25th Annual Meeting of The Fetal and Neonatal Workshop of Australia and New Zealand

Hotel Grand Chancellor
Hobart, Tasmania, Australia
8-9 April, 2011

2011 Organising Committee

Rob De Matteo	Monash University
Valerie Zahra	Monash University
Richard Harding	Monash University

Affiliated with the Perinatal Society of Australia and New Zealand

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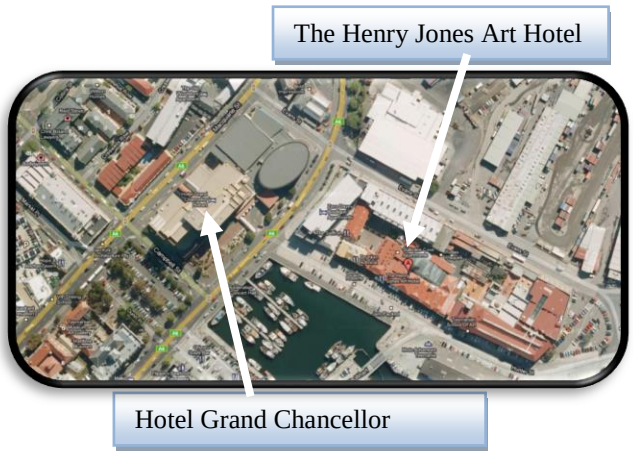
Program Outline

CONFERENCE VENUE

Hotel Grand Chancellor
Chancellor Room 6
1 Davey St

FRIDAY 8TH APRIL

9.00am-10.00am ..... Registration
10.00am-11.30am ..... Session 1
11.30am-12.00pm ..... Morning Tea
12.00pm-1.30pm ..... Session 2
1.30pm-2.30pm ..... Lunch
2.30pm-4.15pm ..... Session 3
4.15pm-4.45pm ..... Afternoon Tea
4.45pm-6.30pm ..... Session 4
7.30pm-10.30pm ..... Conference Dinner
The Henry Jones Art Hotel
The Jones & Co. Room
25 Hunter Street, Hobart



SATURDAY 9TH APRIL

8.30am-9.15am ..... Registration
9.15am-11.00am ..... Session 5
11.00am-11.30am ..... Morning Tea
11.30am-1.15pm ..... Session 6
1.15pm-2.15pm ..... Lunch
2.15pm-4.00pm ..... Session 7
4.00pm-4.30pm ..... Afternoon Tea
4.30pm-6.15pm ..... Session 8
6.15pm ..... Fetal Workshop 2012
6.20pm ..... Presentation of student prizes

Perinatal Society of Australia & New Zealand

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Scientific Program

Hotel Grand Chancellor, Chancellor Room 6

DAY 1- FRIDAY 8th APRIL

Registration: 9.30am-10.00am

E=Early PhD, L=Late PhD

Session 1: Chairs – Vicki Clifton and Tim Moss			
10.00am	**A1**	Laura Bennet	Altered cardiovascular and neural responses of the preterm fetuses to asphyxia after chronic exposure to LPS
10.15am	**A2**	Melinda Dolan (E)	Inflammation-induced fetal lung maturation in glucocorticoid receptor knockout mice
10.30am	**A3**	Robert Galinsky (L)	Mediators of lung injury in preterm sheep ventilated after intrauterine inflammation
10.45am	**A4**	Keiji Suzuki	Effects of antenatal intra-amniotic endotoxin on the development of lung structure in rats
11.00am	**A5**	Alana Westover (L)	The effect of nimesulide on the fetal inflammatory response
11.15am	**General discussion**		

Morning tea: 11.30am-12.00pm

Session 2: Chairs – Barbara Lingwood and Frank Bloomfield			
12.00pm	**A6**	Jacqueline Melville (E)	Antenatal influences on fetal immune function
12.15pm	**A7**	Udani Ratnayake (L)	Immune activation in pregnant spiny mice has a detrimental effect on the behaviour of the offspring
12.30pm	**A8**	Domenic LaRosa (E)	Maternal dietary creatine supplementation protects fetal skeletal muscle from hypoxic damage at birth
12.45pm	**A9**	Hayley Dickinson	Maternal dietary creatine supplementation does not alter the capacity for creatine synthesis and transport in the newborn
1.00pm	**A10**	Laura Hardefeldt (E)	Effects of gestational age on physical findings of dysmaturity, body weight, and survival in neonatal Alpacas and Llamas (2002-2010)
1.15pm	**General discussion**		

Lunch: 1.30pm-2.30pm

Session 3: Chairs – Anne Jaquiery and Alistair Gunn			
2.30pm	**A11**	Anzari Atik (E)	Consequences of high dose caffeine treatment on fetal physiology and the immature brain
2.45pm	**A12**	Aminath Azhan	Investigating the effects of IUGR on GABAergic neurons in the cerebral cortex of the fetal guinea pig
3.00pm	**A13**	Leo Leader	Does antenatal maternal depression, stress and anxiety influence infant development?
3.15pm	**A14**	Stephanie Miller (E)	Changes in $GABA_A$ Receptor α subunit protein expression after neonatal hypoxia-ischaemia
3.30pm	**A15**	Julia Pitcher	Children born preterm have reduced long term depression (LTD)-like neuroplasticity
3.45pm	**A16**	Mary Tolcos	Myelination in IUGR: Not just myelin basic protein
4.00pm	**General discussion**		

Afternoon tea: 4.15pm-4.45pm

Session 4: Chairs – Jane Black and Ian Wright			
4.45pm	**A17**	Jordanna Master (E)	Intergenerational transmission of cardiomyocyte deficits in offspring born small
5.00pm	**A18**	Linda Gallo (L)	Pregnancy in female rats born small: cardiorenal and metabolic adaptations and consequences for next generation fetal growth
5.15pm	**A19**	Anne Jaquiery	Adult body composition after periconceptional events
5.30pm	**A20**	Hong Liu (E)	Neonatal exendin-4 treatment to prevent diabetes after IUGR – preliminary outcomes in the placentally-restricted lamb
5.45pm	**A21**	Zhi Yi Ong (L)	Maternal 'junk-food' diet programs a preference for fat in the offspring: changes in central reward signalling
6.00pm	**A22**	Ana-Mishel Spiroski (E)	The effects of intra-amniotic IGF-I treatment of intra-uterine growth-restricted lambs on growth and body composition between birth and weaning
6.15pm	**General discussion**		

Conference dinner: The Henry Jones Art Hotel, The Jones & Co. Room, 7.30pm

DAY 2- SATURDAY 9th APRIL

Registration: 8.30am-9.15am

E=Early PhD, L=Late PhD, H=Honours

Session 5: Chairs – Sandra Orgeig and Michael Stark			
9.15am	**A23**	Kathryn Gatford	Neonatal exendin-4 treatment in the twin IUGR lamb normalises in vitro islet insulin secretion and expression of its molecular determinants
9.30am	**A24**	Kristina Sobotka (E)	Effect of sustained inflations on asphyxiated near-term lambs
9.45am	**A25**	Chris Maloney	Epigenetic programming of adolescent metabolic phenotypes?
10.00am	**A26**	Megan O'Reilly (L)	Exposure to hyperoxic gas in the neonatal period: effects on the small conducting airways in adulthood
10.15am	**A27**	Hasnah Bahari (E)	Adult onset voluntary exercise ameliorates the impact of maternal obesity on offspring
10.30am	**A28**	Vanni Caruso (L)	Changes in FTO expression in response to maternal obesity in the rat
10.45am	**General discussion**		

Morning tea: 11.00am-11.30am

Session 6: Chairs – Karen Gibson and Rob De Matteo			
11.30am	**A29**	Rebecca Dyson	Development of techniques for measuring hydrogen sulphide in the newborn neonate
11.45am	**A30**	Yvonne Eiby	Coronary and aortic flow in response to changes in preload and afterload in the isolated preterm piglet heart
12.00pm	**A31**	Alistair Gunn	Ontogeny of the heart rate power spectrum in the last third of gestation in fetal sheep
12.15pm	**A32**	Min Kim (L)	Adrenoceptor subtype mRNA expression in the pig heart: developmental changes and effects of maternal glucocorticoid treatment
12.30pm	**A33**	Nayana Parange	Longitudinal sex-specific normograms for Doppler ultrasound indices of intrauterine fetal shunts: the foramen ovale and the ductus arteriosus
12.45pm	**A34**	Stephanie Yiallourou	The effects of dummy sucking on autonomic cardiac control
1.00pm	**General discussion**		

Lunch: 1.15pm-2.15pm

Session 7: Chairs – David Todd and Foula Sozo			
2.15pm	**A35**	Annie McDougall (L)	Trop2 regulates the proliferation and migration of fetal lung fibroblasts and alters activation of the ERK signalling pathway
2.30pm	**A36**	Noreen Ishak (L)	Does surfactant composition play a role in the male disadvantage in respiratory outcome following moderate preterm birth in sheep?
2.45pm	**A37**	Erin McGillick (H)	Role of glucose in surfactant protein mRNA expression in the fetal lung
3.00pm	**A38**	Nadine Brew (L)	The repair genes metallothionein and urokinase receptor are up-regulated 24h after ventilation induced injury in the immature lung
3.15pm	**A39**	Melissa Siew	The effect of positive end-expiratory pressure (PEEP) and rotation on lung ventilation in newborn rabbit pups ventilated lying on their side
3.30pm	**A40**	Patricia Vosdoganes (L)	Human amnion epithelial cells as a treatment for hyperoxia induced neonatal lung injury
3.45pm	**General discussion**		

Afternoon tea: 4.00pm-4.30pm

Session 8: Chairs – Julie Owens and Jon Hirst			
4.30pm	**A41**	Carolyn Mitchell	Epigenetic mechanisms regulating PTGS2 expression in the amnion during gestation
4.45pm	**A42**	Jorge Tolosa	Role of syncytin in placental formation: from transcription factories to maternal immune tolerance
5.00pm	**A43**	Kirsty Pringle	Gestational changes in the expression of the placental renin-angiotensin system and VEGF: roles in placental vascularisation?
5.15pm	**A44**	Michael Stark	Eicosapentanoic acid is more effective than docosahexanoic acid in inhibiting LPS-induced lipid hydroperoxide production and oxidative DNA damage in the placenta
5.30pm	**A45**	Roger Smith	The role of miRNAs in human myometrial activation for labour
5.45pm	**A46**	Toni Welsh	Understanding the role of estrogens in labour onset: mechanisms of non-genomic estrogen signalling in the pregnant myometrium
6.00pm	**General discussion**		
6.15pm	**Fetal and Neonatal Workshop 2012**		
6.20pm	**Presentation of Student Prizes**		
6.30pm	**Close of Workshop**		

ALTERED CARDIOVASCULAR AND NEURAL RESPONSES OF THE PRETERM FETUSES TO ASPHYXIA AFTER CHRONIC EXPOSURE TO LPS

Bennet L, Booth LC, Jensen EC, Naylor A, Mathai S & Gunn AJ.
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Background: Exposure to infection and asphyxia are common during preterm birth and are associated with higher rates of systemic and neural complications. Experimentally, infection can sensitise the fetus to greater injury.

Aims/Hypothesis: To assess whether LPS-sensitisation to increased injury is purely neural or whether infection compromises cardiovascular adaptation of the fetus to insults, such as asphyxia, leading to increased neural injury.

Methods: Chronically instrumented 103 day old (0.7 gestation age: term 147 days) fetal sheep *in utero* were randomized to 4 groups: saline infusion *plus* sham umbilical cord occlusion (saline-sham); saline infusion *plus* asphyxia induced by umbilical cord occlusion for 15 min (saline-occlusion); LPS infusion *plus* sham occlusion (LPS-sham); LPS infusion plus umbilical cord occlusion for 15 min (LPS-occlusion). Fetuses received either LPS as a continuous low dose infusion (100 μg over 24h, followed by 250 μg/24h for 4 days) *plus* boluses of 1 μg LPS at 48 h, 72 h and 96 h of the infusion, or volume equivalent isotonic saline. Asphyxia or sham occlusion was induced on day 5, and fetuses were euthanized 5 days after occlusion and brains taken for histological evaluation. Data are mean±SE.

Results: Baseline fetal heart rate was not different before occlusion (vs vehicle-asphyxia, $P=0.055$) but fell faster at the onset of occlusion (%baseline; $P<0.05$) and was lower mid occlusion ($P<0.05$). In contrast, blood pressure (BP) was significantly lower during the baseline period in the LPS-treated group ($P<0.05$), but increased to a similar peak during occlusion. %baseline BP was not different between the groups in the first 30 s. Carotid blood flow was significantly higher in the LPS-treated group before occlusion (vs vehicle-asphyxia, $P<0.05$) but decreased during occlusion, such that there was no difference between the groups during occlusion. There were no differences in femoral blood flow before or during occlusion between the groups. During occlusion pH was significantly lower in the LPS-treated group compared to vehicle-asphyxia group ($P<0.05$). There was a reduction in reactive microglia ($P<0.05$) and of caspase-3 +ve cells after LPS-occlusion vs vehicle-asphyxia ($P<0.0001$).

Conclusions: Contrary to our initial hypothesis, prolonged exposure to LPS was associated with more rapid cardiovascular adaptation to acute asphyxia and attenuation of the inflammatory reaction to acute severe asphyxia in preterm fetal sheep, and thus may reduce the risk of neural injury.

INFLAMMATION-INDUCED FETAL LUNG MATURATION IN GLUCOCORTICOID RECEPTOR KNOCKOUT MICE

Melinda J Dolan[1], Megan J Wallace[1,2], Annie RA McDougall[1], Valerie A Zahra [1], Timothy J Cole [3] & Timothy JM Moss[1,2].
[1]The Ritchie Centre, Monash Institute of Medical Research, [2]The Department of Obstetrics and Gynaecology & [3]Biochemistry and Molecular Biology, Monash University, Victoria, Australia
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Background: Intra-amniotic inflammation, which is common in preterm births, induces precocious fetal lung surfactant production by an unknown mechanism.

Aim/Hypothesis: We hypothesised that the effects of inflammation on lung development are independent of glucocorticoids, despite their fundamental role in inducing lung maturation.We aimed to use transgenic mice to investigate the role of glucocorticoid signalling.

Method: Pregnant C57BL/6 wild-type mice (n=6 dams) and glucocorticoid receptor (GR) knockout mice (n=2) received injections of either 5 μl of lipopolysaccharide (LPS; 20 pg/μl) or saline into each amniotic sac on embryonic day (E) 15.5. Fetal tissues were collected on E17.5. Lung maturation was assessed by surfactant protein A (SP-A), -B, -C and –D mRNA levels. Lung inflammation was assessed by interleukin-1β (IL-1β) mRNA levels. C57BL/6 wild-type groups were compared using a univariate general linear model with covariates (litter and litter size).

Results: In C57BL/6 wild-type fetuses, IL-1β mRNA expression was >3-fold higher ($p=0.001$) after intra-amniotic LPS injection (2.09±0.43, n=8) than after saline (0.59±0.08, n=18). SP-A, -B, -C and -D mRNA expression was 2-4-fold higher than control after intra-amniotic LPS in C57BL/6 wild-type fetuses. A significant increase in the expression of SP-C (LPS 3.24±1.24; saline 1.00±0.19; $p<0.001$) and SP-D (LPS 4.71±1.71; saline 1.00±0.12; $p<0.001$) was observed. Preliminary data from LPS-exposed GR knockout fetuses (n=3) showed increases in IL-1β and SP mRNA expression greater than those of saline-exposed GR knockout fetuses (n=2), and of similar magnitude to LPS-exposed wild-type fetuses.

Conclusions: Intra-amniotic LPS results in lung inflammation and surfactant production in C57BL/6 wild-type fetal mice. Our preliminary data suggest a similar response in GR knockout fetuses, suggesting the GR pathway is not required for inflammation-induced fetal lung maturation.

MEDIATORS OF LUNG INJURY IN PRETERM SHEEP VENTILATED AFTER INTRAUTERINE INFLAMMATION

Robert Galinsky[1], Alana Westover[1], Graeme R Polglase[1], Stuart B Hooper[1,2], Timothy JM Moss[1,2] & Megan J Wallace[1,2] .
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Background: Inadvertent ventilation-induced lung injury (VILI) in preterm infants may contribute to bronchopulmonary dysplasia (BPD). Intrauterine inflammation is often present before preterm birth and may exacerbate VILI. The early response genes – early growth response 1 (EGR1), cysteine rich-61 (CYR61) and connective tissue growth factor (CTGF) have been identified as potential mediators of VILI, We wanted to determine if they are also unregulated after intrauterine inflammation.

Aims/Hypothesis: We aimed to determine the effect of exposure to intrauterine inflammation before mechanical ventilation on expression of proinflammatory cytokines and the proposed markers of VILI. We hypothesized that expression of interleukin (IL)-1β, IL-6, IL-8, EGR1, CYR61 and CTGF would be higher in the lungs of preterm lambs exposed to intrauterine inflammation before mechanical ventilation.

Methods: At ~118 days of gestation (d; term is ~147 d), pregnant ewes received an intra-amniotic injection of lipopolysaccharide (LPS; *E coli* 055:B5; 20 mg; n=5) to induce intrauterine inflammation. Controls received an intra-amniotic saline (control; n=4). At ~125 d lambs were delivered and mechanically ventilated for 40 minutes before collection of lung tissue for measurement of EGR1, CTGF, CYR61, IL-1β, IL-6 and IL-8 expression using Real Time-Polymerase Chain Reaction.

Results: EGR1 expression tended higher, but IL-1β and IL-8 expression tended lower after mechanical ventilation in LPS-exposed lambs compared to control.

Conclusions: The EGR1 response to mechanical ventilation after exposure to inflammation before preterm birth reinforces that it may be a useful marker of lung injury. The attenuated IL-1β and IL-8 responses may be due to immune tolerance or inflammation-induced ‘maturation’ of the preterm lungs.

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 lung development (chronic lung disease).

Aims: To study effects of antenatal intra-amniotic injection of potent proinflammatory agent, lipopolysaccharide (LPS) on the development of lung structure in rats

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 2, 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%; $p<0.01$). Body weights and lung weights were not different between LPS and control groups. At 2 wk, LPS pups had lower volume density of alveoli (Vv-alv), tendency to higher mean alveolar volume (Valv) and lower numerical density of alveoli (Nv-alv). However, volume density of small arteries (diameter ~50-150μm; Vv-a), ratio of medial and adventitial thickness to diameter of pulmonary arteries (MT/d and AT/d, respectively) were not different between the groups. At 8 wks, there were no differences in all the studied variables between LPS and control groups, except that LPS-exposed females had lower AT/d.

Age	2wk		8wk (male)		8wk (female)	
Group	Control	LPS	Control	LPS	Control	LPS
Vv-alv ($x10^{-2}$)	71.2±1.5	*64.3±2.3	71.6±1.8	72.2±2.1	73.4±2.7	71.7±3.3
Valv ($x10^3 \mu m^3$)	18.3±3.1	27.5±6.9	20.8±1.6	17.0±1.6	20.5±3.9	17.9±1.7
Nv-alv ($x10^3 mm^{-3}$)	45.6±9.1	33.0±8.8	35.1±2.1	44.8±5.0	43.0±6.6	41.6±3.6
Vv-a ($x10^{-2}$)	2.8±0.5	2.6±0.5	1.8±0.3	2.6±0.6	2.1±0.6	2.7±0.6
MT/d ($x10^{-2}$)	4.1±0.2	4.4±0.5	7.3±1.2	7.5±2.0	4.2±0.4	4.0±0.5
AT/d ($x10^{-2}$)	10.5±1.1	10.1±1.4	8.2±1.3	9.2±1.7	8.3±1.1	*5.4±0.7

(mean±SE) *p<0.05

Conclusions: Relatively low dose intra-amniotic LPS (0.1 μg) resulted in higher perinatal mortality in the offspring, but did not influence postnatal body or lung growth. LPS-exposure may have affected development of lung structure (eg. fewer and larger alveoli) transiently, which was mostly reversible toward adulthood.

THE EFFECT OF NIMESULIDE ON THE FETAL INFLAMMATORY RESPONSE

Alana Westover, Stuart Hooper & Tim Moss.
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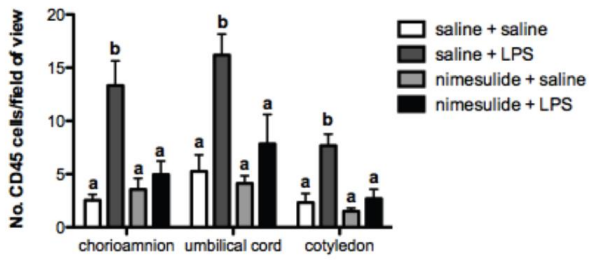
Background: Intra-amniotic (IA) injection of lipopolysaccharide (LPS) induces inflammation and causes profound increases in pulmonary surfactant in the lungs of preterm fetal sheep. Prostaglandins (PGs) are fundamental inflammatory mediators with established roles in fetal lung maturation. We have shown that IA LPS increases PGE_2 in the amniotic fluid and fetal plasma, and that gene expression of PGH Synthase type 2 (PGHS-2) increases in the fetal lung 2 days 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 2 d after LPS administration. We hypothesized that nimesulide would inhibit increases in PGs and alter the inflammatory 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=6) or saline (n=6) was injected via the amniotic cannula. PGE_2 concentrations were measured in the amniotic fluid and fetal plasma by radioimmunoassay. Inflammation was assessed 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.

Results: Maternal nimesulide infusion prevented PGE_2 increasing in response to IA LPS in the amniotic fluid and fetal plasma. Nimesulide significantly reduced the IA LPS induction of CD45-positive cells in the chorioamnion (p=0.01), umbilical cord (p=0.03) and cotyledon (p=0.005) to control levels (Fig 1). In the lungs, nimesulide reduced CD45-positive cell numbers in response to LPS (p<0.016) to the control level, and reduced inflammation in the middle and lower lobes to control levels (p<0.05 v LPS: Fig 2).

1



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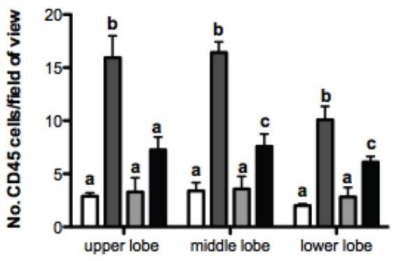

Conclusions: Nimesulide prevented inflammation in the chorioamnion, umbilical cord and cotyledon, and reduces, but does not entirely prevent, the inflammatory response in the lungs. Studies of the effects of infusion of the nimesulide vehicle alone (in animals that receive IA saline or LPS) are ongoing.

NOTES

ANTENATAL INFLUENCES ON FETAL IMMUNE FUNCTION

Jacqueline Melville[1], Robert Bischof[2], Els Meeusen[2] & Tim Moss[1].
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Background: The antenatal environment has the potential to alter the development and maturation of the fetal immune system. Immune development occurs throughout the entirety of gestation and continues after birth. Term neonates have reduced leukocyte pools and reduced immune function. In comparison to adult immune function, at birth there are deficiencies in migration and chemotaxis of monocytes, antigen presentation, cytokine production by T cells, T-B cell interactions and immunoglobulin production by B cells. Preterm birth can exacerbate normal neonatal immune deficiencies, which is a particularly significant problem for morbidity and mortality. Antenatal glucocorticoid treatment is common in preterm birth and its effect on the fetal immune system is not well defined.

Aims/Hypothesis: We aim to determine the effect of antenatal glucocorticoid treatment on fetal immune function. We hypothesise that antenatal betamethasone will initially reduce immune function in the fetus but may subsequently induce precocious maturation.

Methods: Pregnant ewes undergo surgery at ~112 days of gestation (term ~147 days) for the insertion of fetal catheters. At 117 days of gestation the ewe receives an intramuscular injection of betamethasone (0.5mg/kg). A baseline sample of fetal blood is taken before administration, followed by serial blood samples at 6, 24, 48 hours, 7, 14 & 21 days after betamethasone treatment. Leukocytes are isolated from the fetal blood samples for analysis of phenotype by flow cytometry. Fetal macrophages are isolated from the leukocytes through adhesion to a culture plate and incubated with 1μm fluorescent beads for 1 hour. The macrophages are then analysed by flow cytometry to determine their phagocytic capacity. The ewe and fetus are humanely killed at 138 days of gestation.

Results: Preliminary results suggest that the proportions of circulating T cells are reduced 6 hours after exposure to antenatal betamethasone. Functionally there appears to be a reduction in the phagocytic ability of fetal macrophages 48 hours after exposure to antenatal betamethasone.

Conclusions: Our preliminary data suggest that antenatal betamethasone may have a suppressive effect on fetal immune function for at least 48 hours after exposure.

IMMUNE ACTIVATION IN PREGNANT SPINY MICE HAS A DETERIMENTAL EFFECT ON THE BEHAVIOUR OF THE OFFSPRING

Udani Ratnayake, Hayley Dickinson & David W. Walker.
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Background: There is considerable human and animal based evidence to support an association between maternal illness during pregnancy and adverse effects on the long-term health of the offspring. The development of mental health conditions such as attention deficit disorder, autism, and schizophrenia have been linked to sub-optimal fetal growth and development, which arise because of changes in maternal health and the intra-uterine environment. Poly I:C is a viral mimetic that is recognised by TLR3, an innate immune receptor. Poly I:C has been shown to effectively activate the immune system, as would be expected during a period of maternal viral illness.

Aims: The aim of this study was to determine whether our model of viral infection during pregnancy in the spiny mouse (*Acomys cahirinus*) would result in behavioural deficits in the offspring comparable to those seen in humans up to the pre-pubertal stage of development.

Methods: Pregnant spiny mice were injected with 0.5mg/kg Poly I:C (n=12) or PBS (n=8) at 20d gestation (term is 40d). Dams were left to give birth naturally and offspring behaviour was assessed at 20-40d and 60-80d PNA (weaning occurred at 50d). Offspring were assessed on the open field test, novel object recognition test and elevated plus maze.

Results: Open field testing revealed that exposure to Poly I:C during pregnancy reduced activity in the central zone in pre-pubertal offspring. Poly I:C exposed offspring showed a strong tendency ($p=0.07$) toward spending an increased amount of time in the closed or safe arms of the elevated plus maze. However, when Poly I:C offspring reached the post-pubertal age range these deficits seen previously did not reoccur.

Conclusion: Our model of a sub-clinical infection with no obvious signs of sickness behaviours in the pregnant spiny mouse has shown to still result in behavioural deficits in the offspring. This study suggests that even a minor infection during pregnancy may cause changes in the intrauterine environment and subsequently affect the neurodevelopment of the fetus. Ongoing studies are examining the cytokine profile of the mother, fetus and placenta in response to this insult.

MATERNAL DIETARY CREATINE SUPPLEMENTATION PROTECTS FETAL SKELETAL MUSCLE FROM HYPOXIC DAMAGE AT BIRTH

Samir Mesanovic[2], Domenic A LaRosa[1], Hayley Dickinson[1], Zoe J Ireland[1], David Cannatta[2], Rod Snow[3], David W Walker[1] & Jan West[2].
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Background: Fetal hypoxia can result from a range of events including umbilical cord compression, preeclampsia and premature separation of the placenta. Ultimately all causes result in impaired gas exchange between the mother and fetus, and if severe can result in energy failure and damage to the fetal tissues. Recent work by us using a model of birth hypoxia in the precocial spiny mouse has identified that maternal dietary supplementation with creatine increases neonatal survival [1] and protect the brain and the diaphragm [2], from hypoxia induced damage. However, the affect of birth hypoxia and creatine supplementation on the rest of the musculature is unknown.

Aims/Hypothesis: This study investigated the effects of intrapartum hypoxia on the gastrocnemius muscle and the possible role of creatine as a protective agent against hypoxic damage. We hypothesized that birth asphyxia would cause atrophy in this muscle, as it did the diaphragm, and that creatine would protect against this damage.

Methods: Pregnant spiny mice were fed a control or 5% creatine-supplemented diet from day 20 of gestation (term ~39 days). On day 38, pups were delivered by caesarean section, or intrauterine hypoxia was induced, by placing the excised uterus containing all fetuses in a saline bath for 7.5-8mins. The fetuses were then expelled and resuscitation attempted by manual palpation of the chest. Surviving neonates were cross-fostered to a nursing dam for 24h. At post-mortem the gastrocnemius muscle was dissected and frozen. Total creatine content (TCr), proportion of muscle fibre types, cross-sectional area (CSA) of the different muscle fibre types and atrophy regulating gene expression were measured.

Results: Muscle TCr was found to be 27% higher in pups from creatine supplemented dams when compared to control diet, hypoxia-exposed offspring. All fibre types (Type I, IIa and IIb) were found to have significant atrophy after birth asphyxia, with Type I fibres most affected (47% decrease in CSA) compared to control diet, hypoxia-exposed offspring. This atrophy was not observed in the muscles of asphyxiated pups from creatine supplemented dams.

Conclusions: Maternal dietary creatine supplementation attenuates hypoxia-induced atrophy of muscle fibres in the gastrocnemius muscle of newborn spiny mouse pups.

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MATERNAL DIETARY CREATINE SUPPLEMENTATION DOES NOT ALTER THE CAPACITY FOR CREATINE SYNTHESIS AND TRANSPORT IN THE NEWBORN

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Background: We have previously reported that maternal dietary creatine supplementation can protect the neonatal spiny mouse from hypoxia-induced injury to the brain and diaphragm [1, 2]. It is important to consider if a high creatine load during pregnancy has any effects on the development of this system in the fetus.

Aims/Hypothesis: Here we investigated if maternal dietary creatine supplementation from 0.5 gestation to term, a period of 19 days, would alter expression of genes for creatine synthesis (arginine:glycine amidinotransferase [AGAT], guanidinoaceteate methyltransferase [GAMT] and the creatine transporter (CrT1) in the newborn spiny mouse. We hypothesized that maternal dietary creatine supplementation from mid-gestation to term would cause a down-regulation in the expression of the CrT and decrease the expression of AGAT, the first step in the creation synthesis pathway, in the newborn. We also investigated the effect of this creatine supplementation on expression of placental CrT, to determine if supplying extra creatine to the mother was down-regulating the transporter, as might be expected from effects observed in other tissues.

Methods: Pregnant spiny mice were placed on a diet supplemented with 5% creatine monohydrate from day 20 of gestation to term; control dams received a standard rodent diet. Offspring were delivered by caesarean section on day 38 of gestation and cross-fostered to a lactating dam for 24 h. RNA and protein were extracted from placentas collected at the time of delivery and from neonatal kidney, liver, heart and brain collected at 24 h of age for qPCR and western blot analysis respectively.

Results: The maternal creatine supplementation had no effect on expression of AGAT, GAMT, CrT1 in any newborn tissues examined, but CrT1 mRNA expression was significantly increased in the labyrinth region of the placenta; this effect was not observed in the junctional zone of the placenta.

Conclusion: While maternal dietary creatine supplementation from mid-gestation in the spiny mouse does not alter the capacity for the newborn to synthesise and transport creatine, the increased placental expression of the CrT in the exchange region of the placenta suggests an important role for the delivery (or removal) of creatine to (or from) the fetus.

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EFFECTS OF GESTATIONAL AGE ON PHYSICAL FINDINGS OF DYSMATURITY, BODY WEIGHT, AND SURVIVAL IN NEONATAL ALPACAS AND LLAMAS (2002-2010)

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Background: The typically uniparous nature of alpacas and llamas, in addition to their often high individual value, has made neonatal care of these species an area of specialty veterinary care over the past 10 years. Optimising clinical care, and providing information for owners, has been limited by a lack of data regarding outcomes of neonatal intervention. In the camelid population, gestational length is highly variable (normal range 330-360 days) and a definition for prematurity has not been established. Anecdotally, however, it has been noted that crias born outside of this window have an increased incidence of physical findings of dysmaturity (tendon laxity, floppy ears, teeth not erupted) and an increased need for intensive support in the immediate neonatal period.

Aims/hypothesis: The aim of this retrospective study was to investigate the effect of gestational age on physical findings of dysmaturity, body weight, and survival in crias. We hypothesised that low gestational age (<330 days) would be associated with physical findings of dysmaturity, low birth weight, more intensive clinical care, but not with any decrease in short-term survival.

Methods: Medical records were examined of 130 camelids that presented to the University of Wisconsin Madison within the first 30 days of life between 2002 and 2010. For comparing two categorical variables, each with two levels, the Fischer's Exact Test was used to test for a relationship. Where a categorical dependent variable and a continuous independent variable were present, logistic regression was used.

Results: Of the 130 neonatal camelids that presented, 86 had gestational age recorded (range 312-393 days). There were 16 (18.6%) crias with gestational age below 330 days. Clinically dysmature crias had lower birth weights (mean 6.5kg SD 1.5kg) than mature crias (mean 8.8kg SD 3.2kg P=0.05). Similarly, crias born before 330 days were lighter at birth (mean 6.4kg, SD 1.3kg) than those born after 330 days (mean 7.4kg SD 1.7kg, P=0.002). Survival rates of crias were similar in all groups, however. Costs of treatment were higher in dysmature than mature crias and those crias born prior to 330 days of gestation.

Conclusions: Crias born before 330 days and those with physical findings of dysmaturity have a good prognosis but likely require more intensive care, indicated by a higher cost associated with treatment of these cases.

NOTES

CONSEQUENCES OF HIGH DOSE CAFFEINE TREATMENT ON FETAL PHYSIOLOGY AND THE IMMATURE BRAIN

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Background: Apnoea of prematurity (AOP) is common in very preterm infants. It can cause episodes of hypoxemia and bradycardia, which could lead to reduced cerebral oxygen delivery and brain injury. The respiratory stimulant caffeine is the treatment of choice for AOP [1]. In clinical practice, a loading dose of 20mg/kg caffeine (citrate) followed by a daily maintenance dose of 5-10mg/kg is currently used [2]; these doses are not always sufficient to significantly reduce AOP. Therefore, higher doses of caffeine are being used in some centres. However, the effects of higher doses of caffeine on the developing brain have not been subject to rigorous evaluation. Our preliminary evidence in sheep suggests that high-dose caffeine can lead to microgliosis, axonal damage, reduced myelination and an alteration in astrocytic morphology in the subcortical white matter.

Aims: Our principal aim is to determine whether high dose caffeine exposure over an extended period of time results in fetal brain injury. We also aim to examine caffeine-induced changes in cardiovascular physiology and blood chemistry as they may play a role in the effects on the developing brain.

Methods: In order to study the effects of caffeine on the very immature brain, typical of very preterm infants, we have used fetal sheep *in utero*. Pregnant ewes underwent surgery at 99 days gestational age (DGA) for chronic implantation of maternal and fetal catheters (term ~147DGA). A high dose caffeine regimen (50mg/kg loading; 40mg/kg daily maintenance dose; citrate equivalent, n=6) or an equivalent volume of saline (n=6) was administered to the fetus via the maternal circulation each day from 104DGA to 118DGA. Fetal and maternal blood was sampled for the assessment of blood chemistry and caffeine concentration from 104-118DGA. Fetal and maternal arterial pressures were recorded for the first 3 days of the treatment period. At necropsy (119DGA) fetal body and organ weights and body dimensions were measured. Fetal brains were perfused with 4% paraformaldehyde and processed for thionin staining and immunohistochemistry using an antibody against ionized calcium binding adapter molecule 1 (microglia).

Results: There were no significant differences between groups in fetal arterial blood gases (pH, PCO_2, PO_2 or SaO_2) or concentrations of haemoglobin, glucose and lactate throughout the treatment period; plasma caffeine levels are currently being assessed. There were no significant differences in body weight or body dimensions between the control and caffeine-exposed fetuses. There was also no significant difference in brain weight; however caffeine exposed fetuses tended to have heavier brains when brain weight was expressed as a ratio to body weight ($p=0.06$).

Conclusions: Fetal blood gases, blood chemistry and growth were unaffected by high-dose caffeine exposure. Cardiovascular function and the structural analysis for the assessment of fetal brain injury are currently underway. Our study will provide valuable data as to whether high dose caffeine is safe in the developing brain.

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INVESTIGATING THE EFFECTS OF IUGR ON GABAERGIC NEURONS IN THE CEREBRAL CORTEX OF THE FETAL GUINEA PIG

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Background: Intrauterine growth restriction (IUGR) can lead to adverse neurodevelopmental sequelae in postnatal life, ranging from learning and cognitive deficits to cerebral palsy. These sequelae likely result from altered grey and/or white matter development or brain injury. Previously, we have shown that IUGR, induced via uterine artery ligation throughout the second half of gestation in the guinea pig, results in reduced myelination[1,2] and dendritic growth[3] in the fetus, reduced volume of the neocortex in the neonate[2] and functional and behavioural alterations in the long-term[4,]. However we have not yet examined the impact of IUGR on specific subpopulations of neurons in the cerebral cortex.

Aim: In this study we will determine the effects of IUGR on gamma aminobutyric acidergic (GABAergic) interneurons of the cerebral cortex in the growth restricted fetal guinea pig brain. These interneurons represent 20% of brain neurons and are vital for normal cortical function. We will also examine the nature of the glial cell response (microglia and astrocytes) in the cerebral grey and white matter to determine if there is a correlation with alterations to GABAergic interneurons.

Methods: At 30dg (term ~ 67 days of gestation, dg), chronic placental insufficiency was induced by diathermic ablation of half of the branches of the uterine artery supplying the placenta to produce IUGR fetuses (n=8); controls (n=7) were from sham-operated animals. At 52dg, fetal brains were weighed, perfused and processed for H&E staining and immunohistochemistry using antibodies for somatostatin (SST; GABAergic interneuron), calretinin (CR; GABAergic interneuron), glial fibrillary acidic protein (GFAP, astrocytes) and ionized calcium binding adapter molecule 1 (Iba-1; microglia).

Results: In IUGR fetuses at 52dg, there was a significant ($p<0.05$) decrease in crown-rump length (C: 10.84±0.24 vs IUGR: 9.41±0.23), body weight (C: 52.72±2.26 vs IUGR: 40.15±2.53) and brain weight (C: 2.02 ±0.04 vs IUGR: 1.83±0.07). Brain to body weight ratio was increased ($p<0.05$, C: 0.04±0.00 vs IUGR: 0.05±0.00) reflecting relative sparing of the brain in IUGR fetuses.

Conclusions: We are currently assessing the density of SST- and CR-immunoreactive (IR) cells in the visual and motor cortices, and GFAP- and Iba-1-IR neuroglia in WM of IUGR and control fetuses. Difference in cell densities could contribute to cognitive and behavioural abnormalities associated with IUGR.

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DOES ANTENATAL MATERNAL DEPRESSION, STRESS AND ANXIETY INFLUENCE INFANT DEVELOPMENT?

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Background: We have previously shown that antenatal maternal stress and anxiety have an impact on the both the Mental Developmental Index (MDI) and the Behavioural Scales of Bayley Scales of Infant Development (BSID) at 18 months of age

Aim: To examine the effects of maternal depression, stress and anxiety during pregnancy on infant development at 3 years.

Method: One hundred and forty-nine predominantly middle-class Caucasian women were recruited during their first prenatal health care visit. We measured maternal anxiety, using the Spielberger State-Trait Anxiety Inventory (STAI, trait and state), stress using the perceived stress (PSS) /Life events stress (LES) scale and depression using the Edinburgh Scale (EPDS), at 30-32 (time 1) and 36-38 wks of gestation (time 2). Mothers were assessed again at 3 years using the STAI (trait and state) and the Center for Epidemiologic Studies Depression Scale (CES-D). Sixty-nine infants were tested at 3 years. Infant development was evaluated by an independent observer at 18 months of age and 3 years using the Bayley Scales of Infant Development (BSID).

Results: The explanatory variables used were the EPDS and STAI-trait total score at time 2, STAI-State total score at time 2, PSS total score, life event stressors at time 2. The STAI was repeated at 3 years in association with the CES-D to measure depression. Infants whose mothers had STAI scores >45 at time 2 had a significantly lower Mental Developmental Index (MDI 105.72 v 97.25 p=0.023). Mother who were depressed using the EPDS at time 2 also had a significantly lower Mental Developmental Index (MDI 104.71 v 92.8 p=0.031).The Behavioural Rating Scale and the Psychomotor Development Index (PDI) were similar for both groups. No gender differences in development were found.

Conclusion: These results suggest that maternal anxiety and depression in pregnancy does continue to have a significant impact on infant development.

CHANGES IN $GABA_A$ RECEPTOR A SUBUNIT PROTEIN EXPRESSION AFTER NEONATAL HYPOXIA-ISCHAEMIA

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Background: Treatment of seizures after neonatal hypoxia/ischaemia (HI) is one of the few therapeutic options available to doctors in the intensive care nursery. The principal function of the GABA system in mature brain is inhibition, however in the neonatal brain GABA provides much of the excitatory drive. Whilst anticonvulsants augment GABA's inhibitory actions in mature brain, administration of GABAergic drugs to neonates may exacerbate seizures and worsen HI brain injury. Differences in $GABA_A$ receptor expression will influence receptor pharmacology.

Aims: We aimed to assess changes in protein expression of the $GABA_A$ receptor subunits in the neonatal HI piglet model.

Methods: Piglets (n=36) were subjected to a 30min HI insult and euthanased at 24 and 72h. HI animals were grouped based on presence or absence of seizure activity. Cortical brain tissue was collected and $GABA_A$ receptor α_1 and α_3 protein expression levels analysed by western blot.

Results: $GABA_A$ α_1 and α_3 protein expression was altered temporally and regionally following HI. At 24h there were no differences in α_1 expression between HI, HI-seizure and control animals. At 72h α_1 expression was elevated in the parietal, temporal and occipital cortices of HI animals when compared with controls. At 24h α_3 expression was lower in the HI-seizure animals compared with HI animals without seizure, by 72h this reached significance ($p<0.05$).

Conclusions: $GABA_A$ receptor α_3 expression was significantly altered following neonatal HI; presence of seizures further changed this expression. Further investigation into levels of protein expression of other α subunits is ongoing. There is a critical need to develop effective treatment strategies specific to the neonatal brain.

CHILDREN BORN PRETERM HAVE REDUCED LONG TERM DEPRESSION (LTD)-LIKE NEUROPLASTICITY

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Background: Neuroplasticity is the ability of the brain to make short- or long-term changes to the strength of synaptic connections between neurons in response to activity and experiences. It is widely accepted to be the physiological basis for learning and probably memory. Evidence suggests that preterm children have alterations in cortical development, functional connectivity between cortical regions and patterns of neural activation in response to incoming stimuli. This suggests that the capacity for neuroplastic reorganization may be reduced in these children and critically contributes to their common difficulties with learning and memory.

Hypothesis: Compared with their term-born peers, children born before 37 weeks of completed gestation (wks GA) have a reduced response to a non-invasive neuroplasticity induction intervention designed to induce a short-term LTD-like (i.e. inhibitory) change in motor corticospinal excitability.

Methods: Twenty-five members (15 females) of the PREMOCODE study cohort age 12-15 years (13.67 ± 0.48 years) participated; Term born (37-41 wks GA) N=6, Late preterm (32-36 wks GA) N=9 and Early preterm (24-32 wks GA) N=9. Continuous theta burst stimulation (cTBS) was applied to the motor cortex at 80% of active motor threshold (aMT) to induce LTD-like neuroplasticity. To assess changes in corticospinal excitability (an indicator of neuroplasticity), single pulse transcranial magnetic brain stimulation was used to record motor evoked potentials (MEPs) from an intrinsic hand muscle before and at various time points up to 60 min following cTBS.

Results: Term born children showed robust motor cortex inhibition immediately following cTBS and this persisted for at least 60 min. The depth and persistence of the inhibition in this group was greater than previously consistently recorded in adult subjects. In comparison, inhibition in both preterm groups was significantly less than term born children and returned to baseline within 40 min of cTBS ceasing. GA correlated negatively with the mean MEP inhibition following cTBS, i.e. the least inhibition was evoked in the most preterm children.

Conclusions: These preliminary findings provide the first physiological evidence of reduced neuroplasticity in preterm children. While different types of neuroplasticity induction (i.e. LTP-like, behavioural) are yet to be assessed, these results demonstrate that even modest levels of prematurity are associated with significant impairments that persist at least into early adolescence. The underlying mechanisms are not yet clear, but may include synapse specific dysfunction and/or altered cortisol secretion patterns which are known to influence neuroplasticity.

MYELINATION IN IUGR: NOT JUST MYELIN BASIC PROTEIN

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Background: Intrauterine growth restriction (IUGR) can lead to adverse neurological sequelae in postnatal life ranging from learning difficulties and decreased intelligence and cognition in childhood, to cerebral palsy in late-term and term infants. Previously, we have shown that chronic placental insufficiency (CPI) induced via uterine artery ligation (UAL) throughout the second half of gestation in the guinea pig causes a delay in the process of myelination in the brain and spinal cord of the fetal guinea pig (52 days of gestation (dg) and 62dg; term ~ 67dg)[1], but we have not investigated the postnatal and long-term effects on myelination, a vital aspect of neural maturation and function.

Aims: Our objective was to determine whether IUGR, induced by CPI in the guinea pig results in long-term deficits in brain myelination and could therefore contribute to altered neural function.

Methods: At 30dg, CPI was induced in pregnant guinea pigs via UAL to produce IUGR fetuses (60dg), neonates (1 week) and young adults (8 weeks); controls were from the unligated horn or sham-operated animals. At 60dg (control, n=8 and IUGR, n=8), 1 week (control, n=7 and IUGR, n=7) and 8 weeks (control, n=12 and IUGR, n=12) of age the brains were perfused and processed for thionin-staining and immunohistochemistry using antibodies for myelin basic protein (MBP), myelin-associated glycoprotein (MAG), myelin-proteolipid protein (PLP) and oligodendrocyte transcription factor 2 (Olig2). White matter (WM) and cortical volume, MAG- and Olig2-immunoreactive (IR) oligodendrocyte (OL) density and the extent of myelination were determined using image analysis in the cerebral hemispheres and cerebellum.

Results: In IUGR fetuses and neonates, WM volume was reduced ($p<0.05$); this reduction did not persist in young adults however the corpus callosum width was reduced ($p<0.05$). Immunoreactivity for MBP, MAG and PLP, all markers of early myelinating OLs, was reduced in IUGR fetuses compared to controls. Of these markers MBP was the most markedly affected with an abnormal retention of protein in the OL soma and a reduction of its incorporation into the myelin sheath. MAG-IR OL density was reduced ($p<0.05$), while Olig-2-IR OL density was increased ($p<0.05$). MBP-, MAG- and PLP-IR recovered to control levels postnatally.

Conclusions: These results suggest that IUGR transiently delays OL maturation and myelination *in utero* but that myelination and WM volume are restored postnatally. Deficits in myelination are therefore unlikely to be the major factor underlying the altered neurological function, which can be associated with IUGR; we acknowledge that maturational alterations to myelination could have some long-lasting effects on brain function. This study also highlights the need to analyse more than one marker of mature myelinating OLs. Here we show that although IUGR dramatically affects MBP-IR, with what may be interpreted as an absence of myelination, immunoreactivity for MAG and PLP immunoreactivity, although reduced, is present indicating that myelination is still occurring albeit at a reduced level. We suggest that researchers should be circumspect when drawing conclusions about the state of myelination based on MBP-IR alone.

[1]*Nitsos and Rees (1990) Int J Dev Neurosci. 8: 233-244.*

NOTES

INTERGENERATIONAL TRANSMISSION OF CARDIOMYOCYTE DEFICITS IN OFFSPRING BORN SMALL

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Background: Intrauterine growth restriction increases the risk for developing diseases in adult life. Recent evidence suggests that growth restriction and disease risk can be passed to the next generation.

Aims: The aim of this study was to examine the effect of growth restriction on cardiomyocyte nuclei number in F1 and F2 generation male offspring at day 35.

Methods: Uteroplacental insufficiency and sham surgery was induced in F0 WKY pregnant rats on day 18 of gestation giving rise to F1 Restricted and Control offspring. F1 Restricted and Control females gave rise to F2 Restricted and Control offspring without pregnancy interventions. At day 35, F1 and F2 male offspring were perfusion fixed and the heart was collected for stereological analysis.

Results: F1 Restricted males had reduced body weights until day 35 ($p<0.05$). Relative left ventricular weight and volume was increased in F1 and F2 Restricted compared to Control offspring ($p<0.05$). Left ventricular cardiomyocyte nuclei number as a proportion of volume was reduced ($p<0.05$) by 19% in the F1 Restricted, but not F2, compared to Control.

Conclusions: The increase in left ventricular volume and reduced cardiomyocyte number relative to left ventricular volume suggests cardiomyocyte hypertrophy in the F1 generation. The alterations in cardiac structure and reduction in cardiomyocyte nuclei number in the F1 offspring born small are likely to adversely affect cardiac functional reserve and the adaptive capabilities of the heart and has the capacity to be transmitted to successive generations putting subsequent offspring at risk.

PREGNANCY IN FEMALE RATS BORN SMALL: CARDIORENAL AND METABOLIC ADAPTATIONS AND CONSEQUENCES FOR NEXT GENERATION FETAL GROWTH

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Background: Intrauterine growth restriction affects 10% of pregnancies in the Western world and is associated with an increased predisposition to a number of adult diseases. Alterations in cellular number and/or function may be adequately compensated for, particularly in female offspring, until a postnatal stressor or 'second hit' reveals a clinically relevant phenotype. Small birth weight women, compared to those born of normal weight, are more likely to develop hypertension during late pregnancy and studies have reported an increased risk for gestational diabetes. We suggest that the physiological demand of pregnancy is likely to unmask underlying predispositions to disease that would otherwise be absent in females who were small at birth.

Aims/Hypothesis: Whether pregnancy in growth restricted females reveals adverse cardiorenal and metabolic adaptations and consequences for fetal outcomes.

Methods: Uteroplacental insufficiency was induced by bilateral uterine vessel ligation on gestational day 18 in F0 WKY rats, resulting in 10-15% reduction in birth weight (Restricted) compared to sham surgery (Control). Restricted and Control F1 female offspring were mated with normal males at 4 months (Pregnant) and physiological measures were performed in late pregnancy; tail-cuff blood pressure and glucose tolerance test (IPGTT) at E18 and 24h urine collection at E19-20. Post mortem was performed at E20 and various maternal organs, as well as F2 fetal body and placental weights were measured. Maternal glomerular number and volume, and pancreatic β-cell mass were determined. A group of age-matched Restricted and Control F1 females remained as virgins.

Results: Despite a 33% reduction in glomerular number and concomitant glomerular hypertrophy ($P<0.05$) in growth restricted pregnant females, urinary protein excretion, creatinine clearance and systolic blood pressure were not different between groups. Interestingly, Non Pregnant Restricted females had reduced β-cell mass (-36%; $P<0.05$) that was completely restored during pregnancy. However, this did not prevent the development of impaired glucose tolerance during pregnancy in Restricted females compared to Controls ($P<0.05$). F2 male and female fetuses from Restricted mothers at E20 were lighter than those from Controls (-5-6%; $P<0.05$) and placental weights tended to be reduced ($P=0.07$).

Conclusions: Pregnancy in females born small poses significant risk for renal and metabolic dysfunction and programs next generation fetal growth restriction. Adverse pregnancy adaptations, including the development of glomerular hypertrophy may have implications for long-term maternal and offspring health.

ADULT BODY COMPOSITION AFTER PERICONCEPTIONAL EVENTS

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Background: Periconceptional undernutrition alters offspring physiology and adult pathology without affecting birth size. Being born a twin affects birth size and physiology, but data are conflicting about adult outcomes. We have shown recently that being conceived a twin alters intrauterine and postnatal growth, even if the pregnancy is reduced to a single fetus early in gestation. Altered body composition is a possible mechanism by which some of the metabolic changes seen in adulthood after periconceptional events may be mediated.

Aim: To examine the effects of periconceptional undernutrition and twin conception on body composition of adult offspring using dual x-ray absorptiometry (DXA).

Method: DXA scanning was performed on 3-5 year old offspring of ewes well fed at the time of conception (control) or undernourished for varying times around conception: 61d before, to 30d after mating (UN-61-30d); 61d before, to mating (UN-61-0d); or 2d before, to 30d after mating (UN-2-30d), and on 2 year old sheep born a single, born a twin, or conceived as a twin but born a single (one twin ablated in early pregnancy, 'reductions'). Prior to DXA scanning, sheep were weighed and fasted overnight. Sheep were sedated during the DXA scan using an equi-volume mixture of diazepam 5 mg/ml and ketamine 100 mg/ml. Scans were recorded and analyzed using Norland scanner software. Fat and lean mass were expressed as a percentage of body weight and compared between sexes and treatment groups in each study using ANOVA.

Results: Seventy six scans from the periconceptional undernutrition study were analysed. Percentage fat mass was significantly greater in male offspring of all periconceptionally undernourished ewes than in male control offspring (male control 1±0.5%; UN-60-30 8±1; UN-60-0 9±1; UN-2-30 11±1; $p<0.01$).Thirty three scans from the twin study were analysed. Percentage fat mass was significantly higher in sheep born as twins and reductions than those born as singles (male single 7±2 twin 10±1 reduction 11±3; female single, 15±3 twin 20±2; reduction 19±1 $p<0.01$), while % lean mass was higher in singles than in twins and reductions (male single 72±1 twin 70±1 reduction 69±2; female ± single 72±2 twin 69±2 reduction 69± 0; $p<0.05$). There was no relationship between body composition and maternal weight, birth weight or early postnatal growth velocity in either study.

Conclusions: Body composition in adulthood was altered by the periconceptional events of maternal undernutrition and twinning. This was not explained by maternal weight, birth weight or early postnatal growth. This suggests that periconceptional events *per se* may be a determinant of body composition, which may in turn be associated with adverse metabolic outcomes.

NEONATAL EXENDIN-4 TREATMENT TO PREVENT DIABETES AFTER IUGR – PRELIMINARY OUTCOMES IN THE PLACENTALLY-RESTRICTED LAMB

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Background: Low birthweight arising from intrauterine growth restriction in humans and due to placental restriction (PR) in sheep, leads inadequate compensatory increases in insulin secretion and development of insulin resistance, which impairs insulin action in adults and increases the risk of diabetes after human IUGR. Neonatal treatment of PR rat offspring during the first 6 days of life with the GLP-1 analogue, exendin-4, prevented development of diabetes, restored glucose tolerance, and normalised beta-cell mass. In the sheep, treatment of twin IUGR offspring prevented catch-up growth including fat deposition, and restored insulin secretion at the end of treatment at 16d of age. However, whether neonatal exendin-4 restores adult insulin action after IUGR in a species where the majority of pancreatic maturation is prenatal is not yet known. We are therefore investigating long-term effects of neonatal exendin-4 in the placentally-restircted sheep.

Aims: We hypothesise that neonatal exendin-4 treatment will prevent excess neonatal fat deposition during catch-up growth and that this will persist to improve insulin sensitivity. We further hypothesise that neonatal exendin-4 treatment will increase β-cell mass and function and that this will persist to increase insulin secretion and its plasticity in later life. Here we report preliminary data on growth during and after exendin-4 treatment in the PR sheep.

Methods: Weight and size were measured at birth, every 2 days to day 16, and then weekly to weaning, in progeny of unoperated control ewes (CON), in offspring of placentally-restricted ewes (PR) and in offspring of PR ewes who were treated with exendin-4 (EX-4; 1nmol/kg s.c.) from day 1 to 16 postnatal age.

Results: Before treatments began, PR offspring had significantly lower body weight than control offspring (CON, 6.59 ± 0.35 kg, n = 10; PR, 5.06 ± 0.44, n = 15; p = 0.004). Exendin-4 treatment reduced body weight of offspring at day 16 of age when compared with control offspring (CON, 12.23 ± 0.48 kg, n = 10; EX-4 9.54 ± 1.18 kg, n = 7; p = 0.016), while untreated PR offspring had similar body weights as control offspring (PR, 11.70 ± 0.55 kg, n = 4, p = 0.476). At 42 days of age, body weights across all groups were similar (CON, 18.93 ± 0.90, n = 10; PR, 18.10 ± 2.19, n = 8; EX-4, 17.42 ± 1.71, n = 7, p = 0.627). BMI across all groups do not appear to be affected at day 16 (p = 0.14) or day 42 (p = 0.80) postnatal age.

Conclusions: Neonatal exendin-4 treatment prevents catch-up growth of IUGR offspring during treatment. Effects on subsequent growth and body composition up to adulthood, and adult insulin sensitivity and secretion are currently under investigation.

DOES A POST-WEANING LOW-FAT DIET AMELIORATE THE METABOLIC CONSEQUENCES OF A MATERNAL HIGH-FAT, HIGH-SUGAR DIET?

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Background: We and others have previously shown that maternal intake of high-fat, high-sugar diets during pregnancy and lactation alters the development of the central reward pathway of the offspring and programs a preference towards high-fat foods after birth.

Aims/Hypothesis: To investigate whether the introduction of a low-fat diet after weaning can reverse the effects of maternal high-fat, high-sugar diet on offspring food preferences and body fat deposition.

Methods: Rat dams were either fed standard rat chow (control, n=10) or a cafeteria 'junk food' diet (JF, n=10) during pregnancy and lactation. From weaning, all offspring were given free access to control chow. At 6 weeks and 3 months of age, one male and one female offspring from each litter were given free access to both the control and cafeteria diet for 3 weeks and food and macronutrient preferences determined. These offspring were killed at 3 months and 6 months of age respectively and the weight of all fat depots collected for determination of total and percentage body fat mass.

Results: At 6 weeks of age, the percentage of dietary energy derived from carbohydrate was higher (48.6±1% vs 43.2±1.4%; $P<0.01$) and percentage derived from fat was lower (33.7±1.3% vs 39.8±1.6%; $P<0.01$) in female JF offspring compared to controls. At 3 months of age, this was reversed and fat intake was higher (45.5±1.9% vs 40.5±1.4% $P<0.05$) and carbohydrate intake lower (42.0±1.7% vs 46.5±1.2%; $P<0.05$) in the female JF offspring. These differences were not present in males. There was no difference in total body fat between groups at 6 weeks and 6 months of age. However, total body fat was higher in the JF offspring at 3 weeks (Control: 5.9±0.2%, JF: 8.7±0.6%; $P<0.01$) and 3 months of age (Control: 11.0±0.6%, JF: 13.8±0.6%; $P<0.01$) in both males and females.

Conclusions: A low-fat diet after weaning resulted in lower fat intake in female offspring of JF dams at 6 weeks, but not 3 months of age. Importantly, introducing a low-fat diet after weaning resulted in a reduced body fat mass before, but not after, transient introduction of a palatable diet. These findings suggest potential windows of opportunity for intervention.

THE EFFECTS OF INTRA-AMNIOTIC IGF-I TREATMENT OF INTRA-UTERINE GROWTH-RESTRICTED LAMBS ON GROWTH AND BODY COMPOSITION BETWEEN BIRTH AND WEANING

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Background: Intrauterine growth restriction (IUGR) is associated with reduced size at birth and rapid postnatal catch-up growth; both of these are associated with an increased risk of adult chronic disease. Whether antenatal treatment of IUGR to increase fetal growth affects postnatal growth velocity and long-term outcomes is not known.

Aims/Hypothesis: To investigate the effects of intra-amniotic insulin-like growth factor-I (IGF-I) treatment of growth-restricted fetal sheep on size at birth and on growth and body composition from birth to four months of age.

Methods: Singleton-bearing ewes underwent surgery at 97-100 d gestation (term 148 d) to catheterise uterine arteries, the amniotic sac, and fetal vessels. IUGR was induced from 103-107 d gestation by uteroplacental embolization with microspheres, titrated against fetal blood gases. IUGR fetuses were given either 360 µg IGF-I (IUGR-IGF-I; n=15) or saline (IUGR-Saline; n=16) intra-amniotically once a week for five weeks from 107 d. Control animals (n=22) were unoperated and unembolised. Lambs were weighed and measured from birth (T0) through to week 16 (T16) of life. Weaning was at week 12. Lambs underwent a DXA scan at week 1 (T1) and T16 to assess body composition and bone mineralization. Data were analysed by ANOVA or repeated measures ANOVA, followed by Tukey's post-hoc test when appropriate. Statistical significance was set at $p<0.05$. Data are presented as mean (SEM).

Results: Birthweight (BW) in IUGR-Saline lambs (4.67±0.24 kg) was significantly less, and postnatal growth velocity (GV) from birth to 16 weeks of age significantly greater (17.5±0.5 g/kg.d), than in Controls (BW: 5.92±0.20 kg; GV 15.7±0.3 g/kg.d). IUGR-IGF-I lambs were intermediate between Control and IUGR-Saline groups for BW (5.27±0.24 kg) and GV (16.1±0.4 g/kg.d) and were not significantly different from either. Females grew more slowly than males in all groups; however, weight and crown rump length growth were significantly less in IUGR-Saline female lambs compared with Control and IUGR-IGF-I females (both $p<0.001$). Lean mass (LM) and bone mineral content at T7, but not T16, were significantly less in IUGR-Saline lambs than in Controls ($p<0.05$); IUGR-IGF-I lambs were intermediate. LM as a percentage of body weight was not different amongst groups at T7 or T16.

Conclusions: IUGR lambs were born smaller and had accelerated postnatal GV compared with controls. Intra-amniotic IGF-I treated lambs were intermediate for both birth weight and postnatal GV. Ongoing studies will determine whether intra-amniotic IGF-I treatment significantly improves fetal growth, but at the expense of postnatal growth, and whether this has any effect on long-term physiology.

NOTES

NEONATAL EXENDIN-4 TREATMENT IN THE TWIN IUGR LAMB NORMALISES *IN VITRO* ISLET INSULIN SECRETION AND EXPRESSION OF ITS MOLECULAR DETERMINANTS

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Background: Poor growth before birth in humans and in sheep increases the risk of Type 2 diabetes (T2D) in later life, due to development of insulin resistance together with inadequate compensatory increases in insulin secretion. In the placentally-restricted (PR) rat, neonatal treatment with the GLP-1 analogue, exendin-4 (1 nmol/kg, d1 to 6 of age), normalises expression of the β-cell function and mass regulator, *Pdx-1*, by reversing early epigenetic changes in its promoter, and prevents the loss in β-cell mass usually seen after birth. Neonatal exendin-4 also reduces adult size in the PR rat, and may improve insulin sensitivity through preventing excess fat deposition during neonatal catch-up growth. Whether this treatment has similar effects in a species such as the sheep where major events in pancreatic maturation occur before birth is unknown. Here we report the effects of neonatal exendin-4 treatment on insulin secretion and its determinants in the twin IUGR lamb.

Aims: We hypothesise that neonatal exendin-4 treatment of twin IUGR sheep will normalise insulin secretion and its determinants.

Methods: Outcomes were compared in progeny of un-restricted singleton pregnancies (CON) and in twin lambs, injected with exendin-4 (EX-4 IUGR; 1nmol/kg s.c.) or with vehicle (VEH IUGR) from d1 to d16 postnatal age. Lambs were humanely killed at d16, pancreas dissected and islets isolated and used for studies of *in vitro* insulin secretion and gene expression.

Results: Glucose-stimulated insulin secretion from isolated islets was increased (~2.5 fold) in VEH IUGR lambs compared to CON lambs, and exendin-4 treatment normalised this. Islet expression of glucokinase (~4-fold), Slc2a2 (GLUT2, ~10-fold) and p110β (~6-fold) were also increased in VEH IUGR compared to CON lambs, and these were also normalised by neonatal exendin-4 treatment.

Conclusions: Elevated *in vitro* insulin secretion in IUGR lambs may reflect activation of compensatory mechanisms to up-regulate β-cell function in early life. Neonatal exendin-4 treatment normalised glucose-stimulated *in vitro* insulin secretion and expression of these determinants of glucose uptake and metabolism in islets. Neonatal exendin-4 treatment also prevents catch-up growth and fat deposition of twin IUGR offspring during treatment, which might improve later insulin sensitivity. We are currently investigating whether these effects of neonatal exendin-4 on insulin secretion and body composition persist and improve insulin action in later life in the IUGR sheep.

EFFECT OF SUSTAINED INFLATIONS ON ASPHYXIATED NEAR-TERM LAMBS

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Background: Perinatal asphyxia is a common clinical problem in newborns, occurring in 2% of all live births and is associated with adverse neurodevelopment. The most effective way to resuscitate these newborns without contributing to brain injury is not known. An initial sustained inflation (SI) of 20 s duration facilitates lung aeration and improves cerebral blood flow after preterm birth without asphyxia. Current clinical guidelines recommend resuscitation with 5 short (3 s duration) SIs.

Aims/Hypothesis: The aim was to investigate the effects of 5 consecutive SIs of 3 s duration on the respiratory and cerebro-vascular transition after birth in asphyxiated near-term lambs.

Methods: Fetal sheep (139 days of gestation) were instrumented and asphyxiated (induced by reducing maternal inspired oxygen content and delaying the initiation of ventilation after cord clamping) until mean arterial blood pressure was below 20 mmHg. After delivery, lambs were randomized to receive: 5 consecutive SIs (35 cmH_2O, 3 s duration) followed by ventilation for 30 min (SI; n=5); or ventilation for 30 min without Sis (no SI; n=3). Tidal volume, inspiratory pressure, and carotid arterial blood flow and pressure were recorded.

Results: Tidal volumes and inspiratory pressures were similar between groups but arterial oxygen saturation tended to be lower (41.4±15.1 mmHg vs 86±10.8 mmHg at 5 min) and $PaCO_2$ higher in the SI group (79.8±5.4 mmHg vs 63.1±13.0 mmHg at 5 min). Carotid blood flow was higher in the SI lambs and remained elevated throughout the experiment (36.8±8.9 mL/kg/min vs 25.2±4.0 mL/kg/min at 10 min).

Conclusions: Resuscitation with 5 consecutive 3-s SIs doesn't appear to improve oxygenation or stablise blood flow to the brain in asphyxiated newborn lambs.

EPIGENETIC PROGRAMING OF ADOLESCENT METABOLIC PHENOTYPES?

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Background: Modifications to DNA methylation patterns in early development have been proposed as contributing to the early origins of adult diseases such as obesity and diabetes. We fed rats a diet deficient in methyl donors (molecules that donate methyl groups to DNA) during the periconceptional period, when DNA methylation patterns are reset, to determine if nutritional manipulation of the methyl donor pathway predisposes to metabolic syndrome.

Aims/Hypothesis: Inappropriate maternal nutrition alters specific gene loci through an epigenetic mechanism (nutritional programming) mediated by a change in DNA methylation that takes place during early development. These changes are permanent and last throughout the life of the offspring**.**

Methods: Female Wistar rats were fed semi-synthetic diets containing normal or reduced (MD) levels of methionine, choline and folate, for 3 weeks prior to mating until day 5 of gestation; after this time both groups were fed a control diet. From weaning half the offspring were challenged with an obesogenic diet, half were fed control until 3 months of age. Offspring were phenotyped for obesity, glucose intolerance and metabolic disturbance.

Results: Feeding the MD diet during periconception raised plasma homocysteine concentrations five times in the MD dams (control: 4.2 ± 0.2 µmol/L, MD: 19.7 ± 0.7µmol/L, $p<0.0001$). This reduced to a 20% difference within 3 days of reintroducing a balanced diet (control: 6.9 ± 0.4 µmol/L, MD: 8.4 ± 0.3µmol/L, $p<0.01$). The male offspring from MD dams that were fed control diet and those fed control then challenged with obesogenic diet were mildly glucose intolerant when compared to the offspring from control dams fed control diet. However, the MD offspring challenged with an obesogenic diet had both insulin resistance, more significant glucose intolerance and raised liver triglycerides.

Conclusions: Small changes in the methylation potential of the maternal diet during the periconceptional period programs glucose/insulin metabolism in male offspring. A postnatal obesogenic environment interacts with this programming, culminating in offspring gaining aspects of metabolic syndrome and non alcoholic fatty liver disease.

EXPOSURE TO HYPEROXIC GAS IN THE NEONATAL PERIOD: EFFECTS ON THE SMALL CONDUCTING AIRWAYS IN ADULTHOOD

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Background: Very preterm infants usually require prolonged respiratory support, which includes the use of supplemental oxygen therapy with high inspired oxygen concentrations. Numerous follow-up studies have reported poor lung function in children and adults who were born very preterm and received prolonged respiratory support. These studies suggest that very preterm birth, or factors associated with respiratory support, cause long-term changes in the structure of the small conducting airways. We recently showed that inhalation of hyperoxic gas (65% O_2) for 7 days after birth in mice persistently alters the development of the small conducting airways in early adulthood (56 days postnatal age; P56d). We have also recently shown that mice exposed to hyperoxic gas during the neonatal period have altered lung function in "middle-age" (10 months postnatal age; P10mo). However, it is not known if the structural alterations to the airways persist further into adulthood and if this is the cause of the altered lung function observed at this age.

Aim: Our aim was to determine if the alterations we observed at P56d in the structure of the small conducting airways (bronchioles) induced by neonatal exposure to hyperoxic gas persist to "middle-age" (P10mo).

Methods: Neonatal mice (C57Bl/6J) born at term were continuously exposed to hyperoxic gas (65% oxygen) from birth until P7d, after which the mice lived in room air (21% oxygen) until P10mo (n=15). Controls breathed room air from birth (n=16). The structure of the small conducting airways (bronchioles) and lung parenchyma was morphometrically analysed at 10 months of age. In the bronchioles, we measured the thickness of the epithelium, proportion of epithelial cells undergoing proliferation, proportion of ciliated and Clara cells within the epithelium, the amount of collagen and airway smooth muscle (ASM) within the outer airway wall, and the number of alveolar-bronchiolar attachments. In the lung parenchyma, we measured the percent tissue and airspace, and the mean linear intercept (MLI) as an indicator of alveolar diameter.

Results: At P10mo, mice that had been exposed to hyperoxic gas in neonatal life had significantly more ASM in the outer airway wall compared to controls ($p<0.05$). There was no significant difference in the thickness of the airway epithelium or the proportion of epithelial cells undergoing proliferation between hyperoxia-exposed mice and control mice. Additionally, the proportion of Clara cells and ciliated cells in the bronchiolar epithelium was not significantly different. The airway wall collagen, alveolar-bronchiolar attachments, MLI, and tissue and airspace fractions are currently being analysed.

Conclusions: Exposure of the immature lung to hyperoxic gas results in a persistent increase in the amount of ASM surrounding the bronchioles in "middle-age". This increase in ASM, which was also observed at P56d, could be a contributing factor to the altered lung function we see at P10mo.

ADULT ONSET VOLUNTARY EXERCISE AMELIORATES THE IMPACT OF MATERNAL OBESITY ON OFFSPRING

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Background: Maternal obesity leads to changes in metabolism and brain appetite regulators in the offspring at adulthood (Rajia et al. 2010). As maternal obesity is increasing, it will be important to develop interventions to reduce its detrimental impact. Exercise has beneficial cardiovascular and metabolic effects, and we previously showed that exercise reduced adiposity in offspring of obese mothers. However the question of whether exercise implemented in adulthood could also have beneficial effects has not been addressed previously.

Aims/Hypothesis: To examine the effects of short term exercise on adiposity and hormone profile in the offspring of obese rat mothers.

Methods: Adult female Sprague Dawley rats were fed either normal chow or high fat chow diet (HFD) ad libitum for 5 weeks to yield chow and HFD mothers. Next, they were mated with chow fed male Sprague Dawley rats. At weaning, female rats from each litter were separated into 2 diet groups chow (C) or HFD (F) and after 7 weeks on their respective diet, half were exercised (voluntary running wheels, E) for 5 weeks while the remainder were sedentary (S). At 15 weeks, rats were euthanased and the brain was dissected for mRNA analysis.

Results: FS offspring had a significant ($p<0.05$) maternal effect on body weight (BW) and retroperitoneal white adipose tissue (RpWAT) mass regardless of their current diet. HFD consumption significantly increased BW and RpWAT mass in rats from both C and F mothers. Exercise reduced RpWAT mass only in offspring consuming HFD. Energy intake was reduced by exercise in all groups compared to sedentary controls. Brain markers related to appetite regulation are currently under investigation.

Mum's diet	Chow				Fat			
Offspring group	CS	CE	FS	FE	CS	CE	FS	FE
Body weight at death (g)	288 ± 5	278 ± 8	386 ± 13$^{\#}$	335 ± 7$^{\#+}$	326 ± 10	288 ± 6	467 ± 26$^{*\#}$	389 ± 21$^{\#+}$
RpWAT (g)	2.3 ± 0.3	1.5 ± 0.2	8.6 ± 0.5$^{\#}$	4.7 ± 0.6$^{\#+}$	5.0 ± 0.8	2.2 ± 0.4	13.3 ± 1.6$^{*\#}$	8.6 ± 1.4$^{*\#+}$
Energy intake pre-exercise (kJ/rat) x 10^3	10.9 ± 0.2	11.0 ± 0.3	14.0 ± 0.5	14.5 ± 0.5	12.1 ± 0.2	11.9 ± 0.3	14.8 ± 0.4	15.9 ± 0.4
Energy intake during exercise (kJ/rat) x 10^3	16.4 ± 0.1	7.4 ± 0.1^{+}	22.5 ± 0.5	15.2 ± 0.4$^{\#+}$	14.7 ± 0.1	10.9 ± 0.1	22.6 ± 0.2$^{\#}$	13.2 ± 0.3^{+}

Results are expressed as mean ± SEM.
* maternal effect ($p<0.05$), # diet effect ($p<0.05$), + activity effect ($p<0.05$). C: Control, F: Fat, S: Sedentary, E: Exercise

Conclusions: Dietary obesity in mothers led to increased BW and adiposity in offspring who consumed a HFD. A short period of exercise had beneficial effects on offspring from obese mothers, with greater effects in rats fed a HFD.

Reference: Rajia S, Chen H, Morris MJ. Maternal overnutrition impacts offspring adiposity and brain appetite markers-modulation by postweaning diet. J Neuroendocrinology 2010; 22(8):905-914.

CHANGES IN FTO EXPRESSION IN RESPONSE TO MATERNAL OBESITY IN THE RAT

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Background: Intrauterine and postnatal overnutrition program hyperphagia, adiposity and glucose intolerance. Environmental changes during fetal development can reset the expression of genes involved in metabolic function. In humans, recent genome-wide association studies suggested a link between single-nucleotide polymorphisms (SNPs) of the fat mass and obesity associated gene (FTO) and increased risk of obesity. Carriers of the A risk allele exhibited an increased energy intake with no evidence of an effect on energy expenditure. In rodents, FTO is highly expressed in the hypothalamus, in particular in regions critical for the control of energy balance, linking hyperphagic phenotypes with human SNPs. In rodents, knockdown of FTO in the hypothalamic arcuate nucleus increased food intake, while overexpression decreased it, suggesting FTO levels can influence energy intake.

Aims: This study aimed to investigate the changes in FTO mRNA expression in response to maternal obesity, postnatal litter size reduction, and post-weaning high fat diet (HFD) consumption in the brain and liver of offspring.

Methods: Female Sprague Dawley rats were exposed to chow or HFD for 5 weeks before mating, throughout gestation and lactation. On postnatal day 1 (PND1), some litters were adjusted to 3 pups to induce postnatal overnutrition (vs. 12 in control). At PND20, half of the rats from each litter were weaned onto chow or HFD for 15 weeks. FTO mRNA expression in the hypothalamus and liver, as well as markers of lipid metabolism in the liver, were measured by RT-PCR.

Results: At weaning (PND20), maternal HFD feeding increased hypothalamic FTO mRNA expression, whereas in the liver FTO mRNA was decreased, suggesting differential regulation of the gene. Hypothalamic FTO mRNA was positively correlated with fat mass as well as energy intake of HFD in the adult HFD-fed offspring. At day 20, liver FTO mRNA expression was significantly correlated with the lipid oxidative marker carnitine palmitoyltransferase 1a (CPT-1a) and lipid synthesis markers fatty acid synthase (FAS) and sterol regulatory element-binding protein-1c (SREBP-1).

Conclusions: Our results provide evidence that maternal obesity had a significant impact on FTO expression in both hypothalamus and liver. Moreover FTO expression at day 20 was correlated with later food intake of siblings exposed to HFD which suggests increased FTO may lead to subsequent hyperphagia, in line with some human data. These results suggest FTO may also affect hepatic lipid metabolism in offspring.

NOTES

DEVELOPMENT OF TECHNIQUES FOR MEASURING HYDROGEN SULPHIDE IN THE NEWBORN NEONATE

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Background: Previous work shows that microvascular dysfunction characterised by inappropriate dilatation throughout the periphery is associated with illness severity in premature infants. The gasotransmitters nitric oxide and carbon monoxide have been shown to play a role in maintaining vascular homeostasis in the transitional circulation of preterm neonates. In adults, interest is arising in the role of hydrogen sulphide (H_2S) as a vascular mediator important in microvascular tone. As with nitric oxide, H_2S is present in very low concentrations within the microcirculation and is readily exhaled, taken up by a number of molecules including haemoglobin or converted to thiosulphate, making its measurement in biological samples inherently difficult.

Aims/Hypothesis: To assess methods of quantifying H_2S or its stable metabolite (urinary thiosulphate) in preterm biological samples (small volumes of blood or urine) in order to further characterise the microvascular status of the preterm newborn.

Methods: The performance of a commercially available sulphide specific micro ion-selective electrode was investigated and measurement conditions and sample handling procedures for small volume biological samples were optimised. Thiosulphate was detected and measured in neonatal urine samples using a previously published modification of the Sörbo spectrophotometric method (cyanolysis procedure).

Results: With the ion-selective electrode, reproducibility of sample measurement was poor at low concentration ranges, even when measurements were performed under standardised conditions, including sample processing and analysis at 4ºC, addition of a sulphide antioxidant buffer and measurement within 10 minutes of sample collection. Variation was significant in low and medium micromolar ranges, which are now considered to be higher than circulating levels (likely fall within the nanomolar range). The urinary thiosulphate assay is sensitive enough to detect low levels of urinary thiosulphate in samples collected from both term and preterm infants in early extrauterine life. Whether the assay is sensitive enough to detect differences between groups is yet to be determined.

Conclusions: H_2S is a potential important mediator of vascular tone in the neonatal transitional circulation. We are establishing the methods to quantitatively measure its levels and therefore be able to correlate its levels with physiological measures of macrovascular and microvascular flow.

CORONARY AND AORTIC FLOW IN RESPONSE TO CHANGES IN PRELOAD AND AFTERLOAD IN THE ISOLATED PRETERM PIGLET HEART

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Background: Low systemic blood flow occurs in 30% of infants born at less than 30 weeks gestation and is associated with increased morbidity and mortality. We have previously shown in the isolated working heart that preterm piglets have reduced cardiac output (per kg BW) compared to term piglets and importantly were unable to maintain systemic (aortic) flow at high afterloads.

Aims/Hypothesis: To assess in the isolated preterm piglet heart a) the effects of preload and afterload on coronary flow in the isolated heart model and, b) the effects of maternal glucocorticoid treatment on the ability of the preterm heart to produce adequate systemic blood flow.

Methods: Piglets were delivered by caesarean section at term (115d) or preterm (92d) and an additional group of preterm piglets received maternal glucocorticoid treatment similar to current clinical practice (betamethasone, 0.19mg/kg BW at 48h and 24h prior to delivery). An isolated working heart model was used to assess left ventricular function in terms of cardiac output (mL/min/kg BW), aortic flow (mL/min/kg BW), coronary flow (mL/min/g heart), contractility (dP/dt_{max}) and developed pressure (mmHg).

Results: Contractility and pressure development were similar in term and preterm hearts. Preterm hearts had significant preload reserve, increasing cardiac output with preload as predicted by the Starling relationship. However the greater requirement for coronary flow to meet this increased performance meant that increases in aortic flow were small. In preterm hearts elevations in afterload markedly depressed aortic flow, with cardiac output disproportionately redistributed to coronary vessels at high afterloads. As a result, >55% of preterm hearts were unable to maintain adequate aortic flow at afterloads ≥30mmHg. The requirement for coronary flow to perform the same cardiac work was similar in glucocorticoid exposed preterm hearts and term hearts and was less than in untreated preterm hearts. Exposure to maternal glucocorticoids increased the proportion of preterm hearts able to maintain adequate flow at low afterloads from <60% to >80%.

Conclusions: These data suggest the preterm heart may lack the functional capacity to acutely adapt to post-natal afterload as a result of a disproportionate increase in coronary flow at high afterloads. To maximise aortic flow and specifically SBF in preterm infants, treatments limiting afterload while harnessing significant preload reserve should be targeted. Exposure of preterm hearts to maternal glucocorticoids improved systemic blood flow at low to moderate afterloads through either increased of cardiac efficiency or improved coronary vascular tone.

ONTOGENY OF THE HEART RATE POWER SPECTRUM IN THE LAST THIRD OF GESTATION IN FETAL SHEEP

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Background: Due to its non invasive nature, power spectral analysis (PSA) of fetal heart rate variability (HRV) has attracted interest as a potential measure of fetal autonomic balance, that might in turn allow early detection of fetal compromise. It is likely but unproven that both absolute levels of variation and autonomic effects change in the last third of gestation.

Aims/Hypothesis: to quantify changes in spectral power of the FHR and the ratio between low frequency (LF)/ and high frequency power (HF) in the last third of gestation and to test the hypothesis that in late gestation the ratio of LF/HF power is a reliable measure of the balance between parasympathetic and sympathetic autonomic activity.

Methods: Fetal ECG recordings were obtained from chronically catheterised, fetal sheep with normal blood gas values at 0.6 (n=8), 0.7 (n=7) and 0.8 of gestation (n=11), and power at very low (0-0.04 Hz), low (0.04-0.15 Hz) and high (0.15-0.4 Hz) frequencies was calculated. At 0.8 gestation, data were analysed relative to sleep state, defined by low voltage-high frequency (LV) or high voltage-low frequency (HV) EEG activity. In a second study, 0.8 gestation fetuses received either atropine (n=6) or 6-hydroxydopamine (OHDA) (n=6). Atropine was administered as a 4.8 mg bolus, then a 4.8 mg/hr infusion for 30 minutes. 6-OHDA was given as a 20 mg/ml infusion at 2.5 ml/hr for roughly 3 hours. All subjects had normal brachial arterial blood gas values.

Results: Total spectral power increased overall with gestational age ($p<0.05$), while LF/HF decreased from 0.6 to 0.7, but was not different between 0.7 and 0.8 of gestation. In near-term fetuses, although both heart rate and LF/HF were significantly higher during HV sleep ($p<0.05$), spectral power only showed a tendency to be greater during HV sleep in the very low frequency band ($p = 0.056$). Sympathectomy with 6-OHDA was associated with reduced HF spectral power ($p < 0.05$), while atropine was associated with a fall in VLF spectral power ($p<0.05$). There was no significant change in LF/HF after either 6-OHDA or atropine infusion.

Conclusions: FHR power spectra show substantial changes with maturation, which likely in part reflect maturation of autonomic. However, these findings do not support reliable differences in the frequencies of sympathetic compared with parasympathic cardiac activity in late gestation fetal sheep.

ADRENOCEPTOR SUBTYPE mRNA EXPRESSION IN THE PIG HEART: DEVELOPMENTAL CHANGES AND EFFECTS OF MATERNAL GLUCOCORTICOID TREATMENT

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Background: Cardiac sympathetic activity is mediated through binding of catecholamines to G-protein coupled membrane bound adrenoreceptors (ARs) in cardiac myocytes. Sympathetic effects on the fetal heart are predominantly mediated through α adrenoceptors (α-ARs) whereas in adults β adrenoceptors (β-ARs) dominate. The switch in cardiac AR subtype occurs around the time of birth [1, 3]. Hence the ability of the preterm heart to respond to inotropic drugs may depend on the receptor subtype present at the time of birth. The positive effect of maternal glucocorticoid (GC) on the response of the preterm heart to inotropes [2] may be due to GC-induced AR subtype switch.

Aims: To measure and compare mRNA expression of AR subtypes in preterm and term neonatal piglets, and to examine the effects of maternal GC administration.

Methods: Piglets were delivered by Caesarean section at 92 days (preterm group) and 114 days of gestation (term group, full term≈115 days). Glucocorticoids (betamethasone, 0.19mg/kg body wt) were given to mothers of an additional group of preterm piglets at 48 and 24h before delivery. mRNA levels of AR subtypes, normalised to GAPDH, were measured in left and right ventricle (LV and RV) by real-time PCR using Taqman© chemistry (Applied Biosystems). Levels were expressed relative to a calibrator using the comparative C_T ($2^{-\Delta\Delta CT}$) method.

Results: Expression of β_1-ARs was lower in preterm compared to term hearts (0.61±0.19 (mean±SD) vs. 1.60±0.72 in LV and 0.54±0.12 vs. 0.90±0.49 in RV, P<0.001) but α_{1D}- and α_{2A}-ARs mRNAs were higher compared to term hearts (α_{1D}-AR: 0.95±0.28 vs. 0.71±0.42 in LV, 1.01±0.27 vs. 0.47±0.27 in RV, P<0.001 and α_{2A}-AR: 1.84±0.58 vs. 0.79±0.33 in LV, 1.72±0.46 vs. 1.00±0.67 in RV, P<0.001). α_{1A}-AR expression was not different between preterm and term hearts. Maternal GC treatment was associated with a lower expression of α_{1A}-AR mRNA in LV (0.20±0.14 vs. 0.68±0.66, P<0.05) and α_{1D}-AR in both ventricles (0.54±0.41 vs. 0.95±0.28 in LV and 0.60±0.43 vs. 1.01±0.27 in RV, P<0.05). Expression of β_1-AR and α_{2A}-AR was not affected by GC treatment. α_{2B}-ARs were absent in all groups.

Conclusions: The lower expression of β_1-AR in preterm pig hearts compared to term may explain the inefficient cardiac function seen after premature birth and also why some preterm babies do not respond to conventional inotrope treatments that act on β_1 receptors. Maternal glucocorticoids did not affect expression of β_1-AR but reduced expression of some α-ARs.

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LONGITUDINAL SEX-SPECIFIC NORMOGRAMS FOR DOPPLER ULTRASOUND INDICES OF INTRAUTERINE FETAL SHUNTS: THE FORAMEN OVALE AND THE DUCTUS ARTERIOSUS.

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Background: The male disadvantage in fetal and neonatal life has been well established. The developmental biology of this disadvantage is largely unknown and different contributory factors have been proposed such as hormonal, immunological, physiological, genetic, epigenetic and molecular alterations. It is important to delineate physiological differences in both sexes in fetal life, to be able to understand pathophysiological mechanisms underlying fetal and neonatal morbidity and mortality. It may also improve the power of fetal surveillance and management strategies, by more precise sex specific reference values. This may increase the chances of a healthy outcome for both sexes.

Aims: This study was undertaken to establish sex-specific reference ranges for the central intrauterine shunts Ductus arteriosus (DA) and foramen ovale (FO) as well as examine the sex-specific differences in foetal biometric measurements, umbilical artery and fetal middle cerebral artery flows in the same set of foetuses by serial measurements across gestation.

Methods: The fetal FO and the DA were examined in 54 normal singleton pregnancies scanned serially at 9 time points from 16 weeks onwards until delivery. Doppler pulsatility index of the fetal FO, (DA) , pulsatility index (PI) of ductus venosus (DV), resistance index of middle cerebral artery(MCA) and umbilical artery(UA) PI were measured. Cerebroplacental ratio (CPR) was calculated as MCA RI / Umb RI.

Results: Fetal biometric growth parameters were not significantly different between the sexes. FO PI group means increased as gestation progressed in males and this was not observed in females. DA PI was significantly different between males and females at 26 weeks ($p<0.05$).DV pulsatility index reduced with gestation and was significantly different between males and females at 34 weeks ($p<0.05$) DV preload index significantly decreased linearly with gestational age and was significantly different between males and females at 20 weeks . Effect of sex was statistically significant at 20 weeks ($p<0.05$). Significant interaction of effects in group means between 16-20 weeks were observed in females ($p<0.05$), and in males between 20-24 weeks ($P<0.05$).

Conclusion: This study has established sex-specific reference ranges for fetal central intrauterine shunts. Uteroplacental, fetal and fetoplacental haemodynamic variables have also been established.

Key words: Sex-specific, fetus, Doppler, foramen ovale, ductus arteriosus, ductus venosus, normograms, fetal shunts.

THE EFFECTS OF DUMMY SUCKING ON AUTONOMIC CARDIAC CONTROL

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Background: Epidemiological studies have consistently shown that dummy sucking is a protective factor for the Sudden Infant Death Syndrome (SIDS). There is strong consensus that impaired autonomic control plays a major role in the underlying mechanism of SIDS. The protective mechanism by which dummy sucking acts is unknown, however it is thought that dummy sucking may act by enhancing autonomic responsiveness. To date however, there are few studies that have assessed the effects of dummy sucking on autonomic control during infancy.

Aims: We aimed to assess the effects of dummy sucking on heart rate variability (HRV) as a measure of autonomic cardiac control during sleep in infants within the first 6 months of life.

Methods: Term infants were studied longitudinally at 2-4 weeks, 2-3 months and 5-6 months of age using daytime polysomnography. Infants were divided into those who regularly used a dummy (n= 6 at study 1; n= 7 at study 2; n= 7 at study 3) and those who did not (n= 4 at study 1; n= 5 at study 2; n= 4 at study 3). Heart rate was measured continuously during both quiet sleep (QS) and active sleep (AS) in the supine sleeping position. Using spectral analysis, 2 min R-R interval epochs were used to establish HRV indices. Low frequency (LF, reflects baroreflex related changes and sympathertic+parasympathetic activity), high frequency (HF, reflects respiratory related changes and parasympathetic activity), the LF/HF (reflects sympathovagal balance) and Total power (reflecting total variability) spectral indices were calculated. Only periods of non-sucking were analysed. The effect of use of a dummy was compared with one way ANOVA with Student Newman Kuels post hoc analysis.

Results: At 2-4 weeks, infants who sucked on a dummy had a higher LF/HF in AS ($p<0.05$) and higher HF power in QS ($p<0.05$). At 2-3 months, there was strong trend for LF power to be higher during QS in the dummy sucking group, however this just failed to reach significance ($p=0.06$). At 5-6 months, the dummy sucking group had higher Total power ($p<0.05$) and again there was a trend for LF power to also be higher.

Conclusions: This preliminary study has identified that dummy sucking can alter HRV autonomic cardiac control during sleep. Increased Total power and LF power in infants who routinely use a dummy may indicate that autonomic cardiac responsiveness to baroreflex related changes may be enhanced in these infants, however further studies are required to elucidate this.

NOTES

TROP2 REGULATES THE PROLIFERATION AND MIGRATION OF FETAL LUNG FIBROBLASTS AND ALTERS ACTIVATION OF THE ERK SIGNALLING PATHWAY

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Background: The molecular pathways that promote fetal lung growth remain unknown. We have previously shown that the oncogene *Trop2* is positively correlated with cell proliferation in the fetal and neonatal lung during normal lung development and in models of accelerated and delayed fetal lung growth. *Trop2* knockdown in cultured fetal lung cells decreases cell proliferation and cell migration and alters cell morphology. *Trop2* has been shown to regulate migration and proliferation of tumour cells via the ERK1/2 signalling pathway.

Aim: Identify the mechanism by which *Trop2* regulates cell proliferation, morphology and migration in normal fetal lung fibroblasts.

Methods: Primary cultures of fetal lung fibroblasts were generated from E19 rats (term ~E22). *Trop2* expression was reduced (knocked-down) using short-interfering (si)RNA specific for *Trop2* and compared to cells transfected with a control (non-specific) siRNA. Proteins were extracted 48h after siRNA treatment when changes in proliferation and morphology were evident. ERK signalling in control and *Trop2* siRNA-treated cells was examined using an ERK Phospho-Antibody Microarray (Full Moon Biosystems, USA). All values are presented as fold change between fibroblasts transfected with control siRNA or Trop2-specific siRNA.

Results: *Trop2* siRNA reduced *Trop2* mRNA levels by ~70% and substantially altered the levels and phosphorylation state of proteins in the ERK signalling pathway. In fibroblasts with reduced *Trop2* expression, there was a decrease in Elk-1 (0.03), Rac1/cdc42 (0.04) and c-raf (0.1) levels, compared to control cells (1.0 for all proteins). The levels of phosphorylated Elk-1 (0.2) and c-raf (0.4) were also reduced in cells treated with *Trop2* siRNA compared to cells treated with control siRNA (1.0 for all proteins).

Conclusions: *Trop2* alters the levels and activation state of proteins in the ERK signalling pathway in fetal lung fibroblasts, including c-raf, Elk-1 and Rac1/cdc42. C-raf activates the transcription factor Elk-1 via ERK1/2, suggesting that *Trop2* may regulate cell proliferation and migration in the developing lung via the same mechanisms by which it promotes tumourigenesis and metastasis in the adult. The novel *Trop2* downstream target, Rac1/cdc42 is involved in cell proliferation and cytoskeletal organisation and is likely to mediate the effects of *Trop2* on fetal lung fibroblast proliferation, morphology and migration. This is the first study to identify the pathways that mediate the effects of *Trop2* on fetal lung cell proliferation and migration.

DOES SURFACTANT COMPOSITION PLAY A ROLE IN THE MALE DISADVANTAGE IN RESPIRATORY OUTCOME FOLLOWING MODERATE PRETERM BIRTH IN SHEEP?

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Background: Male preterm infants are at a greater risk of respiratory morbidity and mortality than females. The exact mechanisms responsible for this "male disadvantage" in respiratory function are unknown. Previous studies in our lab have found that there are no differences in the structure of the lungs of male and female preterm lambs. It has been suggested that surfactant composition in male preterm infants is less mature than in females. Pulmonary surfactant is comprised of surfactant proteins (SP-A, -B, -C and -D) and 5 classes of phospholipids (PLs). Alterations in surfactant composition may play a role in the poorer respiratory function seen in preterm males.

Aims/Hypothesis: To determine whether there are any differences between male and female preterm lambs in (a) SP mRNA and protein levels in lung tissue, and (b) PL composition in amniotic fluid and bronchoalveolar lavage fluid (BALF).

Methods: At ~125 days of gestation (DG; term ~147DG), 9 female and 9 male fetal sheep underwent surgery for the implantation of catheters (carotid artery, jugular vein, trachea, amniotic sac). Ewes received betamethasone (5.7mg, im) at 131DG. Amniotic fluid was sampled at 131 and 133DG for analysis of surfactant PL composition using liquid chromatography mass spectrometry. At 133DG, lambs were delivered via caesarean section and monitored for 4h. After 4h, lambs were euthanized and lung tissue and BALF were collected for analysis. PL composition in BALF supernatant was determined, as for amniotic fluid. SP mRNA levels and protein levels in lung tissue were determined using qPCR and Western blot analysis, respectively.

Results: No statistically significant differences were observed between males and females in SP mRNA levels in lung tissue. The composition of PLs in the amniotic fluid, just prior to birth, did not differ between the genders. There was a significantly lower percentage of phosphatidylcholine (PC, the major PL in surfactant) in BALF from males (68.0 ± 1.5 %) compared to females (72.6 ± 1.1 %) after preterm birth. Analysis of the species within PC showed a significantly lower percentage of PC32:0 (DPPC; by ~10%) and a higher percentage of PC34:2 (~20%) and PC36:2 (~35%) in males in comparison to females (all $p<0.05$). There was no difference in the composition of other PLs between the genders.

Conclusions: The different PL composition, in particular the lower proportion of DPPC and total PC, between males and females could contribute to the poorer respiratory function seen in preterm males. The similar SP mRNA levels between males and females indicate that SPs are unlikely to play a role in the different respiratory outcomes between genders following preterm delivery. Ongoing analysis includes measuring SP protein levels in lung tissue.

ROLE OF GLUCOSE IN SURFACTANT PROTEIN mRNA EXPRESSION IN THE FETAL LUNG

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Background: Maturation of the surfactant system plays a vital role in preparing a fetus for the transition to air breathing in extrauterine life. Throughout gestation, fetal lung maturation is controlled by a wide variety of factors which can be affected by altered *in utero* conditions. With the world-wide obesity epidemic over the last three decades, there has been a substantial increase in the proportion of women entering pregnancy as overweight or obese which predisposes to multiple obstetric complications including gestational diabetes and preterm birth. Maternal metabolic regulation throughout pregnancy, particularly glucose homeostasis is an important factor affecting fetal growth and development. In a normal pregnancy, maternal insulin sensitivity decreases across gestation. However, obese women are less insulin sensitive than lean and overweight women and are therefore at an increased risk of gestational diabetes. Increased circulating fetal glucose and insulin concentrations are potential inhibitors of fetal lung maturation *in utero* and may contribute to the pathogenesis of respiratory distress syndrome (RDS) observed in infants of diabetic mothers.

Hypothesis: High plasma glucose and insulin concentrations in the late gestation sheep fetus will result in decreased surfactant protein mRNA expression in the lung.

Methods: Vascular catheters were implanted into the ewe and fetus. At 130d gestation (term 150±3d), ewes were assigned to either a saline (n=7) or glucose (n=8) infused group. A glucose solution (50% dextrose 250g/L in saline) was infused continuously at an initial rate of 1.9ml/h for 24h. The infusion rate was increased in a stepwise manner by 1.9ml/h per day for the next 3d. The final infusion rate of 7.5ml/h reached on the 4th day of the infusion was maintained until post mortem at 140d gestation. Saline was infused into control fetuses at corresponding rates. The relative abundance of SP-A, -B, -C and -D mRNA transcripts in fetal lung samples was measured by qRT-PCR. Data were analyzed by a student's t-test and $P<0.05$ was considered statistically significant.

Results: The glucose infused fetuses had higher plasma glucose and insulin concentrations (*P<0.05*) than the saline infused fetuses throughout the infusion period. Fetal weight and relative lung weight were not significantly different between the saline and glucose infused fetuses. Lung SP-A and SP-C mRNA expression were reduced ($P<0.05$) in the glucose infused fetuses.

Conclusions: Increased fetal glucose and insulin concentrations result in a down regulation of surfactant protein mRNA expression in the lung of the late gestation sheep fetus which provides evidence for the link between abnormal glycemic control *in utero* and RDS observed in infants of mothers in obese obstetric populations.

THE REPAIR GENES METALLOTHIONEIN AND UROKINASE RECEPTOR ARE UP-REGULATED 24H AFTER VENTILATION INDUCED INJURY IN THE IMMATURE LUNG

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Background: Very preterm infants often require mechanical ventilation (MV) at birth to overcome respiratory insufficiency. However MV can injure the immature lung and it is associated with bronchopulmonary dysplasia (BPD) which is characterized by abnormal lung development. Even brief MV of immature lungs can activate inflammatory and early response injury genes, which are thought to contribute to injury manifestation. To investigate mechanisms of lung injury and repair we have mechanically ventilated fetal sheep to induce injury in their very immature lungs. We found that brief MV (2h) significantly alters lung morphology within 24h of MV, but this resolved within 15 days, without further treatment.

Aims/Hypothesis: To investigate mechanisms of injury and repair in the very immature lung 24h after brief, injurious MV.

Methods: Pregnant sheep underwent aseptic surgery to exteriorize the fetal head and chest at either 110d gestational age (GA; n=6) during the canalicular stage of lung development, or at 125d GA during the early alveolar stage of lung development (n=6; term ~147dGA). Fetuses were intubated and ventilated for 2h with an injurious ventilation protocol (end expiratory pressure=0cmH_2O, peak inspiratory pressure=40cmH_2O, Tidal Volume=~5ml/kg) and then returned to the uterus. Unventilated sham-operated fetuses were used for control tissue (n=7-8). Ewes and fetuses remained healthy until necropsy, 24h later. Gene expression was measured by qRT-PCR. Protein deposition was determined by immunohistochemistry.

Results: At 24 hours after injurious ventilation, there was no difference in the relative mRNA levels of the early response genes Connective Tissue Growth Factor, Cysteine Rich 61, and Early Growth Response 1, between the control and ventilated lungs at either stage of lung development. Similarly, levels of gene expression for the pro-inflammatory cytokines Interleukins -1β, -6, -8 and Tumor Necrosis Factor-α were also not different at 24h between control and ventilated lungs at either stage of lung development. Nor were there differences in the protein abundance of EGR1 and CYR-61 at either stage of lung development. The expression of metallothionein and urokinase receptor mRNA levels were increased in MV lungs at the canalicular (2.7±0.4 and 2.9±0.8 fold increase respectively) and early alveolar (2.6±0.7 fold and 1.7±0.3 fold increase respectively) stages of development, relative to levels in control lungs.

Conclusions: Lung repair processes commence with normalisation of early response and inflammatory gene expression and the activation of repair genes, within 24h of brief, injurious MV, in the very immature lung.

THE EFFECT OF POSITIVE END-EXPIRATORY PRESSURE (PEEP) AND ROTATION ON LUNG VENTILATION IN NEWBORN RABBIT PUPS VENTILATED LYING ON THEIR SIDE

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Background: It is well known that the position of the newborn causes dependent lobes to ventilate better than non-dependent lobes of the lungs. This could cause injury by over-inflating the non-dependent lobes whilst causing atelectrauma in the dependent lobes. Positive end-expiratory pressure (PEEP) increases lung gas volumes but it is unknown if PEEP reduces the difference in lung ventilation between the dependent and non-dependent lobes. It is also unknown if rotating the newborn promotes a more uniform distribution of ventilation.

Hypothesis:PEEP and rotating the newborn can reduce the difference in lung ventilation between the non-dependent and dependent lobes in the ventilated newborn rabbit pup lying on its side.

Methods: Rabbit pups (30dGA; term=32dGA) were delivered by c-section and were surgically intubated with an ET tube (18G). Pups were positioned lying on their right side and in a horizontal head out water-filled plethysmograph. Pups were ventilated with a constant PIP (30cmH_2O) whilst PEEP was changed according to the following sequence; 0-3-5-0cmH_2O. The plethysmograph was then rotated 180°. Phase contrast (PC) X-ray images were acquired throughout ventilation. Pups were then killed and plethysmograph recordings analysed to determine changes in lung gas volumes. PC X-ray images were observed for the distribution of ventilation.

Results: At 0PEEP, lungs could not develop a functional residual capacity (FRC) but lost FRC (-0.8±0.6ml/kg). Tidal volume (V_T) increased to 12.4±4.4ml/kg. At 3PEEP, FRC and V_T increased by 3.9±1.1ml/kg and 9.9±2.0ml/kg, respectively. At 5PEEP, FRC and V_T increased by 2.0±0.8ml/kg and 2.6±0.8ml/kg, respectively. At all PEEPs, non-dependent lobes were better ventilated than dependent lobes but this difference was less at 5PEEP than at lower PEEPs. After rotating the pup, non-dependent and dependent lobes were similarly aerated at FRC. V_T ventilated the dependent lobe (previously the non-dependent lobe) more than the non-dependent lobes (previously the dependent lobe).

Conclusions: Rotating the pup quickly reduced the difference between ventilation in the non-dependent and dependent lobes. Only high PEEP (i.e.5PEEP) was able to reduce this difference.

HUMAN AMNION EPITHELIAL CELLS AS A TREATMENT FOR HYPEROXIA INDUCED NEONATAL LUNG INJURY

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Background: While often necessary to support respiration, exposure of the preterm neonatal lung to supplemental oxygen may cause lung injury and contribute to bronchopulmonary dysplasia (BPD). Human amnion epithelial cells (hAECs) have been previously shown to modulate pulmonary inflammation and aid in lung tissue repair.

Aims/Hypotheses: We hypothesised that hAECs would attenuate hyperoxia-induced changes in neonatal lung structure and development.

Method: Within 24 hours of birth, neonatal C57Bl/6 mice were randomly assigned to either normoxia (FiO_2 = 21%) or hyperoxia (FiO_2 = 83%). Animals received either hAECs at a daily intraperitoneal dose of $1.5x10^6$ cells or saline, in 50mL, on postnatal days 5, 6 and 7. Lungs were collected at postnatal day 14 for morphometric assessment.

Results: Hyperoxia exposure resulted in altered lung structure, with fewer, larger alveoli; loss of septal crests; oedematous basement membrane; and patchy interstitial fibrosis. Intraperitoneal delivery of hAECs increased septal crest density and reduced interstitial oedema and alveolar enlargement but did not normalize overall lung structure.

Conclusions: Systemic administration of human amnion epithelial cells may mitigate oxygen-induced neonatal lung injury. Ongoing studies aim to assess a) the impact of concurrent intranasal administration of hAECs on lung injury, and b) the impact of systemic hAECs administration on the neonatal brain and liver.

NOTES

EPIGENETIC MECHANISMS REGULATING PTGS2 EXPRESSION IN THE AMNION DURING GESTATION

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Background: In the amnion PTGS2 expression increases with advancing gestation and labour. Epigenetic mechanisms involving histone modifications have been implicated in influencing gene expression. Acetylation and methylation of lysine residues on the N-terminal tail regions of histone H3 have been shown to activate and repress genes. We were therefore interested in the role these modifications may play in regulation of PTGS2 gene expression in the amnion during pregnancy.

Aim: To examine the levels of activating and repressive histone modifications located at the PTGS2 gene at early and late pregnancy and their relationship to PTGS2 expression.

Methods: Amnion tissues were collected at 11-18 weeks of pregnancy (n=3) and after elective Caesarean section (n=3) at term. Chromatin immunoprecipitation (ChIP) was carried out using cross-linked chromatin in amnion tissue, with antibodies recognizing the activating epigenetic marks acetylated histones-3 (acH3) and 4 (acH4), histone H3 tri methyl K4 (H3K4me3), histone H3 tri methyl K36 (H3K36me3) and the repressive histone marks histone H3 tri methyl K9 (H3K9me3) and histone H3 tri methyl K27 (H3K27me3). The immunoprecipitated DNA was assessed by real-time PCR using 12 primer pairs spanning the PTGS2 gene.

Results: AcH3, acH4 and H3K4me3 accumulated in the first 1000bp region of the PTGS2 promoter and levels for both acH3 and H3K4me3 increased as gestation advanced. H3K27me3 spanned the promoter and transcribed region of the gene with levels decreasing around the TATA site. H3K27me3 levels were high early in gestation and decreased at term. The ratio of H3K4me3 to H3K27me3 increased at term. H3K36me3 levels peaked in the 3' region of the gene. H3K9me3 was present on the gene and levels increased at term.

Conclusions: The PTGS2 gene is marked with both active and repressive histone modifications. The changes occurring with advancing gestation are consistent with a switch from a repressive to a permissive chromatin structure. This suggests that the PTGS2 gene is activated by epigenetic mechanisms at term allowing increased expression and prostaglandin production promoting the onset of labour.

ROLE OF SYNCYTIN IN PLACENTAL FORMATION: FROM TRANSCRIPTION FACTORIES TO MATERNAL IMMUNE TOLERANCE

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Despite intensive research during the last decade, the molecular mechanisms of cytotrophoblast-syncytiotrophoblast fusion in the human placenta and its control are still poorly understood. Cell fusion processes are fundamental for the renewal of the syncytiotrophoblast which depends on continuous fusion of cytotrophoblast cells. Two endogenous retroviral envelope fusogenic proteins, syncytin-1 and -2, have been related to cell-cell fusion between cytotrophoblast cells. Syncytin-1 appears to have a key role in this process; however syncytin-1 topological expression in human placental villi is controversial, as syncytin-1 is predominantly expressed in the syncytiotrophoblast, which would imply that syncytin-1 expression occurs in the already syncytialized syncytiotrophoblast. Syncytin-1 receptor expression is restricted to the cytotrophoblast compartment of the placental villa and largely absent from the syncytiotrophoblast. Also, it has suggested that the fusion of placental trophoblast cells is not regulated by local or temporal variations of syncytin-1 receptor expression in villous cytotrophoblast cells. On the other hand, syncytin-2 expression is restricted to cytotrophoblast cells and its receptor is only expressed in the syncytiotrophoblast.

The eukaryotic nucleus consists of highly organized structures where active gene transcription units can be clustered in discrete sites called transcription factories. In the present study we present a model for the study of transcription factories during the process of cytotrophoblast cell differentiation and fusion into the syncytiotrophoblast. While syncytin-1 and -2 are differentially expressed in the two morphologically contrasting cell types, they use the same transcription factor (GCMa) and their genes are located in different chromosomes which make them an excellent model for the study of transcription factories. Also, syncytiotrophoblast transcriptional activity is very restricted in comparison to other cell types and this allows a more direct correlation between transcription factories and gene expression.

Here we also present a model for syncytin-1 cell trafficking and its sorting into the exosomal pathway that could explain the controversial distribution observed for syncytin-1 in the syncytiotrophoblast, its involvement in the regulation of syncytialization and also present a mechanism through which syncytin-1 can become exposed to the maternal immune system and exert its immunosuppressive role.

GESTATIONAL CHANGES IN THE EXPRESSION OF THE PLACENTAL RENIN-ANGIOTENSIN SYSTEM AND VEGF: ROLES IN PLACENTAL VASCULARISATION?

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Background: Although the renin-angiotensin system (RAS) is classically viewed as an endocrine system that regulates blood pressure homeostasis, a placental RAS has been described and may be involved in development of the placental vasculature. Prorenin is inactive until bound to the (pro)renin receptor (P)RR, then it can generate angiotensin I (Ang I) from angiotensinogen (AGT) or stimulate intracellular signaling independent of Ang I. Ang I is cleaved to Ang II by angiotensin-converting enzyme (ACE). Ang II acts via the type 1 or type 2 angiotensin receptors (AT_1R or AT_2R), which have opposing effects. An alternate ACE, ACE2, is able to terminate the action of Ang II by converting it to Ang 1-7, which has actions opposite to those of Ang II on the AT_1R.

Aims/Hypothesis: We aimed to examine the expression and localization of components of the RAS as well as the angiogenic factor, vascular endothelial growth factor (VEGF) in early gestation (6-16 weeks) and term placentae in order to gain insights into the potential roles of the RAS in placental growth and vascularisation.

Methods: Early gestation and term placentae were collected from women undergoing elective termination of pregnancy (6-16 weeks gestation, n=33) or elective caesarean section at term (>37 weeks, n=10), respectively. Prorenin, (P)RR, AGT, ACE1, ACE2, AT_1R, AT_2R and VEGF mRNAs were measured by qPCR. Comparisons between samples were made by determining $\Delta\Delta$CT relative to β-actin. Immunohistochemistry to localize RAS proteins (Prorenin, (P)RR, ACE, ACE2, AT_1R, AT_2R) and VEGF was performed using commercial antibodies.

Results: Renin mRNA levels were highest at 6-9 weeks compared to second trimester and term samples ($P<0.02$) and lowest at term ($P\leq0.03$). (P)RR mRNA, was also lowest in placentae collected at term compared to early gestation placentae (P=0.000). In contrast, levels of VEGF and AT_1R mRNA were not altered throughout gestation. Renin, (P)RR and AT_1R mRNA levels were highly correlated with VEGF mRNA abundance (P=0.000, r=0.606; P=0.000, r=0.703, and P=0.001, r=0.478, respectively). AGT mRNA was low in all samples. Analyses of ACE, ACE2 and AT_2R mRNA levels are currently underway.
Prorenin and AT_1R protein were localized to the cytotrophoblasts (CTBs), syncytiotrophoblast (STBs) and extravillous trophoblast cells (EVTs) whereas (P)RR protein was localized to the STB and EVTs, but not CTBs. ACE2 was also localized to STB and CTB whereas ACE was only localized to the fetal endothelium. There was very low immunostaining of AGT protein in trophoblast cells but there was dense staining in term placentae.

Conclusions: This is the first study to identify the placental expression of (P)RR and other RAS components throughout gestation. Here we have demonstrated that the expression of both renin and (P)RR mRNA are highest in very early gestation placentae when vasculogenesis is maximal, and they and AT_1R are highly correlated with VEGF. This is the first evidence that the placental RAS may play a role in regulating placental angiogenesis.

EICOSAPENTANOIC ACID IS MORE EFFECTIVE THAN DOCOSAHEXANOIC ACID IN INHIBITING LPS-INDUCED LIPID HYDROPEROXIDE PRODUCTION AND OXIDATIVE DNA DAMAGE IN THE PLACENTA

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Background: Clinical, experimental, and epidemiologic evidence supports the anti-inflammatory actions of omega-3 (n-3) fatty acids, such as docosohexanoic acid (DHA) and eicosapentaenoic acid (EPA). However, the relative action of each and concerns about their susceptibility to lipid peroxidation and secondary oxidative damage to proteins and DNA remain unanswered.

Aims/Hypothesis: As the placenta is a major source of oxidative stress and excessive oxidative stress is causally involved in patho-phsyiologic processes affecting the fetus and neonate, we investigated the effects of DHA and EPA on oxidative stress and pro-inflammatory cytokine production induced by lipo-polysaccharide (LPS) in a placental explant model.

Method: Placental explants (n=8) obtained from non-labour elective caesarean sections, were pre-treated with either DHA or EPA (1mM, 10mM and 100mM) prior to LPS (1ng) exposure or co-exposed to LPS. Malondialdehyde (MDA, lipid peroxidation) and 8-hydroxy-2-deoxy Guanosine (8-OH-dG, oxidative DNA damage) were measured by ELISA.

Results: Duration of incubation did not influence MDA and 8-OH-dG production. LPS increased both MDA ($p<0.001$) and 8-OH-dG ($p=0.01$) production. **Low concentration (1mM-10mM):** DHA treatment alone increased both MDA and 8-OH-dG production ($p=0.01$) with the degree of lipid peroxidation, but not oxidative DNA damage, lower when compared to LPS exposure ($p<0.05$). EPA treatment increased 8-OH-dG production ($p<0.05$), with both MDA and 8-OH-dG levels lower than LPS exposure ($p<0.01$). Co-treatment with EPA+LPS was associated with decreased MDA and 8-OH-dG production ($p=0.01$), an effect not observed with DHA+LPS. Pre-treatment with DHA or EPA inhibited of LPS induced MDA and 8-OH-dG production ($p=0.01$) to levels similar to controls with the reduction in 8-OH-dG greater for EPA ($p<0.05$). **High concentration (100mM):** For all experimental conditions oxidative stress was higher than LPS treatment alone. The DHA and EPA mediated effects onoxidative stress were not paralleled by alterations in TNFα and IFNγ production.

Conclusions: Pre-treatment with low dose n-3 fatty acids limits LPS induced oxidative stress, but high dose exposure accelerates production of reactive oxygen species. EPA exerts a greater protective effect, a process not mediated by alterations in pro-inflammatory cytokine production. n-3 fatty acids exert anti-inflammatory actions via several pathways including up-regulation of antioxidant enzyme gene expression and alteration in the production and balance of n-6-derived eicosanoids, pathways currently being investigated in this placental explant model. With increasing evidence supporting beneficial effects of the n-3 LCPUFA's on neonatal neuro-developmental outcome and the incidence of inflammatory morbidities such as chronic lung disease, characterisation of the mechanisms through which these effects are mediated will accelerate their implementation into clinical practice.

THE ROLE OF miRNAS IN HUMAN MYOMETRIAL ACTIVATION FOR LABOUR

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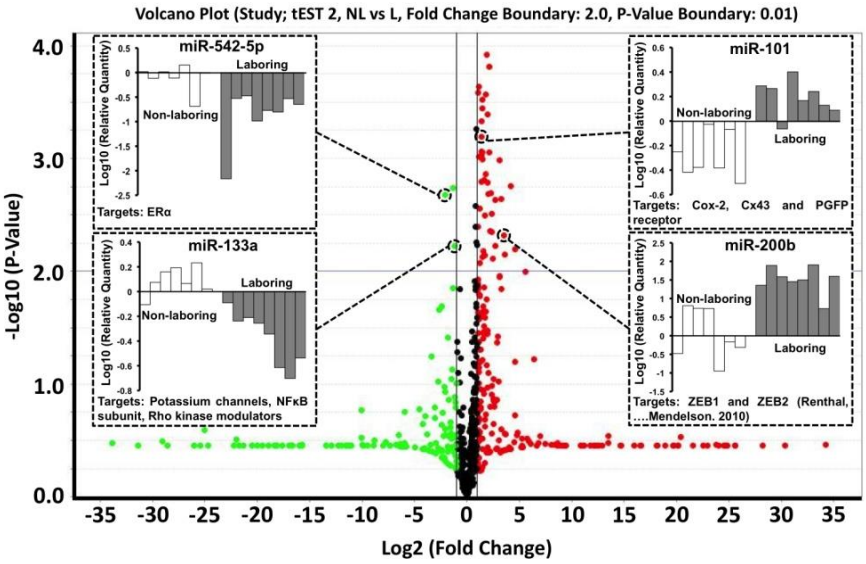
Background: Recently, Carole Mendelson and colleagues have explored the role of miRNA in the regulation of progesterone responsive genes in mouse myometrial tissue(Nora E. Renthala et al., PNAS). They demonstrated that as labour approached, the level of a group of miRNAs called the miR-200 family greatly increased blocking the production of two proteins called ZEB1 and ZEB2 that inhibit contraction. However the changes in miRNAs and the role of estrogens in regulating miRNA are unexplored.

Aims/Hypothesis: Using miRNA arrays and human myometrial tissue obtained at caesarean section prior to or after the onset of labour to determine which species of miRNA change with labour. To use the identified miRNA species to identify novel target genes that may be relevant to parturition.

Methods: We have performed miRNA expression profiling of 754 miRNA species using the Taqman® miR Array with microfluidic cards (Taqman® Low Density Array [TLDA] Plates A & B, Sanger version 10, Applied Biosystems). Total RNA extracts of myometrial tissues from 8 subjects at term prior to the onset of labour and 8 subjects in whom labour had begun were reverse-transcribed using Taqman® MegaPlex Pools A or B primers and real-time PCR performed.



Differential miRNA expression in non-labouring and labouring human myometrium.

Results: Each spot represents a miRNA species in the array experiment: green are miRNA species that decreased, and red are those that rose, with labour in the myometrial tissue. Insets show 4 examples of specific miRNA expression in 16 individual subjects (n = 8 NL, n=8 L).

Conclusions: Human labour is associated with a dramatic increase in miRNA expression suggesting a repression of expression for a wide range of genes.

UNDERSTANDING THE ROLE OF ESTROGENS IN LABOUR ONSET: MECHANISMS OF NON-GENOMIC ESTROGEN SIGNALLING IN THE PREGNANT MYOMETRIUM

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Background: Estrogen activation is a crucial event that precedes the onset of labour in mammals, however the genomic targets and signalling pathways controlled by estrogens in the pregnant uterus are largely unknown. In addition to the classic genomic mode of steroid hormone action, estrogens are known to rapidly activate multiple cytoplasmic signalling cascades. This non-genomic activity of estrogens may be mediated by various estrogen receptors (ERs) including the classical receptors ER-α and -β, and the seven-transmembrane G-Protein Coupled Receptor-30 (GPR30).

Aims/Hypothesis: The purpose of this study was to determine: 1) whether estradiol (E2) stimulates activation of the extracellular-regulated kinase (ERK) MAPK signalling cascade in the pregnant human myometrium, in order to affect the downstream contractile capacity of the tissue, and 2) which ERs are involved in mediating rapid non-genomic estrogen signalling in the myometrium.

Methods: Myometrial tissue samples were obtained from women undergoing caesarean section at term before labour onset. A portion of each tissue sample was snap-frozen, with the remainder dissected into explants and cultured overnight in serum-free and phenol red-free media. Fresh media was then added and the explants incubated with inhibitors (10 μM U0126 or 1 μM ICI 182,780) where required for 30 minutes. E2 (1-100 nM) or vehicle were then added and the explants incubated for 10 minutes (for determination of ERK1/2 phosphorylation) or six hours (for changes in gene expression). Immunoblotting was performed to determine the levels of phosphorylated- and total-ERK1/2, and real-time RT-PCR was used to measure oxytocin receptor (OTr) mRNA levels. ERα, ERβ and GPR30 mRNA levels were measured in snap-frozen tissues.

Results: Estradiol stimulated rapid non-genomic signalling in myometrial explants, as evidenced by increased phosphorylation of ERK1/2 within 10 minutes of steroid addition. E2-stimulated ERK phosphorylation was inhibited by pre-treatment with the classical ER antagonist ICI 182,780, indicating that these rapid effects of E2 were likely mediated by ER-α or -β, and not by GPR30. Using quantitative RT-PCR we found that mRNA encoding ERα was the predominant ER transcript in pregnant human myometrium. The median GPR30 mRNA level was 7.5-fold lower than the median ERα level, while ERβ mRNA was virtually undetectable. Furthermore, E2 induced a significant increase in OTr mRNA expression in myometrial explants after six hours ($P<0.05$), which was abrogated by pre-treatment with the MEK (MAPK kinase) inhibitor U0126, indicating that E2 regulates OTr gene expression indirectly *via* activation of the ERK pathway

Conclusions: We conclude that ERα, upon ligand activation, promotes contractility in myometrial cells *via* rapid extranuclear activation of the ERK MAPK signalling cascade, leading to up-regulation of genes encoding contraction-associated proteins. These findings reveal a novel pathway in which to explore estrogen signalling in the pregnant myometrium.

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