall survival rate for both genders of 50% (8 out of 16)). There was no difference in birth weight between LPS treated and control lambs (Controls = 3.56±0.29 kg, LPS = 3.30±0.28 kg, p=0.280), however LPS treated lambs were on average 5.3kg heavier than preterm controls at post mortem at 9 weeks post-term equivalent age (Controls = 18.20±1.21 kg, LPS = 23.53±2.10 kg , p=0.05). There was no difference between preterm controls and LPS treated preterm lambs on the basis of heart, left ventricle and right ventricle weights. After correcting for body weight, LPS treated animals had significantly smaller right ventricles (Controls = 1.13±0.06 g/kg, LPS = 0.79±0.11 g/kg, p=0.044) and atria (Controls = 0.73± 0.06 g/kg, LPS = 0.42±0.05 g/kg, p=0.0.01) compared with preterm controls. There was no difference between groups in the weights of the left ventricles. Future studies will include a detailed assessment of cardiac muscle development.

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THE RENAL RESPONSE TO WATER RESTRICTION IS UNIMPAIRED IN THE OFFSPRING OF EWES WITH RENAL INSUFFICIENCY

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Background: There is evidence that osmoregulation may be programmed by the intrauterine environment. Lambs born to ewes that were water restricted for the last third of pregnancy had a higher plasma osmolality threshold for AVP secretion and increased arterial blood pressure in response to infusion of hypertonic saline[1]. In contrast to maternal water restriction, in which the transplacental fluid flux to the fetus is reduced, we have developed a model of maternal renal insufficiency in which fetuses are exposed to high rates of fluid flux across the placenta, and consequently have high urine flow rates.[2]

Hypothesis: Offspring of ewes with renal insufficiency would have an impaired homeostatic response to water restriction.

Methods: Non-pregnant ewes were subtotally nephrectomised prior to mating as previously described.[3] Female offspring (n=5 STNx and n=5 controls) were studied at 6 months of age. At least 4 days prior to the first experiment, femoral arterial and venous catheters were inserted under general anaesthesia. On the evening before each experiment an indwelling bladder catheter was inserted via the urethra. Baseline measurements were conducted when the animals had access to water *ad libitum.* Water restriction measurements were made at the same time of day at ~ 43 h after water had been removed from the metabolic cage.

Results: During water restriction, plasma sodium, chloride, osmolality and protein levels increased by similar amounts in both groups ($P<0.05$). Glomerular filtration rate (measured as the clearance of endogenous creatinine) and urine flow rate fell in both groups ($P<0.05$) while urinary osmolality rose ($P<0.05$). Plasma AVP levels rose in both groups as did plasma renin levels, although the rise in plasma renin levels tended to be greater in the STNx offspring ($P=0.09$). Although mean arterial pressure did not change in either group, heart rate fell in both groups ($P<0.05$). In the STNx offspring only, there was an inverse relationship between plasma AVP levels and heart rate ($r=0.84$, $n=10$, $p=0.002$)

Conclusions: It is concluded that the renal response to absence of drinking water for ~ 43h was unaltered in 6 month old offspring of ewes with renal insufficiency. However, there may be subtle differences in cardiovascular control in these offspring which warrant further exploration.

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THE EFFECT OF CHORIOAMNIONITIS ON THE DEVELOPING HUMAN KIDNEY

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Background: Chorioamnionitis is one of the major causes of preterm birth. A major concern associated with fetal exposure to chorioamnionitis is the accompanying systemic fetal inflammatory response which may subsequently lead to tissue injury. Whether fetal exposure to chorioamnionitis adversely impacts on nephrogenesis *in utero* is currently unknown. Investigation of its effects on the developing kidney is of great importance as the vast majority of the functional units of the kidney (nephrons) are formed during the third trimester of gestation when chorioamnionitis is commonly present.

Aim: The aim of this study was to examine the affect of chorioamnionitis *in utero* on nephrogenesis in the developing human kidney.

Methods: Archived human kidneys of stillborn preterm and term fetuses that displayed evidence of chorioamnionitis (n = 40) and appropriately grown gestational controls that had died acutely *in utero* (n = 23) were examined. The subjects were divided into four gestational age categories (18-22 weeks, 23-29 weeks, 30-36 weeks and ≥37 weeks). Glomerular generations, renal corpuscle size, nephrogenic zone thickness and glomerular maturation were examined using image analysis.

Results: The nephrogenic zone thickness within the kidneys of fetuses exposed to chorioamnionitis *in utero* in the 18-22 and 23-29 week gestational age categories was approximately double that of the gestational controls ($P<0.0001$). In the later gestational age categories (30-36 and ≥37 weeks) there was no difference in nephrogenic zone thickness in the kidneys of fetuses exposed to chorioamnionitis when compared to their gestational controls. There were no differences in the number of glomerular generations, renal corpuscle size and glomerular maturation between fetuses exposed to chorioamnionitis and their gestational controls across all gestational age categories.

Conclusions: Chorioamnionitis exposure in earlier time-points in gestation appears to influence fetal nephrogenesis leading to an increase in the thickness of the nephrogenic zone. The consequences of this on nephron number are currently being investigated.

THE EFFECTS OF HYPEROXIA ON KIDNEY DEVELOPMENT

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Background: Preterm birth is associated with many developmental complications, especially in organs that continue to develop following preterm delivery such as the lung and kidney. Nephrogenesis occurs in late gestation at a time when preterm infants are already delivered. Recent studies in our laboratory have shown that many of the glomeruli located in the outer renal cortex of preterm kidneys are abnormal, suggesting that glomeruli formed in the extra-uterine environment are vulnerable to preterm birth. The cause of these abnormalities is unknown but may relate to factors in the postnatal care of the preterm infant leading to deleterious effects in the kidney.
In this regard, hyperoxia in the preterm neonate has been previously shown to disrupt lung and eye organogenesis, resulting in bronchopulmonary dysplasia and retinopathy of prematurity. However, the effects of hyperoxic exposure on renal development has not been explored.

Aim: The aim of this study was to determine the effects of hyperoxia on nephrogenesis in the mouse kidney at postnatal day 7. Similar to the preterm infant nephrogenesis is ongoing at birth in the mouse.

Methods: BLK6 mice pups were delivered at term, and maintained in a controlled normoxic (21% oxygen) or hyperoxic (65% oxygen) environment for 7 days postnatally. One male and one female pup from each litter was then culled at postnatal day 7 (control n=10; hyperoxic n=7). The right kidney from each pup was embedded in paraffin and sections were analysed using Image Pro analysis software to determine average glomerular size and stage of maturity (graded from stage 0 to 3).

Results: There was no statistically significant difference in glomerular size between hyperoxic and control treatment groups, and no influence of gender. In all control kidneys nephrogenesis was complete at 7 days after birth. Interestingly, however, in many of the male hyperoxic kidneys there was still evidence of a nephrogenic zone at postnatal day 7 and a number of the glomeruli were observed to be in the most immature stage of development, thus suggestive of delayed maturation in the hyperoxic kidneys. In two of the three female hyperoxic kidneys, there was a high proportion of abnormal glomeruli, with the majority of glomeruli exhibiting a dilated Bowman's space (Figure 1).

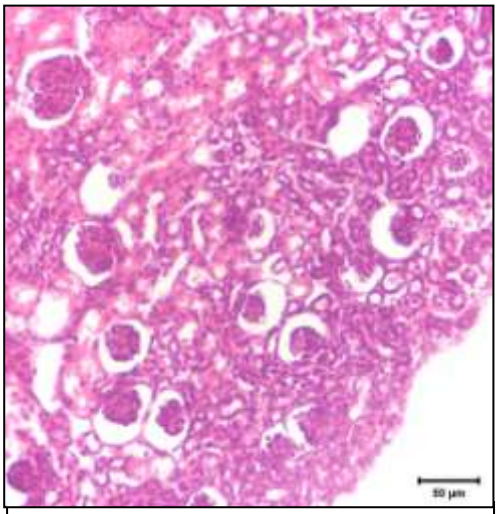

Figure 1: Abnormal glomeruli in the renal cortex of a hyperoxic exposed mouse kidney.

Conclusion: Exposure to hyperoxia postnatally appears to delay glomerular maturation in the mouse kidney and sometimes leads to abnormalities in glomerular morphology.

NOTES

EFFECTS OF UTERINE DEVELOPMENT ON PULMONARY VASCULAR DEVELOPMENT AND FUNCTION – THE ORIGIN OF POSTNATAL CARDIOPULMONARY DISEASE?

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Background: Cardiopulmonary disease is the greatest contributor to neonatal morbidity and mortality in Australia's >21,000 preterm infants born annually. The focus on the development of diseases such as bronchopulmonary dysplasia (BPD) and persistent pulmonary hypertension of the newborn (PPHN) has been on the immediate postnatal period. Recent studies suggest that the uterine environment can alter pulmonary vascular development predisposing the preterm infant to cardiopulmonary disease.

Aims/Hypothesis: We aimed to investigate the effects of inflammation and corticosteroids *in utero*, on pulmonary vascular development and function in preterm lambs. We hypothesise that the antenatal environment can alter pulmonary vascular development and function, subsequently altering cardiopulmonary haemodynamics, thus predisposing the preterm to BPD and PPHN.

Methods: Preterm lambs were exposed to intra-amniotic (IA) injection of ureaplasma serovars 3 and 6 at 55d GA (term ~150 d; chronic inflammation), IA lipopolysaccharide at 121d (10mg; acute inflammation) or maternal intramuscular corticosteroids (celestone chronodose 0.5 mg/kg) at 121 or 126 d GA. All lambs were delivered at 128d, samples collected for morphometry and molecular assessment, and compared to relevant saline controls. Morphometric analyses of pulmonary resistance arterioles (<50 μm) was conducted on 5 μm paraffin sections stained with smooth muscle α-actin. Adventitial fibrosis was determined on lung sections stained with Trichome blue as assessed using qualitative scoring. Endothelial proteins PECAM-1, VEGF -165 and -188 and Tie-2 mRNA expression was measured using RNAse protection assay. eNOS, VEGFR-2 and protein expression was quantitated using ELISA. One-way ANOVA was used to compare groups, with significance accepted at $p<0.05$.

Results: Acute Inflammation: Medial smooth muscle hypertrophy and increased adventitial fibrosis were observed 7 days after IA LPS. Endothelial protein expression was decreased within 2-4 d after IA LPS but was largely resolved by 7 d. Lambs delivered and ventilated at 128 d had pulmonary and systemic hypertension, right ventricular hypertrophy and maintained a right-to-left shunt through the ductus arteriosus.
Chronic Inflammation: Medial smooth muscle area and thickness was not different between chronic inflammation and controls. Level of adventitial fibrosis was not different between groups, nor was endothelial protein mRNA expression.
Corticosteroids: Medial smooth muscle area and thickness were significantly decreased 2 and 7 d after celestone chronodose compared to controls. Adventitial fibrosis was not different between groups.

Conclusions: Acute, but not chronic inflammation *in utero* alters pulmonary vascular development which results in physiological consequences at birth in preterm lambs. Corticosteroids 2 or 7 d prior to delivery results in altered pulmonary vasculature which may also have consequences for the preterm neonate. The uterine environment is critical for pulmonary vascular development and function.

EFFECT OF LIFE-LONG VITAMIN D DEFICIENCY ON CARDIAC FUNCTION AND SUSCEPTIBILITY TO ISCHEMIA/REPERFUSION INJURY IN THE ADULT RAT HEART

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Background: We have recently demonstrated that life-long vitamin D deficiency increases left ventricular wall volume, cardiomyocyte number and size, and the proportion of immature mononucleated cardiomyocytes at 4 weeks of age. Thus, exposure to vitamin D deficiency *in utero* and early life leads to delayed maturation and subsequent enhanced growth (proliferation and hypertrophy) of cardiomyocytes in the left ventricle. The implications of these changes on cardiac function later in life are unknown.

Aim: The aim of the present study was to investigate the effect of vitamin D deficiency in adult rats on cardiac function and the susceptibility to ischemia/reperfusion injury.

Methods: Four week old Sprague-Dawley female rats were fed either a vitamin D deplete or vitamin D replete (control) diet for 6 weeks prior to pregnancy, during pregnancy and throughout lactation. At weaning the offspring remained on their respective diets until adulthood. Hearts of 16 week old male and female offspring (*n* = 8/group) were mounted on a Langendorff apparatus. Basal heart rate (HR), coronary flow, rate of contraction (+dp/dt) and relaxation (-dp/dt) and response to isoprenaline were recorded. The hearts were then subjected to 20 minutes of ischemia and 1½ hours of reperfusion. At the end of the reperfusion period the left ventricle was sliced and incubated in 1% 2, 3, 5 triphenly tetrazolium chloride solution (TTZ), to determine infarct area using computerized planimetry.

Results: Left ventricle (LV) weight of vitamin D deficient females was increased (p=0.02), but unaltered in the males. Basal cardiac function (HR, +dp/dt, -dp/dt) was not different. However, basal coronary flow tended to be lower in hearts of vitamin D deficient rats and this was significant in males (p=0.01). Isoprenaline increased HR, +dp/dt and -dp/dt. The isoprenaline-induced, increase in HR tended to be greater in vitamin D deficient males (p=0.06), but there was no differences in contractile function between groups. After 55 minutes of reperfusion, HR had declined by 30% of that before ischemia, in both males and females, with HR being higher in vitamin D deficient males compared with control males; there were no differences between the female groups. Basal coronary flow was halved and now not different between control and vitamin D deficient groups. Basal cardiac function -dp/dt had declined significantly, but was not different between groups, although the rate of relaxation was significantly slower in the vitamin D deficient males (p=0.04), but not females. Strikingly, infarct area was 2-fold greater in vitamin D deficient hearts of both males and females (p=0.006 and p=0.03, respectively) compared with their control counterparts.

Conclusion: Basal and stimulated heart function was not altered although coronary flow was significantly reduced in vitamin D deficient rats. The hearts of vitamin D deficient rats were particularly susceptible to ischemia/reperfusion injury. Dysregulation of coronary flow and the extent of vascularisation may be factors which contribute to the increased susceptibility to ischemia/reperfusion injury.

CONTRACTILE PROPERTIES OF EXCISED MURINE PULMONARY ARTERIES TO VARIOUS VASOACTIVE AGENTS COMMONLY USED IN NEONATAL UNITS

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Background and Objectives: Proper management of pulmonary circulation is crucial in treating sick newborn infants. However, there is only limited basic knowledge on how pulmonary arteries react to inotropes. The aim was to study contraction/relaxation responses of excised murine pulmonary arteries to various inotropic agents commonly used in neonatal units.

Materials and Methods: First main branch of pulmonary arteries (PA) were dissected from the lungs of adult female SD rats (~45 weeks old) and cut into rings (diameter, ~1mm; length, ~1mm) in modified Krebs-Ringer bicarbonate buffer. Artery rings were suspended in organ chambers filled with 4mL of buffer maintained at 39 ℃ aerated with gas of 95% O_2 and 5% CO_2. Changes in vessel tension of PA were measured with incrementally increasing doses of each agent: dopamine (DOA), epinephrine (EP), norepinephrine (NE), isoproterenol (ISP) or arginine vasopressin (AVP), and compared them with responses of iliac arteries (IA; representing systemic arteries). All values of tension changes were expressed as %tension (in reference to the tension change after depolarizing contraction induced by K^+ 60mEq/L).

Results: Iliac arteries showed contraction with all the tested agents. Pulmonary arteries contracted with DOA, EP and NE, but not with ISP or AVP. Both EC_{50} (concentration of 50% maximum contraction) and %tension at EC_{50} of pulmonary arteries to DOA, EP and NE were lower than those of iliac arteries.

Agents	EC_{50} IA	(1) %tension at EC_{50} IA	EC_{50} PA	(2) %tension at EC_{50} PA	(2)/(1)
DOA	$10^{-4.8}$	84	$10^{-5.4}$	21	0.25
EP	$10^{-7.2}$	156	$10^{-7.6}$	37	0.24
NE	$10^{-7.1}$	125	$10^{-7.6}$	39	0.31
ISP	$10^{-4.6}$+	66+	-	0	0
AVP	$10^{-9.5}$	86	-	0	0

Conclusions: Pulmonary arteries contracted with DOA, EP and NE at concentrations presumably achieved by clinical dosing regimen which are higher than normal physiological plasma levels. Both ISP and AVP induced contraction with iliac arteries but not with pulmonary arteries. AVP had the lowest PA to IA contraction ratio at clinically achievable plasma levels and may be efficacious in treating persistent pulmonary hypertension of the newborn.

BEING BORN SMALL REDUCES THE NUMBER OF CARDIOMYOCYTES WHICH CAN BE INCREASED BY IMPROVING POSTNATAL NUTRITION

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Background: Cardiomyocytes cease proliferating soon after birth when they become terminally differentiated. A reduced complement of cardiomyocytes at birth can adversely affect the functional and remodelling capacity of the heart.

Aim: We aimed to determine whether uteroplacental insufficiency and fetal growth restriction reduces the number of cardiomyocytes in hearts of rat offspring, and if so, whether this can be overcome by restoring early postnatal nutrition during lactation.

Methods: We studied male offspring from mothers that underwent bilateral uterine vessel ligation (Restricted) or sham surgery (Control) on day 18 of gestation. Control and Restricted pups were cross-fostered onto Control (normal lactation) or Restricted (impaired lactation) mothers 1 day after birth. At 7 days of age cardiomyocyte number was determined stereologically.

Results: There was a significant reduction ($p=0.02$) in body weight and heart weight of Restricted-on-Restricted offspring; this was accompanied by a 28% reduction ($p<0.05$) in total cardiomyocyte number. Providing a normal lactational environment to restricted offspring (Restricted-on-Control) improved postnatal growth such that body weights, heart weights and cardiomyocyte number were not significantly different compared to Control-on-Control offspring.

Conclusion: Growth restriction due to uteroplacental insufficiency adversely impacts on heart cardiomyocyte number postnatally. Importantly, improvement of lactational nutrition, at a time when the cardiomyocytes are still undergoing proliferation, prevents the deficit in cardiomyocyte number associated with growth restriction.

ASSESSMENT OF BAROREFLEX SENSITIVITY IN THE FREQUENCY AND TIME DOMAINS DURING SLEEP IN TERM INFANTS

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Background: Non-invasive assessment of baroreflex sensitivity (BRS) during sleep relies on techniques that assess spontaneous blood pressure (BP) and heart period (HP) fluctuations, such as spontaneous sequence analysis (time domain) and cross-spectral analysis (frequency domain). However, in infants the sequence method may be limited as analysis is based on adult criteria. Of major concern is the HP time delay of the baroreceptor loop (Δt) (equal to 1 beat in adults) which may differ in infants. As cross-spectral analysis can be used to estimate both BRS and Δt, the application of Δt estimated in the frequency domain in spontaneous sequence analysis could potentially improve BRS estimated in the time domain.

Aims: The aims of this study were to: 1) assess BRS and Δt in the frequency domain; 2) determine the effect of postnatal age (PNA), sleep state and sleeping position on Δt; 3) apply the estimated Δt in the sequence method; and 4) compare BRS estimated in the time and frequency domains.

Methods: Polysomnography was performed on 20 term infants at 2-4 wks, 2-3 mo and 5-6 mo PNA. BP was measured continuously for 2 min during both quiet sleep and active sleep in both supine and prone positions. Cross spectral analysis between systolic arterial pressure (SAP) and HP fluctuations was used to estimate BRS(sp) and Δt in the frequency domain. Based on the estimated Δt, spontaneous sequence analysis was performed using three different criteria. BRS(i) Δt = 1 beat, BRS(ii) Δt = 5 beats and BRS(iii) Δt is variable (1-12 beats, selected based on peak r^2). Effects of sleep state, position and PNA were tested using one way ANOVA. Correlations between BRS(sp) and BRS (i, ii, & iii) were tested using Spearman correlation. Correlation coefficients were compared with a Fisher's Z test.

Results: Using cross spectral analysis we observed that Δt = 3.4±0.2s (~5-6 beats) for all epochs analysed (n=100). Sleep state and sleeping position had no effect on Δt. However, Δt was shorter at 2-4 wks PNA (3.0 ± 0.4s) compared to 5-6 mo PNA (3.8±0.3s), $p<0.05$. There was marked variability in values of Δt with estimates ranging from 0.1–8.4s (n=100). BRS(sp) had the strongest correlation with BRS(iii) $r^2=0.526$, $p<0.001$, followed by BRS(ii) $r^2=0.462$, $p<0.001$ and then BRS(i) $r^2= 0.359$, $p<0.001$. There was no significant difference between the r^2 values.

Conclusions: For the first time we have shown that BRS estimated from time and frequency domains correlate significantly and can be applied in infant studies. We found that the Δt in infants was longer compared to that reported in adults, and therefore sequence analysis criteria should account for these differences. Together these methods could potentially provide a useful analytical tool to provide information on sleep-related pathologies in which impaired cardiovascular control has been implicated.

NOTES

CARDIOVASCULAR AUTONOMIC FUNCTION IS ALTERED IN PRE-PUBERTAL LAMBS BORN PRETERM

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Background: Adults born even moderately preterm have higher blood pressure and greater cardiovascular morbidity and mortality than those born at term. Elevated resting heart rate (HR) and reduced heart rate variability (HRV) are indirect measures of autonomic function, and also predict those at risk. HRV may be assessed in the time domain, using the standard deviation of differences in successive RR intervals on ECG (SD delta NN, where reduced HRV reflects reduced parasympathetic activity) and in the frequency domain, using power spectral analysis of RR variation. Low frequency [LF (0.04 – 0.14 Hz), reflecting sympathetic drive] and high frequency [HF (0.15-0.4 Hz), reflecting parasympathetic drive] contributions to overall HRV are converted to normalised units (LFnu and HFnu).

Aims / Hypothesis: To compare resting HR and HRV in lambs born preterm and at term.

Methods: Ewes carrying singleton lambs were either given dexamethasone to induce preterm delivery or allowed to deliver spontaneously at term. At 4 months subcutaneous ECG leads were placed and resting ECG recordings were obtained using LabChart6 (ADInstruments, Dunedin, NZ). Resting HR and frequency and time-domain analyses of HRV were calculated from a five minute rhythm strip. Data were analysed using ANOVA with Fisher *post hoc* test and multiple linear regression and are presented as mean±SE.

Results: We studied 11 preterm (5 female, 6 male) and 15 term lambs (5 female, 10 male). Preterm lambs delivered at 137 ± 0.2 d; term lambs delivered at 147 ± 0.3 d. Compared with lambs born at term, lambs born preterm had higher resting HR (120 ± 5 vs 103 ± 4 beats per minute, p=0.02), lower SD delta NN (26.3 ± 5.6 vs 42.6 ± 4.8 ms, p=0.04) and higher LFnu (51 ± 6 vs 30 ± 5 normalised units, p=0.006). There were no differences between groups in HFnu or LFnu/HFnu, and no relationships between any measures of HRV and sex, birth weight, early growth velocity or current weight.

Conclusions: Pre-pubertal lambs born preterm have increased heart rate, reduced heart rate variability and evidence of reduced parasympathetic and increased sympathetic drive. These changes are not explained by sex, size at birth or postnatal growth. Altered cardiovascular autonomic function may be a mechanism through which preterm birth predisposes to later hypertension and cardiovascular morbidity and mortality.

EFFECT OF VENTILATOR GAS FLOW RATE AND MODE OF VENTILATION ON EARLY LUNG INJURY MARKERS IN THE PRETERM LUNG

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Background: We previously have shown that low bias gas flow rates in ventilated preterm lambs lengthen inspiratory time (Ti), improve the ventilator efficiency index, and reduce histological evidence of lung injury and mRNA levels of genes reflective of early lung injury compared with high flow rates. However, a long Ti, as may occur with ventilation strategies involving a plateau inspiratory phase, has been associated with increased risk of pneumothoraces in preterm neonates.

Aims: To determine the effect of bias gas flow rate and a plateau inspiratory phase on histological and molecular markers of lung injury.

Methods: Following antenatal glucocorticoids to the ewe, 31 lambs (110-112 d gestation, term=147 d) were randomised to low or high bias gas flow rates (4 or 18 L/min) with either PSV+VG (inspiration terminates once flow falls to 15% of peak inspiratory flow) or SIPPV+VG (inspiration maintained for the set Ti) mode. Lambs' heads were delivered via a hysterotomy, but placental circulation was maintained. Lambs were ventilated with air for 2 hr (Dräger Babylog 8000*Plus*, tidal volume 10 ml (~5ml/Kg), PEEP 6 cmH_2O, rate 30 breaths/min, set Ti 1 sec). Lungs were pressure fixed and tissue was prepared for histological analysis or measurement of early growth response (*EGR)-1*, connective tissue growth factor (*CTGF*) and cysteine-rich (*CYR)-61* by quantitative RT-PCR with 18s as the housekeeping gene. Data were analysed by nested ANOVA or two-way ANOVA with Tukey HSD as appropriate.

Results: Ventilation up-regulated mRNA levels of all three genes, with levels highest in the PSV18 and SIPPV4 groups for *CTGF* and *CYR61*. The interaction effect between flow rate and mode of ventilation was significant for *CTGF* (p=0.02). A similar pattern of interaction was seen for septal crest density and space occupied by lung tissue, which were both impaired in the PSV18 and SIPPV4 groups.

	Control(n=5)	PSV4(n=5)	PSV18(n=5)	SIPPV4(n=6)	SIPPV18(n=5)
EGR1	1 (0.07)	5.3 (1.7)	9.0 (2.2)**	7.4 (0.9)**	10.4 (3.2)**
CTGF	1 (0.18)	0.8 (0.3)	1.9 (0.4)	1.9 (0.3)	1.2 (0.5)
CYR61	1 (0.2)	1.8 (0.4)	2.7 (1.0)	2.7 (0.6)	1.9 (0.1)
Septal crest	5.2 (1.2)	5.1 (1.24)	3.6 (1.6)*	4.2 (1.2)*	7.0 (1.7)**
Lung tissue	36.3 (3.6)	39.8 (4.3)*	43.4 (5.3)**	44.0 (4.4)**	38.1 (4.2)

Table 1: mRNA levels for *EGR1*, *CTGF* and *CYR61* expressed as fold change of controls, septal crest density and space occupied by lung tissue (expressed as % of total area) [mean (SEM)]. $^{*}p$<0.05 and $^{**}p$<0.01 compared with unventilated controls.

Conclusion: These data suggest that duration of exposure influences the effects of bias gas flow rate on lung injury. This requires further investigation.

HOW MIGHT MOTOR CORTEX DEVELOPMENT INFLUENCE SPECIFIC LEARNING DIFFICULTIES IN CHILDREN BORN PRETERM?

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Background and Aims: The PREMOCODE study has been investigating motor cortical and cognitive development in 300 12 year old children born after 24 – 41 weeks gestation at the Women's & Children's Hospital. Emerging evidence suggests even the mildly preterm experience co-morbid motor and cognitive dysfunction at school age, which affects their educational achievement. The major aims of PREMOCODE are to differentiate the relative influences of gestation length (GA) and fetal growth (i.e. birthweight centile; BW%) on motor and cognitive development in childhood, and to determine if the motor and cognitive dysfunction seen in non-cerebral palsy preterm children shares a common underlying physiology.

Methods: Assessments have included transcranial magnetic brain stimulation (TMS) to assess motor cortical (M1) neurophysiology and the Woodcock Johnson III Tests of Cognitive Abilities. BW% is calculated using the GROW centile calculator.

Results: For every week GA is shortened or centile BW% is reduced, M1 excitability is reduced in both motor hemispheres at age 12 years. The neural projection from the left hemisphere M1 to the right hand is preferentially weakened by shortened GA, while reductions in BW% preferentially weaken the right M1. Multiple linear regressions show that GA and BW%-associated reductions in M1 function predict poorer language comprehension, concept formation, auditory working memory and lower general intellectual ability. Low BW% but not GA affects phonetic coding.

Conclusions: Even in the mildly preterm, reductions in GA and BW% are associated with delayed or abnormal M1/corticospinal development that is still evident at the end of the first decade. This abnormal development of cortical motor areas is associated with dysfunction in a number of cognitive abilities required for reading and language development. Based in these preliminary findings, we have newly hypothesised that the specific learning difficulties preterm children have in the reading and language domains are directly related to the abnormal development of their brain motor areas, including M1 and the ventral premotor cortex i.e. preterm children may have abnormally-developed mirror neuron systems. Though still controversial, M1 is now thought to contribute to language perception in humans *via* either intrinsic mirror neurons or by input to M1 from mirror neurons in the premotor and/or Broca's areas. In this talk, I will discuss the rationale for these new hypotheses based on the findings from the PREMOCODE study.

THE EFFECT OF VOLUME LOADING ON CARDIOPULMONARY HAEMODYNAMICS IN VENTILATED PRETERM LAMBS

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Background: Pulmonary edema is thought to be a major contributor to the development of pulmonary hypertension in preterm infants exposed *in utero* to inflammation. Volume loading has been used extensively in animal models as a model of pulmonary edema, but recently has been adopted in clinical practice to improve cardiovascular output in preterm infants.

Aims/Hypothesis: We investigated the effect of volume loading on oxygenation, pulmonary haemodynamics and cardiovascular output in preterm ventilated lambs. We hypothesized that volume loading would improve cardiopulmonary haemodynamics and cardiovascular output, but have deleterious effects on oxygenation and pulmonary haemodynamics.

Methods: Preterm lambs (~128 days gestation) were exposed via laparotomy and instrumented with flow probes and catheters. Lambs were then delivered and placed on positive pressure ventilation, with peak inspiratory pressure adjusted to maintain tidal volume of ~7 mL/kg, and F_iO_2 was adjusted to maintain S_aO_2 above 80%. At 15 min lambs were randomized to 1 of 3 groups: 1) 50 mL/kg bolus of saline (Vol50, n=7); 2) 80 mL/kg bolus of saline and subsequent maintenance infusion (Vol80, n=3); and 3) Controls (no volume given, n=7). At 30 min all groups underwent a ramp airway recruitment maneuver to test cardiopulmonary and respiratory indices. Blood flows and pressures were recorded in real-time and all ventilation/respiratory parameters and blood-gas parameters were conducted at 10 min intervals. The ratio of shunting through the ductus arteriosus (DA shunt) and left ventricular output (LVO) was measured using Doppler Echocardiography.

Results: Vol80 had significantly higher wet-dry lung weight ratio compared to Vol50 and Controls ($p<0.05$). Vol80 and Vol50 lambs had worse oxygenation ($p=0.024$) and had a tendency for lower static compliance and ventilator efficiency index than Controls during ventilation. Pulmonary blood flow was not different between groups, but pulmonary vascular resistance was higher in Vol80 and Vol50 groups compared to Controls ($p=0.06$). Systolic arterial pressure and heart rate was significantly higher in Vol80 and Vol50 groups compared to Controls ($p<0.05$). LVO and pulmonary arterial pressure was higher in Vol80 lambs compared to both Controls and Vol50 whilst DA shunt was similar between groups.

Conclusions: Volume loading improves cardiovascular output and arterial pressures, but results in worse oxygenation and increased pulmonary vascular resistance compared to Controls. The volume infused, and the subsequent Respiratory and pulmonary haemodynamic consequences need to be considered before adopting volume loading into clinical practice.

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PRE-ECLAMPSIA: EFFECTS ON THE PRETERM INFANT CARDIOVASCULAR SYSTEM

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Background: Epidemiological studies have identified long-lasting effects of maternal pre-eclampsia on the cardiovascular system in offspring. Maternal pre-eclampsia is associated with increased blood pressure (BP) in offspring which persists into adulthood.[1,2] Whilst the mechanisms involved in altered cardiovascular control in the offspring are unknown, fetal hypoxia and growth restriction are believed to play key roles. Also unknown is whether the BP changes in sleep that typify preterm birth are exaggerated in the offspring of pre-eclamptic pregnancies.

Aim: To assess the effect of maternal pre-eclampsia during pregnancy on BP and heart rate (HR) during sleep in infants born preterm, across the first 6 months of term-corrected age (CA).

Methods: Twenty-five preterm infants (28-32 wk GA) were studied using daytime polysomnography, at 2-4 wk, 2-3 mo and 5-6 mo CA. Infants were divided into control and pre-eclamspia groups based on reporting of maternal pre-eclamspia in infant medical histories. BP was recorded using a photoplethysmographic cuff (Finometer™). BP and HR were assessed during both quiet (QS) and active (AS) sleep. Infants were also tilted 15° head-up to assess BP and HR responses to a cardiovascular challenge.

Results: There were no differences in HR during QS or AS between the infant groups. BP at 2-3 mo CA was significantly increased (by 10mmHg) in the pre-eclampsia group when compared to the control group, during both QS and AS ($p<0.05$). There were no effects of group on BP at either 2-4 wk or 5-6 mo CA.
Infants in the pre-eclampsia group had altered cardiovascular responses to head-up tilting at all three ages studied, whereby peak increases in BP were on average 2% larger and the return of BP to baseline was significantly delayed by an average of 14 beats.

Conclusion: Pre-eclamspia is associated with increased infant BP at 2-3 mo CA and altered cardiovascular control during sleep across the first 6 months of CA. Further investigation is warranted to assess the effects of pre-eclampsia and other causes of fetal hypoxia on offspring cardiovascular control and the impending effects on the cardiovascular system later in life.

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[2]Tenhola *et al. Pediatr Res* 2006 59; 320-324
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NOTES

DETERMINANTS OF INTER-UTERINE GROWTH IN PATHO-PHYSIOLOGICAL PREGNANCY

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Background: Our research has focused on sex specific alterations in maternal physiology in pregnancy complicated by asthma and the effects on placental function and fetal development. More than a third of Australian women of child bearing age are overweight or obese with growing evidence supporting an association between obesity and asthma. In addition, obesity is associated with more frequent and severe asthma exacerbations during pregnancy and adverse pregnancy outcomes, including altered fetal growth.

Aims: As part of a longitudinal study of prenatal determinants of fetal growth and development, we sought to examine the interaction of maternal asthma and body mass index (BMI) on fetal growth with respect to sex.

Methods: From 1998 to 2005 pregnant women were prospectively recruited at the John Hunter Hospital, Newcastle, NSW. Women were stratified according to presence of asthma and BMI (<25 kg/m^2 normal; 25-30 kg/m^2 overweight; >30 kg/m^2 obese) resulting in six groups (reference group: non-asthmatic normal BMI). Sex-specific neonatal weight and head circumference centiles were calculated at birth. Data for 458 mother-infant pairs was analysed by hierarchical multiple regression to investigate the interaction of maternal asthma and obesity with respect to fetal growth, controlling for pregnancy induced hypertension (PIH) and smoking.

Results: Within the asthmatic group, the incidence of PIH (13%) and proportion of overweight (26%) and obese (32%) women was not significantly different from the reference group although a larger number smoked (28% vs 11%, $p<0.001$). Whilst smoking and PIH significantly influenced birth weight centile in both sexes (males: $R^2=0.031$, $F(2,217)=3.514$, $p=0.031$, females: $R^2=0.081$ $F(2,217)=9.58$, $p<0.001$), maternal asthma and BMI did not make a significant independent contribution to the regression equation in males. Conversely, in females the addition of asthma and BMI to the regression model significantly predicted birth weight centile ($R^2=0.121$, $F(7,212)=4.17$, $p<0.001$), with a BMI>30 in the presence of asthma associated with a 16 centile increase in birth weight ($p=0.021$). Smoking and PIH did not significantly influence head circumference centile in either sex. For females only, the addition of asthma and BMI to the regression model again predicted head circumference centile ($R^2=0.34$, $F(7,240)=2.24$, $p=0.032$), with BMI>30 in the presence of asthma associated with a 13 centile increase ($p=0.011$). Within the asthmatic group, BMI was positively correlated with birth weight centile for both males ($R=0.157$, $p=0.041$) and females ($R=0.211$, $p=0.005$). BMI was observed to be positively correlated with maternal systolic blood pressure at 18 ($R=0.444$, $p<0.001$) and 30 ($R=0.33$, $p<0.001$) weeks gestation and inversely correlated with umbilical arterial S:D ratio at 30 weeks gestation ($R=-0.31$, $p=0.035$) in females, but not males.

Conclusions: Metabolic derangements observed with higher BMI have been proposed to promote increased nutrient transfer, fetal hyper-insulinemia and accelerated fetal growth. Yet obesity is also characterised by maternal endothelial dysfunction favouring a vasoconstricted state. Relationships between BMI, blood pressure, umbilical arterial S: D ratio and fetal growth were observed only in females of asthmatic women. Therefore, alterations in maternal vascular control, secondary to obesity, may counter-balance known sex-specific alterations in placental function and fetal growth secondary to maternal asthma, ensuring normal female fetal growth is maintained.

INSULIN RESPONSE TO GLUCOSE TOLERANCE TEST IS ALTERED BY PRETERM BIRTH, TWINNING, SEX AND POSTNATAL NUTRITION IN LAMBS

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Background: Preterm birth, twins, low birth weight and altered postnatal growth are all associated with impaired glucose tolerance in later life. However the causal relationships between these factors have not been established.

Aim: To investigate the effects of preterm birth, twins, birthweight and postnatal nutritional supplements on insulin response to a glucose tolerance test (GTT) in prepubertal lambs.

Methods: Singleton bearing ewes were allowed to deliver spontaneously at term. Twin bearing were either given dexamethasone to induce preterm delivery or allowed to deliver spontaneously at term. Lambs were randomly assigned to receive nutritional supplements (protein, carbohydrate, vitamins) or water supplements for the first two weeks after birth. At 4 months of age lambs underwent a 3 hour intravenous GTT. Area under the curve (AUC) for glucose and insulin was calculated using triangulation. Groups were compared using ANOVA with Fisher *post hoc* test. Relationships between AUC and growth parameters were analysed using linear regression. Values are mean±SEM.

Results: GTT was performed on 65 lambs (term, single n=31; term, twin n=13, preterm, twin n=21). Peak insulin concentration tended to be greater in males than females. Peak insulin concentration and insulin AUC were greater in single than in twin lambs (1.7±0.2 vs 1.1±0.1ng.ml^{-1} p=0.04; and 125±8 vs 84±8 ng.min.ml^{-1}, p=0.0007, respectively). Higher current weight was also associated with greater insulin AUC only in term single male lambs (R^2=0.3, p=0.04). Higher birth weight was associated with increased AUC in term twins (R^2=0.44 p=0.01), but not in preterm twins or term singletons. Early nutritional supplements were associated with increased insulin AUC in males, but decreased AUC in females, in all groups.

Conclusions Preterm birth, twinning, birth weight and postnatal nutrient intake all alter the insulin response to GTT in lambs, but the way in which this occurs is different between the sexes. Any proposed interventions to ameliorate the effects of low birth weight or prematurity on later health may have to be sex specific.

THE EFFECT OF MATERNAL UNDERNUTRITION ON GENE EXPRESSION IN VISCERAL FAT OF THE LATE GESTATION GUINEA PIG

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Background: Epidemiological studies have demonstrated that exposure to a reduced nutrient supply before birth is associated with an increased risk of abdominal obesity in later life; however the mechanisms which underlie this association are poorly understood.

Hypothesis: In the present study, we have investigated the hypothesis that maternal undernutrition results in increased expression of lipogenic genes in the fetal guinea pig in late gestation. This study investigated the development of adipose tissue in the guinea pig and the impact of maternal undernutrition on the structural and functional characteristics of adipose tissue in the dam and fetus.

Methods: Date-mated guinea pigs were provided with either *ad libitum* feed (Control, C) or 85% of food intake per body weight of the Control diet (Undernutrition, UN). Maternal (C, n = 6 and UN, n = 7) and fetal perirenal adipose tissue (PAT) was collected at 50d (C, n = 4) and 60 d (C, n = 8 and UN, n = 7) gestation. The expression of stearoyl-CoA desaturase (SCD-1), fatty acid synthase (FAS), lipoprotein lipase (LPL) and leptin mRNA was determined by Real Time PCR and normalised to the housekeeper RPPO.

Results: Total PAT mass increased from 50d gestation to adulthood, whilst relative PAT mass was highest at 58-62d gestation ($P<0.01$). FAS expression was highest at 48-55d gestation, but there was no effect of development on the expression of SCD-1 or LPL. Maternal UN resulted in reduced maternal (C = 1141 ± 29 g, UN = 985 ± 43 g, $P = 0.01$) and fetal body weight (C = 74 ± 2 g, UN = 62 ± 3 g, $P = 0.004$) in late gestation. There was no effect of maternal UN on total or relative PAT mass in either the dam or the fetus, however the UN fetuses had a higher percentage of large lipid locules in their PAT compared to Controls ($P < 0.05$). The expression of FAS, LPL, SCD-1 and leptin mRNA was not different between the Control and UN groups in either the dam or the fetus ($P > 0.05$).

Conclusion: These data suggest that maternal UN promotes lipid storage within adipose cells before birth, and provide important insights into the mechanisms through which maternal UN alters the structural and functional characteristics of adipose tissue in the dam and her fetus and can program an increased risk of obesity.

INTRAUTERINE GROWTH RESTRICTION DELAYS MATURATION OF THE PULMONARY SURFACTANT SYSTEM IN THE SHEEP FETUS

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Background: Postnatal lung function is dependent on adequate lung growth and surfactant maturation during fetal development. Placental insufficiency is a major cause of intrauterine growth restriction (IUGR) and neonatal morbidity due to restricted fetal substrate supply. Chronic fetal hypoxemia in the sheep fetus alters surfactant protein expression, but changes are not consistent between models. Surfactant proteins are critical for airbreathing, as they aid surfactant function by promoting lipid adsorption to, and reducing surface tension, at the air-liquid interface of the lung.

Aims: We determined whether the expression of surfactant proteins is delayed or enhanced during late gestation by chronic hypoxemia caused by chronic placental insufficiency in the carunclectomy sheep model.

Methods: IUGR was induced by surgical removal of endometrial caruncles from ewes prior to mating. Post-mortem sampling of lung tissue was conducted on 15 pre-term fetuses at 130-135 days' gestation (control n=9, IUGR n=6) and 29 fetuses at 139-145 days' gestation (control n=15, IUGR n=14). Quantitative real-time PCR (qRT-PCR) and semi-quantitative western blot analyses detected the relative abundance of SP-A, -B and -C mRNA transcripts and proteins in fetal sheep lung.

Results: SP-B and –C mRNA and SP-A protein expression increased with gestational age. All three surfactant protein genes and the proteins demonstrated decreased expression in response to IUGR. This reduction was more enhanced at the younger gestational age. Normalised surfactant protein expression correlated with mean gestational pO_2 across both age and treatment groups.

Conclusions: Chronic hypoxemia in the carunclectomy model of IUGR represents a significant inhibitor of surfactant maturation, suggesting that preterm IUGR fetuses may be at increased risk of respiratory distress.

NOTES

WHO DETERMINES THE LENGTH OF GESTATION – MOTHER, FETUS OR PLACENTA? (A STUDY IN PROGRESS)

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Background: Reproductive physiologists have long pondered the question; who determines the length of gestation? Is it determined by a biological clock, or does pregnancy end when the fetus reaches a size that places physical and nutritional loads on the mother that can no longer be met? We propose to transfer embryos between two rodent species (F1 mouse, *Mus musculus* and spiny mouse*, Acomys cahirinus*) of similar adult size, but which have quite different gestational periods (19 and 39 days respectively), to investigate this phenomenon.

Aims: The primary aim of this project is to determine whether gestation length is determined by the mother, placenta, or the fetus. Specifically we aim to:
1. Perform embryo transfer between *Mus* and *Acomys* to determine whether gestation length is controlled by the mother or the fetus (embryo).
2. Elucidate the maternal, fetal and placental interactions that control gestation length by developing and transferring *Mus/Acomys* chimeric embryos.
3. Monitor fetal growth under each of our experimental manipulations (Aims 1 & 2) to determine the contribution of the mother, placenta and/or fetus to the eventual offspring size.
4. Assess the methylation pattern of imprinted genes (*Igf2r*, *Mest*, *Peg1/3, Ins1/2, H19*) known to regulate fetal growth in fetuses and placentas from each of our experimental manipulations (Aims 1 & 2).

Methods: Given the limited knowledge of the reproductive axis of the spiny mouse it has been necessary for us to optimise protocols, routinely used in the mouse, to allow these experiments to proceed. These protocols include superovulation, vasectomy (to induce pseudopregnancy in females for embryo transfer), embryo culture and vitrification of oocytes, embryos and sperm. Once established, pseudopregnancy will be induced in female *Mus* and A*comys* and embryo transfer performed as described for mouse. Chimeric embryos will be developed by the transfer of the inner cell mass of a *Mus* blastocyst into the cavity of an *Acomys* blastocyst and vice versa.

Results/Conclusions/Upcoming Experiments: To date we have successfully achieved superovulation, vasectomy and vitrification in the spiny mouse, which have not been previously described. We are currently optimising culture conditions for spiny mouse embryos by analysing their *in vitro* metabolic activity and nutrient preferences. The next phase of our experiments will optimise a pseudopregnancy protocol for the spiny mouse, which will allow us to perform *Acomys*-to-*Acomys* embryo transfers before embarking on *Acomys*-to-*Mus* embryo transfers and vice versa.

THE MAINTENANCE OF PREGNANCY IN THE SPINY MOUSE (A STUDY IN PROGRESS)

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Background: The spiny mouse is a precocial rodent species with a relatively long gestation (38-39 days) compared to other rodent species such as mice and rats (19-21 days). However, the endocrine changes that occur during pregnancy, and the importance of the placenta and/or ovary, have not been defined for this species.

Aims: To investigate the changes in serum oestrogen and progesterone during pregnancy in the spiny mouse, and to determine if pregnancy is maintained by the placenta (as in the human) or the ovary (as in the mouse).

Methods: Pregnant spiny mice underwent ovariectomy or sham surgery at 30 days gestation (n=6). Animals were monitored and their weight recorded every 12 h. Radioimmunoassays will be used to measure blood and tissue levels of oestrogen and progesterone and immunohistochemistry used to identify the cell populations producing these hormones within the ovary and/or placenta.

Results: Pregnant dams that underwent the ovariectomy procedure were not able to maintain pregnancy after surgery, and abortion of fetuses occurred within 24-48 h postoperatively. Studies are currently ongoing to describe the hormone profile and the sites of steroid hormone synthesis in these organs during pregnancy.

Conclusions: The maintenance of pregnancy in the spiny mouse requires hormonal support produced by the ovary.

A NEW METHOD FOR QUANTIFYING STABILITY DURING RESPIRATORY INSTABILITIES AND DETERMINING THE EFFICACY OF INTERVENTION

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Background: Breathing instabilities, such as periodic breathing (PB) and apnea, are common in the newborn, particularly those born preterm. Although instabilities may be a reflection of immaturity, and therefore a normal developmental phenomenon, they sometimes cause profound recurrent hypoxaemic episodes that animal studies implicate in neuro-developmental impairment. Attempts to stabilise the respiratory control system using continuous positive airway pressure (CPAP) and supplemental inspired O_2 are not always effective, for reasons poorly understood. We propose the engineering concept 'loop gain' (LG), a measure of the stability of the feedback loop controlling ventilation, can provide important insights into causes and treatments for respiratory instabilities, but its utility is unproven because currently there is no easy method for its measurement. Here, we present a novel method for the determination of LG that is mathematically precise and readily calculated from the duty ratio (DR) of PB, which is the ventilatory duration of each cycle of PB divided by total cycle duration.

Aim: To validate our method of determining LG by testing whether predicted changes in LG occur when CPAP and supplemental O_2 are adjusted in an established newborn lamb model of PB.

Hypotheses: As predicted by theory, LG (as measured using our method) will fall in proportion to CPAP-induced increases in lung volume. LG will also fall with the application of supplemental inspired O_2 (which stabilises control in the lamb) in a manner proportional to the inspired-arterial PO_2 difference (PIO_2-PaO_2).

Methods: Anaesthetised newborn lambs (10-20 d of age) were tracheostomised and intubated. Functional residual capacity was calculated from O_2 washout, and changes in lung volume were measured using a calibrated transthoracic circumference band. PB was induced by switching the lamb into a ventilatory circuit and hyperventilating for >5 min; on cessation of ventilation a period of apnea is usually followed by an epoch of PB. To alter LG, various levels of CPAP (0-10 cmH_2O) and FIO_2 (10-40%) were applied. The DR of the resulting breathing pattern was measured and LG calculated.

Results: LG, as calculated using our method, fell in proportion to the CPAP-induced increase in lung volume ($n=8$, $p<0.0001$) and in proportion to decreased (PIO_2-PaO_2) in the single lamb studied ($p<0.01$).

Conclusions: Our measure of LG from DR demonstrated the predicted changes in LG when CPAP and inspired O_2 were altered, hence validating our simple-to-apply mathematical method. Our new method provides clinicians with powerful means to track the effectiveness of interventions aimed at resolving respiratory instabilities in the newborn.

IMAGING LUNG MOTION USING PHASE CONTRAST X-RAY IMAGING

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Lung disease is one of the leading causes of morbidity and mortality in humans and is particularly prevalent in infants born very preterm. These infants usually suffer respiratory failure at birth and require assisted ventilation to survive. This can injure their lungs leading to the development of chronic lung disease, particularly bronchopulmonary dysplasia. However, the mechanisms by which assisted ventilation causes lung injury are unknown, although understanding the mechanisms involved is vital for the development "safer" ventilation strategies. We have recently developed an imaging technique that can image the lung as it aerates at birth, allowing us to determine the temporal and spatial pattern of lung aeration as well as the distribution of ventilation within the lung during the first breaths after birth. We have also developed a technique, called particle image velocity (PIV), which measures the velocity and direction of tissue movement across the lung as it expands and deflates throughout a breath (Figures 1 & 2; velocities at mid-inspiration). Using this technique, we can measure regional shear stress, differential distribution of ventilation as well as compliance differences across the lung, particularly in response to different ventilation strategies. These techniques have been used to identify the effect of positive end-expiratory pressure (PEEP), an initial 20 second sustained inflation and prophylactic surfactant administration on the rate of lung aeration and the distribution of ventilation within the lung during the immediate new born period. All treatments had different but beneficial effects on lung aeration and the distribution of ventilation. By measuring differences in lung motion, indicated by regions with abnormal velocities and direction of movement, we can identify lung regions with altered compliance caused by injury as well as identifying ventilation strategies most likely to cause injury.

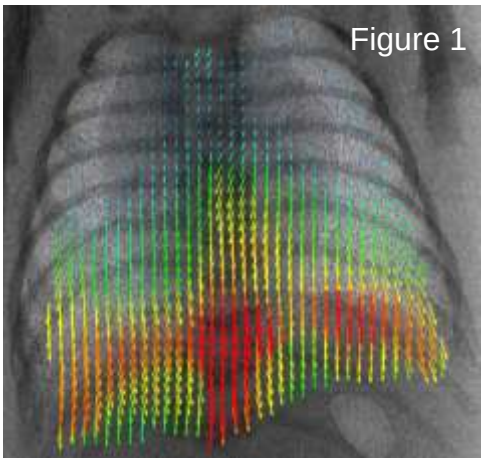
Figure 1

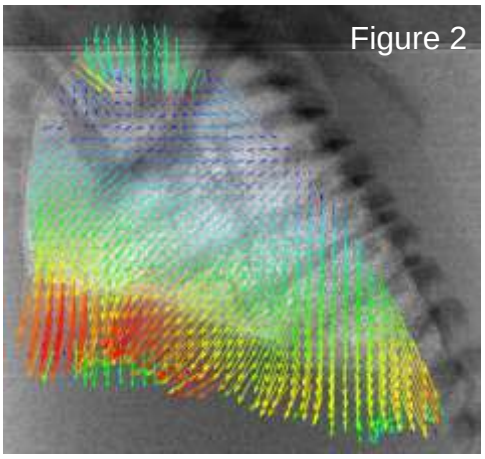
Figure 2

MATERNAL FOLIC ACID SUPPLEMENTATION AND ALTERED METABOLIC HEALTH OF OFFSPRING: MOLECULAR BASIS

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Background: Exposure to increased folic acid intake during pregnancy is common and increasing. Adequate folate is required for DNA and histone methylation in the epigenetic control of gene expression. Maternal folic acid supplementation (MFAS) may therefore influence epigenetic state as it is established in early life and later phenotype. Recently, high maternal folate status was found to be predictive of insulin resistance in Indian children.

Aims/Hypotheses: We therefore determined the effect of MFAS before and throughout pregnancy on glucose tolerance and insulin action in young adult progeny and expression of proximal insulin signalling molecules and other determinants of glucose and lipid control, in liver and skeletal muscle.

Methods: Moderate MFAS was imposed before and throughout pregnancy in the rat (6mg/kg versus 2mg/kg diet), at levels mimicking pregnancy recommendations for women, and glucose tolerance, insulin secretion and insulin tolerance, assessed in young adult progeny (~90 days of age). Gene expression of selected molecules in liver and skeletal muscle was measured by Multiplex RTPCR.

Results: MFAS improved glucose tolerance in young adult progeny ($p=0.044$), but altered insulin sensitivity differently in male and female progeny ($p=0.017$). MFAS increased whole body insulin sensitivity and maximal insulin action (insulin sensitivity x insulin secretion) in males, but impaired these in females. MFAS did not change tissue expression of insulin signalling molecules, but did alter indirect modulators of this and glycaemia:

- reduced hepatic peroxisome proliferator–activated receptor coactivator-1(PGC-1α) expression (- 16%, $p=0.048$) and increased glucokinase expression (only in females) (+ 38%, $p=0.043$), consistent with improved glucose tolerance. In liver, PGC-1α coactivates transcription factors regulating gluconeogenic genes and glucose production, and glucokinase, the latter.
- increased hepatic stearoyl-coenzyme A desaturase 1expression (+ 44%, $p=0.01$), mainly in females, which would increase lipogenesis, triglycerides and cholesterol and lipid induced insulin resistance.
- decreased skeletal muscle PGC-1α expression in females, but not males, (-25%, $p=0.09$), reducing lipid oxidative capacity and increasing likelihood of lipid induced insulin resistance.

Conclusions: MFAS improves glucose tolerance in adult progeny, in part via alterations in some hepatic molecular determinants, but adversely alters those of lipid metabolism in both glucoregulatory tissues, mainly in females. This may increase susceptibility of MFAS female progeny, to further impaired insulin action and glucose homeostasis when challenged, such as with an obesogenic diet or pregnancy

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