24th Fetal and Neonatal Physiology Workshop
Of Australia and New Zealand

Lion Harbourview Lounge
Michael Fowler Centre
111 Wakefield Street, Wellington
New Zealand
26-27 March 2010

2010 Organising Committee

Frank Bloomfield	University of Auckland
Serina Digby	University of Auckland
Ruth Simons	University of Auckland
Rob de Matteo	Monash University
Richard Harding	Monash University

Fetal and Neonatal Physiology Workshop 2010
Programme Outline

Friday 26 March

8:00am – 8:45am	**Registration**
8:00am	Tea/Coffee
8:45am	**Session 1,** Chair Prof Laura Bennet
10:30am	Morning Tea
11:00am	**Session 2,** Chair Dr Jon Hirst
12:30pm	Lunch
1:30pm	**Session 3**, Dr Rob de Matteo
2:45pm	Afternoon tea
3:15pm	**Session 4**, Chair Dr Kathy Gatford
4:30pm	**Close**
6:00pm	**Drinks and dinner**
	Mac's Brewery Bar
	Taranaki Street Wharf (next to Te Papa)

Saturday 27 March

8:30am – 9:00am	**Registration**
8:30am	Tea/Coffee
9:00am	**Session 5**, Chair Dr Beverly Mühlhausler
10:30am	Morning Tea
11:00am	**Session 6**, Chair Dr Karen Moritz
12:30pm	Lunch
1:30pm	**Session 7**, Chair Dr Anne Jaquiery
2:45pm	Afternoon Tea
3:15pm	**Session 8,** Chair Dr Beth Allison
4:45pm	**Close**

FETAL AND NEONATAL WORKSHOP SCIENTIFIC PROGRAMME

DAY 1- FRIDAY 26th MARCH

Registration 8:00 am – 8:45 am

8:00 am Tea/Coffee

Session 1 Chair: Prof Laura Bennet			
8:45 am	A1	Annie McDougall	TROP2 regulates proliferation and morphology of fetal lung fibroblasts
9:00 am	A2	Amy Sutherland	The effects of betamethasone on lung morphology in the growth restricted ovine fetus
9:15 am	A3	Beth Allison	Investigating the effect of different peak sustained inflation pressures on lung aeration
9:30 am	A4	Anzari Atik	Does intra-uterine inflammation have a persistent effect on the lung following preterm birth?
9:45 am	A5	Keiji Suzuki	Effects of antenatal intra-amniotic endotoxin on the development of lung structure in rats
10:00 am	A6	Megan O'Reilly	Exposure of the immature mouse lung to hyperoxic gas: do structural changes in the lung cause long-term changes in lung function?
10:15 am			Panel discussion

Morning tea: 10:30 am-11:00 am

Session 2 Chair: **Dr Jon Hirst**			
11:00 am	A7	Paul Drury	Braxton-Hicks' contractures are associated with cerebral deoxygenation and increased cerebral blood volume in the preterm sheep fetus
11:15 am	A8	Tamara Yawno	Protective effects of melatonin on brain injury in the newborn lamb
11:30 am	A9	Alistair Gunn	Can insulin like growth factor-1 improve white matter protection with delayed cerebral hypothermia?
11:45 am	A10	Joanne Davidson	The effect of dexamethasone on brain activity in the fetal sheep
12:00 pm	A11	Heidi Richardson	Does sleep position affect arousal from sleep pathways in infants born preterm?
12:15 pm			Panel discussion

Lunch 12:30 pm – 1:30 pm

Session 3 Chair: **Dr Rob De Matteo**			
1:30 pm	A13	Rhiannon Coster	Different fetal and postnatal growth trajectories in twins, twins reduced to singletons in early gestation, and singletons lead to similar adult body size
1:45 pm	A14	Maggie Honeyfield-Ross	Glucose tolerance during pregnancy in ewes born to mothers who were adolescent (12 months old) or mature (36 months old) at mating
2:00 pm	A15	Kathryn Gatford	Tissue-specific changes in insulin signaling in the lamb after placental-restriction *in utero*
2:15 pm	A16	Kyungjoon Lim	Vulnerability of intrauterine growth restricted rat offspring to adult hyperglycemia: effects on renal function
2:30 pm			Panel discussion

Afternoon tea 2:45 pm to 3:15 pm

Session 4 Chair: **Dr Kathy Gatford**			
3:15 pm	A17	Amanda Boyce	Maternal obesity and early postnatal overnutrition: programming the intrarenal renin-angiotensin system?
3:30 pm	A18	Zhi Yi Ong	Programming a taste for fat: the effect of maternal junk food feeding on food preferences in the offspring
3:45 pm	A19	Beverley Mulhausler	Maternal omega-3 supplementation alters fat distribution in the offspring
4:00 pm	A20	Margaret Morris	Impact of maternal obesity on offspring behavior and metabolic risk
4:15 pm			Panel discussion

DAY 2- SATURDAY 27 MARCH

Registration 8:30 am – 9:00 am

8:30 am Tea/Coffee

Session 5 Chair: **Dr Beverly Mühlhausler**			
9:00 am	A21	Megan Probyn	Establishing a relevant model of chronic, low dose gestational ethanol consumption
9:15 am	A22	Mary Berry	Influence of preterm birth and antenatal corticosteroid exposure on early growth patterns and response to growth hormone administration in lambs
9:30 am	A23	Kathy Gatford	Maternal folic acid supplementation and abundance and expression of insulin-like growth factor-II in offspring
9:45 am	A24	Anne Jaquiery	Periconceptional undernutrition affects the relationship between early growth and later glucose tolerance in lambs
10:00 am	A25	Mark Oliver	Reduced cortisol response to AVP+CRH challenge in adult offspring of ewes undernourished around the time of conception
10:15 am			Panel discussion

Morning tea: 10:30 am-11:00 am

Session 6 Chair: **Dr Karen Moritz**			
11:00 am	A26	Min Kim	Developmental changes in maturity of cardiomyocytes
11:15 am	A27	Lee O'Sullivan	Surgical stress alters gene expression independent of dexamethasone administration in the mouse
11:30 am	A28	Lindsea Booth	Baroreflex control of heart rate and renal sympathetic nerve activity (SNA) in preterm and near-term fetal sheep
11:45 am	A29	Yvonne Eiby	Cardiac function in preterm piglets
12:00 pm	A30	Stephanie Yiallourou	Effects of gender on the maturation of heart rate variability in term and preterm infants
12:15 pm			Panel discussion

Lunch 12:30 pm – 1:30 pm

Session 7 Chair: **Dr Anne Jaquiery**			
1:30 pm	A31	Kirsty Pringle	Promyelocytic zinc finger: the missing link in decidualisation?
1:45 pm	A32	Michael Stark	Oxidative stress and the inter-relationship of nitric oxide and carbon monoxide in the preterm placenta
2:00 pm	A33	James Cuffe	Short term maternal glucocorticoid exposure alters placental shape and gene expression in the mouse
2:15 pm	A34	Nicolette Hodyl	Regulation of the placental glucocorticoid barrier in human preterm pregnancy
2:30 pm			Panel discussions

Afternoon tea 2:45 pm – 3:15 pm

Session 8 Chair: **Dr Beth Allison**			
3:15 pm	A35	Luke Weaver-Mikaere	Repeated exposure to TNF-α in an *in vitro* ovine model of preterm infection/inflammation-mediated brain injury: effects on MMP, TIMP and TACE expression
3:30 pm	A36	Alana Westover	The role of prostaglandins in the fetal response to intra-uterine inflammation
3:45 pm	A37	Rob Galinsky	Intra amniotic inflammation; a stereological analysis of the kidney
4:00 pm	A38	Rob Galinsky	Postnatal Cerebral and pulmonary haemodynamic consequences of intrauterine inflammation
4:15 pm	A39	Hiroshi Masaki	How do arteries contract to arginine vasopressin (AVP)? Comparison between pulmonary and iliac / femoral arteries in juvenile and adult rats
4:30 pm			Panel discussion

Close 4:45 pm

TROP2 regulates proliferation and morphology of fetal lung fibroblasts

Annie RA McDougall, Stuart B Hooper, Valerie A Zahra and Megan J Wallace.
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Background: During fetal life lung growth is critically dependent on the high degree of fetal lung expansion. However, the pathways mediating the effect of lung expansion on lung growth are not known. We have demonstrated that a calcium regulator, *TROP2,* is positively correlated with cell proliferation rates during normal fetal lung development and following altered lung expansion and that *TROP2* expression is localised to cell types that proliferate in response to increased lung expansion, including fetal lung fibroblasts.

Aim: To determine the effects of inhibition of *TROP2* expression on cell proliferation of cultured fetal lung fibroblasts**.**

Methods: Primary cultures of fetal lung fibroblasts were generated from E19 rats (term ~E22). *TROP2* expression was knocked down by transfection with short-interfering (si)RNA specific for *TROP2*. *TROP2* mRNA levels were measured by real-time PCR. Proliferation and cell morphology were assessed by Ki-67 immunolabelling and haematoxylin & eosin staining, respectively.

Results: Transfection with *TROP2* siRNA caused ~65% decrease in *TROP2* mRNA levels (0.4 ± 0.2) compared to levels in cells transfected with control siRNA (1.0 ± 0.0; $p<0.01$). Transfection with *TROP2* siRNA caused a significant decrease in the percentage of cells proliferating (11.4 ± 1.4% vs 21.0 ± 2.0%; $p=0.001$) and tended to decrease cell number (82.0 ± 4.4/mm^2 vs 102.3 ± 10.7/mm^2; $p=0.09$) compared to control siRNA. Cells treated with *TROP2* siRNA also had altered morphology; cells were flatter and had reduced cytoplasmic extensions.

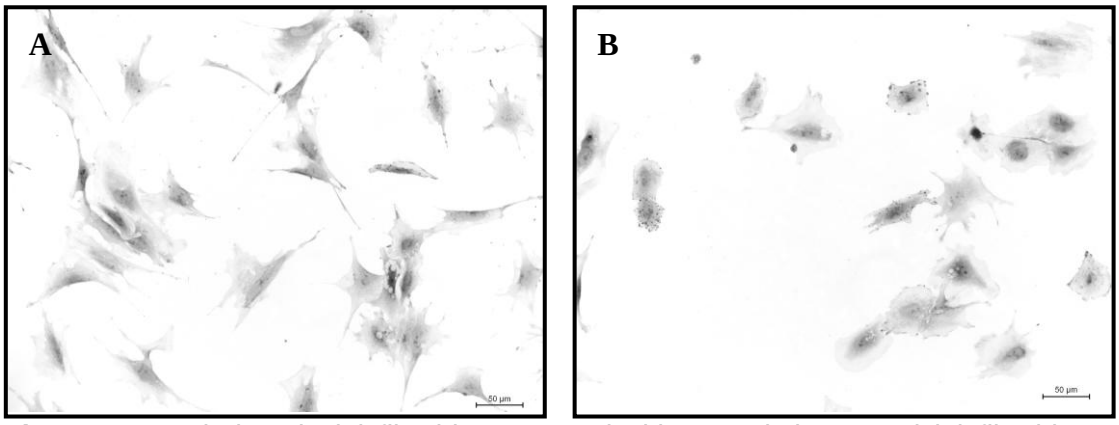

Figure 1. Morphology in (A) fibroblasts treated with control siRNA and (B) fibroblasts treated with siRNA specific for *TROP2*.

Conclusions: Inhibiting *TROP2* expression caused a reduction in the number and proliferation of cultured fetal lung fibroblasts. This finding supports the hypothesis that *TROP2* regulates proliferation of fetal lung cells. *TROP2* may also regulate fibroblast cell morphology.

The Effects of Betamethasone on Lung Morphology in the Growth Restricted Ovine Fetus

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Background: Intrauterine growth restriction (IUGR) is associated with fetoplacental hypoxemia and increased incidence of respiratory morbidity and mortality. The IUGR fetus has an increased risk of preterm birth therefore these fetuses are likely to be exposed to glucocorticoids.
Aims/Hypothesis: This project aims to examine the lung morphology in IUGR fetuses following glucocorticoids. IUGR fetuses are expected to show simplified morphology and glucocorticoids should improve this.
Methods: Time mated twin pregnant ewes underwent surgery at 105-110 days gestation (term ~147d), with one fetus undergoing single umbilical artery ligation (SUAL) to induce IUGR and the other acting as an age-matched control. Betamethasone (BM; 11.4mg i.m. to ewe; n=7) or vehicle (n=7) was given on days five and six following surgery and post mortem was conducted on day seven. The lungs were pressure fixed via the trachea and processed for light microscopy.
Results: Compared to controls (28.2±0.5%), lung tissue volume was significantly reduced following SUAL (25.7±0.6%), BM (23.9±0.7%) and in SUAL+BM fetuses (22.8±0.5%, $p<0.05$). Preliminary analysis suggests that compared to controls, secondary crest density is reduced in SUAL fetuses and following exposure to BM in both control and SUAL fetuses. The proportion of Ki67 labeled proliferating cells was significantly decreased compared to controls (6.1±0.5%) following BM alone (3.1±0.3%) and SUAL + BM (3.8±0.4%), but not in SUAL alone (6.7±0.5%).
Conclusions: Betamethasone and/or SUAL-IUGR increase the proportion of airspace within the developing lung and reduce secondary septal crest development. We will further determine whether BM exacerbates altered lung development in IUGR fetuses.

Investigating the effect of different peak sustained inflation pressures on lung aeration.

Arjan Te Pas[1], Beth Allison[2], Melissa Siew[2], Marcus Kitchen[3], Kentaro Uesugi[4], Naoto Yagi[4], Rob Lewis[5], Megan Wallace[2], Stuart Hooper[2]

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Background: Establishing adequate lung aeration is key to the successful resuscitation of a preterm infant. Many preterm infants need ventilation to aerate their lungs and overcome the high surface tension and frictional forces caused by liquid-filled immature airways. Initiating ventilation with a sustained inflation (SI) may improve lung aeration from birth by allowing time for the air/liquid interface to move into the distal airways. We have previously demonstrated that in ventilated preterm rabbit pups at birth, a SI improves lung aeration. However, little is known about the appropriate level of pressure to apply during a SI to achieve adequate ventilation without overinflating the delicate preterm lung.

Aims: This study aimed to determine the relationship between lung aeration and the pressure applied during a SI.

Methods: Preterm rabbit pups (28 d) were randomized at birth into four groups (n = 6 for each): 1) 20 second SI @ 20 cmH_2O, 2) 20 second SI @ 30 cmH_2O 3) 20 second SI @ 40 cmH_2O and 4) No SI (IPPV). Pups received an initial inflation as dictated by their group and were subsequently ventilated to achieve a tidal volume of 7.5 mL/kg and 5 cmH_2O PEEP. Functional residual capacity and tidal volume were measured by plethysmography and uniformity of lung aeration was assessed using phase contrast X-ray imaging.

Results: Altering the peak SI pressure altered lung aeration. Pups of the 40 cmH_2O SI group achieved the target tidal volume (7.5 mL/kg) by the end of the SI. Target tidal volume was not achieved until the 30th breath in all other groups. However, only pups which received 30 cmH_2O SI accumulated a functional residual capacity by 180 breaths.

Conclusions: Different peak SI pressures can alter the manner in which the lung aerates following birth in preterm rabbit pups. Further experiments are required to fully understand the role of a SI in lung aeration.

Does intra-uterine inflammation have a persistent effect on the lung following preterm birth?

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Background: Fetal exposure to intra-uterine inflammation and infections such as chorioamnionitis can lead to preterm birth and may also have a detrimental effect on the developing lung. Exposure of the fetus to intra-uterine inflammation improves lung function by increasing surfactant production[1,2]; however it is also associated with a decrease in alveolarization which could increase the risk of chronic lung disease[3]. In rats born at term, the alveolar simplification persists up to postnatal day 60[3], but no studies have examined the postnatal effects of prenatal LPS exposure on the conducting airways or lung parenchyma after preterm birth.
Aims: To determine the effects of intra-uterine inflammation on the structure of the conducting airways and lung parenchyma in postnatal lambs that were born preterm. We have also determined whether intra-uterine inflammation affects postnatal growth and cardiovascular function.
Methods: Fetal sheep were exposed to lipopolysaccharide (LPS, 1 mg/day, n=6) or saline (n=9) administered to the amniotic sac from 110 days gestational age (DGA) until induced preterm birth at ~133 DGA (term ~147d). After birth lambs were weighed daily and body dimensions measured weekly until necropsy at 11 weeks postnatal age. At necropsy the lung was pressure-fixed via the trachea (20cmH_2O) and lung structure analysed morphometrically, focussing on the parenchyma and bronchioles. Arterial pressure was recorded via a chronically implanted catheter at 10 weeks postnatal age.
Results: There were no differences in body weight and body dimensions from birth to necropsy between the LPS and control groups. In LPS-exposed lambs, the spleen and kidneys were significantly lighter relative to body weight compared to control lambs. In the lungs, we found no significant differences in the bronchioles in terms of epithelial area, alveolar-bronchiolar attachments and collagen deposition and smooth muscle content in the outer airway wall. In the lung parenchyma, we found no significant difference in percent tissue and airspace, alveolar size (mean linear intercept), elastin deposition and collagen deposition. There was no significant difference in mean arterial pressure between groups.
Conclusions: The lungs of LPS-exposed preterm lambs are structurally similar to control lungs at 11 weeks postnatal age, suggesting that the lungs recover structurally with continued development. The decreases in kidney and spleen weights could influence disease susceptibility in later life and thus require further investigation.

1. MOSS, T. J. et al. (2002) Early gestational intra-amniotic endotoxin: lung function, surfactant, and morphometry. *Am J Resp Crit Care Med,* 165**,** 805-11.
2. KRAMER, B. W. et al. (2002) Injury, inflammation, and remodeling in fetal sheep lung after intra-amniotic endotoxin. *Am J Physiol Lung Cell Mol Physiol,* 283**,** L452-9.
3. UEDA, K. et al. (2006) A rat model for arrest of alveolarization induced by antenatal endotoxin administration. *Ped Res,* 59, 396-400.

EFFECTS OF ANTENATAL INTRA-AMNIOTIC ENDOTOXIN ON THE DEVELOPMENT OF LUNG STRUCTURE IN RATS

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Background: Chorioamnionitis is one of the major causes of preterm delivery. It may also be associated with impairment of developing organs such as brains and lungs.

Aims: To study effects of antenatal intra-amniotic injection of potent proinflammatory agent, lipopolysaccharide (LPS) on fetal and neonatal growth and development of the lungs.

Methods: At 20 d gestation, pregnant SD rats were anesthetized and the uterus exposed under general anesthesia. The uterine wall was punctured and 0.1 μg LPS; *E. coli* endotoxin (O55:B5; Sigma, St. Louis, MO, USA) dissolved in 0.1mL saline injected into each amniotic cavity. In the control group, 0.1mL saline was injected. At 22 d (term), the fetuses were delivered spontaneously and vaginally. The newborn pups were breast fed and nursed by their own mother. At 4 and 8 weeks, the pups were euthanized and the lungs harvested, perfused, pressure-fixed (10cmH_2O) through the airways and pulmonary arteries and processed for morphometric analyses.

Results: LPS-exposed pups had higher perinatal mortality rate (58% vs 15%; $p<0.01$). There were no significant differences in body weights and lung weights between LPS and control groups. At 4 wk, LPS pups tended to have lower alveolar surface density (Sv-alv) and higher ratio of medial thickness/diameter of pulmonary arteries (MT/d). The mean alveolar volume (Valv), numerical density of alveoli (Nv-alv) and volume density of small arteries (diameter ~50-150μm; Vv-a) were not different between the two groups. At 8 wks, there were no differences in all the variables between LPS and control groups.

	4 weeks			8 weeks (female)		
	Control	LPS	p	Control	LPS	p
Valv (μm^3)	11800+/-1800	12400+/-1200	0.79	17000+/-5700	14500+/-700	0.63
Nv-alv (mm^{-3})	70000+/-10400	62000+/-5300	0.51	49000+/-10400	48000+/-3000	0.87
Sv-alv (mm^{-1})	95+/-2	90+/-2	0.12	76+/-4	75+/-5	0.85
Vv-a	0.026+/-0.006	0.026+/-0.002	0.39	0.033+/-0.011	0.045+/-0.016	0.90
MT/d	0.045+/-0.004	0.053+/-0.004	0.18	0.039+/-0.004	0.037+/-0.004	0.72

Conclusions: Antenatal intra-amniotic LPS (0.1 μg) resulted in higher perinatal mortality in offspring. In surviving pups however, LPS did not influence postnatal body and lung growth. LPS-exposure may have affected lung development of both air sacs (fewer and larger alveoli) and vasculature (thicker media of pulmonary arteries) transiently, which was reversible toward adulthood.

Exposure of the immature mouse lung to hyperoxic gas: do structural changes in the lung cause long-term changes in lung function?

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Background: Very preterm infants are born with immature lungs and often require supplemental oxygen therapy to survive. Numerous studies have reported poor lung function in children and adults who were born very preterm and received respiratory support; however the contribution of inhalation of hyperoxic gas is not clear. We have recently shown that exposure of the immature mouse lung to physiologically high concentrations of oxygen results in persistent alterations to the structure of the small conducting airways and the lung parenchyma; these alterations in the lung structure could increase the risk of poor lung function later in life.

Aim: To determine if the structural alterations in the lung induced by neonatal exposure to hyperoxic gas affect adult lung function.

Methods: Neonatal mice (C57Bl/6J) born at term were continuously exposed to hyperoxic gas (65% oxygen) from birth until postnatal day 7 (P7). At P7, mice were allowed to live in room air (21% oxygen) until adulthood at P56 (n=32). Age-matched controls received only room air from birth (n=32). At P56, lung function was assessed by measurement of transpulmonary resistance and dynamic compliance in response to increasing doses of the bronchoconstrictor methacholine using whole-body invasive plethysmography. Pulmonary resistance and compliance were calculated at each dose and values expressed as the percent change from control (saline). Mice were sacrificed, bronchoalveolar lavage fluid (BALF) collected and the number of total and differential immune cells enumerated using standard morphologic criteria.

Results: There was no significant difference between hyperoxia-exposed mice and control mice in baseline pulmonary resistance and compliance. In adulthood (P56), mice that were exposed to hyperoxic gas tended to have a smaller increase in pulmonary resistance (p=0.105) and a greater increase in pulmonary compliance (p=0.085) in response to methacholine than controls. In adulthood, the total number of immune cells in BALF was significantly greater (p<0.05) in mice exposed to hyperoxic gas compared to controls; >95% of immune cells were macrophages.

Conclusions: Structural alterations in the adult lung following exposure to a moderate level of hyperoxic gas for the first 7 days after birth does not have major effects on airway resistance or compliance in adulthood. However, the presence of increased immune cells within the lung suggests that neonatal exposure to hyperoxic gas can induce low-grade chronic inflammation, which could re-program the immune response and affect the ability of the lungs to clear pathogens.

NOTES

Braxton-Hicks' contractures are associated with cerebral deoxygenation and increased cerebral blood volume in the preterm sheep fetus

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Background: Braxton-Hicks' contractures occur from around 20 weeks gestation in the human fetus and increase in frequency thereafter. These contractures are associated with a fall in fetal Po_2 secondary to increased placental vascular resistance and a fall in uterine blood flow. Although pronounced uterine contractions during labour are associated with fall in cerebral oxy- and total haemoglobin; there are limited data on the effects of the smaller Braxton-Hicks' contractures on cerebral blood flow and oxygenation during gestation.

Aim: To determine the cerebrovascular and electrocortical effects of Braxton-Hicks' contractures in the preterm sheep fetus.

Methods: Experiments were carried out in eight preterm (100-104 days gestation, term is 140 days) chronically instrumented fetal sheep. Braxton-Hicks' contractures were identified from the amniotic pressure (AP) trace and changes in arterial blood pressure (BP), venous BP (VP), carotid blood flow (CaBF), electrocorticogram (ECoG), total cerebral haemoglobin (Hb) and delta Hb (HbO_2-Hb) were recorded.

Results: Contractures ranged from 4-8 minutes duration and changes up to the first 4 min across all contractures were used for consistency. AP increased by 3.0 ± 0.65 mmHg ($p < 0.01$) and BP and VP increased by 3.6 ± 0.88 and 2.5 ± 1.4 mmHg respectively ($p < 0.01$). HR increased by 6.6 ± 5.0 bpm at 2 min, and then fell to 5.0 ± 5.9 above baseline at 4 min ($p < 0.05$, 2-4min). CaBF fell to a nadir of -1.6 ± 2.1 mL/min at 2 min (NS), with no change in femoral blood flow. ECoG activity fell but was only significant at 3 min. Total Hb content increased significantly to 2.9 ± 2.4 µM at 4 min ($p < 0.05$) and delta Hb fell significantly to -3.9 ± 1.9 µM at 4 min ($p < 0.05$) driven by a rise in cerebral deoxyhaemoglobin (3.3 ± 1.4 µM, $p < 0.005$) and no change in HbO_2. Cytochrome oxidase also increased significantly ($p < 0.05$).

Conclusions: These data show Braxton-Hicks' contractures in the preterm fetus are associated with cerebral deoxygenation, increased cerebral blood volume and a fall in ECoG activity and CBF, consistent with intact flow-metabolism coupling. Ongoing analysis will reveal whether coupling remains intact after asphyxia.

Protective Effects of Melatonin on brain injury in the Newborn lamb

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Background: Intrauterine growth restriction (IUGR) is a serious complication of pregnancy, associated with brain injury, congnitive dysfunction and cerebral palsy.
Aims: This study aims to examine whether chronic maternal administration of melatonin reduces brain injury and neonatal behavioural deficits, which can occur as a consequence of IUGR.
Method: Ewes with a single fetus underwent surgery at 110 days gestation. We performed single umbilical artery ligation (SUAL) in fetuses to induce chronic hypoxemia and IUGR. Melatonin (MEL) was administered continuously (0.25 mg/hr from surgery until term). Sham IUGR was undertaken for controls. Following delivery, lambs were monitored closely for 24 hours after birth to determine the time taken to reach behavioural milestones.
Results: At term (~145 days), SUAL (3.0±0.4kg) and SUAL+MEL lambs (3.4±0.1kg) weighed less than controls (4.5±0.2kg; $P<0.05$). SUAL lambs took longer to reach all neonatal milestones compared with controls; eg time to stand (58±20min *v* 23±5min) and to find the ewe's udders (81±15min *v* 40±6min). Melatonin infusion improved the time taken to reach milestones (stand 39±12min, find udder 60±11min). Within the SUAL-IUGR brains, 4-HNE (lipid peroxidation) staining was high in the hippocampus, cortex and thalamus compared with controls. Melatonin reduced 4-HNE immunoreactivity. SUAL resulted in infiltration of inflammatory cells, which was greatest in the subcortical white matter and hippocampus. No inflammatory cells were found in these brain regions from control or SUAL+MEL lambs.
Conclusion: Maternal melatonin administration in IUGR pregnancies improves indices of brain injury and newborn behaviour.

CAN INSULIN LIKE GROWTH FACTOR-1 IMPROVE WHITE MATTER PROTECTION WITH DELAYED CEREBRAL HYPOTHERMIA?

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Background. Cooling the brain after perinatal hypoxia-ischemia can significantly reduce injury and improve long-term neurological outcome. However, both clinically and experimentally, protection is only partial. One likely reason is the progressive reduction in effectiveness of cooling with greater delay after injury. Potentially combining hypothermia with another agent could further improve outcome of delayed treatment. We and others have previously shown that insulin-like growth factor-1 (IGF-1) can reduce neuronal loss and promote oligodendrocyte survival and myelination after ischemia; in particular IGF-1 promotes proliferation of oligodendrocytes and other glia.

Hypothesis. The combination of IGF-1 with therapeutic hypothermia would improve oligodendrocyte survival compared with hypothermia alone.

Material and Methods. Unanesthetized near-term fetal sheep *in utero* were subjected to 30 minutes of cerebral ischemia. Fetuses were then randomized to receive either cooling from 5.5 to 72 hours (n = 12) or an infusion of 3 μg of IGF-1 intracerebroventricularly from 4.5 to 5.5h plus cooling from 5.5 to 72h (n=8), or sham cooling plus sham infusion (n=12), or sham ischemia (n=5). Cooling was induced by circulating cold water through a coil around the fetal head. The water temperature was titrated to reduce fetal extradural temperature from 39.1 +/- 0.1 °C to between 30 and 33 °C, while maintaining esophageal temperature >37°C. Fetuses were killed after 5 days for histological assessment. Data are mean±SD.

Results. 30 min of cerebral ischemia was associated with severe white matter damage in a watershed pattern, with loss of CNPase positive intragyral oligodendrocytes compared with sham controls (380±138 *vs* 1180± 152 cells/field, $p<0.001$). Delayed hypothermia alone reduced this loss (847±297 cells/field, $p<0.01$ *vs* ischemia alone). There was no significant difference between hypothermia alone and hypothermia plus IGF-1 infusion (1015±211 cells/field, NS). Ischemia was associated with marked activated caspase-3 expression in white matter (216±41 *vs* 19±18 cells/field, $p<0.001$). Delayed hypothermia was associated with a reduction in activated caspase 3 positive cells (116±81 cells/field, $p<0.05$), with no significant difference between hypothermia alone and hypothermia plus IGF-1 infusion (91±27 cells/field, NS).

Conclusions. Partial white matter protection with delayed, moderate cerebral hypothermia is associated with reduced caspase-3 activation after 5 days recovery. There was no apparent further improvement from combination therapy with delayed IGF-1 infusion, potentially suggesting that hypothermia and IGF-1 are at least in part protecting oligodendroglia through overlapping anti-apoptotic mechanisms.

The effect of dexamethasone on brain activity in the fetal sheep

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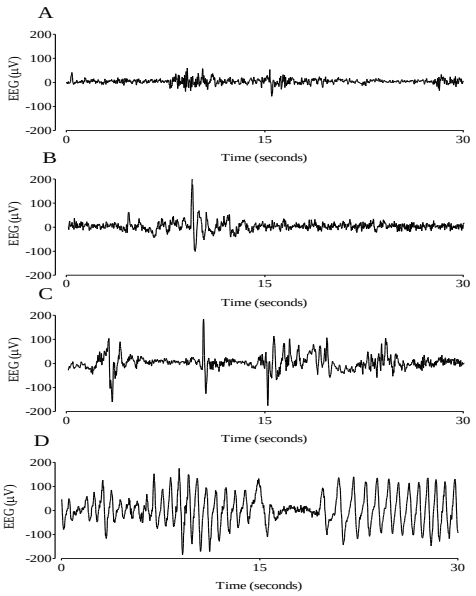
Background: Antenatal glucocorticoid therapy enhances fetal lung maturation in pregnancies at risk of preterm delivery, but may alter the development and function of other organs such as the brain.

Aims: To examine the effects of a single course of maternally administered dexamethasone (DEX) on fetal brain activity.

Methods: Ewes at 103 days gestation, received two intramuscular injections of either DEX (12 mg/2 ml, n = 8) or saline vehicle (2 ml, n = 7) 24 hours apart. Fetal EEG activity was monitored continuously from 24 h before until 120 h after the first injection.



Results: Dex injection 1 caused a significant rise in EEG amplitude, peaking around 12 h ($P<0.01$), with values returning to control by 24 h. A smaller significant rise ($P<0.05$) occurred after injection 2, with a return to control values by 30 h. Analysis of the raw EEG showed that normal sleep (characterised as discontinuous mixed amplitude and frequency activity with transients, i.e. spikes, sharp and slow waves) (**Fig A**) changed to a more continuous pattern of higher amplitude lower frequency (delta) activity, and greater numbers of transients (**Figs B&C**). Seizures were observed from 4-5 h (**Fig D**). Similar patterns were observed after injection 2, but the effect on amplitude was less marked. Seizures continued infrequently for 48 h after injection 2, there was sustained EEG continuity, and elevated alpha wave frequency activity. No cell death or microglial activation was seen 5 days after the first injection.

Conclusions: Dexamethasone appears to induce short-term seizure activity in the fetus at an age which is equivalent to the 28-30 week human in terms of neural maturation. Studies show that glucocorticoids can facilitate a sustained calcium influx through glutamate receptors and this may mediate the epileptiform activity we observed. Dexamethasone also appears to alter sleep patterns, changing activity from a discontinuous to a more continuous state, with increased alpha activity observed suggesting enhanced cortical maturation. These electrographic changes were not associated with neuronal cell death or microglial activation. EEG monitoring is increasingly being used to monitor infants at risk of neural injury, and it is important to know how routinely administered drugs affect the EEG. Our data highlight the need for clinical assessments of infants to determine whether similar effects are seen.

Does sleep position affect arousal from sleep pathways in infants born preterm?

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Background: Sudden Infant Death Syndrome (SIDS) is thought to occur when an infant is unable to respond appropriately to a life-threatening stress; an impaired progression of arousal responses from the brainstem to the cortex, is considered to play an important role. Epidemiological studies have shown that infants born preterm are more likely to succumb to SIDS, and it has been demonstrated previously that both spontaneous and induced arousal responses were decreased in preterm infants when compared with term infants. These studies, however, did not account for sub-cortical and cortical elements of the arousal pathway separately. As the final cortical element of the arousal process may be the most critical for survival, we hypothesised that the increased vulnerability of preterm infants to SIDS could be partly explained by depressed cortical arousal responses when compared with term infants.

Aim: To evaluate the effects of preterm birth on stimulus-induced arousal processes in both prone and supine sleeping positions.

Methods: 10 healthy ex-preterm infants were studied with daytime polysomnography, in both supine and prone sleeping positions, at 2-4 wk, 2-3 mo and 5-6 mo post-term. Arousal from sleep was induced using a pulsatile jet of air to the nostrils at increasing pressures. Arousals were scored as sub-cortical activations (SCA) and cortical arousals (CA) using standard criteria and were expressed as proportions of total arousal responses. Data were then compared with previously-presented data from 13 healthy term infants.

Results: In term infants, prone sleeping was associated with increased CA when compared to the supine position, but only at 2-3 mo of age. By contrast, in preterm infants this positional effect of increased CA when prone was evident at all three ages studied, during both active sleep (AS) and quiet sleep (QS) (see table).

Table: Proportions of CA from total arousal responses in preterm infants

	AS – Supine	AS – Prone	QS – Supine	QS - Prone
2-4 wk	32%	47% *	22%	35% *
2-3 mo	20%	47% *	18%	38% *
5-6 mo	14%	33% *	3%	34% *

* $p<0.05$ vs. supine, Chi-square analysis

Conclusions: These preliminary findings suggest that prone sleeping in preterm infants promotes cortical arousal responses throughout the first six months of post-term age. We have previously suggested that effective CA normally represents a critical protective response to a potentially harmful situation, and one that is lost in the SIDS infant.

NOTES

Different fetal and postnatal growth trajectories in twins, twins reduced to singletons in early gestation, and singletons lead to similar adult body size

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Objective: Twins are born both earlier and smaller than singletons, but adult size in humans is not different. Reduced size at birth is associated with an increased risk of adverse health outcomes such as obesity, type 2 diabetes and cardiovascular disease. Postnatal growth trajectories, particularly in early postnatal life, may also affect these risks. We have previously shown that reducing twin pregnancies to singletons in early gestation does not alter the reduced size at birth. Here we report the postnatal growth trajectories and adult body size in sheep born as twins, born as a singleton following fetal reduction from a twin conception in early pregnancy and in singletons conceived as singletons.
Methods: The study consisted of three groups: Singletons (n = 23), Twins (n = 19 pairs) and those conceived as twins but reduced to singletons in early gestation (Reductions; n = 30). Growth velocity was determined from birth to weaning (3 months) and from weaning to young adulthood (12 months). At 12 months a randomly selected subset (T_n = 8 males, 7 females; R_n = 5 males, 4 females; S_n = 2 males, 6 females) underwent post mortems to obtain tissue weights and to determine the proportionality of growth between all three groups. Data were compared by ANOVA followed by post hoc correction with data presented as mean (SEM). Statistical significance was taken when $P<0.05$.
Results: Twins and Reductions were significantly lighter than singletons at birth. From birth to weaning, Reductions had a greater growth velocity (21.0 (0.3) g/kg.d; P=<0.05) than Twins (20.0 (0.3 g/Kg.d)) and Singletons (19.9 g (0.4) g/Kg.d), which had similar growth velocities. Milk intake in the second week of life was not different amongst groups. From weaning through to young adulthood Twins had a greater growth velocity (2.1 (0.1) g/Kg.d; P<0.05) than Singletons (1.6 (0.1) g/Kg.d) and Reductions (1.6 (0.1) g/Kg.d).
At 12 months of age, body weight (Singletons 52.0 (7.4) Kg; Reductions 51.5 (8.0) Kg; Twins 47.9 (7.6) Kg) and size were similar amongst groups, although twins had significantly greater chest girth and rump height than singletons and Reductions.
Ovaries were heavier (P = 0.04) and right ventricular wall thickness less (P = 0.006) in twins than in Reductions and Singletons. Other organ weights were similar amongst groups.
Conclusion: These data demonstrate that twins experience constraint of growth both *in utero* and through to weaning, with release of constraint and thus accelerated growth post weaning. Reducing twins to singletons in early pregnancy does not relieve the constraint on fetal growth, but removes the postnatal constraint on growth. This is not due to increased milk intake. Thus, Twins, Singletons and Reductions attain similar adult size, but after following quite different growth trajectories. The implications of these different growth trajectories for endocrine function and body composition in adulthood are currently under investigation.

Glucose tolerance during pregnancy in ewes born to mothers who were adolescent (12 months old) or mature (36 months old) at mating.

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Background: In humans, offspring of adolescent mothers are at increased risk of reduced birth weight and preterm birth. In sheep, data are conflicting. Very little is known, in either species, on the effect of adolescent pregnancy on the daughter's ability to adapt her metabolism to pregnancy and produce offspring within the normal weight range.

Aims/Hypothesis: Determine the effect grand-maternal (G0) age at pregnancy has on G1 and G2 birth weights and on glucose tolerance in pregnant G1 females.

Methods: Singleton offspring born to 36 month old mature (Mat) or 12 month old immature (Imm) ewes were mated at 18 months of age. Before, and 60 to 70 d after, mating G1 ewes carrying singleton fetuses (Mat: n=11; Imm=8) were tested for glucose tolerance (GTT, 0.5 g/kg glucose iv) after overnight fasting and blood sampled over 180 min for plasma glucose and insulin concentrations. Area under the glucose response curve (AUC) was calculated. Lamb weights were recorded from G1 and G2 generations. Data are means±SEM and were analysed by ANOVA.

Results: Imm ewes were 36% lighter than Mat ewes at mating (39±2 vs. 61±3 kg, $p < 0.0001$) and had singleton offspring 20% lighter at birth (4.1±0.2 vs 5.2±0.3, $p < 0.01$). At mating G1 daughters were identical in weight (both groups 51±1 kg), but at 60-70 d of pregnancy Imm ewes were slightly lighter than Mat ewes (57±1 vs. 59±1 kg, $p < 0.05$). However, G2 birth weight was not different (Imm: 5.7± 0.3 vs. Mat: 5.4±0.2 kg). In G1 mothers, fasted baseline plasma glucose was slightly higher in Imm than Mat ewes before mating (3.7±0.1 vs 3.4±0.1 mM, $p < 0.05$) but there was not a significant difference in glucose AUC during GTT (1739±52 vs1619±46 mM.min^{-1}, $p = 0.1$). At 60-70 d of pregnancy baseline glucose concentration and glucose AUC during GTT in Imm ewes were similar to those in Mat ewes (baseline glucose concentration: 3.3±0.1 vs. 3.3±0.1 mM; glucose AUC 1639±48 vs 1673±56 mM.min^{-1}).

Conclusions: Grand-maternal age at mating had no effect on birth weight in the G2 offspring despite a marked effect on G1 birth weight. Offspring of Imm ewes had higher fasting glucose concentrations prior to pregnancy, but no difference in fasting glucose concentration or glucose tolerance in mid-pregnancy. The moderate difference in weight gain during pregnancy suggests some aspects of maternal partitioning are influenced by grand maternal age. These findings have important implications for the sheep production industry in NZ but their relevance to human reproductive biology has yet to be determined.

Tissue-specific changes in insulin signaling in the lamb after placental-restriction *in utero.*

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Background: Intrauterine growth restriction (IUGR) in humans increases the risk of developing diabetes in later life. We have shown that the placentally-restricted (PR) and low birth weight sheep, like the IUGR human, has enhanced whole-body insulin sensitivity in neonatal life, during catch-up growth, but then reverses to impaired insulin sensitivity subsequently. Decreased insulin sensitivity of muscle and enhanced insulin sensitivity of adipose tissue are implicated in the impaired glucose homeostasis and enhanced fat deposition characteristic of human IUGR and ovine PR, but the mechanisms for this are not clear.

Aims/Hypothesis: The aim of this study was to determine how PR alters expression of key determinants of proximal insulin signaling in liver, skeletal muscle and adipose tissue.

Methods: Whole-body insulin sensitivity was measured by hyperinsulinaemic euglycaemic clamp at 30 d of age, in lambs from ewes in which placental growth and function were surgically-restricted from before conception (PR, n=12) and from un-operated control ewes (control, n=13). Liver, skeletal muscle (vastus lateralis) and perirenal adipose tissues were snap-frozen at post-mortem at 43 d of age. Expression of insulin signaling determinants was quantitated by RT-PCR.

Results: PR reduced whole-body insulin sensitivity (↓57%, P<0.001). In skeletal muscle, PR reduced expression of the insulin receptor (↓35%), IRS-1 (↓24%), GSK3□ (↓23%), and glycogen synthase (↓28%), and tended to reduce expression of AMPK□2 (p=0.054) in PR females. In liver, PR tended to increase IRS-1 gene expression overall (p=0.059), reduced AMPK□3 expression (p=0.030) in PR females, but increased GLUT2 expression (p=0.047) in PR males. In adipose tissue, PR reduced leptin gene expression (↓74%), but did not alter expression of a preliminary set of insulin signaling pathway genes investigated to date.

Conclusions: PR reduces expression of multiple components of the insulin signaling pathway, and to a greater extent in muscle than liver in the lamb. This suggests that skeletal muscle insulin resistance is the primary or initial site of impaired insulin sensitivity in postnatal life after IUGR. Interventions to improve metabolic outcomes after PR and IUGR may therefore be more effective if targeted to earlier developmental windows, before onset of impaired insulin signaling in skeletal muscle.

Vulnerability of intrauterine growth restricted rat offspring to adult hyperglycemia: effects on renal function

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Background: Intrauterine growth restriction (IUGR) leads to a reduced nephron endowment at birth and is associated with an increased risk of renal disease later in life.

Hypothesis / Aims: We propose that the reduction in nephron endowment at birth in IUGR infants renders the kidneys vulnerable to secondary postnatal insults. Hence, the aim of this study was to investigate whether a postnatal insult of mild or moderate hyperglycemia in rats leads to greater renal dysfunction in IUGR rat offspring compared to normal offspring.

Methods: Female WKY rats were fed either a normal protein diet (NPD, 20% casein) or low protein diet (LPD, 8.7% casein) 2 weeks prior to mating, during pregnancy and lactation. After weaning, all male offspring were fed a normal rat chow *ad libitum*. At 23 weeks of age, streptozotocin (STZ; 50mg/kg) was administered intraperitoneally to induce hyperglycemia. From 5 days after STZ injection, blood glucose levels were measured daily; long acting insulin (protophane) was injected (1-2U) daily to stratify blood glucose levels (mild, 7-10 mmol/L and moderate, 10-15 mmol/L; n=8 males per group). At 32 weeks of age, conscious mean arterial blood pressure and heart rate was measured using a tail artery catheter. Using clearance techniques of ^{3}H inulin and ^{14}C para-aminohippurate, effective renal blood flow (RBF), glomerular filtration rate (GFR), renal vascular resistance (RVR) and filtration fraction (FF) were determined.

Results: LPD offspring were born with reduced body weight and they remained significantly smaller than NPD offspring throughout the experimental period ($P < 0.001$). Induction of hyperglycemia (mild or moderate) led to a further decrease in body weight ($P < 0.001$). There was no difference in relative kidney weight between LPD and NPD offspring. However, offspring with hyperglycemia exhibited a significant increase in relative kidney weight in both the mild and moderate groups ($P < 0.0001$). There were no significant differences in mean arterial blood pressure and heart rate between LPD offspring and NPD offspring at 32 weeks of age. There was no significant difference in GFR adjusted for kidney weight between LPD and NPD offspring. However, there was a significant decrease in GFR in all hyperglycaemic offspring ($P < 0.0001$). RVR was significantly increased ($P = 0.005$, $P = 0.02$ and $P < 0.0001$, respectively) in LPD offspring and this was exacerbated in hyperglycemic offspring ($P < 0.0001$). RBF adjusted for kidney weight was significantly decreased ($P = 0.006$) in LPD offspring and this was again, exacerbated in hyperglycaemic offspring ($P < 0.0001$).

Conclusions: Induction of hyperglycemia leads to a marked decline in renal function. Although glumerular filtration rate was maintained in IUGR offspring compared to non-IUGR offspring, renal vascular resistance was significantly increased and exacerbated with hyperglycemia. As the animals age this is likely to adversely impact on glomerular filtration rate.

NOTES

Maternal obesity and early postnatal overnutrition: programming the intrarenal renin-angiotensin system?

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Background: The intrarenal renin-angiotensin system (RAS) is critical for normal renal development, but overactivity in the adult kidney can lead to renal injury and accelerate disease progression. In obese adults, the RAS is highly active and contributes to hypertension and renal pathology. While a number of models of maternal undernutrition, and of IUGR, have reported programming of the intrarenal RAS, we do not know whether maternal obesity or early life overnourishment have any immediate or lasting effects on this system.

Aims: To examine and compare the impact of maternal obesity and early postnatal overnutrition in the rat on expression of the intrarenal RAS at weaning and in adulthood.

Methods: Maternal obesity was induced in Sprague-Dawley dams by offering a palatable high fat diet (34% fat) from 5 weeks prior to conception and throughout pregnancy and lactation. Control dams ate standard chow (14% fat). In a third group, early postnatal overnutrition was induced in control offspring by reducing litter size (from 12 to 3/litter) on postnatal day 1. All groups were weaned onto standard chow at 20 d; males were studied at weaning and at 16 weeks (n=8-9/group). mRNA levels for components of the RAS, normalised to 18S, were measured in homogenised whole kidney by real-time PCR.

Results: At weaning, offspring from obese mothers (MatOb) or small litters (postnatal overnourishment; PostON) were 45-50% heavier than controls and kidney weights were 30-40% greater (P<0.05). Maternal obesity was associated with suppression of renal angiotensinogen (AGT) mRNA levels (P<0.05) and upregulation of ACE2. By contrast, litter size reduction led to a 1.3-fold increase in renal prorenin mRNA (P<0.05), and (pro)renin receptor (PRR) mRNA levels tended to be higher (P=0.07). ACE2 mRNA levels were also upregulated (P<0.05). As adults, MatOb and PostON were ~10% heavier than controls (P<0.05), but kidney weights were higher in MatOb only (~10%, P<0.05). TGF-β1 expression was ~60% of controls in this group (P<0.05). AT_{1a} receptor mRNA tended to be suppressed in adult PostON (n.s.). No other differences in gene expression were found in adult offspring.

Conclusions: Pre- and postnatal overnutrition had a very different impact on the developing renal RAS. In weanlings exposed to maternal obesity, suppression of AGT, combined with increased ACE2, could be predicted to reduce intrarenal angiotensin II (Ang II) generation or oppose its actions. By contrast, upregulation of prorenin and PRR by postnatal overnutrition suggests increased activity of the RAS, either by increased Ang II generation or by increased PRR signalling. These effects were not sustained to adulthood.

Programming a taste for fat: the effect of maternal junk food feeding on food preferences in the offspring

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Background: Maternal high-fat feeding is a major risk factor for the subsequent development of obesity in the offspring. This may be due to the effect of maternal diet on the development of systems which control appetite and food preferences. The present study investigated the hypothesis that maternal junk food feeding is associated with an increased preference for high-fat foods in the offspring.

Methods: Rat dams were either fed standard rat chow (control, n=6) or a cafeteria 'junk food' diet (JF, n=6) during pregnancy, and lactation. From weaning, all offspring were given free access to both control and junk food diets. Food intake was monitored daily and used to calculate food and macronutrient preferences in juvenile (3 to 6 weeks) and adult (6 weeks to 3 months) offspring. Body composition and plasma concentrations of glucose, free fatty acids, insulin and leptin were determined at 3 weeks, 6 weeks and 3 months of age.

Results: At 3 weeks, JF offspring had a higher percentage body fat (Control: 5.7±0.3%; JF: 8.3±0.7%, $P<0.05$) and higher plasma leptin concentrations (Control: 8.3±0.5µg/L; JF: 14.8±1.5µg/L, $P<0.05$) compared to controls. Both male and female JF offspring had a higher intake of fat (Male: Control: 16.5±1.53g/kg/day, JF: 23.1±0.37g/kg/day; Female: Control: 17.5±1.04g/kg/day, JF: 23.0±1.13g/kg/day) and protein (Male: Control: 19.8±0.92g/kg/day, JF: 23.4±0.83g/kg/day; Female: Control: 18.1±1.17g/kg/day, JF: 22.3±0.98g/kg/day) during the juvenile period ($P<0.05$), and a higher intake of fat (Male: Control: 9.17±0.54g/kg/day, JF: 12.3±0.37g/kg/day; Female: Control: 11.6±0.33g/kg/day, JF: 16.7±1.92g/kg/day) throughout adulthood ($P<0.05$). Total energy and carbohydrate intake were not different between groups.

Conclusions: These data provide evidence that excessive intake of junk foods during pregnancy can program an increased preference for fat in the offspring. This may be one mechanism through which prenatal exposure to a high-fat diet increases the subsequent risk of obesity.

Maternal omega-3 supplementation alters fat distribution in the offspring

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Background: Exposure to an increased nutrient supply before birth is associated with an increased risk of obesity in postnatal life. This is due, at least in part, to altered development of fat cells, since fat cell formation (adipogenesis) and lipid accumulation (lipogenesis) are both highly sensitive to the nutritional environment during the period of fat cell development. Omega-3 long chain polyunsaturated fatty acids (LCPUFA) inhibit adipogenesis and lipogenesis in adult rats, however it is not known whether supplementing the maternal diet with omega-3 LCPUFA results in reduced fat deposition in the offspring.

Aims/Hypothesis: To determine the effect of maternal omega-3 supplementation on fat deposition in the offspring in a rodent model

Methods: Female Wistar rats were fed either a standard chow (Control, n=8) or chow supplemented with 25mg/kg/day of the omega-3 LCPUFA Docosahexaenoic acid (22:6n-3, DHA) during pregnancy and lactation (Omega-3, n=8). Tissues were collected from pups at 6wk of age and weights of visceral and subcutaneous fat depots were recorded. Blood samples were collected for the determination of fatty acid status (fatty acid composition of erythrocyte membranes) and plasma concentrations of glucose, insulin and free fatty acids in the offspring.

Results: Omega-3 fatty acid status (as a % of total fatty acids) was higher in omega-3 offspring (6.7 ± 0.2 % vs 5.6 ± 0.2%, $P<0.001$). Birth weight and 6wk body weight were not different between groups. The relative mass of subcutaneous fat was higher in male (0.019 ± 0.001 g/g vs 0.014 ± 0.002 g/g, $P<0.04$) and female (0.018 ± 0.001 g/g vs 0.013 ± 0.002 g/g, $P<0.04$) omega-3 offspring. Whilst there was no difference in the relative mass of visceral fat, the ratio of visceral to subcutaneous fat mass was lower in omega-3 offspring compared to controls in both males (1.48 ± 0.12 vs 2.19 ± 0.4, $P<0.02$) and females (1.83 ± 0.27 vs 1.58 ± 0.13, $P<0.05$). There was no effect of maternal omega-3 supplementation on circulating concentrations of glucose, insulin or free fatty acids in either male or female offspring.

Conclusions: These data suggest that whilst maternal omega-3 supplementation does not result in an overall reduction in body fat mass in the offspring, it may be associated with a change in body fat distribution. Specifically, maternal omega-3 supplementation resulted in a reduction in the amount of visceral fat relative to subcutaneous fat, which is a pattern of fat deposition which has previously been shown to be associated with a more favourable metabolic phenotype.

Impact of maternal obesity on offspring behaviour and metabolic risk

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Background: Changes in maternal nutrition during gestation and lactation can exert profound long-term effects on offspring. The obesity epidemic threatens future generations, as maternal obesity increases the risk of offspring obesity. Maternal obesity is also linked to metabolic syndrome in offspring. We have shown that high-fat-diet (HFD) consumption after weaning exacerbates offspring metabolic risk. This study set out to explore behavioural impacts, and to test an exercise intervention.

Aims/Hypothesis: Our aims were two-fold. 1. Obesity has been shown to influence behaviors such as learning, but data are conflicting. We tested the effects of maternal obesity on offspring anxiety levels, learning and memory. 2. Physical exercise improves diet-induced obesity, insulin resistance, dyslipidemia and hypertension. We tested whether voluntary exercise would ameliorate the adverse effects of maternal obesity on lipid homeostasis and blood pressure in offspring.

Methods: Female Sprague Dawley rats were fed chow (C) or HFD for 6 weeks before mating, throughout gestation and lactation. At 20 days, female pups were weaned onto either C or HFD, yielding CC, CH, HC and HH groups. Pups (12 per group) underwent anxiety testing (elevated plus maze, EPM) and forced swim test (FST) from 9-19 weeks. In a separate cohort, half the rats were provided with running wheels enabling voluntary exercise.

Results: Offspring of obese mothers (HC) were significantly heavier at weaning (3 weeks) with increased adiposity and this was amplified by HFD (HH). At 11 weeks, HC and HH offspring spent more time, and made more entries, in the open arm of the EPM, with more exploratory behaviour, compared to CC and CH offspring ($P<0.05$). Post-weaning diet had no significant impact. While some effects of maternal diet were observed on FST, with increased immobility in offspring of obese mothers, this appeared to be related to overall adiposity. At 21 weeks plasma leptin was markedly increased in HH versus HC offspring.
While exercise had no impact in CC rats, it reduced the detrimental effects of maternal obesity in HC and HH offspring. Larger effects were observed in HH rats, where exercise decreased body weight, food intake, fat mass, plasma triglyceride and leptin concentrations (HH_{EX} vs HH, $P < 0.01$). Blood pressure was elevated in HH offspring (vs HC; $P <0.001$) and this was reversed by exercise.

Conclusions: Maternal obesity appears to reduce female offspring anxiety-like behaviour; ongoing work is aimed at dissecting the underlying mechanisms. Exercise had no obvious beneficial effects in offspring of lean mothers consuming a low fat diet, who had a low risk of metabolic disease. However voluntary exercise implemented at weaning reduced the deleterious effects of maternal obesity, with greater beneficial effects in offspring of obese mothers consuming HFD (HH).

NOTES

Establishing a relevant model of chronic, low dose gestational ethanol consumption.

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Background: The effects of prenatal ethanol (EtOH) exposure on the fetus and offspring need to be determined as many women continue to drink alcohol throughout pregnancy. Most studies in this field have used models of high EtOH exposure (35% calories derived from EtOH) however the applicability of this to human society is questionable.
Aims: The aim of this study was to determine the drinking pattern of pregnant rats fed a liquid diet containing a low dose of EtOH (6%v/v) and measure their plasma ethanol concentration (PEC).
Methods: Time-mated Sprague-Dawley rats were fed an ad-libitum liquid diet ±6% EtOH for 21 hours a day throughout pregnancy (n=39 control, n=39 EtOH); the diets were approximately isocaloric. Diet consumption and weight gain were recorded daily. In a subset of EtOH fed dams (n=7) blood samples were collected from the tail 15 minutes after the diet was offered at 8±1 day of gestation and PEC determined using a colourimetric assay (EnzyChrome™ Ethanol Assay, BioCore, Sydney).
Results: During the 21 hour period, EtOH fed rats consumed 29.1±0.6mL diet/day and control rats consumed 31.4±0.7mL diet/day (61±1 calories/day for EtOH fed dams and 65±2 calories/day for control dams; ~7% difference in daily caloric intake). For the EtOH group, 9 calories/day (15% calories) were provided by EtOH. During the first 5 hrs of the diet being offered, 60±13% of the diet was consumed by EtOH dams compared to 53±5% for control dams, with most of the diet being consumed within the first 15 minutes of the diet being offered (12±1% for EtOH dams versus 14±1% for control dams). Following this time of maximal dietary consumption, PEC reached 0.024±0.008g/dL (0.03±0.01%). Throughout gestation total maternal weight gain was not different between groups (98±4g for both groups). For the dams that were allowed to deliver their pups, there was no difference in gestational length (22.5±1 days) or the number of male and female pups per litter (EtOH: 6±1 male and 6±1 female pups; control: 6±1 male and 5±1 female pups) between groups.
Conclusion: Pregnant rats are not adverse to a 6% (v/v) EtOH liquid diet and share the same pattern of dietary consumption as rats fed a similar isocalorically equivalent liquid diet. Our 6% EtOH liquid diet maintains a low plasma ethanol level that does not induce preterm birth or alter litter size or composition. Hence, this is an appropriate model in which to study the effects of low prenatal EtOH exposure of offspring.

Influence of preterm birth and antenatal corticosteroid exposure on early growth patterns and response to growth hormone administration in lambs.

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Background: Growth hormone (GH) and Insulin like Growth Factors (IGF) are key mediators of postnatal growth. Maturation of the fetal somatotrophic axis close to term is dependent on the late gestation increase in endogenous corticosteroids. Preterm infants are frequently exposed to exogenous corticosteroids *in utero* and often have poor postnatal growth. It is unknown whether poor growth is due to preterm birth *per se* or effects of antenatal glucocorticoids on the somatotrophic axis.

Hypothesis: Preterm birth, and not antenatal steroid exposure, alters postnatal growth trajectory and somatotrophic axis function in pre-pubertal lambs.

Methods: Singleton bearing ewes were randomised to Dexamethasone induced labour at term (IOL; d147, n=6) or preterm (Preterm; d137, n=15) gestations, or delivered spontaneously at term (Term; d147 n=15). Lambs were weighed and measured weekly until weaning (12 weeks of age), and monthly thereafter. At 4 months, lambs received GH (0.15 mg/kg i.m.) twice daily, for 4 days. Blood samples were taken at 0, 8, 24, 48, 72 and 96 hr following the first injection. Lambs were weighed before and after the GH test and fed *ad libitum* throughout. Data were analysed by repeated measures (RM) ANOVA or multiple regression analysis with post hoc correction where indicated.

Results: Preterm lambs were smaller and lighter at birth than IOL and Term lambs, which were of similar size, and remained significantly smaller and lighter until weaning ($p<0.05$) despite having a greater growth velocity (GV) (20.8±0.2 vs 19±0.2 (IOL) vs 19.5±0.2 (Term) g/kg.d; $p<0.05$). IOL lambs had reduced weight gain and linear growth between birth and weaning than Term lambs ($p<0.05$). By 4 months of age, body weight was similar in all three groups. IOL lambs had a significantly greater IGF1 response to GH than Term, but not Preterm, lambs (areas under the curve 16,500 vs 10,500 (Term) vs 12,100 (Preterm) ng/mL.hr; $p<0.05$). IGF1 response was not related to sex, GV, current weight or birth weight z-score. Relative weight gain and feed intake during GH exposure were not different amongst groups.

Conclusions: Antenatal corticosteroid exposure at term leads to significantly reduced early growth but increased IGF1 response to GH. Lambs born preterm, following equivalent exogenous antenatal corticosteroid exposure, have significantly greater early GV, but similar IGF1 responses to those of lambs born spontaneously at term. Postnatal somatotrophic axis function may be influenced by gestational age at time of corticosteroid exposure. The physiological basis for this is as yet undetermined.

MATERNAL FOLIC ACID SUPPLEMENTATION AND ABUNDANCE AND EXPRESSION OF INSULIN-LIKE GROWTH FACTOR –II IN OFFSPRING

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Background: Folic acid supplementation (FAS) is recommended for pregnant women in order to prevent neural tube defects in babies. By supplying methyl groups, folic acid may also affect methylation of DNA and histones thus affecting gene expression. Maternal exposure to famine periconceptionally or maternal choline and methyl nutrient deficiency has been shown to affect methylation status and expression of the imprinted gene, insulin-like growth factor-II, IGF-II. Whether increasing methyl group supply through maternal FAS (MFAS) could also alter tissue expression and circulating abundance of IGF-II in offspring postnatally is unknown.

Aims/Hypothesis: MFAS will increase IGF-II expression in skeletal muscle, increase plasma IGF-II and reduce adiposity in offspring.

Methods: Female Wistar rats were fed either a control ($2mg.kg^{-1}$) (n=22) or a folic acid supplemented diet ($6mg.kg^{-1}$) (n=23) beginning two weeks prior to mating until offspring were born. Offspring were sacrificed at postnatal days 1, 7, 14, 21, 28, 40 and 90 and plasma and tissues collected. Plasma IGF-II was measured by specific radioimmunoassay (RIA) after molecular sieving by high performance liquid chromatography (HPLC). Quantitative RT-PCR was used to measure *igf2* gene expression in skeletal muscle of offspring at days 7, 14 and 90 normalized to the expression of β-actin.

Results: MFAS increased plasma IGF-II in offspring overall, due to mainly increased plasma IGF-II in females at day 7 of age, but did not alter *igf2* expression in skeletal muscle in offspring. While skeletal muscle *igf2* could account for ~36% of variation in plasma IGF-II in control offspring, this is reduced to ~16% in MFAS offspring, suggesting that there was an increased contribution from other tissue sites of *igf2* expression, particularly in females.

Conclusions: MFAS does increase IGF-II abundance in offspring, but transiently in a sex dimorphic manner and possibly through actions on tissues other than skeletal muscle.

Periconceptional undernutrition affects the relationship between early growth and later glucose tolerance in lambs

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Objective The aim of this experiment was to determine whether relationships between early postnatal growth, later weight and glucose tolerance are affected by maternal periconceptional undernutrition.
Methods Singleton lambs of normally nourished (N, n=30; male (M) n=10, female (F) n=20) or peri-conceptionally undernourished (UN, n=29; M n=14, F n=15) ewes were weighed weekly to weaning at 12 weeks, then at 4 and 10 months of age. Exponential growth velocity for weight was calculated from birth to 6 weeks of age (postnatal GV). At 4 and 10 months of age, intravenous glucose tolerance tests were performed on a subset of lambs and area under the curve (AUC) for both glucose and insulin was calculated using a triangulation method. Data were analysed using ANOVA with Fisher post hoc test and groups compared using regression. Results are expressed as mean±SEM.
Results Offspring of N ewes had a lower birth weight than those of UN ewes (p=0.02), and males were heavier than females in both nutritional groups (p=0.005) (N M 5.8 ± 0.3, F 5.2 ± 0.2 kg; UN M 6.4 ± 0.2 kg, F 5.7±0.2 kg). Postnatal GV was greater in offspring of N than UN ewes (N 28 ± 1; UN 25 ± 1 $g.kg^{-1}.d^{-1}$, p=0.008) and tended to be greater in females than males (p=0.05), so that by 6 weeks of age there was no longer any weight difference between lambs of either nutrition group or sex (N M 18 ± 1, F 17 ± 1 kg; UN M 17 ± 1, F 18 ± 1 kg). This remained the case at 12 weeks (weaning) and at 4 months (juvenile). At 10 months (post puberty), offspring of N ewes were lighter than those of UN ewes (p=0.04), and males were heavier than females in both nutritional groups (p=0.0007) (N M 50 ± 4, F 39 ± 1 kg; UN M 57 ±3 kg, F 44 ± 2 kg). Birth weight was inversely related to postnatal GV in all lambs (R^2 = 0.4, p<0.0001). Postnatal GV was positively associated with 4 month weight in offspring of N, but not UN, ewes (N R^2 = 0.7, p=0.004; UN R^2 = 0.1, p=0.3). In lambs of N, but not UN ewes, postnatal GV was positively associated with glucose and insulin AUC at 4 months in lambs of both sexes, significant only for insulin (N R^2 = 0.9, p=0.01; UN R^2 = 0.4, p=0.1). There was no association between postnatal GV and either glucose or insulin AUC at 10 months in either nutritional group. There was no association between weight at 4 and 10 months in lambs of either sex or nutritional group, and no association between weight change between 4 and 10 months and glucose or insulin AUC at 10 months.
Conclusion Maternal undernutrition around conception results in altered regulation of the relationships between postnatal growth and carbohydrate metabolism. Intrauterine events may contribute to the effects of early postnatal growth on later health, which may in turn be modified by pubertal changes in growth and metabolism.

Reduced cortisol response to AVP+CRH challenge in adult offspring of ewes undernourished around the time of conception.

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Background: Undernutrition of ewes from 61 d before to 30 d after mating advances development of the fetal hypothalamic-pituitary-adrenal axis (HPAA) in late gestation. The consequences for adult HPAA function are not known.
Aim: To investigate the development of HPAA function through to 18 mo of age in offspring of ewes undernourished around the time of conception.
Methods: Ewes were undernourished from 61 d before to 30 d after mating (PCUN) to maintain a 10-15% loss in weight, or were well fed (N). Offspring were challenged at 4, 10 and 18 mo of age with CRH (0.5 µg/kg) and AVP (0.1 µg/kg). Plasma samples were collected over 60 min. Plasma cortisol was measured by mass spectrometry, ACTH by RIA. Areas under the curve (AUC) for cortisol and ACTH responses were calculated. Data were analysed by ANOVA and multiple regression and are expressed as mean ± SEM.
Results: Birth weight was not affected by maternal PCUN (N; 5.4±0.1 vs. PCUN; 5.3±0.2 kg), sex or single/twin status. At 4, 10 and 18 mo males were heavier than females (p<0.05) but there was no effect of maternal PCUN or twinning on weight. Plasma cortisol AUC was not affected by twinning, birth weight or current weight but was higher in females at 10 and 18 mo (Table, both p<0.05). In females the ratio of cortisol to ACTH AUC was higher in N than PCUN at 18 mo (p<0.05).

	Ewes		Rams	
	N	PCUN	N	PCUN
Cortisol AUC (ng.ml.min^{-1})				
4 mo	1784±82(23)	1658±111(19)	1647±112(18)	1414±106(14)
10 mo*	2614±137†(22)	2023±91§†(18)	1771±134(17)	1374±50§ (12)
18 mo*	2507±109(17)	1985±78§ (16)	1426±112†(16)	1173±113(9)
ACTH AUC (ng.ml.min^{-1})				
4 mo	6.4±0.8	7.8±1.2	6.6±0.7	7.8±0.9
10 mo*	9.3±0.7†	8.7±1.0	7.1±0.6	6.9±0.7
18 mo$^{\Psi}$	6.6±0.5†	9.1±1.0§	7.9±1.1	7.4±0.7
Cortisol ÷ ACTH ratio				
4 mo	344±31	299±42	299±49	204±21
10 mo	311±22	279±28	267±23	234±42
18 mo*	401±24†	257±30§	221±31	162±18

Data are mean±SEM (n). § p<0.05 for treatment effect within sex. * p<0.05 for sex effect. $^{\dagger}p$<0.05 for difference from value at previous age. $^{\Psi}p$<0.05 treatment and sex interaction. N, well nourished; PCUN, periconceptionally undernourished.

Conclusions: Despite advancement of fetal HPAA development in PCUN offspring, by 10 mo of age cortisol response to AVP+CRH stimulation was depressed in both sexes. In female PCUN offspring, this reduced cortisol response was maintained at 18 mo. In contrast, the cortisol response to ACTH in N ewes became more sensitive with increasing age. In male PCUN offspring, there was no change in cortisol response to ACTH with increasing age, whereas in N rams the cortisol response decreased at 18 mo. PCUN leads to a general depression in HPAA responsiveness postnatally but there are clear sex differences in how this occurs.

NOTES

Developmental changes in maturity of cardiomyocytes

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Background: Cardiac myocytes develop early in life by both hyperplasia and hypertrophy. Hyperplasia of myocytes ceases either before or shortly after birth so that further increases in cardiac muscle mass depend on cardiac cell hypertrophy. Terminal differentiation is characterised by the appearance of cardiac myocytes with multiple nuclei. The timing of the transition from proliferative cardiac growth to hypertrophic growth varies between species. The degree of maturity of cardiac myocytes may affect cardiac function in early postnatal life.
Aim: To measure cardiac myocyte maturity in fetal and neonatal pig hearts.
Methods: We studied Landrace/Large White crossbreed piglets. Term piglets (Term=115 days) were killed within 12 h of birth while preterm piglets were delivered by caesarean section at 92 days. Cardiac myocytes were isolated from right and left ventricular free walls (RVFW; LVFW). Ratios of uninucleated to bi- or multinucleated myocytes were determined and myocyte volumes of uni- and binucleated cells measured using confocal microscopy (Burrell et al, 2003).
Results: The %RVFW/kg body weight was greater than %LVFW/kg body weight in term piglets (0.22±0.01% (mean±SEM) vs. 0.17±0.01%, P=0.045) but they were of similar mass in preterm piglets. At 92 days (0.8 of term), there was only an occasional binucleated cardiomyocyte, whereas at term, LVFW binucleates were 19.7±4.2%, P=0.004 and RVFW binucleates 20.7±2.3% (P<0.001, compared to preterm). Uninucleated cardiac myocytes from the RVFW were bigger than LVFW myocytes at both ages (P<0.001). RVFW uninucleated myocytes were bigger in term hearts (P=0.005) compared to preterm but LVFW uninucleated myocytes were a similar volume in preterm and term piglet hearts.
Conclusion: The proportion of mature myocytes is greater in term than in preterm piglet hearts, but only about 20% of myocytes are mature at term whereas in the lamb, 78% of myocytes are mature (Burrell et al, 2003). In pigs, myocyte maturation starts late in gestation (>0.8 term); in sheep it begins at approximately 0.7 term. RVFW myocytes were bigger than those in the LVFW, probably because the right ventricle contributes more to the cardiac output during intrauterine life. RVFW myocytes continue to increase in size during the last 3 weeks of gestation in the pig, but LVFW uninucleated myocytes remain the same size, possibly to provide a larger reserve of myocytes for hyperplasic growth after birth.
Burrell et al (2003) Anatomical Record, 274, 952-61.

Surgical stress alters gene expression independent of Dexamethasone administration in the mouse.

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Background: Excess prenatal glucocorticoid (GC) exposure has been shown to be detrimental to adult health, resulting in diseases such as hypertension, by a process commonly known as 'developmental programming'. This study investigated the effects of short-term exposure to the synthetic GC dexamethasone (DEX) on cardiac gene expression in the developing fetus.
Method: C57BL/6 mice were either left untreated (CON), or underwent surgery for the subcutaneous implantation of an osmotic mini-pump which either infused DEX (1µg/kg/h) or saline (SAL) for 72 hours from embryonic day 12.5 (E12.5). Hearts were collected at E17.5. Expression of genes related to cardiac growth (angiotensin II receptor subtypes a [AT1a] and b [AT1b] as well as vascular endothelial growth factor A [VEGF-A] and mitogen activated protein kinase 1 [Map2k1]) in addition to apoptotic related genes (B-cell CLL/lymphoma 2 [Bcl-2], Bcl-2–associated X protein [Bax] and interleukin-1 receptor-associated kinase 1 [Irak]) were analysed by real-time PCR.
Results: The body weight of the embryos across all groups was similar. No significant changes ($p < 0.05$) were observed between groups in VEGFa. Significant changes in relative gene expression were seen between CON and DEX groups in each of AT1a , AT1b, Irak, Map2k1 and Bcl-2, as well as between SAL and DEX groups in Irak and Map2k1 with tending towards significance ($p = 0.05$). Interestingly, changes were also observed between the CON and SAL groups in AT1b, Irak, Bax and Bcl-2 with Map2k1 also tending towards significance ($p = 0.06$).
Conclusions: In addition to the anticipated changes in relative gene expression due to prenatal DEX exposure, the observed changes between CON and SAL groups imply that the act of surgery per se is a stressor capable of up-regulating endogenous GC production enough to alter the expression of genes involved in cardiac growth and development. These results highlight the importance of selecting the most appropriate control group(s) when quantifying changes in gene expression after a surgical procedure. Poor choice of control groups may either exaggerate or understate the 'true' change in the measured outcome.

Table 1. Changes in relative gene expression between groups

	CON	SAL	DEX
AT1a	1.01 ± 0.15	0.90 ± 0.10	1.95 ± 0.45 *
AT1b	1.21 ± 0.20	1.87 ± 0.22 *	2.61 ± 0.47 *
Irak	1.02 ± 0.06	1.33 ± 0.07 *	1.73 ± 0.12 †
Map2k1	1.09 ± 0.12	1.37 ± 0.06	1.62 ± 0.09 †
Bax	1.04 ± 0.09	0.79 ± 0.06 *	1.44 ± 0.44
Bcl-2	2.00 ± 0.27	4.14 ± 0.69 *	2.86 ± 0.26 *

*/† Significantly different to CON/SAL groups respectively.

Baroreflex control of heart rate and renal sympathetic nerve activity (SNA) in preterm and near-term fetal sheep

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Background: The arterial baroreflex is a fundamental reflex that buffers rapid changes blood pressure (BP) by regulating heart rate (HR) and sympathetic nerve activity (SNA) to the vasculature. In adults a sigmoidal relationship between BP – HR and SNA is well documented. Preterm babies have a high incidence of low or unstable blood pressure especially in the first few days of life and treatments are not clearly effective. Potentially, immaturity of the baroreflex may contribute to unstable BP in the preterm infant.

Aims/Hypothesis: To determine whether renal SNA and HR are under baroreflex control in preterm fetal sheep and to assess if the baroreflex matures in late gestation.

Methods: Seven preterm (90-100 days gestation; term 140 days) and ten near-term fetal sheep (118-127 days gestation) were chronically instrumented. BP was manipulated using vasoactive drugs, sodium nitroprusside (SNP) and phenylephrine (PE) and the resultant changes in HR and renal SNA measured.

Results: In preterm fetal sheep the cardiac baroreflex was active, with reciprocal changes in HR when BP was manipulated ($P<0.005$); however, the hypotensive response was significantly slower than the response to hypertension ($P<0.05$). In near-term fetal sheep the cardiac baroreflex was highly sleep state dependent, with reciprocal changes in HR in response to BP changes during low-voltage, high frequency (LV) sleep that were similar to the preterm, but limited changes in HR during high-voltage, low frequency (HV) sleep.

In contrast, renal SNA did not appear to be under baroreflex control in preterm fetal sheep. Near-term, the only significant change in renal SNA during baroreflex challenges was a significant decrease ($P<0.05$) in response to hypertension during HV sleep.

Conclusions: The results indicate that *in utero* the cardiac baroreflex is active at 0.7 of gestation, although the response is not as rapid as in adults. Further, even near-term the cardiac baroreflex was highly sleep state dependent and no faster than in preterm fetal sheep. The striking lack of response in sympathetic outflow to changes in BP in both preterm and near-term fetal sheep support the hypothesis that an immature baroreflex may contribute to BP instability in newborn preterm infants and raises the question as to when during gestation or indeed after birth tight baroreflex control of SNA develops.

Cardiac function in preterm piglets

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Background: Low systemic blood flow (SBF) occurs in 30% of infants born <30wk gestation and is resistant to current therapeutic and preventative interventions. These infants have a high risk of neurodevelopmental disability. Inability of the preterm heart to adapt to the challenges that occur at birth may contribute to low SBF.

Aim: Our aim was to compare the function of term and preterm hearts *in vitro,* by assessing their ability to maintain systemic blood flow in the face of physiological challenges that may be encountered in the adaptation to *ex utero* life.

Methods: We developed an isolated working heart preparation (antegrade perfusion) to test the cardiac function of preterm (0.8 of term) and term piglet hearts. Preload (pressures stretching the left ventricle) and afterload (analogous with vascular resistance) were varied by adjusting the height of reservoirs. Hearts were paced and cardiac output, coronary flow and ventricular and arterial pressures were measured.

Results: Preterm piglets had poorer cardiac function compared to term piglets at a moderate preload (12mmHg; see Table), with reduced cardiac output, cardiac power, stroke volume and arterial flow per gram of tissue, and reduced contractility. Raising the preload to 20mmHg increased the average cardiac output of preterms by 123%, but only 15% in term hearts. Preterm hearts did not maintain positive arterial flow at afterloads above 40mmHg whereas term hearts maintained positive flow at 65mmHg.

	Preterm (n=4)	Term (n=4)	P value
heart mass (g)	5.7 ± 0.3	13.0 ± 1.7	0.003
cardiac output (mL/min/g)	11.9 ± 6.7	27.8 ± 3.6	0.044
cardiac power (mmHg/mL/min/g)	324 ± 210	1134 ± 147	0.010
stroke volume (mL/mg)	75 ± 40	153 ± 20	0.139
arterial flow (mL/min/g)	3.6 ± 1.2	13.3 ± 3.4	0.019
max +dP/dt (mmHg/s)	551 ± 124	1820 ± 189	<0.001

Data expressed as mean ± SEM.

Conclusions: In terms of therapeutic interventions, these results suggest that preterm infants with low system blood flow could benefit from increased preload in conjunction with decreased afterload.

Effects of Gender on the Maturation of Heart Rate Variability in Term and Preterm Infants

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Background: Male gender is associated with increased neonatal mortality and long-term morbidity in infants born preterm. In addition, infants born preterm have been reported to exhibit an increased risk for hypertension and cardiovascular disease later in life. During the first 6 months of life autonomic cardiac control undergoes significant maturation and these changes are sleep state dependant. Assessment of autonomic control can be made with the use of heart rate variability (HRV) analysis. To date the effects of gender on the maturation of autonomic cardiac control have not been assessed in preterm infants.

Aims: To assess the effects of gender on autonomic cardiac control using spontaneous heart rate variability (HRV) across the first 6 months of life in both term and preterm infants during sleep.

Methods: 30 term infants (15 males/16 females) and 25 preterm infants (14 male/11 female) born at 28-32 weeks of gestational age were studied using daytime polysomnography at 2-4 wks, 2-3 mo and 5-6 mo term-corrected age (CA). HRV was assessed using spectral analysis, during 1-2 min epochs during both quiet and active sleep in the supine sleeping position at each age (n=3 in each infant in each sleep state). Low frequency (LF, attributed to the baroreflex mediated changes and sympathetic activity), high frequency (HF, attributed to respiratory related changes), LF/HF (indicates sympathovagal balance) and total power (indicates total variability). The effects of gender and sleep state were compared with two way ANOVA with Student Newman Kuels post hoc analysis.

Results: In term infants, there was no effect of gender on any of the HRV indices analysed at any age. However, in preterm infants at 5-6 mo CA, during AS only LF power was lower in males (310 ± 80 ms^2) compared to females (602 ± 75 ms^2, $p<0.05$). Total power was also lower in males (525 ± 142 ms^2) compared to females (930 ± 134 ms^2, $p<0.05$) at this age. Also at 5-6 mo CA there was an overall effect of gender on HF power ($p<0.05$), however these differences were too marginal to isolate which sleep states differed.

Conclusions: Our study has assessed gender differences in both term and preterm infants matched for gestational age. At 5-6mo (CA) gender differences begin to emerge between males and females in preterm infants which are not present at the same age in term infants. Lower HRV in male infants suggests that male infants may have higher sympathetic activity or reduced vagal tone compared to female infants at this age. Higher sympathetic tone at such an early age may increase the risk of forming hypertension and cardiovascular disease later in life in males born preterm.

NOTES

PLZF: the missing link in decidualisation?

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Background: Prorenin, despite being inactive, is the major form of renin found in tissues. Prorenin becomes active if it binds to the novel prorenin receptor (ATP6AP2) found by Nguyen et al (2002). The prorenin-ATP6AP2 complex has been found to stimulate translocation of Promyelocytic Zinc Finger (PLZF) protein to the nucleus where it increases expression of the p85α subunit of PI3 kinase (PI3K-p85α) and represses the expression of ATP6AP2 (Schefe et al., 2006). This renin/ATP6AP2/PLZF/PI3K-p85α signalling pathway was shown to stimulate proliferation of cardiac myofibroblasts.
Aims/Hypothesis: We aim to determine whether decidualisation of endometrial stromal cells by progesterone regulates PLZF and its downstream targets PI3K-p85α and ATP6AP2.
Methods: Human Endometrial Stromal Cells (HESC) were cultured in DMEM/F12 medium with 2% fetal bovine serum, with or without 1μM medroxyprogesterone acetate, 10nM estradiol-17β and 0.5mM dibutyryl cyclic AMP (MPA) or 15μM 5-aza-2-deoxycytidine (N=3 experiments in triplicate). Total RNA was extracted using TRIZol® and converted to cDNA for quantitative real-time PCR using SuperScript III and random hexamers. mRNA abundances for PLZF, PI3K-p85α and ATP6AP2 were calculated relative to Alien RNA using the ΔΔCT method.
Results: Exposure of HESCs to MPA or AZA resulted in an increase in markers of decidualisation (IGFBP-1 and prolactin) and increased prorenin mRNA (Lumbers et al., 2010), whilst ATP6AP2 mRNA abundance was unchanged. PLZF expression could not be detected in control samples nor was its expression affected by exposure of cells to AZA. PLZF was expressed in all cells that were incubated with MPA ($P<0.01$). PI3K-p85α mRNA was detected in all samples but in MPA treated samples levels were much greater than in control samples ($P<0.05$).
Conclusions: Our preliminary data show that HESCs express both prorenin and ATP6AP2. On decidualisation of HESCs by incubation with progesterone and cAMP prorenin mRNA levels are increased (Lumbers et al, 2010). In addition PLZF is expressed and PI3K-p85α expression is also increased. It is known that the interaction of prorenin with ATP6AP2 stimulates translocation of PLZF to the nucleus and stimulates PI3K-p85α expression, which is important in growth and differentiation. We believe that we have described a novel signalling pathway in HESCs which has the potential to modulate decidualisation.

Lumbers ER, Pringle KG, Logan PC, Mitchell MD. PSANZ, March 2010.

Schefe, J. H., Menk, M., Reinemund, J., Effertz, K., Hobbs, R. M., Pandolfi, P. P., Ruiz, P., Unger, T., Funke-Kaiser, H. Circ. Res.99, 1355-1366.

Oxidative stress and the inter-relationship of nitric oxide and carbon monoxide in the preterm placenta

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Background: Sexual dimorphisms in the incidence of patho-physiological pregnancy, and neonatal outcome are well recognised. Feto-placental production of reactive oxygen species (ROS), principally the superoxide anion (0_2^-) and nitric oxide (NO), is central to patho-physiologic conditions precipitating preterm birth and the pathogenesis of common preterm neonatal morbidities. The interactive relationship between the NOS/NO system and the anti-inflammatory/anti-oxidant heme oxygenase (HO) system may be critical in the placenta.
Aims/Hypothesis: The aim of this study was to characterise the relationship between the NOS/NO and HO/CO systems with respect to redox balance in the preterm placenta with respect to fetal sex.
Method: Placentae were collected from 32 women who delivered <29 weeks gestation. Oxidative and nitrative stress (nitrotyrosine, protein carbonyl, and lipid hydroperoxide concentration), anti-oxidant enzyme activity (glutathione peroxidase , thiredoxin reductase, and superoxide dismutase) and reactive oxygen species (nitric oxide (NO) and carbon monoxide (CO) concentration) where determined by ELISA and spectrophotometry. HO-1 and 2 mRNA expression was determined by qRT-PCR.
Results: Placental NO production was greater in males than females (p=0.03). While there was no difference in HO-1 expression between the sexes, HO-2 expression was significantly higher in females ($p<0.05$). Sex also influenced placental CO production, the bioactive product of HO, with levels higher in males (p=0.03). Sex-specific differences were evident in oxidative stress with levels greater in males (protein carbonyl p=0.04, nitrotyrosine p=0.018, lipid hydroperoxide p=0.03). While superoxide dismutase and thioredxin reductase activity were not influenced by sex, glutathione peroxidase activity was higher in females (p=0.05).
Conclusion: This data supports sex-specific differences in reactive oxygen species production and markers of oxidative stress in placentae from pregnancies complicated by preterm delivery. The regulatory pathways controlling feto-placental responses to inflammatory stimuli and the homeostatic balance between ROS production and anti-oxidant defence are yet to be fully delineated These molecular and cellular mechanisms represent potentially targetable pathways in patho-physiologic pregnancy and are the focus of on-going studies.

Short term maternal glucocorticoid exposure alters placental shape and gene expression in the mouse

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Background: Maternal glucocorticoid (GC) exposure during pregnancy has been shown to increase the incidence of hypertension in offspring. The placenta plays a vital role in protecting the foetus from many potentially harmful substances in the maternal blood supply, including GCs. Foetal protection from GCs is primarily brought about by the actions of the enzyme 11βHSD2 which breaks down GCs into their 11-keto metabolites. This study investigates the effects of GCs on placental formation during a "critical period" of development known to result in programming of disease. A mouse model was used to allow investigation of molecular mechanisms to be more easily undertaken.
Aims: This study aims to investigate changes in placental gross morphology and gene expression after short term maternal exposure to the synthetic glucocorticoid dexamethasone.
Methods: Pregnant C57/BL/6 mice were infused with dexamethasone (DEX-1µg/kg/h) for 72 hours via osmotic minipump beginning at embryonic day (E) 12.5. At 14.5 (during treatment) and E17.5 (after treatment), animals were killed and foetuses removed. Foetal and placental dimensions were taken before placentae were collected and frozen. The gene expression of the angiotensin II receptors (AT1a and At1b) and 11βHSD2 were examined by real time PCR
Results: At E17.5, foetal weight, placental weight and the placental weight to foetal weight ratio were unchanged as were placental length and width. Placental thickness was increased in foetuses prenatally exposed to DEX (control= 1.71±0.15 mm, DEX= 2.06±0.06 mm, $P<0.01$) while the placental length to width ratio was decreased (control=1.12±0.02, DEX= 1.08±0.03, $P<0.05$). The gene expression of AT1a in foetuses prenatally exposed to DEX was not changed at E14.5 but was decreased at E17.5 ($p<0.05$). Expression of AT1b was increased at E14.5 ($p<0.05$) in dexamethasone exposed foetuses but was not different at E17.5. 11βHSD2 gene expression was increased in females during DEX exposure at E14.5 ($p<0.05$) but not different in males or in either sex at E17.5.
Conclusion: This study indicates that while short term GC exposure during mid gestation in mice does not affect foetal or placental weights, changes in placental dimensions suggests DEX alters placental morphology. Gene expression changes in AT1a and AT1b suggest age specific alterations in placental angiogenesis and proliferation. This supports previous findings in our lab which have shown short term DEX exposure to alter the expression of other vasculogenesis genes including members of the VEGF family. The sex specific changes in 11βHSD2 expression suggest a greater effort by the placenta to protect the female foetus from GCs. Thus alterations in placental morphology and gene expression may contribute to changes in placental function which may in turn impact on foetal development and later adult health.

Regulation of the placental glucocorticoid barrier in human preterm pregnancy

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Background: Antenatal administration of synthetic glucocorticoids (such as betamethasone) to women at risk of preterm birth results in beneficial effects on both fetal maturation and postnatal adaptation of preterm infants. Both animal and human data support a sex-specific response to glucocorticoid exposure with greater prophylactic effect in preterm female infants. Fetal exposure to glucocorticoids is regulated in part by the placental barrier, comprised of placental 11β-hydroxysteroid dehydrogenase 2 (11βHSD2) and multi-drug resistant proteins (P-glycoprotein, P-gp; and multi-drug resistant protein-1, MRP-1).

Aims/Hypotheses: We aimed to examine components of the placental barrier in preterm deliveries with respect to betamethasone exposure and fetal sex. We hypothesise that 11βHSD2, P-gp and MRP1 exhibit sexually dimorphic responses that act together to confer a female advantage in the neonatal period.

Methods: Placental samples were collected from women who delivered preterm (24-28 weeks n=23, 29-34 weeks n=19). P-gp and MRP-1 mRNA was measured by qRT-PCR and 11βHSD2 activity by radiometric conversion assay. Betamethasone exposure was classified as delivery <72 or >72 hours after maternal steroid administration (based on the known bioactive life of betamethasone in circulation).

Results: In placenta from preterm infants, P-gp expression decreased with advancing gestation (r=-.337, p=0.029) and was correlated with 11βHSD2 activity in females (r=.664, p=0.018) but not in males (r=.333, p=0.35). An interaction between fetal sex, gestation and time of delivery from betamethasone exposure was observed (p=0.008). In infants born 24-28 weeks gestation and <72 hours after maternal betamethasone administration, P-gp was higher in females compared to males (p=0.009). P-gp expression was also higher in this very preterm group compared to those born 29-34 weeks, but only in females (p=0.01). MRP-1 expression did not change over gestation in the preterm placenta, nor was it affected by fetal sex and betamethasone exposure.

Conclusions: As an active glucocorticoid transporter, the sex-specific response in P-gp to exogenous glucocorticoid exposure prior to preterm delivery may protect against fetal hypothalamic-pituitary-adrenal axis suppression. In this study, the placental glucocorticoid barrier appeared more protective in preterm females than males, and may explain greater female neonatal physiological stability following premature delivery.

NOTES

REPEATED EXPOSURE TO TNF-α IN AN *IN VITRO* OVINE MODEL OF PRETERM INFECTION/INFLAMMATION-MEDIATED BRAIN INJURY: EFFECTS ON MMP, TIMP AND TACE EXPRESSION

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Background: Fetal brain injury associated with maternal infection and inflammation has a unique predilection towards injury of the white matter characterised by focal necrosis or diffuse injury in the periventricular region, so-called white matter injury (WMI). WMI is now the leading cause of brain injury and a common cause of neurodevelopmental impairment in preterm infants; however, the precise pathways involved are poorly understood. Experimental evidence supports inflammatory mediators, in particular the cytokine tumour necrosis factor alpha (TNF-α) produced in response to infection, as strongly related to cell death of oligodendrocytes [1]. In addition, these cytokine-mediated events may also be associated with release of matrix metalloproteinases (MMPs). It has recently been demonstrated that inappropriate activity of these proteinases, particularly the gelatinases (MMP-2 and MMP-9), can have detrimental consequences, as demonstrated by their destructive effects on the extracellular matrix (ECM) in the brain [2].
Aims/Hypothesis: Given that ECM disruption and increased cytokine levels are frequently observed following injury to the developing brain, this project aimed to investigate the association of MMP-2 and -9 with inflammatory-mediated WMI in a preterm ovine *in vitro* model. We hypothesised that TNF-α-induced expression and activation of the gelatinases plays a pivotal role in the pathogenesis of WMI of the preterm brain.
Methods: Primary mixed glial cultures were derived from preterm (0.65 gestation, term is 147 days) ovine forebrains. Infection/inflammation was induced in cultures by exposure to a chronic treatment regime of 100ng/mL of ovine recombinant TNF-α for 5 days. The time-course and duration of the *in vitro* effects of TNF-α treatment on genes encoding the gelatinases, their endogenous inhibitors (tissue inhibitor of metalloproteinases; TIMP-1 and -2) as well as TNF-α converting enzyme (TACE) were determined using quantitative real-time PCR. Gelatinase activity of MMP-2 and -9 was assessed by gelatin zymography.
Results: Gene expression was markedly increased and paralleled observations of increased cell death in treated cultures by 72 hours for MMP-2 ($P < 0.001$) and 12 hours for MMP-9 ($P < 0.001$). Activity of MMP-2 progressively increased across time while MMP-9 activity first increased and later decreased to levels of controls. TIMP-1 expression following TNF-α exposure increased after 96 hours ($P < 0.01$). The increase in TIMP-2 expression did not reach significance across the 5 days and expression of TACE was persistently increased in all treated cultures ($P < 0.001$).
Conclusions: In conclusion, mixed glial cultures established from the fetal ovine forebrain provide a highly reproducible model of preterm WMI. Our findings of TNF-α induced changes in expression of MMPs *in vitro* indicate that MMP-2 and MMP-9 may contribute to the pathogenesis of injury in response to infection/inflammation.

1. Nakazawa, T., et al., *Tumor necrosis factor-{alpha} mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma.* Journal of Neuroscience, 2006. 26(49): p. 12633.
2. Ranasinghe, H.S., et al., *Proteolytic activity during cortical development is distinct from that involved in hypoxic ischemic injury.* Neuroscience, 2009. 158(2): p. 732.

The role of prostaglandins in the fetal response to intra-uterine inflammation

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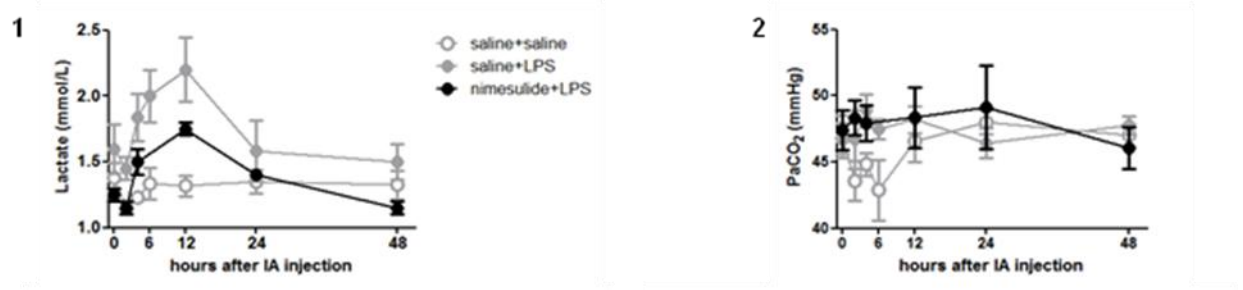
Background: Intra-amniotic (IA) injection of lipopolysaccharide (LPS) induces inflammation and causes profound increases in pulmonary surfactant in the lungs of preterm fetal sheep. Prostaglandins (PGs) are fundamental inflammatory mediators with established roles in fetal lung maturation. We have shown that IA LPS increases PGE_2 in the amniotic fluid and fetal plasma, and that gene expression of PGE synthase (PGES) increases in the fetal lung 7 days (d) after IA LPS.
Aims/Hypothesis: We aimed to block the IA LPS-induced increase in PGE_2 with a PGHS-2 inhibitor (nimesulide) and examine the effects 2d post-administration. We anticipate that nimesulide will inhibit inflammation-induced increases in PGs and alter the fetal response to IA LPS.
Methods: Pregnant ewes underwent surgery at ~112 days of gestation (d; term is ~147d) for cannulation of the amniotic cavity, fetal trachea and a fetal carotid artery and jugular vein, and the maternal jugular vein. At ~117d, a continuous maternal intravenous infusion of saline (2mL/hr) was started and LPS (*E. coli* 055:B5; 20mg; n=6) or saline (n=6) was injected via the amniotic cannula. In other sheep a continuous maternal intravenous infusion of nimesulide (50mg/hr) was started and LPS (n=3) or saline (n=3) was injected via the amniotic cannula. Nimesulide has very poor solubility. We are dissolving it in a solution of 90% polyethylene glycol 400:10% ethanol at a solubility of 65mg/mL. Fetal blood gases were sampled before and at 2, 4, 6, 12, 24 and 48 hours after IA injection. Inflammation was assessed (in maternal saline groups) by immunohistochemistry using an antibody to CD45 (leukocyte common antigen). CD45-positive cells were counted in fetal lung tissue, chorioamnion, umbilical cord and cotyledon samples.
Preliminary results: IA LPS increased fetal arterial lactate (Fig 1) and $PaCO_2$ (Fig 2) during maternal saline infusion. Maternal nimesulide infusion appeared to attenuate fetal lactate responses to IA LPS (Fig 1) but did not affect $PaCO_2$ responses (Fig 2). In fetuses from saline-infused ewes, IA LPS increased the number of CD45-positive cells in lung tissue ($p<0.003$), chorioamnion ($p=0.001$), umbilical cord ($p=0.004$), and cotyledon ($p<0.001$) samples.



Conclusions: This is the first study to show placental inflammation after IA LPS injection and to quantify the umbilical inflammatory response. Nimesulide appears to alter fetal responses to IA LPS, but it is currently unknown how nimesulide affects the fetal inflammatory response and its effect on lung development.

Intra Amniotic Inflammation; A Stereological Analysis of the Kidney

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Background: Intra amniotic inflammation (IAI), often manifest as chorioamnionitis, is a major cause of preterm birth and is often accompanied by fetal inflammatory response syndrome leading to tissue and organ injury. Whether IAI adversely impacts kidney development is unknown. The majority of the functional units of the kidney (nephrons) are formed during the third trimester of gestation, when IAI is commonly present, making nephrogenesis potentially vulnerable to effects of IAI.

Aim: To examine the effect of IAI on nephrogenesis in fetal sheep.

Methods: IAI was induced in pregnant ewes bearing singleton or twin fetuses via intra-amniotic injection of *E coli* lipopolysaccharide (LPS: serotype 055:B5; 10 mg) at ~122 days of gestation (d; term is 150 d). Fetal lambs exposed to LPS (n=7) and gestational controls (n=10) were delivered by caesarean section 1 week later at ~129d. Half of one kidney was embedded into resin before being sectioned and stained with haematoxylin and eosin for stereological estimation of glomerular number, volume and density using the physical disector/fractionator approach.

Results: Glomerular number and density in LPS-exposed fetuses tended lower than the controls for twins (P=0.20) but not singletons (P=0.77). Renal corpuscle volume in LPS fetuses tended larger than control for singletons (P= 0.09) but not twins (P=0.78).

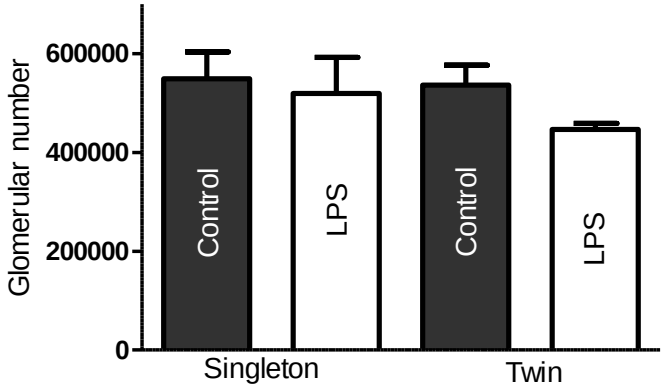

Figure 1 data are mean ± SEM.

Conclusions: Glomerular number and density may be reduced, and renal corpuscle volume may be higher after IA LPS. Thus, nephrogenesis may be influenced by IAI. We will assess nephrogenesis in a further 3 control fetuses to provide sufficient statistical power for definitive results.

Postnatal Cerebral and Pulmonary Hemodynamic Consequences of Intra-uterine Inflammation

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Background: Inflammation *in utero* often manifest as chorioamnionitis has adverse affects on lung and brain development. Inflammation *in utero* causes adverse vascular development in the fetal lungs and results in pulmonary hypertension of the newborn. Decreased left ventricular output is characteristic of neonatal pulmonary hypertension and may directly inhibit cerebral blood flow. Little is known about the effect of inflammation *in utero* on cerebral perfusion and oxygen delivery before or after birth.

Aim: To examine the affect of inflammation *in utero* on pulmonary and cerebral blood flows and oxygenation.during the neonatal period

Methods: At ~112 days of gestation (d) fetal surgery was performed on ewes bearing singleton fetuses for implantation of arterial and venous catheters and Transonic flow probes. Inflammation *in utero* was induced by a single intra amniotic infusion of *E coli* LPS (serotype 055:B5; 10 mg) at ~118 d (term is 150 d). Fetuses exposed to LPS (n=3) or saline (n=4) were delivered by hysterotomy and ventilated for 40 min at ~125 d. The pulmonary vasculature was challenged by altering positive end-expiratory pressure during ventilation. Cerebral and pulmonary arterial pressures, flows and blood-gas status were measured.

Results: During ventilation, pulmonary blood flow (PBF) tended lower in LPS-exposed lambs than controls but cerebral blood flow (CBF) was similar between groups

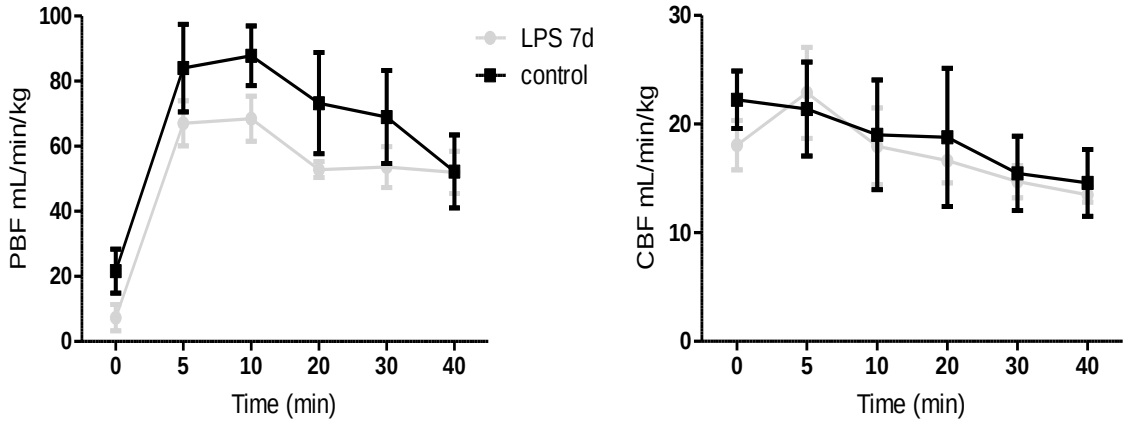

Figure 1 data are mean ±SEM

Conclusions: PBF tended lower in LPS-exposed lambs, consistent with previous observations. CBF appeared unaffected, suggesting that cerebral autoregualtion is unaffected 7 days after exposure to inflammation *in utero.*

How do arteries contract to arginine vasopressin (AVP) ---- comparison between pulmonary and iliac/femoral arteries in juvenile and adult rats

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Background and Objectives: AVP is a strong vaso-constrictor of systemic blood vessels. There are some reports that AVP might decrease pulmonary vascular resistance. However, effects of AVP on PA and their maturational changes are not well known. The aim was to study contraction/relaxation responses of excised PA and systemic arteries (IA and FA) in juvenile and adult rats.
Methods: First main branch of PA, IA or FA were dissected from adult (~48 weeks) or juvenile (~4 weeks) female SD rats, and cut into rings 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 were measured with incrementally increasing doses of AVP. For IA and FA, after inducing the maximal contraction with 10^{-8}M AVP, endothelium-dependent relaxation was also measured with stepping up doses of Ach (10^{-9} to 10^{-3} M). For PA, relaxation response to AVP ($10^{-11}$to 10^{-5} M) was examined after pre-contraction with 10^{-7}M NE.
Results: IA and FA contracted with AVP in both juvenile and adult rats. Maximum contraction was similar between juveniles and adults (p=0.60). However, relaxation of IA by Ach was less in juveniles than in adults (%relaxation at 10^{-3}M; 82±5 vs 59±5; p=0.027). PA showed no responses in all concentrations of AVP (10^{-11} to 10^{-5} M). PA relaxed with AVP (> 10^{-8} M) after pre-contraction with NE in both juvenile and adult rats.
Conclusions: AVP induced contraction with IA and FA but not with PA at clinically achievable concentration levels. In systemic (and pulmonary) arteries, maturation of capacity of relaxation may be delayed compared with capacity of contraction. This study provides a basis for possibility of treating pulmonary hypertension with AVP.

NOTES

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