

THE SEVENTEENTH NATIONAL WORKSHOP ON FETAL AND NEONATAL PHYSIOLOGY

Clinical School
University of Tasmania
Hobart, Tasmania
March 8-9, 2003



Organising Committee:
Dr Megan Cock and Prof Richard Harding
Department of Physiology
Monash University

Program

Saturday 8th	Sunday 9th
10.30-11.30 Registration	
11.00-11.30 Morning tea	10.30-11.00 Morning tea
SESSION 1	SESSION 4
11.30-11.45 (A1)	11.00-11.15 (A15)
11.45-12.00 (A2)	11.15-11.30 (A16)
12.00-12.15 (A3)	11.30-11.45 (A17)
12.15-12.30 (A4)	11.45-12.00 (A18)
12.30-1.00 general discussion	12.00-12.30 general discussion
1.00-2.00 Lunch	12.30-1.30 Lunch
SESSION 2	SESSION 5
2.00-2.15 (A5)	1.30-1.45 (A19)
2.15-2.30 (A6)	1.45-2.00 (A20)
2.30-2.45 (A7)	2.00-2.15 (A21)
2.45-3.00 (A8)	2.15-2.30 (A22)
3.00-3.15 (A9)	
3.15-3.45 general discussion	2.30-3.00 general discussion
3.45-4.15 Afternoon tea	3.00-3.30 Afternoon tea
SESSION 3	3.30 Presentation of Student Prizes
4.15-4.30 (A10)	
4.30-4.45 (A11)	
4.45-5.00 (A12)	
5.00-5.15 (A13)	
5.15-5.30 (A14)	
5.30-6.00 general discussion	
6.00 Drinks & Nibbles at Clinical School	6.00 PSANZ Welcome Reception
8.00 Dinner at "Mures"	

Saturday 8th March

10.30-11.30 Registration / Morning tea

Session 1: Chair: Dr Kelly Crossley			
11.30	A1	Yan , Melendez, Smythe, Walker	Peripheral infusion of quinolinic acid increases albumin, 4-HNE and GFAP immunoreactivity in late gestation fetal sheep brain.
11.45	A2	Rose , Gibbs, Rodricks, Wallace, Jenkin, Miller	Effects of developmental insults on the structure and function of the developing chick brain: role of activin?
12.00	A3	Scott , Wilkinson, Billiards, Walker, Hirst	Deoxycorticosterone in the ovine fetus and neonate, and its role in the stress response.
12.15	A4	Leader , Dolby	Fetal habituation to vibroacoustic stimulation as a predictor of development and IQ at 7-8 years of age
12.30-1.00 General discussion			

1.00-2.00 Lunch

Session 2 Chair: Prof Bill Parer			
2.00	A5	Thavaneswaran , Horton, Kind, Grant, Robinson, Owens	Sexual dimorphism in the effect of fetal growth restriction on the development of the insulin resistance syndrome in the aged guinea pig
2.15	A6	Johnston , Sloboda, Moss, Waddell, Newnham	The effect of maternal betamethasone administration on renal mineralocorticoid receptor and sodium-potassium-adenosine triphosphatase levels in adult sheep offspring
2.30	A7	Horton , Thavanswaran, Kind, Martella, Robinson, Owens	Interaction between the effects of fetal growth restriction and aging on the insulin sensitivity of glucose metabolism in the guinea pig
2.45	A8	Dodic , Jefferies, Cock, Wintour, May	Cardiovascular responses to intracerebro-ventricular Angiotensin II infusion in adult sheep prenatally exposed to dexamethasone
3.00	A9	De Blasio , Hoebee, Walker, Gatford, McMillen, Robinson, Owens	Placental restriction of fetal growth decreases size at birth and increases growth rate and suckling in the neonatal lamb
3.15-3.45 General discussion			

3.45-4.15 Afternoon tea

Session 3 Chair: Assoc. Prof Stuart Hooper			
4.15	A10	Thiel , Hooper	Effect of increased fetal lung expansion of Aquaporin-5 expression in sheep
4.30	A11	Hanna , Sozo, Cock, Wallace, Suzuki, Hooper, Harding	The effects of preterm birth on postnatal respiratory function
4.45	A12	Sozo , Hanna, Wallace, Cock, Harding, Hooper	Preterm birth alters postnatal lung growth
5.00	A13	Suzuki , Hooper, Harding	Fetal lung hypoplasia effects on pulmonary circulation and respiration in neonatal sheep
5.15	A14	Polglase , Hooper	The role of intraluminal pressures in regulating PVR before and after birth.
5.30-6.00 General discussion			

6.00 Pre dinner Drinks- Clinical School

8.00 Workshop dinner - Mures Restaurant

Sunday 9th March

10.30-11.00 Morning tea

Session 4 Chair: Dr Megan Wallace			
11.00	A15	Mason, Sloboda, Moss, Newnham.	The effects of maternal betamethasone administration on GLUT-1 expression in the sheep placenta
11.15	A16	Morrison, Chien, Riggs, Gruber, McMillen, Rurak	Fetal hypothalamic-pituitary-adrenal axis response to maternal Prozac treatment
11.30	A17	Mitchell, Harding, Cock, Bertram, Black.	Nephron endowment in the lamb following growth retardation in utero
11.45	A18	Zimanyi, Denton, Forbes, Widdop, Bertram, Black	Administration of advanced glycation endproducts to rats with a congenital nephron deficit
12.00-12.30 General discussion			

12.30-1.30 Lunch

Session 5 Chair: Prof Richard Harding			
1.30	A19	O'Connell, Lumbers, Boyce, Gibson	The late gestation consequences in cardiovascular and renal function of a midgestational asphyxial episode in fetal sheep
1.45	A20	Parer, Blanco, Cabello, Giussani, Hanson, Herrera, Pulgar, Reyes, Riquelme, Sanhueza, Llanos	Physiologic strategies for tolerance to hypoxia in fetal sheep and llamas
2.00	A21	Supramaniam, Jenkin, Wallace, Miller	Activin A in the intra-uterine growth restricted ovine fetus
2.15	A22	Moss, Nitsos, Newnham, Ikegami, Jobe	Effects of subchorionic endotoxin infusion in sheep
2.30-3.00 General discussion			

3.00-3.30 Afternoon tea

3.30 Presentation of:

1. Student Prize for Best Oral Presentation
2. Student Prize for Best Discussant

Abstracts

A1

PERIPHERAL INFUSION OF QUINOLINIC ACID INCREASES ALBUMIN, 4-HNE AND GFAP IMMUNOREACTIVITY IN LATE GESTATION FETAL SHEEP BRAIN.

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Quinolinic acid (QUIN) is an endogenous kynurenine metabolite of L-tryptophan which is increased in blood and brain in inflammatory states. QUIN is a weak agonist at N-methyl-D-aspartate (NMDA) receptors, and in high concentrations or after prolonged exposure induces cell death, lipid peroxidation and oxidative stress (1,2). In fetal sheep QUIN is increased in plasma 2-fold and in the brain 3 to 5-fold (depending on region) in response to endotoxin treatment. In adult rats intracerebroventricular injection of QUIN causes increased permeability of the blood-brain barrier (BBB) (3). The aim of this study was to determine if a prolonged increase of plasma QUIN in the fetus had effects on the BBB, on lipid peroxidation of cells and fibre tracts (measured as 4-hydroxynonenal, 4-HNE), and on astrocytes (measure as glial fibrillary acidic protein, GFAP).

Carotid artery and jugular vein catheters were surgically implanted into 6 fetal sheep at 124-6 days gestation, and at 134-6 days fetuses received a continuous i.v. infusion of either QUIN (0.1 mmol/h; n=3) or an equivalent volume of saline (6.7 ml/h; n=3) for 12 h. Fetal and maternal arterial blood samples were taken before, during and after the infusion for determination of blood gases and pH, and recovery of plasma to measure QUIN by GC/MS. The ewe was killed after 24 h by pentobarbitone sodium overdose and the fetal brain was perfusion fixed in situ with 4% paraformaldehyde. Endogenous albumin, 4-HNE and GFAP were visualised by immunohistochemistry on 10 μ m fixed paraffin embedded tissue sections using appropriate polyclonal or monoclonal primary and biotinylated secondary antibodies.

QUIN infusion had no effect on fetal blood gases or pH. Plasma QUIN concentrations increased from 22.3 ± 6.0 to 144.2 ± 28.0 μ M by the end of the 12h infusion (mean \pm sem, $P < 0.05$); maternal plasma QUIN concentrations (1.2 ± 0.6 μ M) did not change. GFAP immunoreactivity was significantly increased in cerebral white matter, striatum and the granule cell layer of cerebellum in the QUIN infused fetuses. Extravasation of albumin into the perivascular space was not observed in any brain region, and 4-HNE immunoreactivity was not increased. However, albumin and 4-HNE were present in the Purkinje cell bodies of cerebellum, whereas both were absent in control fetal brains.

These results show that QUIN does not cross the placenta. As for the adult brain, it is unlikely to cross the intact BBB in the fetus. The increase of GFAP immunoreactivity (regarded as a marker of reactive astrogliosis) in many regions of the QUIN treated brain may therefore result from an action of QUIN on cerebral blood vessels, blood flow, or oxygenation. The increased 4-HNE and albumin staining in the cerebellum also suggests oxidative stress due to QUIN. The increase of intracellular albumin may be neuroprotective as shown for adult brain following ischaemia (4).

References

1. Schwarcz et al. 1983 Science 219:316-8
2. Santanar@a et al. 2001 Neuroreport 12:2693-6
3. Št'astný et al. 2000 Brain Res. Bull. 53:415-20
4. Liu et al. 2001 Eur. J. Pharmacol. 428:193-201

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A2

Effects of Developmental Insults on the Structure and Function of the Developing Chick Brain: Role of Activin? Ilona Rose^a, Marie Gibbs^b, Candice Rodricks^a, Euan Wallace^c, Graham Jenkin^a & Suzanne Miller^a. Departments of Physiology^a, Pharmacology^b and Obstetrics & Gynaecology^c, Monash University Victoria 3800

Background: During prenatal development, reductions in oxygen supply, malnutrition, or infection are significant factors that may result in intrauterine growth restriction (IUGR), perinatal morbidity, or mortality. IUGR in infants has been associated with various neurological defects such as cerebral palsy and learning disorders. The mechanisms that underlie the neurological impairment and increased instance of neonatal mortality caused by fetal compromise remain unclear. The protein hormone activin A has been found in the maternal circulation and fetal membranes in human and ovine pregnancy, and levels are elevated in the ovine fetus in response to reduced uterine blood flow and subsequent fetal hypoxia. Previous studies confirm that levels of activin A may be useful in predicting compromised pregnancies, fetal distress and, therefore potential poor neonatal outcome.

Aims: 1) To determine the effect of in ovo compromise on chick physical and neurological development. 2) To determine tissue and blood activin A concentrations during normal chick development. 3) To determine whether activin A expression is altered in response to developmental insults such as hypoxia, malnutrition or infection.

Methods: Experiment #1: Hypoxia versus Control. Eggs were incubated in normoxia (control) or in 14% oxygen (hypoxia) between days 10-14 (of 21). Experiment #2: Malnutrition. Protein malnutrition was induced by removing 5%, 7.5% or 9% of the available albumin on day 0 of incubation. Experiment #3: LPS. Lipopolysaccharide (LPS) (200, 400, 500 & 750 µg) was administered onto the chorioallantoic membrane of the egg on day 12 of incubation. To assess how the insults affected brain function, memory testing was undertaken in chicks soon after hatch, using the Discriminated Avoidance training.

Results: It was found that the hypoxic, malnourished and LPS-treated embryos, all hatched earlier (d20) than the controls (d21). There was no difference in hatch weights in hypoxic chicks ($35.93 \pm 0.55\text{g}$) compared to control ($36.84 \pm 0.37\text{g}$) chicks. The 5% and 7.5% malnourished chicks ($34.0 \pm 0.59\text{g}$ & $34.31 \pm 0.57\text{g}$ respectively) weighed significantly less than controls (9% malnourishment was too severe- 11.9% hatch rate). The LPS (750 µg) chicks, however, weighed ($40.65 \pm 0.33\text{g}$); more than the controls. Chicks were tested for memory retention (at 30 & 120 mins) after training. Hypoxic chicks displayed memory deficits at 30 and 120 minutes indicating that labile memory was impaired. There were no memory deficits seen in either the 5% and 7.5% malnourished chicks, or the LPS (up to 750 µg) chicks. Chicks were decapitated after testing; whereby blood, brains and hearts were collected. Activin A assays on brains taken from day 10, 12, 14, 16, 18 and 20 embryos showed that activin levels in the controls ranged from 13.1 to 5.07 ng/g brain. Activin A in hypoxic embryos ranged from 5.88 to 6.29 ng/g brain.

Discussion: The developing chick is an excellent model for research into prenatal compromise leading to preterm delivery, and investigation of the neuroprotective role of activin A. Activin A is abundant in the chick brain and levels appear to be dependent on tissue oxygenation. In terms of memory testing, only chicks incubated in hypoxia between days 10-14 displayed memory deficits, which supports previous research that indicates restriction of prenatal gas exchange impairs memory consolidation in the chick.¹ Prenatal malnutrition and infection did not affect postnatal memory function. Hypoxic, malnourished and LPS-treated embryos all hatched earlier than the controls, demonstrating that in the developing chick, prenatal compromise results in preterm delivery. This supports clinical findings where research has shown that preterm labour is correlated with intrauterine infection. We are currently undertaking histological examination of brains from each group.

¹ E.J. Camm et al. / *Developmental Brain Research* (2001) 132: 141-150

A3

DEOXYCORTICOSTERONE IN THE OVINE FETUS AND NEONATE, AND ITS ROLE IN THE STRESS RESPONSE

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Background: Neuroactive steroids are synthesized in the brain from cholesterol or from peripheral precursors. Deoxycorticosterone is the obligate precursor for the potent GABA_A receptor agonist tetrahydroDOC (THDOC), and may determine THDOC concentrations in the brain. DOC production is increased in response to stress, and this may raise brain THDOC leading to sedative and sleep-inducing effects. We determined DOC concentrations in the fetal and neonatal sheep brain, and then examined the effects of endotoxin challenge on DOC concentrations in the newborn brain.

Methods: Fetal and newborn lambs were killed humanely by pentobarbitone overdose at 139-140, 144 days gestation (GA) and 3 and 20 post natal days (PND) of age (n=3-4). Brains were removed immediately, divided into gross anatomical regions, and stored at -70°C. In addition, a group of newborn lambs at 10-20PND were given a 5ml bolus injection of either LPS-endotoxin (E-Coli, 0.7µg/kg; n=5) or saline only (n=4). LPS or saline were each given 3 times with at least 2 days between each administration. Arterial blood samples were taken at regular intervals before, and after administration of LPS or vehicle for measurement of blood gases and pH, and for neurosteroid concentrations in plasma. Brain tissue was removed 3 h after the third LPS or saline administration and stored -70°C for subsequent analysis of steroids by RIA.

Results: DOC content in the brain ranged from 1-5 pmol/g wet weight. There were significantly higher levels of DOC content in the fetal Primary Motor Cortex at 139-140 GA compared to the other fetal and neonatal ages, but there were no other age-related changes of DOC in the other brain regions examined. Following birth, DOC content decreased slightly in all brain regions, but this reached statistical significance only for the medulla at 3 PND. By 20 PND the brain DOC content had returned to levels similar to those at 144GA. Following LPS treatment DOC concentrations increased significantly in all brain regions examined (p<0.05) except the PMC. The PMC did however show a trend toward higher DOC concentrations following LPS administration.

Conclusions: The DOC content in the fetal brain was lower than for other potent neuroactive steroids such as allopregnanolone and pregnenolone sulphate. Whereas brain allopregnenolone content decreases markedly at parturition (1), DOC content decreased only slightly or not at all in most brain regions examined. DOC was also elevated in response to endotoxin exposure. DOC may become the important precursor for inhibitory steroid synthesis in the brain after birth, taking over from placental progesterone and pregnenolone as the major precursors in the fetus.

1. Nguyen P et al. 2003. Changes in 5 α -pregnane steroids and neurosteroidogenic enzyme expression in the perinatal sheep. *Pediatr. Res.* (*in press*).

A4

Fetal Habituation to vibroacoustic stimulation as a predictor of Development and IQ at 7-8 years of age

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We have previously demonstrated that fetal heart rate habituation < 2 weeks before birth predicts infant development at 18mths and 36 months of age using the Bayley Scales of Infant Developmental.

The fetus was stimulated for 1 second every 60 seconds using a Corometrics vibroacoustic stimulator. An increase in heart rate of 10 or more beats per minute was considered a response. Failure to respond to five successive stimuli was regarded as habituation.

Thirty-two children (18 girls and 14 boys) were assessed at 7-8 (± 3 mths) years of age by an experienced clinical psychologist using the Stanford-Binet Scale of Intelligence. The psychologist was blinded to the clinical and habituation details of all children. Twenty-two children were from low risk pregnancies and 10 were from high risk pregnancies.

We found that children who habituated prenatally achieved higher scores in most aspects of their tests.

Test	All Children		Males		Females	
	Habit	No Habit	Habit	No Habit	Habit	No habit
Verbal reasoning	117	106*	115	108.2	118.8	104.5*
Abstr/visual reasoning	113	101.5	108.4	112.6	117.1	93.5
Quantit reasoning	112.6	108	114.4	112.4	111	105.1
Short term memory	117.9	103.3*	117.5	103.6	118.3	103.1*
Composite Score	118	108	116.4	111.2	119.5	105.7*
Behavioural Score Total	35.6	27.3*	30.4	30.8	25	39.1**

* P<0.05

**P<0.01

These preliminary results suggest that prenatal habituation testing predicts I-Q at 7-8 years of age.

SEXUAL DIMORPHISM IN THE EFFECT OF FETAL GROWTH RESTRICTION ON THE DEVELOPMENT OF THE INSULIN RESISTANCE SYNDROME IN THE AGED GUINEA PIG

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Epidemiological studies have demonstrated that impaired growth in early life, as indicated by being light or thin at birth, is associated with an increased risk of developing the Insulin Resistance Syndrome (IRS) in adult life. The aim of the current study was to determine whether the guinea pig that undergoes spontaneous fetal growth restriction subsequently develops the IRS as an adult. Pregnant guinea pigs were allowed to deliver and size at birth was measured in offspring, which then had vascular catheters inserted into the carotid artery and jugular vein under general anaesthesia and strict asepsis at 400 days of age. All studies were approved by the Animal Ethics Committee of the University of Adelaide.

Fasting plasma glucose concentrations increased with decreasing weight at birth (B_W) in females ($r=-0.70$, $p<0.001$), but not males. Glucose tolerance, calculated as the area under the glucose concentration curve during an intravenous glucose tolerance test (IVGTT) (0.5g/kg dextrose), increased with decreasing B_W in females ($r=-0.45$, $p<0.05$), but not males. Whole body insulin sensitivity of glucose metabolism, as indicated by the steady state glucose infusion rate achieved during the hyperinsulinaemic euglycaemic clamp (HEC) (120 minutes, 7.5mU insulin/min/kg), decreased with decreasing B_W in females ($r=-0.61$, $p<0.05$), but not males. Post-hepatic insulin clearance rate during the HEC decreased with decreasing nose-rump length (B_{NRL}) ($r=0.70$, $p<0.01$) and head length at birth (B_{HL}) ($r=0.68$, $p<0.02$) in females, but not males. Fasting plasma insulin levels increased with decreasing B_{NRL} ($r=-0.46$, $p<0.05$), B_{HL} ($r=-0.51$, $p<0.03$) and head width at birth (B_{HW}) ($r=-0.55$, $p<0.02$) in females, but not males. Systolic blood pressure increased with decreasing abdominal circumference at birth (B_{AC}) ($r=-0.67$, $p<0.05$) in females, but not males. Heart rate increased with decreasing B_{AC} ($r=-0.56$, $p<0.05$) and B_{HW} ($r=-0.57$, $p<0.05$) in females, but not males. Pulse pressure increased with decreasing B_{AC} ($r=-0.59$, $p<0.05$) and B_{HW} ($r=-0.61$, $p<0.05$) in females, but not males.

In conclusion, the effect of fetal growth restriction on the development of the IRS in the aged guinea pig exhibits a sexually dimorphic pattern, producing female offspring that are insulin resistant, hyperinsulinaemic and diabetic, and hypertensive and male offspring with unaltered insulin sensitivity, glucose tolerance and blood pressure.

THE EFFECT OF MATERNAL BETAMETHASONE ADMINISTRATION ON RENAL MINERALOCORTICOID RECEPTOR AND SODIUM-POTASSIUM-ADENOSINE TRIPHOSPHATASE LEVELS IN ADULT SHEEP OFFSPRING

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Introduction: The aim of this study was to investigate the effects of single or repeated doses of maternally administered betamethasone on mineralocorticoid receptor (MR) and sodium-potassium-adenosine triphosphatase (Na⁺/K⁺-ATPase) protein levels in the adult ovine kidney. The action of aldosterone on MR facilitates sodium reabsorption in renal tubular cells through activation of the epithelial sodium channel and basolateral membrane pump, Na⁺/K⁺-ATPase. Both aldosterone and glucocorticoids activate transcription of the human Na⁺/K⁺-ATPase- α 1 subunit gene.

Methods: Tissue for this study was collected from a larger cohort of animals (Moss *et al.*, 2001; Sloboda *et al.*, 2002). Pregnant ewes were injected intramuscularly with 150mg of medroxyprogesterone acetate at 100 days of pregnancy (d). Animals were randomly assigned to receive repeated injections of saline (MS) or betamethasone (M4) at 104, 111, 118 and 125d, or a single dose at 104d, followed by saline at 111, 118 and 125d (M1). Lambs were born naturally and all animals were killed at 3.5 years of age and kidneys collected for subsequent analysis (MS: n = 5 [3 male, 2 female]; M1: n = 4 [2 male, 2 female]; M4: n = 5 [2 male, 3 female]). Maternal betamethasone caused dose-dependent reductions in birthweight (Moss *et al.*, 2001). Western blotting was used to determine MR and Na⁺/K⁺-ATPase- α 1 protein levels in the kidneys of adult offspring.

Results: Body weight and kidney weight of offspring at 3.5 years of age were not different between groups. No significant changes in renal protein levels of Na⁺/K⁺-ATPase- α 1 were detected in M1 or M4 animals. In the MS group, males demonstrated significantly higher MR protein levels compared with females (p<0.001). Renal MR levels in M1 males were lower than in MS and M4 males (p<0.05). There was no effect of prenatal betamethasone treatment on renal MR levels in females.

Conclusions: The results of this study suggest that renal MR levels are differentially regulated in male and female adult sheep. Prenatal exposure to a single dose of maternal betamethasone in males may permanently program a reduction in renal MR levels that could lead to alterations in tissue sensitivity to glucocorticoids and mineralocorticoids and/or renal electrolyte balance. Previous studies have found sex-specific differences in brain MR levels following prenatal glucocorticoid treatment (Dean *et al.*, 1999; McCabe *et al.*, 2001). Na⁺/K⁺-ATPase- α 1 levels were similar between groups, consistent with previously published results that these animals at 3, 6, and 12 months of age demonstrated no change in arterial sodium concentrations and basal mean arterial pressure following maternal betamethasone treatment (Moss *et al.*, 2001).

References:

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- 2) McCabe, L., Marash, D., Li, A. and Matthews, S. G. (2001). *J Neuroendocrinol* **13**, 425-31.
- 3) Moss, T. J. M., Sloboda, D.M., Gurrin, L.C., Harding, R., Challis, J.R.G., and Newnham, J.P. (2001). *Am J Physiol (Regulatory Integrative Comp Physiol)* **281**, R960-R970.
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A7

Interaction between the effects of fetal growth restriction and aging on the insulin sensitivity of glucose metabolism in the guinea pig

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Studies have shown a decline in whole body insulin sensitivity of glucose metabolism with aging, using both the minimal model approach and the hyperinsulinaemic euglycaemic clamp (HEC). In addition, fetal growth restriction as indicated by being light, short or thin at birth, is also a risk factor for insulin resistance later in life. However the interaction between fetal growth restriction and postnatal aging on insulin resistance has not been fully investigated. Some studies have suggested that the link between small size at birth and postnatal insulin resistance is due to genetic polymorphisms that impair insulin action and thus growth *in utero* as insulin is a potent stimulator of fetal growth. This hypothesis has been described as the “Fetal Insulin” or “Thrifty Genotype” hypotheses. These polymorphisms may also lead to postnatal insulin resistance in adulthood. Therefore the aims of this study were to examine the associations of small size at birth and the how aging alters these associations. Guinea pigs of known size at birth were allocated to age groups of 30 days (weanling), 100 days (young adult) and 400 days (old adult). Insulin sensitivity was assessed using HEC (insulin infusion 7.5mU/min/kg). The steady state glucose infusion rate (adj ssGIR), adjusted for the plateau plasma insulin concentrations achieved during the HEC, was higher in the weanlings ($0.13 \pm 0.01 \text{ mg}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}/\text{mU}\cdot\text{ml}^{-1}$), compared to the young ($0.039 \pm 0.003 \text{ mg}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}/\text{mU}\cdot\text{ml}^{-1}$) and old ($0.026 \pm 0.03 \text{ mg}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}/\text{mU}\cdot\text{ml}^{-1}$) ($p < 0.0005$ for both). However there was no further impairment in insulin sensitivity from young to old adulthood. In weanlings the adjusted ssGIR correlated negatively with birth weight ($r = -0.69$, $p < 0.002$), while in young adults correlated positively with birth weight ($r = 0.56$, $p < 0.0005$). There was no association between size at birth and insulin sensitivity in the old adult cohort suggesting other factors like increasing adiposity, reduced activity or other lifestyle factors play a greater role in this age group. This switch from a highly sensitivity state to insulin resistance does not support the “Fetal Insulin Hypothesis” as these low birth weight animals are not insulin resistance through out their entire postnatal life. The mechanisms by which this switch from insulin sensitive to insulin resistance occurs is unknown, however the increased insulin sensitivity, accelerated growth postnatally and hyperphagia in early life in these low birth weight animals may all contribute to the change that occurs before puberty in this species.

Cardiovascular Responses to Intracerebroventricular Angiotensin II Infusion in Adult Sheep Prenatally Exposed to Dexamethasone

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Abstract

There is increasing evidence to suggest that fetuses exposed to a suboptimal intrauterine environment may develop hypertension in adulthood (1). This type of hypertension may be caused by excess exposure to glucocorticoids. We have developed an animal model, sheep, in which brief exposure to glucocorticoids (dexamethasone or cortisol) administered to the mother between 26-28 days of gestation results in high blood pressure in the adult offspring (2,3). We have shown previously that in these adult animals elevated arterial pressure amplifies with age, and is associated with increased cardiac output, left ventricular hypertrophy and reduced cardiac functional reserve (4). In addition, when these animals were killed we found increased expression of AT₁ mRNA in the medulla oblongata (5).

We aim to determine the possible role of the brain renin-angiotensin system (RAS) in adult hypertension programmed by brief prenatal dexamethasone treatment. We used a group of sheep that were prenatally exposed to either saline (control) or dexamethasone (0.48mg/h) at 26-28 days of gestation. These sheep are now 3-4 years of age and have shown to have high blood pressure (5). The sheep were instrumented with lateral ventricle guide tubes, and allowed two weeks recovery. A one hour control period was followed by a one hour intracerebroventricular infusion of Angiotensin II (1, 3.8 or 10µg/h) and a one hour post infusion period. Various cardiovascular parameters (blood pressure, cardiac output, stroke volume and total peripheral resistance) and water intake were measured, during the 3h study period. Our preliminary results suggest that the dexamethasone treated animals have an increased blood pressure response to Angiotensin II (10µg/h) (Δ MAP=19mmHg, n=3), compared to that of the control group (Δ MAP=12mmHg, n=3). Further studies are needed to show conclusively if the brain RAS plays a sufficient role in this type of hypertension.

References

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PLACENTAL RESTRICTION OF FETAL GROWTH DECREASES SIZE AT BIRTH AND INCREASES GROWTH RATE AND SUCKLING IN THE NEONATAL LAMB

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Intrauterine Growth Restriction (IUGR) in infants results in a reduction of weight, length, and/or increased thinness at birth for a given gestational age. Infants born small, undergo “catch-up” growth¹ which can continue until the child has “caught up” to their normal growth curve. Some infants (~15%) do not catch-up however and may have a reduced final adult stature, while those who do, may have increased adiposity^{2,3}. The mechanism causing catch-up growth and associated increased adiposity is not known, but we have hypothesised that it may be due in part to hyperphagia in the lamb following placental restriction. Following birth, lambs of low birth weight due to multiple pregnancies with ad libitum access to milk replacer have increased feed intake for their body weight than high birth weight lambs. These hyperphagic low birth weight lambs were relatively fatter at the same live weight⁴. Previously we have shown that placental restriction (PR) in the sheep limits delivery of oxygen and nutrients to the fetus resulting in disproportionate growth restriction, similar to that of much human IUGR. We hypothesised that placental and hence fetal growth restriction would reduce size at birth, increase postnatal growth rate, and increase appetite and adiposity in the young lamb.

In a subset of animals, feed (milk) intake of lambs was measured (15 days of age), and blood samples taken every 15 minutes to assess endocrine and metabolic responses to feeding. Lambs were fasted for 1 hour, weighed, and then returned to the ewe and the number and length of successful suckling events was recorded over the next 90 minutes. Suckling was defined as when the lamb is in the correct position for at least 5 seconds, and head movements were appropriate for suckling.

PR did not alter gestational age, but reduced survival rate and size at birth for: weight (-26%), crown-rump length (CRL) (-11%), body mass index (-9.5%), tibia and metatarsal lengths (-7%), hind limb (-17%) and abdominal circumference (-12%) (all $p < 0.05$). PR did not alter absolute growth rates (AGR), but increased neonatal fractional growth rates (FGR) for weight (+24%), tibia (+15%) and metatarsal (+18%) lengths, hind limb (+23%), and abdominal circumference (+19%) from birth to 45 days. PR ($n=29$) lambs “caught-up” to controls ($n=31$) by 30 days of age in terms of weight, CRL, shoulder height, tibia and metatarsal length, skull length, abdominal and hind limb circumference, but not for skull width. PR increased adiposity in terms of perirenal and omental fat weight relative to body weight ($p < 0.05$), with a tendency for increased retroperitoneal fat weight ($p=0.07$). When analysed according to gender, total omental and visceral fat mass was significantly increased in the males compared to the females ($p < 0.05$). PR males had increased total visceral fat mass, and total perirenal, omental and visceral fat mass when corrected for live weight ($p < 0.05$).

PR increased the total number of suckling events (control: 6.3 ± 2.2 events, PR: 7.7 ± 1.5 events, $p=0.045$), and tended to increase total suckling time during the same period (control: 154.0 ± 8.0 secs, PR: 190.0 ± 11.0 secs) during the first 30 minutes after being returned to the ewe. Total suckling time and mean suckling time in the first 30 minutes, relative to body weight, increased or tended to increase with decreasing weight, crown-rump length and tibia length at birth ($r=-0.4$ to 0.52 , $p=0.03$ to 0.09). Fractional growth rate for weight and for tibia length also tended to increase with increasing total suckling time and mean suckling time relative to body weight ($r=0.40$, $p < 0.08$).

We have shown that placental restriction of fetal growth decreases size at birth, and increases suckling by the young lamb. This predicts and may contribute to the catch-up growth and eventually increased adiposity that are evident after placental restriction.

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Effect of increased fetal lung expansion on Aquaporin-5 expression in sheep

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Background: The alveolar epithelium is composed of type-I and type-II alveolar epithelial cells (AECs). Type-I AECs provide a large surface area for gas exchange whereas the Type II AECs produce and secrete surfactant. Despite the importance of both cell types for effective gas exchange after birth, little is known of the factors controlling their phenotype before and after birth. We have shown that, in the fetus, the AEC phenotype is strongly influenced by the degree of stretch imposed by altering the level of lung expansion. Prolonged increases in fetal lung expansion induce most (>90%) AECs to differentiate into type-I AECs and, as a result, the proportions of type-II AECs are severely reduced (to <2%). Expression of the surfactant proteins (SP-A, SP-B and SP-C) by type II AECs follows the same pattern, but currently no cell marker has been identified for type-I AECs in sheep. Aquaporin 5 (AQP-5) is a membrane water-transport protein that is thought to be specific to type-I AECs. Although, AQP-5 is also expressed by bronchial airway epithelia and cells of the mucosal gland, it has been suggested that AQP5 may be an effective cell marker for type-I AECs. Our aim was to determine if changes in AQP-5 expression parallel the changes in type-I AEC proportions induced by increased lung expansion in fetal sheep lung.

Methods: Fetal lung tissue was collected from fetal sheep exposed to no treatment (control), or to 2, 4 or 10 days (n=5 for each group) of tracheal obstruction (TO). TO causes liquid to accumulate within the fetal lungs, causing the lungs to expand with secreted liquid. AQP-5 expression levels in fetal lung tissue were quantified by Northern blot analysis using a specific ³²P-labelled ovine cDNA probe; minor loading differences between lanes were standardized using a 18S rRNA cDNA probe. The density of each band was measured and the results expressed as a percentage of control. Protein levels were determined by Western blot analysis using 10% SDS-PAGE gels and an AQP-5 antibody. Both cDNA and Antibody were supplied by E.M.Wintour.

Results: After 2 days of TO, AQP-5 mRNA levels in fetal lung tissue decreased from $100.0 \pm 9.3\%$ in control fetuses to $77.6 \pm 7.6\%$ ($p < 0.003$). Compared with control values, AQP-5 levels remained reduced at 4 days ($60.0 \pm 4.0\%$) and 10 days ($59.0 \pm 6.7\%$) $p < 0.003$, of increased lung expansion. Preliminary analysis of the western blots suggests that there is no change in AQP5 protein levels following increased lung expansion.

Conclusion: These results indicate that the changes in AQP-5 expression in fetal lung tissue induced by an increase in fetal lung expansion do not parallel the changes type-I AEC proportions. It is unlikely, therefore, that changes in AQP-5 expression can be used as a marker for the appearance of the type I AEC phenotype.

A11

THE EFFECTS OF PRETERM BIRTH ON POSTNATAL RESPIRATORY FUNCTION

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Introduction: In preterm infants, respiratory insufficiency is a major problem. Preterm birth causes abrupt changes in the physical, endocrine and metabolic environment of the developing lungs. These changes are likely to affect lung structure and function. In clinical studies, the use of assisted ventilation and supplemental oxygen have confounded our understanding of the structural and functional abnormalities that occur within the lung as a result of preterm birth and the extent to which they are reversed during postnatal life.

Aims: Our aim was to determine whether postnatal respiratory function was affected by preterm birth in the absence of ventilatory support.

Methods: Preterm birth was induced by Epostane (50 mg, maternal i.v.) Antenatal betamethasone (3mg, maternal, i.m.) was administered to ensure neonatal survival. Twelve preterm lambs were born vaginally 2 weeks before term, at 133 days of gestation (term ~147d); the control group (n=12) were born normally at full term. For measurements of lung function, lambs were mechanically ventilated with a Drager ventilator whilst intubated and sedated. The lambs were ventilated at 30 breaths/min and tidal volume of 10ml/kg or 50 breaths/min and tidal volume of 6ml/kg. Lambs were humanely killed at the equivalent of full term, that is, at 148 days post-mating in preterm lambs and 24 hours after birth in term (control) lambs.

Results: At term equivalent age, measurements of compliance, airway resistance and ventilatory efficiency index (VEI) did not significantly differ in preterm lambs, relative to their gestational age-matched controls. However, alveolar arterial O₂ (AaDO₂), oxygenation index (OI) and weight-adjusted airway conductance were significantly lower in the preterm lambs than in controls.

Conclusion: These results suggest that the preterm lung has developmental limitations that compromise pulmonary gas exchange; however, some aspects of pulmonary function appear to mature normally at term-equivalent age. Further long-term studies are required to determine the capacity of lungs of preterm lambs to recover from early deficits associated with preterm birth.

A12

PRETERM BIRTH ALTERS POSTNATAL LUNG GROWTH AND LUNG STRUCTURE

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Background: Preterm birth shortens the period during which the lungs develop *in utero*. At birth, the recoil of the lung is increased due to the creation of surface forces within the air-spaces; this increase in recoil reduces the level of lung expansion. As lung growth is intimately linked to the degree of lung expansion, the premature reduction in the basal lung expansion caused by preterm birth is likely to affect the growth and development of the lungs.

Aim: To determine the effects of preterm birth, in the absence of postnatal ventilatory support, on postnatal lung growth and lung structure.

Methods: Four groups of ewes received betamethasone (3mg i.m.) at 131d gestational age (term ~147d). In two groups, preterm birth was induced using Epostane (50mg, maternal i.v.), with vaginal delivery occurring at 133d. The other two groups were allowed to deliver normally at term. One preterm and one term group (both n=6) were euthanised at term equivalence (TE; ie at ~6h after spontaneous delivery at term or 14d after preterm birth). The other preterm and term groups (both n=5) were humanely killed at 6 weeks post-TE. The lungs were fixed at 20 cm H₂O via the trachea. Total pulmonary DNA and protein contents were measured to assess lung growth and measurements of lung architecture were made using light microscopy and standard stereological techniques.

Results

Term equivalent age: At term-equivalent age, preterm lambs weighed significantly more than control lambs ($5.80 \pm 0.48\text{kg}$ vs $4.42 \pm 0.18\text{kg}$; $p < 0.05$). Pulmonary DNA content ($119.4 \pm 10.9\text{mg/kg}$) and protein content ($2441.6 \pm 154.0\text{mg/kg}$) in preterm lambs were similar to those in control lambs (DNA $136.1 \pm 6.1\text{mg/kg}$ and protein $2902.4 \pm 521.6\text{mg/kg}$).

Six weeks after term equivalent age: By 6 weeks after TE, body weights and total lung DNA content (preterm $64.5 \pm 6.8\text{mg/kg}$ vs control $65.3 \pm 4.9\text{mg/kg}$) were not different between control and preterm lambs. At this age preterm lambs had a significantly greater total lung protein content compared to control lambs ($2674.3 \pm 280.3\text{mg/kg}$ vs $1937.1 \pm 47.8\text{mg/kg}$, $p < 0.05$). The proportions of lung air space and tissue space in preterm lambs (air $74.3 \pm 1.2\%$; tissue $25.7 \pm 1.2\%$) were similar to levels in control lambs (air $76.3 \pm 2.6\%$; tissue $23.7 \pm 2.6\%$). Alveolar size was not different between preterm lambs ($48.3 \pm 1.2\mu\text{m}$) and control lambs ($46.5 \pm 1.3\mu\text{m}$). However, preterm lambs tended to have a greater alveolar wall thickness compared to control lambs (preterm $6.0 \pm 0.4\mu\text{m}$ vs control $5.0 \pm 0.0\mu\text{m}$; $p = 0.06$) at 6 weeks PTE.

Conclusions: Preterm birth results in abnormal lung growth and a disruption in the structural development of the lungs, which only become apparent further into postnatal life. Whether these changes persist to adult life remains to be established.

A13

FETAL LUNG HYPOPLASIA: EFFECTS ON PULMONARY CIRCULATION AND RESPIRATION IN NEONATAL SHEEP

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Background: Lung hypoplasia (LH) is defined as impaired lung tissue growth and may also be associated with impaired development of the pulmonary vascular bed. Reduced pulmonary vascular development may be a major cause of respiratory insufficiency in LH. However, relatively little is known about pulmonary vascular development in LH.

Aims: The objective of this study was to characterise pulmonary hemodynamics in newborn lambs with LH and to compare them with alterations in lung mechanics and ventilation.

Methods: LH was induced in 6 ovine fetuses by tracheo-amniotic shunt and amniotic fluid drainage beginning at 98 -112 days of gestation (term ~147days). At 138-140 days, fetuses were exteriorised under maternal-fetal anaesthesia to implant an ultrasonic flow probe around the left pulmonary artery and to catheterise the main pulmonary artery, the carotid artery and the jugular vein. The lambs were then delivered and ventilated for 2 hours, during which we recorded systemic and pulmonary arterial pressures, left pulmonary artery blood flow, airway pressure and air-flow. During the experiment, the lambs were sedated and ventilator settings were adjusted according to the systemic arterial blood gas data. At autopsy, lungs were weighed, fixed (at 20 cmH₂O pressure) and volume measured. We calculated oxygenation index (OI), total respiratory system compliance (Crs), mean pulmonary blood flow (PBF), and pulmonary vascular resistance (PVR).

Results: In LH lambs, lung weights (21.7+/-3.1 vs 30.7+/-4.0 g/kg) and volumes (23.2+/-3.0 vs 37.9+/-0.8 mL/kg) were smaller than in controls (both p<0.01). FiO₂, mean airway pressure (MAP), PaO₂ and OI were not different between the groups. LH lambs had lower Crs than controls (0.40+/-0.08 vs 0.62+/-0.12 mL/cmH₂O/kg, p<0.05). They also had lower PBF (42.3+/-13.1 vs 104.3+/-7.5 mL/min/kg) and higher PVR (0.88+/-0.19 vs 0.24+/-0.03 mmHg/mL/min*kg) than controls (both p<0.01). The table summarises functional differences between the groups.

	LH (n=6)	Control (n=3)
FiO ₂ 2h after birth	0.35+/-0.05 (ns)	0.33+/-0.06
MAP 2h after birth (cmH ₂ O)	9.7+/-1.1 (ns)	5.8+/-3.2
PaO ₂ 2h after birth (mmHg)	93.6+/-22.8 (ns)	76.4+/-21.3
OI 2h after birth	4.0+/-1.0 (ns)	2.7+/-2.1
Crs 2h after birth (mL/cmH ₂ O/kg)	0.40+/-0.08*	0.62+/-0.12
PBF 2h after birth (mL/min/kg)	42.3+/-13.1**	104.3+/-7.5
PVR 2h after birth (mmHg/mL/min*kg)	0.88+/-0.19**	0.24+/-0.03

**p<0.01, *p<0.05

Conclusion: In LH lambs, a reduction in lung weight of ~30% was associated with a ~60% decrease in PBF and a ~270% increase in PVR at 2h after birth, despite similar oxygenation levels. LH induced a change in PVR that was much greater than the change in Crs (35% decrease).

A14

THE ROLE OF INTRALUMINAL PRESSURES IN REGULATING PULMONARY VASCULAR RESISTANCE BEFORE AND AFTER BIRTH.

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Introduction: Pulmonary vascular resistance (PVR) is high in the fetus and markedly decreases at birth. This decrease in PVR has been attributed to many factors, including an increase in oxygenation, vasodilation of the pulmonary vascular bed and a non-specific effect of ventilation; the latter can account for >65% of the decrease in PVR at birth. The mechanisms by which ventilation effects PVR are unclear, but has been linked to changes in intraluminal pressures caused by the entry of air into the lungs with the onset of air-breathing. Our aim was to examine the role of intraluminal pressures in regulating pulmonary blood flow (PBF) and PVR in both the fetus and newborn, particularly during the transition to extra-uterine life. We hypothesized that intraluminal pressures, by regulating the alveolar/capillary transmural pressure, is a primary factor regulating PVR before and after birth.

Methods: Study 1. We measured left atrial pressure, pulmonary arterial pressure, tracheal pressure and pulmonary blood flow (PBF) in chronically catheterised fetal sheep (116-128 days gestation n=6). Measurements of PVR and PBF were made during periods of fetal breathing movements (FBM) and compared to values measured during the immediate preceding apneic period. FBM were divided into periods of vigorous (amplitude of >3mmHg) and non-vigorous periods. Study 2. Measurements of PBF and PVR were made in 128 day preterm lambs, ventilated with 0, 4, 8 and 12 cmH₂O PEEP for 20 minutes in a random sequence, using a Drager neonatal ventilator delivering a guaranteed V_T of 5ml/kg. A 2-way ANOVA was used to compare mean PBF, PBF_{min}, PBF_{max} and PVR during the different FBM periods, and at the different PEEP levels.

Results: During accentuated, but not during normal FBM, PVR was reduced by $13.8 \pm 6.4\%$ ($p < 0.025$) below the preceding apneic period value. Conversely, increasing PEEP from 4 to 8 (4-8) cmH₂O and from 4 to 12 (4-12) cmH₂O in the preterm lamb increased PVR by $23.1 \pm 5.5\%$ and $46.9 \pm 14\%$ respectively.

Conclusion: We conclude that, in the fetus, PVR is decreased during accentuated episodes of FBM and, in the newborn, is increased with increasing airway pressure. These findings support the concept that alveolar/capillary transmural pressures play an important role in regulating PBF and PVR both before and after birth.

THE EFFECTS OF MATERNAL BETAMETHASONE ADMINISTRATION ON GLUT-1 EXPRESSION IN THE SHEEP PLACENTA

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Introduction: Maternal intramuscular administration of betamethasone reduces birthweight and placental weight in sheep, potentially due to impaired placental function (Newnham *et al.*, 1999). Fetal growth is reliant on the efficient maternal-fetal transfer of glucose via placental glucose transporters (GLUTs). GLUT-1 and GLUT-3 are preferentially localised in human (Knipp *et al.*, 1999), rodent (Hahn *et al.*, 1999; Langdown & Sugden, 2001) and sheep (Currie *et al.*, 1997; Dandrea *et al.*, 2001) placentae. The effects of maternal glucocorticoids on sheep placental GLUT-1 are unknown. In the rat, placental GLUT-1 mRNA and protein expression levels have been reported to be up-regulated (Langdown & Sugden, 2001) and down-regulated (Hahn *et al.*, 1999) by maternally administered glucocorticoids. We have previously reported that a single maternal intramuscular injection of betamethasone on day 117 of pregnancy was sufficient to significantly reduce total placental weight and the average weight of B subtype placentomes within seven days (Mason *et al.*, 2002). Therefore the aim of the present study was to determine the effect of glucocorticoids on GLUT-1 protein levels in B subtype placentomes of the sheep placenta. **Methods:** Pregnant ewes bearing single fetuses received a single intramuscular injection of betamethasone (0.5mg/kg bodyweight, n=8) or saline (n=7) on 117 days of pregnancy (d, term is ~150d). At 124d animals were killed and all placentomes were collected, weighed and classified (A, B, C & D subtype). Western blotting was used to determine GLUT-1 protein levels in B subtype placentomes. **Results:** Total placental weight and the average weight of B subtype placentomes were significantly lower 1 week after a single maternal intramuscular injection of betamethasone than after maternal saline (Mason *et al.*, 2002). GLUT-1 protein levels in B subtype placentomes were not significantly different between betamethasone and saline treatment groups ($p = 0.39$). **Conclusions:** The results of this study demonstrate that maternal betamethasone treatment does not significantly alter GLUT-1 protein levels in B subtype placentomes 1 week after treatment, despite a significant decrease in total placental weight and the average weight of B subtype placentomes. Future investigations will address the possibility that maternal antenatal betamethasone treatment alters the proportion of fetal and maternal tissue within the sheep placenta. It is possible that growth factors involved in placental and fetal growth may be vulnerable to maternal betamethasone treatment and may account for reduced placental and fetal weights observed in our sheep studies. Future analyses will determine the effects of maternal betamethasone administration on placental IGF-II and VEGF.

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A16

FETAL HYPOTHALAMIC-PITUITARY-ADRENAL AXIS RESPONSE TO MATERNAL PROZAC TREATMENT

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Clinical depression is diagnosed in 10-15% of women during pregnancy and exposes the mother and fetus to health risks such as poor self-care and nutrition, disturbed sleep patterns, lack of prenatal care, increased exposure to alcohol and drugs and increased risk of suicide. Thus, treatment of depression during pregnancy is crucial.

Pharmacological interventions have a faster and more consistent onset of action than psychotherapy and thus are preferable. Prozac (fluoxetine, (Fx)), a selective serotonin reuptake inhibitor is often prescribed due to its efficacy, high margin of safety and mild side effects. Serotonin interacts with glucocorticoids in the development of the hypothalamic-pituitary-adrenal (HPA) axis and chronic FX treatment alters cortisol and adrenocorticotropin hormone (ACTH) responses to serotonin agonists. Excess exposure to glucocorticoids programs fetal development with negative consequences in adulthood. We hypothesise that treatment with FX will increase fetal exposure to serotonin and thus HPA activity. The University of British Columbia Animal Care Committee approved all procedures. Pregnant ewes were infused with either fluoxetine (98.5ug/ml.d, n=7) or sterile water (n=8) for 8 d at 122-126 d gestation with hormone measures taken at 0700 daily. FX did not change maternal plasma ACTH and cortisol concentrations. In the FX group, fetal plasma ACTH concentration doubled on Infusion Day 7 whilst fetal plasma cortisol concentration increased by 58 ± 19 nmol/l on Infusion Day 8 from Preinfusion Day. Interestingly, in both groups fetal plasma cortisol concentration was doubled in male compared to female fetuses on Infusion Days 7 and 8. This study shows that while maternal drug treatment affects the fetus, fetal gender also impacts on cortisol concentration and must be considered in determining the treatment effects.

A17

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Intrauterine growth restriction (IUGR) is linked with hypertension and cardiovascular disease in later life, suggesting that changes in fetal life can be later reflected in the adult pathology. The kidney plays a primary role in regulating the osmolarity of the blood and is implicated in the development of hypertension. It is likely that IUGR will affect nephron endowment, as nephrogenesis is complete in humans by 36 weeks of gestation. Previous studies have addressed this issue in rodent animal models where nephrogenesis is incomplete at birth and so are difficult to relate to humans. A closer model to kidney development in humans is seen in sheep where nephrogenesis is complete *in utero*.

The aim of this study was to investigate the effect of IUGR on nephron endowment.

IUGR was induced experimentally in Border Leicester X Merino fetal lambs by umbilico-placental embolisation (from day 120 of gestation) or naturally through twinning. At sacrifice (140 days gestation), the right kidneys were removed and perfusion fixed with 4% paraformaldehyde and 0.2% glutaraldehyde. The kidneys were sampled using a smooth fractionator approach and processed in glycolmethacrylate. The embedded tissue was exhaustively sectioned at 20 μ m, every 10th and 11th section collected, and stained with haematoxylin and eosin. Using an unbiased physical disector / fractionator technique the number of glomeruli (and thereby nephrons) in the kidneys were estimated.

Delivery weights were significantly reduced ($p < 0.01$) by approximately 30% in both the twin (3.72 ± 0.27 kg) and embolised-lambs (3.41 ± 0.28 kg) compared to the controls (5.13 ± 0.22 kg). Kidney weights were reduced ($p < 0.05$) proportionally to body weight, with kidney weights in the twins and embolised-animals averaging 9.05 ± 0.75 g and 10.36 ± 1.27 g, respectively and control lambs averaging 14.22 ± 0.76 g. Nephron endowment was significantly reduced in the twin animals ($344,100 \pm 43,100$ nephrons) compared to the controls ($558,800 \pm 80,900$ nephrons). Whereas, nephron endowment was not affected in the lambs that were embolised, with nephron number averaging $507,300 \pm 59,100$. The average volume of a glomerulus in the twins ($5.91 \times 10^{-4} \pm 6.56 \times 10^{-5}$ mm³) was significantly larger ($p < 0.05$) when compared to glomeruli in both the controls ($3.88 \times 10^{-4} \pm 5.04 \times 10^{-5}$ mm³) and embolised-lambs ($3.33 \times 10^{-4} \pm 2.81 \times 10^{-5}$ mm³).

In conclusion, IUGR due to twinning leads to a reduced nephron endowment at birth and this is accompanied by compensatory glomerular hypertrophy. Alternatively, nephron endowment is not affected by umbilico-placental embolisation, from 120 days of gestation, as nephrogenesis is complete in the fetus by this time. Hence, reduced birth weight *per se* does not directly imply reduced nephron endowment but rather is dependent on the timing of IUGR *in utero*.

A18

ADMINISTRATION OF ADVANCED GLYCATION ENDPRODUCTS TO RATS WITH A CONGENITAL NEPHRON DEFICIT

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Introduction: Intrauterine growth retardation, due to low protein diet (LPD) exposure during early development, has been shown to result in low birth weight offspring with significant nephron deficits. In a previous study we have shown that the expected increase in systolic blood pressure, in response to a nephron deficit (Brenner *et al.* 1988) does not occur. This suggests that the kidney has the ability to successfully compensate a primary insult, such as a congenital reduction in nephron endowment, however, a secondary insult may lead to the development of hypertension and compromised kidney function if the glomeruli reach their limit of compensation.

The aim of this study was therefore to investigate the effect of administration of advanced glycation end-products (AGE) (a secondary insult) to rats with a congenital nephron deficit (a primary insult) as a result of exposure to a LPD during kidney development on blood pressure levels and kidney function in adult rats.

AGEs are associated with the pathogenesis of diabetes and with the cardiovascular changes observed in aging. In this study AGEs were administered to rats via an osmotic mini-pump, at a dose equivalent to AGE levels after 16 weeks of streptozotocin induced diabetes.

Methods: Female Wistar-Kyoto rats were fed either a low (9% casein) or a normal (20% casein) protein diet two weeks prior to mating, throughout pregnancy and for a further two weeks after birth (nephrogenesis is complete in the rat at postnatal day 10). Male offspring were allowed to grow to 20 weeks of age, at which time osmotic mini-pumps containing AGEs or bovine serum albumin (BSA) were implanted subcutaneously. At 24 weeks of age, mean arterial blood pressure, glomerular filtration rate and renal blood flow were determined.

Results: Offspring of rats fed the LPD during pregnancy were significantly smaller at birth than the offspring of rats fed a normal protein diet (NPD) throughout gestation (3.80 ± 0.49 g and 3.94 ± 0.36 g, respectively), and remained significantly smaller throughout the experimental period. In the animals at 24 weeks, there was a significant difference in body weight, kidney weight and body weight: kidney weight ratios between the offspring of rats fed the LPD and NPD during pregnancy. However, kidney function was not affected by either the protein diet exposure *in utero* or secondary treatment, with no significant differences detected between the 4 treatment groups in mean arterial pressure, glomerular filtration rate and renal blood flow.

Conclusion: In this study the offspring of rats fed the LPD during pregnancy had low weight at birth with small kidneys, however in response to the AGE administration, kidney function in these rats was not compromised.

THE LATE GESTATION CONSEQUENCES IN CARDIOVASCULAR AND RENAL FUNCTION OF A MIDGESTATIONAL ASPHYXIAL EPISODE IN FETAL SHEEP

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Introduction: We have recently demonstrated that fetuses asphyxiated at midgestation retained relatively normal kidney structure and function¹. However, they were unable to increase their urine flow rate enough to prevent the formation of hydrops. We postulated that as the kidney matures they will be able to excrete the excess fluid, so that resolution of hydrops will occur. However, the fetus will be left with structural and functional changes to the heart and kidneys as well as the renal and cardiac renin angiotensin systems (RAS).

Methods: Chronically catheterised fetal sheep will be subjected to 30 min of complete cord occlusion at 90 days gestation (term 150 days) and studied at ~130 days. The asphyxial episode is achieved by inflation of an occluder, secured around the umbilical cord, with a known quantity of saline. A successful occlusion is demonstrated by an initial increase in fetal mean arterial pressure (MAP) and decrease in heart rate (FHR) with hypoxaemia, hypercapnia and acidaemia at 5 min of occlusion². One group of fetuses will be sacrificed at 130 days for morphological measurements and tissue studies. These will include, kidney renin and angiotensinogen levels, AT₁ and AT₂ receptor densities, gene expression of components of the RAS, and myocyte nucleation and number. Another cohort of animals will undergo functional studies at 130 days including basal measurements of kidney and cardiac function and RAS activity as well as the responses to challenges (amino acid infusion and hypertension).

Results: So far at 130 days we have studied 3 fetuses asphyxiated at 90 days and 6 sham fetuses. Two of the three asphyxiated fetuses were not hydropic, while the other showed signs of hydrops including subcutaneous oedema. Overall body weight however, did not differ between groups (asphyxia 3360 ± 360g SEM; sham 3160 ± 197g). Heart weight (asphyxia 27.0 ± 2.6g; sham 31.0 ± 3.2g) and total kidney weight (asphyxia 22.9 ± 3.6g; sham 24.2 ± 2.4g) also did not differ. However, brain weight was lower (p<0.01) and lung weight tended to be lower (p=0.06).

Conclusion: The hydrops that results from an asphyxial episode at midgestation seems to resolve as the fetus matures. However, a consequence of the presence of excess fluid in fetal body cavities during development, is restricted growth of some organs. This may have long term consequences after birth.

1. O'Connell, A *et al* (2003), *7th Annual Congress of the PSANZ, Hobart (Poster)*
2. Bennet, L *et al* (1999), *Journal of Physiology* 517, 247-257

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Physiologic strategies for tolerance to hypoxia in fetal sheep and llamas

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Objective: Vertebrates have evolved many different mechanisms for surviving periods of oxygen insufficiency. We are studying fetal responses to hypoxia in 2 ungulates, 1 from the lowlands (sheep) and the other adapted to high altitude (llama).

Materials & Methods: Chronically prepared llama fetuses were studied in Santiago at 585 m altitude, and sheep in San Francisco. Maternal FiO₂ was reduced for up to 1 hr, giving a fetal arterial PO₂ of 10-12 mm Hg, and cardiorespiratory variables measured.

Results:	Sheep		Llamas	
	<u>Control</u>	<u>Hypox</u>	<u>Control</u>	<u>Hypox</u>
Art BP mm Hg	54±9	61±10*	47±2	54±2*
FHR bpm	181±28	146±21*	118±6	95±7*
CVO ml/min/kg	450±69	406±63	279±27	258±21
VRcarc mmHg/ml/min/100g	1.9±0.5	3.9±2.7*	3.4±0.4	8.2±1.8*
Fetal O ₂ cons ml/min/kg	8.4±1.9	4.8±1.2*	4.5±0.5	N/A
Q cereb ml/min/100g	125±23	203±70*	126±14	123±8
Cereb VO ₂ ml/min/100g	4.1±0.1	N/C	1.6±0.3	0.9±0.3*

*p<0.05

Fetal sheep achieve vasoconstriction by alpha-adrenergic activity, catecholamines, neuropeptide Y, AVP, Ang and cortisol, and vasodilatation by beta adrenergic activity, NO, PGs and adenosine. Fetal llamas have greater vasoconstriction of the carcass and viscera due to alpha adrenergic enhancement, with little change in umbilical resistance; these regions include over 80% of the fetal vascular beds.

Conclusions: The most striking differences are the decrease in cerebral metabolism and the more intense vasoconstriction of the muscle, skin, bone & viscera of the llama. We have data which suggest that the fetal llama, unlike the sheep, becomes hypometabolic without cerebral damage (absence of seizures).

The protective mechanisms used by the llama are not yet determined, but other vertebrates survive hypoxia and energy limitation by blood flow redistribution and O₂ stores (seals), hibernation (bears), metabolic arrest (freshwater turtles), and alternate metabolic endproducts (ethanol instead of lactate, crucian carp).

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Activin A in the Intra-Uterine Growth Restricted Ovine Fetus

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Background: A small, but significant, number of human pregnancies are complicated by factors such as maternal hypertension, pre-eclampsia, maternal smoking and anaemia. These complications may result in placental insufficiency and may compromise the wellbeing of the developing fetus, often resulting in intrauterine growth restriction (IUGR) and sometimes in fetal death. In recent years, evidence has accumulated to suggest that the protein hormone activin may be used as a marker for gestational disease during human pregnancy. In human pregnancies complicated by pre-eclampsia or IUGR, circulating activin A levels are significantly increased; suggesting that this protein may be a useful endocrine marker for fetal compromise.

Aims: (a) Use the model of single umbilical artery ligation (SUAL) in mid-late pregnant sheep to severely disrupt placental function, thus producing chronic hypoxia and IUGR, and (b) to investigate the levels of activin A, PGE₂ and cortisol in SUAL fetuses.

Methods: Surgery was performed at gestational age 105-110 day, where catheters were inserted into the fetal femoral artery (FFA), fetal jugular vein (FJV), amniotic fluid (AF), maternal carotid artery (MCA) and maternal jugular vein (MJV). Flow probes were placed around the fetal carotid artery (FCA) and FFA. The two umbilical arteries were located, and one was ligated using a tight silk tie. In control experiments the umbilical artery was manipulated but not ligated. A blood sample was taken from the MCA, FFA and AF at the time of surgery. Following surgery, samples were routinely taken from the FFA, AF and MCA for activin A, PGE₂ and cortisol assays. Fetal heart rate (HR), mean arterial pressure (MAP), and fetal blood flows were monitored twice a week for 4 hours. Post mortem was performed when the ewe entered labour (SUAL n=3) or at 135 days (Control; n=1). A complete post mortem was performed to examine indices of fetal and placental growth.

Results: SUAL resulted in chronic fetal hypoxaemia (%SaO₂= 40.94 ± 4.40%) compared to that in the control animal (%SaO₂ =66.28%). Within 48 hrs after surgery, amniotic fluid activin A concentrations were significantly increased in SUAL animals and remained elevated over the experimental period. Mean activin A concentrations in SUAL fetuses (503.92 ± 165.26 ng/ml), compared to SUAL samples taken at surgery (100.59 ± 75.39 ng/ml) and to the mean control value (74.47 ± 3.50 ng/ml). Glucose concentrations were unchanged by SUAL, however fetal plasma lactate concentrations in the SUAL animals (1.52 ± 1.40 mmol/l) appear to be elevated compared to the control animal (0.99 ± 0.05 mmol/l). Preliminary analysis of fetal HR and MAP indicates that they are not altered in response to SUAL, however compared to control values, carotid blood flow was greater at 116 ± 4 days and femoral blood flow was reduced. At post mortem, IUGR fetuses weighed less than the control fetus (2.03 ± 0.09 Kg versus 3.89 Kg) and less than normal age matched controls (3.13 Kg)¹. SUAL fetuses had an increased brain to body mass ratio (1.77×10⁻² ± 2.10×10⁻⁴) compared to that of the control (1.32×10⁻²) and the adrenal weight to body mass ratio was greater in SUAL fetuses (8.65 × 10⁻⁵) compared to that of the control (5.71 × 10⁻⁵).

Discussion: Umbilical artery ligation in mid-late gestation fetal sheep provides a clinically relevant model for chronic placental insufficiency and resulting fetal hypoxaemia and IUGR. Preliminary observations indicate that SUAL produces a redistribution of blood flow to essential organs, resulting in heavier brains and adrenals, relative to their body weight. This model produces a significant increase in amniotic fluid activin A levels within 48 hrs after surgery and levels are then maintained for the duration of the experiment (approximately 3 weeks). Further animals are required to validate these findings however, these ovine studies, combined with observations in the human, strongly suggest that activin A could be used as a marker of fetal compromise.

Effects of subchorionic endotoxin infusion in sheep.

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Chorioamnionitis is implicated in the pathogenesis of preterm labour and reduces the incidence of respiratory distress syndrome in humans. Consistent with these observations, our previous experiments in sheep have demonstrated that intra-amniotic administration of endotoxin causes chorioamnionitis and improves lung function in preterm sheep. However, in human pregnancies, chorioamnionitis likely results from ascending infectious organisms that might not enter the amniotic fluid. Therefore we aimed to determine if endotoxin infusion outside the fetal membranes would cause chorioamnionitis and lung maturation in preterm sheep.

Pregnant ewes underwent surgery at 118 days of pregnancy (d, term is 150d) for placement of osmotic pumps into the amniotic sac or subchorionic space (under the myometrium but outside the fetal membranes) to infuse endotoxin (subchorionic dose 7.2 mg/day, n=7; intra-amniotic dose 2.4 mg/day, n=6) or saline (n=7), or received a single intra-amniotic injection of saline (n=2) or endotoxin (10 mg, n=7) at 118d. Fetuses were delivered by caesarean section at 124d, at which time we collected samples for measurement of amniotic fluid endotoxin levels and umbilical arterial pH and blood gases. Fetuses were painlessly killed, the chest was opened and we determined the pressure-volume relationship of the lungs during deflation from 40cmH₂O. We counted inflammatory cells in bronchoalveolar lavage fluid, and scored the severity of inflammation in H&E stained sections of fixed chorioamnion.

Ewes remained well during subchorionic endotoxin infusion. Umbilical arterial pH at delivery was lower after subchorionic endotoxin infusion (7.197 ± 0.044) than in controls (7.314 ± 0.013 , $P=0.056$) and PCO₂ was higher (73.0 ± 5.7 mmHg) than in controls (55.3 ± 2.4 mmHg, $P<0.05$). Inflammatory scores for the chorioamnion were comparable after intra-amniotic endotoxin infusion (2.3 ± 0.1 units/ml) or subchorionic endotoxin infusion (2.1 ± 0.1 unit/ml), and were greater in these groups than in controls (1.47 ± 0.1 units/ml, $P<0.05$). Numbers of inflammatory cells in alveolar lavage fluid were greater, and hydrogen peroxide production was greater than control after intra-amniotic endotoxin infusion or injection ($P<0.05$), but not after subchorionic endotoxin infusion. Lung function (indicated by pressure-volume curves) was greater than control after intra-amniotic endotoxin infusion or injection ($P<0.05$) but was not different from control after subchorionic endotoxin infusion. Endotoxin concentrations in amniotic fluid after subchorionic endotoxin infusion were not significantly different from control.

Our data demonstrate that 6 days of subchorionic endotoxin infusion in sheep causes chorioamnionitis, which is tolerated by the ewe but may have mild adverse effects on the fetus. In contrast to the effects of intra-amniotic endotoxin, 6 days of subchorionic endotoxin infusion does not improve preterm lung function. Therefore chorioamnionitis may be caused by inflammation originating from various sites and different sites of origin are associated with different effects on the fetus. Our findings suggest that preterm lung maturation accompanies intrauterine inflammation only if the proinflammatory stimulus responsible is present in the amniotic fluid. It remains possible that longer-term subchorionic endotoxin infusion could result in entry of sufficient amounts of endotoxin into the amniotic fluid to cause preterm lung maturation, but this might be accompanied by adverse fetal effects.

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