C145

The effects of short-term infusion of fenoldopam on systemic haemodynamics and indices of renal function in the normotensive neonatal foal

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The effects of fenoldopam mesylate, a dopamine-1 receptor agonist, are unknown in the foal, as is the distribution of dopamine-1 receptors. The aim of this study was to document the effects of fenoldopam on the systemic circulation and renal function of the normotensive neonatal foal. Fenoldopam is licensed as an anti-hypertensive drug for humans in the USA. It decreases systolic and diastolic arterial pressure in hypertensive humans at doses of 0.04mcg/kg/min to 0.8mcg/kg/min (Taylor et al. 1999). However, no change in haemodynamics at a dose of 0.03mcg/kg/min were seen in normotensive humans, and a dose of 0.3mcg/kg/min caused a moderate decrease in diastolic pressure but no change in systolic pressure (Mathur et al. 1999). Both doses increased renal blood flow, but not creatinine clearance, in normotensive humans (Mathur et al. 1999). There are species differences in the renal response to fenoldopam. Dogs showed an increase in renal blood flow, but this effect could not be documented in the sheep (Lass et al. 1988) or in neonatal piglets (Pearson et al. 1996).

Six Thoroughbred foals, 87-122 h in age, were studied. All were considered normal on the basis of clinical examination, haematology and baseline measurements of cardiac output and arterial pressure. The foals were sedated with 5-10mg intravenous diazepam, and instrumented with jugular venous, dorsal metatarsal arterial and urinary catheters for the study. The foals were allowed to stand and nurse from the dam, and given a recovery period of 1 h from the administration of diazepam. The foals were then restrained in lateral recumbency on a foal mat and given two doses of fenoldopam (low dose: 0.04mcg/kg/min and high dose: 0.4mcg/kg/min) and a control dose of saline, in a double blind, randomized study.The infusion was maintained for 30 min, and measurements were performed during the last 20 min of infusion. The washout period was 40 min between infusions. Heart rate, arterial blood pressure and cardiac output (lithium dilution) were measured, and systemic vascular resistance, stroke volume, cardiac index and stroke volume index calculated. Renal function was estimated by urine output and endogenous creatinine clearance. Repeated measures and one-way ANOVA tests were used to compare these parameters between each drug and placebo.

Compared with saline, high dose fenoldopam resulted in a significantly increased heart rate (from 104±14 bpm to 143±22bpm), and decreased mean (from 89±6mmHg to 72±7mmHg) systolic and diastolic arterial blood pressure. There were no changes in renal indices. Low dose fenoldopam had no significant effect on systemic haemodynamics, but increased urine output. There was a trend to increased creatinine clearance with low dose fenoldopam, which was significant when compared with high dose fenoldopam. These data suggest that, unlike in the normotensive human, high dose fenoldopam results in a significant decrease in arterial blood pressure in the neonatal foal. Low dose fenoldopam results in no significant changes in haemodynamics, with increased urine output. Low dose fenoldopam therefore has a potential clinical application in neonatal foals with acute renal failure, and this warrants further investigation.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C146

Effects of dysmaturity on pancreatic βcell function in newborn foals

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Neonates must have functional pancreatic βcells responsive to glucose(G)(Fowden et al. 1982). Newborn foals, especially premature, have poor regulation of glycaemia. This study examined effects of dysmaturity on pancreatic βcell responses & anatomical development in foals during the 1st 10 days postpartum.

Over 48h periods at 3 time intervals (days 1-2, 5-6 and 9-10), βcell responses to iv G (0.5g/kg, 40%G, days 1, 5 and 9) or saline (20ml 0.9% NaCl, days 2, 6 and 10) were examined in pony foals delivered either spontaneously (n=7) or induced 2-3 days before fullterm (n=7). Venous jugular blood samples were taken from an indwelling catheter at 5-15 min intervals for 30 before and 60 min after administration. Foals were muzzled throughout this period. Plasma G levels were determined enzymatically. Plasma insulin(I) and total proinsulin(PI) levels were measured by immunoassay validated for equine plasma (Hemmilä et al. 1984). On day 11, all foals were killed. Each pancreas was analysed using stereological methods validated for equids (Beech, 1998). Statistical analyses were ANOVA, paired/unpaired t tests. Data are means±SEM.

Induced foals were all classified as dysmature (Rossdale et al. 1984). There were no significant differences in basal levels of G, I and PI between fullterm and dysmature foals on any days. Clearance of iv G was also similar in the 2 groups at all ages studied. However, the maximum increment in plasma I in response to iv G was greater ($p<0.02$) in dysmature foals on days 1 and 5. On day 1, I levels also remained elevated for longer after iv G in dysmature foals and the area under the I curve was greater ($p<0.01$). In contrast, PI responses to iv G were similar in the 2 groups. On day 11, pancreatic mass was similar in the 2 groups but total islet volume and percentage of βcells in islets were greater in dysmature foals (Table).

Results show that dysmaturity of the foal is associated with enhanced I secretion and resistance. Greater percentage of βcells in islets and total islet volume suggests that the neonatal pancreas is trying to compensate for I resistance by βcell proliferation.

Means (sem) pancreatic stereological analyses of fullterm (n=7) and dysmature (n=7) foals

	Pancreas mass(g)	%βcells	Islet vol. µm3 x10-5	Total islet no.x10-7	Total islet vol.µm3 x10-13
FullTerm	19.9±1.1	37.9±2.4	4.6±2.1	5.9±1.5	1.2±0.7
Dysmature	21.5±1.6	47.9±2.5*	8.1±1.7	7.3±3.0	3.4±0.4*

***sig. diff between the 2 groups(p<0.02)**

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C147

The influence of genotype and environmental temperature on colonic temperature and adipose tissue (AT) uncoupling protein (UCP) 2 expression in neonatal pigs

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It is well established that neonatal mortality is greater in the leaner commercial (CB) porcine genotypes compared to the ancient Meishan (MB) breed, which has a higher percentage of body fat. MB piglets, despite being considerably lighter at birth than commercial breeds, are resistant to hypothermia and hypoglycaemia. Pigs are one of the few mammals that do not express UCP1 which is used for heat production during the neonatal period. UCP2 is a mitochondrial protein proposed to be involved in energy metabolism. It is unknown whether an environmental challenge, such as a low temperature, would influence UCP2 expression in adipose tissue of newborn piglets.

On the first day of life, piglets from 12 MB and 12 CB litters were ranked according to birth weight and the median piglet was randomly assigned to warm (MB n=6, CB n=6) (24°C) (W) or cool (MB n=6, CB n=6) (14°C) (C) treatment. In order to standardise milk intake, all piglets were given 3ml of commercial sow milk replacement. Colonic temperature was measured, piglets were placed in a temperature controlled chamber and maintained for 180 min after which time they were humanely killed with an overdose of barbiturate (100 mg kg^{-1} pentobarbital sodium: Euthatal). UCP2 expression in AT was measured using RT-PCR as described previously (Mostyn A *et al.*, 2004). Results are shown as means ±SEM; significant differences between breeds at each sampling age were assessed by GLM.

Cool maintained CB piglets were colder than W maintained after 20 minutes (20 minutes W, 38.2±0.1; C, 37.3±0.4 °C (P=0.026)) an effect not apparent until 90 minutes in the MB piglets (90 minutes W, 38.7±0.2; C, 37.6±0.3 °C (P=0.004)). Environmental temperature had no effect on UCP2 expression in CB piglets, which had significantly higher UCP2 expression compared to MB, a difference which was greatest in C maintained (C: MB, 3.8±2.9; CB, 58.11±12.7 % of reference (P=0.019); W: MB, 44.1±13.5; CB 75.69±12.6 % of reference (P=0.037)).

In conclusion, despite MB piglets having a substantially lower abundance of UCP2 compared to CB piglets, they have no difficulty maintaining a normal body temperature. UCP2 therefore does not appear to compensate for the absence of UCP1 in promoting heat production in neonatal piglets.

Mostyn, A., Litten, J. C., Perkins, K. S., Alves-Guerra, M., Pecqueur, C., Miroux, B., Symonds, M. E. & Clarke, L. (2004). Influence of genotype on the differential ontogeny of uncoupling protein 2 and 3 in subcutaneous adipose tissue and muscle in neonatal pigs. Journal of Endocrinology In Press.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C148

Effect of acute and chronic leptin administration on uncoupling protein-2 mRNA abundance in ovine neonatal adipose tissue.

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In the neonate, plasma leptin peaks one week after birth and both acute and chronic exogenous administration promotes the loss of the brown adipose tissue (BAT) specific uncoupling protein (UCP)-1 (Mostyn *et al.* 2001). Recently, increased proinflammatory pathways and macrophage numbers in adipose tissue have been implicated in the metabolic complications of obesity (Weisberg SP *et al.* 2003). UCP2, another inner mitochondrial protein, is genetically linked to obesity and type 2 diabetes, and has been implicated in macrophage-mediated immunity (Arsenijevic D *et al.* 2000). The effect of acute and chronic leptin administration on UCP2 and glucocorticoid receptor (GCR) mRNA abundance in neonatal BAT has not been determined.

For the acute study, 7 pairs of 1-day old, triplet lambs received sequentially 10, 100 and 100ug leptin (L) or vehicle (V), before humane euthanasia 4 hours from start of the study and BAT sampling. While in the chronic study, 9 pairs of 1-day old lambs received either 100ug L or V daily for 6 days, before humane euthanasia and BAT sampling on day 7. All procedures were carried out according to UK animal legislation. BAT total RNA was isolated and mRNA abundance was measured by RT-PCR using oligonucleotide primers designed specifically to ovine UCP2 and GCR. Results are given as means (±SEM) relative to 18S rRNA. Statistical differences between groups were analysed by Mann-Whitney U test.

Lamb body and BAT weights, and BAT per kg body weight were similar between groups and increased appropriately with postnatal age. Acute leptin administration decreased both UCP2 (V 96.7±5.1, L 73.3±5.0, $p<0.05$) and GCR (V 72.7±1.4, L 44.3±2.7, $p<0.05$) mRNA abundance, while this pattern was reversed with chronic leptin administration (UCP2: V 73.8±5.3, L 97.0±6.4, $p<0.05$; GCR: V 53.4±4.1, L 67.8±3.3, $p<0.05$). Overall, there was a positive correlation between UCP2 and GCR mRNA (Spearman's Rank Order Test R=0.62, $p<0.0001$).

In conclusion, acute and chronic administration of leptin had differential effects on UCP2 and GCR mRNA abundance in neonatal BAT. In particular, the chronic leptin effects may be important in promoting adipose tissue inflammation and subsequent excess growth of fat.

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Arsenijevic D *et al.* (2000). *Nature Genetics* **26**, 435-439.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C149

Effects of extracellular alkalinisation and K^+ channel blockers on spontaneous contractions in isolated human myometrium

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Understanding the mechanisms regulating the contractility and relaxation of uterine smooth muscle has important therapeutic implications for the treatment of preterm or dysfunctional labours. Modest external alkalinisation has been shown to be a potent modulator of uterine contractility; the spontaneous contractile function of uteri from rats was altered in a gestational-dependent manner by raising extracellular pH (pH_e) from 7.4 to 8.0 (Taggart *et al.*, 1997). One possible mechanism of action of altered pH_e is to affect uterine smooth muscle electrical excitability by targeting K^+ channels. In this study, therefore, we wished to investigate the effect of external alkalinisation on spontaneous contractions of human myometrium. In addition, we examined if any effect of external alkalinisation was modified by blockers of Ca^{2+}- or voltage-activated K^+ channels (TEA or 4-aminopyridine (4-AP) respectively).

Human myometrial tissues from non-pregnant (hysterectomy, 17 strips n= 9) and pregnant women (elective Caesarean delivery at term, 14 strips n = 8) were obtained following informed written consent, dissected and mounted for isometric force recording in an organ bath and equilibrated in physiological saline (pH_e7.4) until regular spontaneous contractions occurred (1-2 hours). Extracellular alkalinisation to pH_e8.0 reduced spontaneous contractions in 5/8 myometria from pregnant women. Overall, contractile frequency was significantly reduced to 0.43±0.05-fold of control (mean±sem, n=8; $p<0.05$, paired t-test). When a contraction did occur in pH_e8, the amplitude was similar to control (1.00±0.03-fold, n=8). In the continued presence of pH_e8, application of the K^+channel blockers TEA (5mM) or 4-AP (1mM) reversed the inhibition of spontaneous contractions such that the amplitudes were 1.45±0.07-fold (pH_e8/TEA, n=3; $p<0.05$) and 1.79±0.66-fold (pH_e8/4-AP, n=3), and frequencies were 1.21±0.61-fold (pH_e8/TEA, n=3) and 1.04±0.10-fold (pH_e8/4-AP, n=3) of control (pH_e7.4). In myometria of non-pregnant women, raised pH_edid not significantly reduce spontaneous contractions.

These data suggest that in human myometrium, like rat uteri, there is a change in the sensitivity of uterine contractions to alkalinisation of pH_e8 with pregnancy. Furthermore, as the inhibitory influence of pHe8 on spontaneous contractions of myometria from pregnant women can be reversed by K^+ channel blockers, this implicates that changes in membrane potential underlie the mechanisms of action of external alkalinisation.

Taggart MJ *et.al.* (1997) Am. J. Obstet. Gynecol. **177**, 959-963.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C150

Characteristics of catecholamine release from fetal rat chromaffin cells

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Catecholamines (CA) secreted by fetal adreno medullary chromaffin cells (AMCs) play an essential role during the adaptation to extrauterine life. Experiments were performed on primary culture of AMC from E15 and E19 rat fetuses. Animals were humanely killed. The techniques of Ca2+ current, amperometry and capacitance measurements were used. Data are presented as mean +/- SEM. Statistical significance was assessed with unpaired Student's t-test.

At E15, application of digitonin (20 microM) and 10 microM free Ca2+ induce secretion of CA. At both stages, the exocytotic process requires the expression of HVA calcium channels, this current density is not significatively different and its exocytotic efficiency remains the same.

The quantal secretion of CA during this prenatal period, was compared by the cumulative integral of amperometric currents induced by depolarisation with 20 s exposure to 70 mM KCl, together with the characteristics of individual spikes. The value of cumulative integrals of amperometric currents at 1 min after KCl application were 3.47 +/- 0.9 pC for E15, (n=8) and 7.24 +/- 0.7 pC for E19 (n=8), $p<0.01$; such difference is the consequence of the increase in the number of molecules of CA per granule (E15: 164842 +/- 4712, (n= 566); E19: 277067 +/- 9737 (n= 600), $p< 0.001$). The time delay to release 50% of the total amount of quanta mobilized by KCl was shorter at E19 (n=8) than at E15 (n=8): 5.58 +/- 4.42 s, and 11.49 +/- 16.04 s respectively ($p<0.001$).

During the perinatal period, CA release in AMC is supposed to be controlled by an oxygen sensing mechanism. At E19, hypoxia induces a membrane depolarisation and CA release but at E15,

hypoxia induces membrane hyperpolarisation. This dual effect over membrane potential is related to the changing contribution of different types of K+ channels. We compared the effect of glibenclamide (50 microM) over the total K+ current with or without external Ca2+. At E19, IKCa is the main component with 61.19% +/- 7.38 (n = 10) and IKATP: is 14.25 % +/- 2.72, n=10 ($p<0.001$) of the total IK+ currents. At E15, the predominant K+ current is IKATP with 48.7 % +/- 4.7, n=9 and IKCa is 29.46% +/- 4.94, n = 8, ($p<0.001$) of the total. At E19, both Apamin (400 nM) and Iberiotoxin (100 nM) induce membrane depolarisation, but hypoxia failed to generate further membrane depolarisation in the presence of apamin.
These data show that at E15, the exocytotic process is functional but the quantal size is small compared to E19. The non-neurogenic CA secretion appears when the Apamin-sensitive K+ current is the main component of the total K+ current.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C151

A Possible Role for the Carotid Body in Fetal Nutrient Detection

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Clinically, Doppler blood flow measurements in growth restricted human fetuses suggest a redistribution of combined ventricular output in favour of 'vital' organs, probably due to hypoxia. However, there is no direct evidence to date that similar fetal responses occur in the face of undernutrition. The carotid body is well established as a mediator of fetal cardiovascular responses to hypoxia (Giussani *et al* 1993). In adults, the carotid body is sensitive to blood glucose (Pardal & Lopez-Barneo 2002) and is involved in glucose homeostasis (Alvarez-Buylla *et al* 1997). The purpose of this study was to investigate the role the carotid body plays in fetal nutrient detection and growth.
Pregnant Merino ewes (Animal Experimentation Ethics Committee, Department of Agriculture, Western Australia) of uniform body weight were housed individually and fed 100% total nutritional requirement, supplemented with 0.5kg barley straw per day, for 2 weeks. At 111 to 115 days gestation (dGA) the ewes were anaesthetised (3% halothane/O2) for fetal surgery. The fetal carotid sinus nerves were sectioned bilaterally (CSD, n= 19) or the nerve was identified but not cut (sham, n= 23). After 5 days of recovery, ewes were fed 100% (CSD, n= 10, sham, n= 12) or 50% (CSD, n= 9, sham, n= 11) total nutritional requirements for three weeks, until post-mortem (140 to 145 dGA). Placental and fetal organ weights and fetal size data were analysed by two-way ANOVA with post-hoc Student's t-tests, and by linear regression (LR) analysis against ewe weight change over the experimental period.
There was an interaction between the effects of CSD and diet on kidney and adrenal weights ($p<0.05$ ANOVA). In all sham animals, ewe weight change over the experimental period was negatively correlated with fetal adrenal weight ($r^2=0.19$ $p<0.05$ LR) and positively correlated with fetal liver weight ($r^2= 0.18$ $p<0.05$ LR), both as percent fetal body weight. This effect was not observed in CSD animals. In sham males, but not sham females, heart weight was positively correlated with ewe weight change ($r^2= 0.24$ $p<0.05$ LR). This effect was not observed in CSD animals.
This study suggests an organ specific redistribution of nutritional resources following a late-gestation maternal nutrient restriction. This is the first study to show that this depends in part on the carotid body.

Giussani *et al* 1993 J Phys 461:431-49
Pardal & Lopez-Barneo 2002 Nat Neurosci 5:197-8
Alvarez-Buylla *et al* 1997 J Phys 272:R392-R399

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C152

Melatonin inhibits the sympatho-adrenomedullary response to acute hypoxaemia in the late gestation ovine fetus

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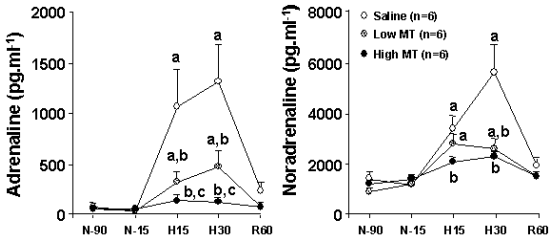
The actions of melatonin on fetal physiology have mostly been investigated at the level of the suprachiasmatic nucleus in relation to circadian rhythmicity (Serón-Ferre *et al.* 2003). However, the presence of melatonin receptors in diverse fetal tissues including the brain and adrenal gland suggest that melatonin may be involved in a wider array of fetal functions. Recent studies have reported that fetal exposure to elevated concentrations of melatonin suppresses stimulated adrenocortical function (Torres-Farfán *et al.* 2003). However, the effects of melatonin on the sympatho-adrenomedullary axis in the fetus remain unknown. This study investigated the effects of fetal treatment with low and high doses of melatonin on the plasma catecholamine response to acute hypoxaemia in fetal sheep.
Under halothane, 6 fetal sheep at 0.8 of gestation were chronically catheterised. Five days later all animals were subjected to 1.5 h of normoxia, 0.5 h of hypoxaemia and 1 h of recovery either during fetal i.v. infusion with saline (vehicle) or during fetal i.v. treatment with low (0.15 $mg.min^{-1}$) or high (1.5 $mg.min^{-1}$) melatonin. The doses of melatonin were designed to mimic circulating concentrations of melatonin in the ovine fetus measured at night (low dose; Yellon & Longo, 1988) or that which may result in the human fetus following typical therapy to avoid jet lag in travelling prospective mothers (high dose; Okatami *et al.* 1998). Fetal arterial blood samples were taken during normoxia, hypoxia and recovery for measurement of blood gases and plasma con-

centrations of catecholamines by HPLC. At the end of the experiment, animals were humanely killed with pentobarbitone (200 mg kg^{-1} i.v.). All values are means ± S.E.M.; a two-way RM ANOVA with post hoc Tukey's test was used.

Fetal treatment with melatonin did not alter basal blood gases or plasma catecholamine concentrations. In hypoxaemia, a similar fall in fetal P_aO_2 occurred during saline (20.8±0.4 to 10.7±0.6), low (20.5±0.6 to 11.0±0.3) or high (21.2±0.5 to 11.2±0.3 mmHg) melatonin treatment. Melatonin markedly suppressed the plasma concentrations of adrenaline and noradrenaline during hypoxaemia in a dose-dependent manner (Figure). These data show that fetal treatment with melatonin markedly suppresses the fetal plasma catecholamine response to acute hypoxaemia.



Serón-Ferre *et al.* (2003) *Ann Rev Physiol* **43**,141.
Torres-Farfán *et al.* (2003) *J Physiol* **554**,841.
Yellon & Longo (1988) *Biol Reprod* **39**,1093.
Okatami *et al.* (1988) *J Pineal Res* **25**,129.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C153

EGF stimulated trophoblast differentiation is dependent on activation of the MAPK p38

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Placental cytotrophoblast cells differentiate into the multi-nucleated syncytiotrophoblast and it has been suggested that this process is abnormal in placental tissue from pre-eclamptic/IUGR pregnancies [1]. Although there is an incomplete understanding of the differentiation process in normal cytotrophoblasts, it has been shown previously that EGF is necessary for efficient formation of syncytial trophoblasts in vitro[2]. We recently demonstrated that EGF activates the MAPK p38 and that this effect is independent of the growth factors ability to inhibit apoptosis [3]. In this study we hypothesized that inhibition of EGF induced p38 activation using two specific inhibitors, SB203580 and SB202190, would prevents EGF induced cytotrophoblast differentiation in vitro as judged by both β-HCG production and cell multi-nucleation.

Cytotrophoblasts isolated from term human placentas were cultured with and without EGF (10ng/ml). EGF treated cells were also incubated with or without p38 inhibitors (SB203580 (10uM) and SB202190 (10uM)), in a controlled 5%CO2/20%O2 atmosphere for 5 days. Media was changed on days 1,3 and 5 of culture and β-HCG assayed. On day 5 cells were fixed with methanol:acetone and stained with anti-desmoplakin (cell membrane marker) and DAPI (nuclear marker) for immunofloures-cence, prior to imaging and manual counting to determine the number of nuclei per trophoblast.

Trophoblast like cells incubated with EGF demonstrated increased multi-nucleation as compared to control cell cultures (EGF 53%±2.1% versus control 14%±2% $p<0.05$, after 5 days of culture) in agreement with previously published data [2]. This effect was reversed by the addition of either p38 inhibitor to EGF containing cultures (EGF+SB203580 20%±1.1% versus 53%±2.1% $p<0.05$, EGF+SB202190 9%±0.4% versus 53%±2.1% $p<0.05$).β-HCG concentrations were also reduced in EGF treated cells with the addition of either SB203580 (β-HCG undetectable) or SB202190 (β-HCG reduced by 73%).

Stimulation of p38 by EGF may be responsible for its pro-differentiation actions on cytotrophoblasts. This may be as a result of downstream signalling to cAMP response element [4], a known trophoblast differentiation stimulator in vitro, or an as yet unidentified downstream substrate of p38.

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All tissue was obtained in conjunction with local ethics committee approval.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C154

Programming of fetal adipose tissue sensitivity to insulin by maternal nutrient restriction between early to mid gestation in sheep

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Maternal nutritional restriction between early to mid gestation results in greater perirenal adipose tissue deposition in the fetus at term in conjunction with an increased mRNA for the insulin-like growth factors-I and II (Bispham et al 2003). The ability of adipose tissue to take up glucose in conjunction with insulin sensitivity can also contribute to increased fat growth. The aim of the present study was to determine the effect of nutritional restriction on fetal adiposity and expression of key insulin signaling proteins in fetal adipose tissue.

Fourteen singleton-bearing sheep of similar body weight and parity were entered into the study. Six of these were nutrient

restricted (NR) between 28-80 days gestation and consumed 60% of their total calculated metabolisable energy (ME) requirements for body weight and pregnancy whilst the remainder were fed to appetite (Controls; C) and consumed 150% of calculated ME requirements. After 80 days gestation all ewes were fed to appetite and consumed very similar amounts of feed. At 140 days gestation all sheep were humanely euthanased (IV Na pentobarbitone, 170mg/kg) before fetal adipose tissue sampling. Protein was extracted from adipose tissue and the expression of the insulin receptor β subunit, the p85 regulatory subunit of phosphoinositide (PI) 3- kinase, the p110 β catalytic subunit of PI 3-kinase and the glucose transporter GLUT4 was determined by Western blotting. Results in all cases are given as mean arbitrary unit (AU) ± SEM. Significant differences between groups were assessed by the Students unpaired t-test. A P value of <0.05 was considered statistically significant.

Fetuses of NR mothers possessed more perirenal adipose tissue per kg body weight than C (C 3.7 ± 0.2; NR 5.1 ± 0.5 g/kg $p<0.05$). Protein expression of both the insulin receptor (C 6474 ± 785; NR 11310 ± 1362 AU $p<0.01$) and p85 (C 38456 ± 2217; NR 44764 ± 1818 AU $p<0.05$) was significantly increased in NR fetuses. In contrast GLUT 4 (C 34452 ± 4097; NR 36018 ± 2557 au) and p110β (C 17749 ± 1956; NR 21018 ± 1625 AU) protein expression was similar between groups.

In conclusion, fetuses of mothers who were nutrient restricted during early pregnancy show increased adiposity compared to controls. This is associated with increased expression of the insulin receptor and regulatory subunit of PI3-kinase. This could contribute to an increase in insulin sensitivity and an increased propensity to store fat.

Bispham J., Gopalakrishnan G.S., Dandrea J., Wilson V., Budge H., Keisler D.H., Broughton Pipkin F., Stephenson T. & Symonds M.E. (2003) Endocrinology 144: 3575-3585

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C155

Glucose metabolism and body composition in mature adult sheep following early life undernutrition

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Birth size and early postnatal growth influence the risk of adverse adult health such as obesity and metabolic disorders. Previously, we have shown that postnatal undernutrition (UN) improves glucose tolerance, while combined pre- and postnatal UN increases fatness, in young adult (1.5 year-old) female sheep (Poore *et al.*, 2003, 2004). In the current study, the interaction between poor development in early gestation and adverse postnatal conditions on adiposity and metabolism has been examined in mature adult sheep.

Ewes received either 100% (group C, $n = 39$) or 50% of global nutritional requirements (group U, $n = 41$) from conception—day 30 gestation, and 100% thereafter (Animals (Scientific Procedures) Act 1986). Offspring were fed *ad lib.* (CC, $n = 22$; UC, $n = 19$) or to reduce body weight to 85% of target (predicted from 0—12 weeks growth trajectory) from 12—25 weeks postnatal age and *ad lib.* thereafter (CU, $n = 17$; UU, $n = 22$). At age 2.5 years, body condition score (BCS) was assessed and fat depth measured by ultrasound then corrected for body weight. Arterial and venous catheters were inserted under general anaesthesia (3% halothane/O_2). Glucose handling was determined by areas (AUC) under the glucose and insulin curves following *i.v.* glucose (0.5 g/kg). Plasma insulin was measured by ELISA. Data were analysed by linear regression (population) and ANOVA (4 groups).

In all female, but not male, adult sheep, glucose tolerance at 2.5 years worsened (increased glucose AUC) with increasing BCS ($r^2 = 0.26$, $p < 0.005$). However, glucose AUC was reduced ($p < 0.05$) by postnatal UN in 2.5 year-old females, not males, regardless of previous prenatal nutrition (CU and UU). The increased fat depth in 1.5 year-old UU females was no longer evident at 2.5 years, although fat depth at this age was related to 12—25 week growth ($r^2 = —0.10$, $p = 0.05$) and fatness at 1.5 years ($r^2 = 0.19$, $p < 0.05$). Pre- or postnatal UN had no effect on insulin AUC at 2.5 years in either sex.

These findings show that the association between relatively poor glucose tolerance and higher body condition in female sheep is disrupted by early postnatal UN. The improvement in glucose handling in female sheep who faced UN in early postnatal life persists until 2.5 years of age and is likely due to an increase in insulin sensitivity rather than secretion. Improved glucose utilisation may aid subsequent recovery of body weight, although it is unclear whether this strategy would be beneficial in the longer term.

Poore *et al.* (2003) *Ped Res* **53(6)**:12A Part 2 Suppl

Poore *et al.* (2004) *J Phys* **555P** C96

Supported by the British Heart Foundation

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C156

The renin-angiotensin system in adult sheep following moderate postconceptional undernutrition and undernutrition in early postnatal life

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The renin-angiotensin system (RAS) is a candidate mechanism linking altered nutrition in early life to cardiovascular dysfunction. Previously we have shown that in 1 year-old sheep the postnatal blood pressure response to frusemide is modified by nutrient restriction from conception to day 30 of gestation (Cleal *et al.*, 2003). In this study we investigated the effects of postcon-

ceptional and postnatal nutrient restriction on RAS function in adult sheep at 2.5 years of age.

Welsh Mountain ewes (UK Animals Scientific Procedures Act 1986) received 100% (group C, *n*=37) or 50% of global nutrient requirements (group U, *n*=40) from conception to day 30 of gestation, and 100% thereafter. Offspring were then fed either *ad libitum* (CC, *n*=20 and UC, *n*=19) or at a level that reduced body weight to 85% of individual target weight (predicted from 0-12 week growth trajectory) from 12-25 weeks postnatal age and *ad libitum* thereafter (CU, *n*=17 and UU, *n*=21). Each group contained approximately equal numbers of males and females. At ~2.5 years of age catheters were inserted into the carotid artery and jugular vein under general anaesthesia (3% halothane/O_2). RAS function was assessed using an angiotensin II bolus (0.05μg/kg; *i.v.*) and a frusemide challenge (5mg/kg; *i.v.*) and heart rate (HR), mean arterial blood pressure (MAP), diastolic (DBP) and systolic blood pressure (SBP) were monitored. Data (mean±S.E.M.) were expressed as area under the curve (AUC) and maximum response, and were analysed by ANOVA.

In males, but not females, postconceptional nutrient-restricted offspring (UC and UU) had a smaller MAP, DBP and SBP response to angiotensin II at 2.5 years of age ($P<0.05$) compared with controls (CC and CU). In addition, UC males had a greater decrease in HR in response to angiotensin II ($P<0.05$) compared with CC, which was not seen if they received a subsequent postnatal nutrient restriction (UU). Moreover, male offspring that received a postconceptional nutrient restriction (UC) had a greater maximum HR decrease and HR AUC in response to frusemide ($P<0.05$) compared with CC, but not if they also received a postnatal nutrient restriction (UU).

This study suggests that RAS mechanisms of cardiovascular control in adulthood are influenced by diet in early life. The sex-specific interaction between postconceptional and postnatal nutrition on heart rate responses to RAS challenge may indicate altered baroreflex mechanisms and could have consequences for renal/cardiovascular function in later life.

Cleal JK *et al.* (2003) *Ped Res* **53**: 18A

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C157

Unidirectional maternofetal calcium clearance across the placenta of the anaesthetised PTHrP knockout mice.

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Recent evidence from both humans and mice suggests parathyroid hormone related protein (PTHrP) is a key regulator of placental calcium flux. The PTHrP knockout mouse ($PTHrP^{-/-}$) suffers from severe skeletal dysplasia and neonatal morbidity (Karaplis *et al.*, 1994). Using a technique recently devised for artificially perfusing the fetal circulation of the mouse placenta (Bond *et al.*, 2004) we measured unidirectional maternofetal calcium clearance ($^{Ca}K_{mf}$) across placentas of $PTHrP^{-/-}$ versus $PTHrP^{+/+}$ and $PTHrP^{+/-}$ fetuses. Expression of the placental calcium binding protein, calbindin-D_{9k} was also determined.

Heterozygote mice were mated and on day 18 of gestation (term = 19-20d) mice were anaesthetised (i.p. fentanyl citrate/fluanisone: 24 and 750μg) and the uterus delivered into a bath of 40°C saline. A fetus was selected, the umbilical artery and vein catheterised and perfused with Krebs Ringer (pH 7.4) at 60 $\mu l.min^{-1}$. $^{45}CaCl_2$ (2μCi/50 μl saline) was injected via a maternal tail vein. Perfusate samples were collected every 5 mins for 45 mins. $^{Ca}K_{mf}$ was calculated as: perfusate [^{45}Ca] x perfusion rate/maternal plasma [^{45}Ca] x placental weight. Calbindin-D_{9K} protein expression was measured in placentas using Western blotting and signal measured in arbitrary density units. Results for the perfusion are expressed as the mean value over 45 mins as there was a tendency, although not significant, for $^{Ca}K_{mf}$ to decline over time in all genotypes. Results are shown in Table 1, Mean±SEM, n=number of litters.

$^{Ca}K_{mf}$ was significantly higher across the placentas of the $PTHrP^{-/-}$ relative to the $PTHrP^{+/-}$ and $PTHrP^{+/+}$ fetuses (***$P<0.001$, repeated measures ANOVA followed by Bonferronis post hoc). Calbindin-D_{9k} protein expression is possibly increased in the placentas from the $PTHrP^{-/-}$ fetus (*$P<0.07$, Kruskal-Wallis). These data provide further evidence that PTHrP has an important role in regulating calcium transport across the placenta and therefore in fetal development, thus emphasising the importance of direct measurements of the unidirectional components of net flux across the placenta.

All work was in accordance with the U.K. Animals (Scientific Procedures) Act 1986.

Table 1

Genotype	$PTHrP^{+/+}$	$PTHrP^{+/-}$	$PTHrP^{-/-}$
$^{Ca}K_{mf}$ (μl/min/g placenta)	125.3±10.0 (n=4)	114.9±10.9 (n=11)	197.6±13.2 (n=5)
Calbindin-D_{9k} (Signal Intensity, arbitrary units)	6.7±0.9 (n=9)	6.7±0.7 (n=9)	9.2±0.9 (n=9)

Karaplis AC *et. al.* (1994) *Genes Dev* **8**: 277-289.
Bond H *et. al.* (2004) *Placenta* **25**: A43.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C159

Nifedipine-insensitive calcium entry is associated with constriction of pressurised human chorionic plate arteries.

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Thromboxane A2 (TXA_2) is a potent constrictor of fetoplacental arteries but little is known of the electromechanical events of smooth muscle contraction in these vessels. We reported previously that constriction of chorionic plate arteries (CPA's) with U46619 (TXA_2 mimetic) is partially inhibited by nifedipine, a blocker of L-type voltage gated Ca^{2+} channels (VGCC's) (Cooper *et al.* 2003). In this study we measured $[Ca^{2+}]_i$ and vessel diam-

eter in pressurized CPA's to test the hypothesis that TXA_2-induced constriction can by mediated by Ca^{2+} influx through nifedipine-sensitive and -insensitive pathways.
CPA's (199±17μm) were dissected from placentae at term following informed consent, loaded with the Ca^{2+} indicator dye Indo-1 (2 hours, 21°C) and mounted on an arteriograph at 25mmHg. Simultaneous recordings of intraluminal diameter and $[Ca^{2+}]_i$ (400:500nm fluorescence ratio at 360nm excitation) were made at 37°C. The effect of 10^{-6}M U46619 or 60mM KCl (high-K) (5 min) was examined in the presence and absence of extracellular Ca^{2+} with and without 10^{-4}M nifedipine. Statistical analyses were performed with Kruskal-Wallis and Dunns post hoc tests with $p<0.05$ taken as significant.
High-K induced a maintained decrease in diameter of 10±4% and a concomitant increase in $[Ca^{2+}]_i$ (n=17;mean±SEM). Nifedipine significantly inhibited both responses by >95%. High-K had no effect on either vessel diameter or $[Ca^{2+}]_i$ in the absence of extracellular calcium. U46619 induced a 62±6% constriction (Fig.1B) and a peak increase in $[Ca^{2+}]_i$ followed by an elevated plateau, which were 51±11% and 50±10% of the high-K response respectively. Under Ca^{2+}-free conditions, U46619 caused a small but significant peak increase in $[Ca^{2+}]_i$ probably due to Ca^{2+} release from intracellular stores. Re-addition of Ca^{2+} induced a large increase in $[Ca^{2+}]_i$ and a sustained constriction (Fig 1A,B). Nifedipine did not alter the responses to U46619 when extracellular Ca^{2+} was absent. When Ca^{2+} was re-introduced, nifedipine significantly reduced, but did not completely prevent, the rise in $[Ca^{2+}]_i$ and constriction (Fig 1A,B).
We propose that constriction of human CPA's in response to U46619 is mediated by nifedipine-sensitive VGCC's and by nifedipine-insensitive Ca^{2+} entry.

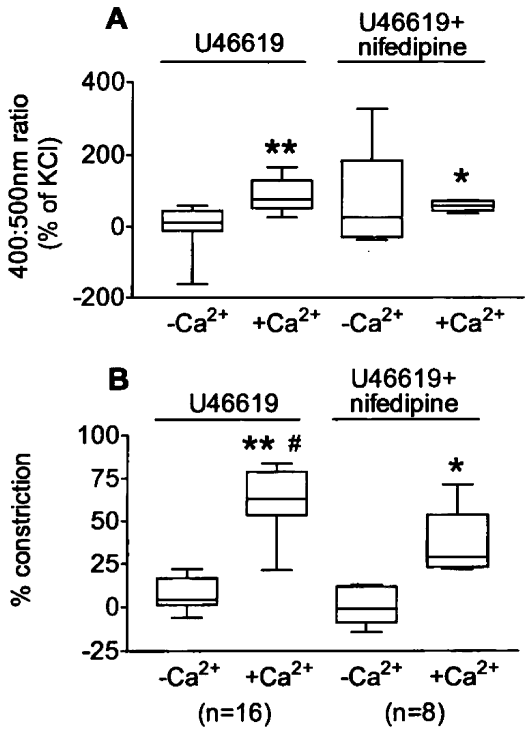

Figure 1. Effect of 10-4M nifedipine on (A) [Ca2+]i (% of the KCl-induced response) and (B) vessel constriction (% resting diameter) in response to U46619 (10-6 M) in the presence (+) and absence (-) of extracellular Ca2+. n=no of vessels. ** p<0.001, * p< 0.05 vs –Ca2+; # p<0.001 vs nifedipine.

Cooper EJ et al (2003). J. Soc. Gynecol. Invest. 10 (2) 210

Supported by Tommy's the Baby Charity

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C160

Characterisation of serine uptake into microvillous membrane vesicles of the human placenta

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On a molar basis, the human fetus requires more glycine than any other amino acid but direct placental transfer of glycine is not thought sufficient to meet fetal demand (Widdowson et al. 1979). $_L$-Serine is a major metabolic source of glycine for the fetal sheep (Chung et al. 1998) and this may also be true in human pregnancy. We have characterised the amino acid transport systems involved in the uptake of serine by the microvillous membrane of the human placental syncytiotrophoblast and compared the uptake rates to those of glycine.
Term placentas were collected within 30 min of delivery from women who had provided written informed consent. Using standard techniques (3,4), we prepared microvillous membrane (MVM) vesicles and measured uptakes of ^{14}C-$_L$-serine and ^3H-glycine into these vesicles at room temperature. $_L$-Serine uptakes were performed for 15 s (characterisation) or 20 s (kinetics) and glycine uptakes were performed for 60 s, times when uptake had been shown to be linear.
Na^+-dependent uptake accounted for 40 ± 4% (mean ± SEM, n=14) of $_L$-serine uptake, of which 29 ± 10% was attributable to system A (MeAIB inhibitable). Na^+-independent uptake accounted for 60 ± 3% of $_L$-serine uptake, of which 51 ± 3% was attributable to system L (BCH inhibitable). BCH inhibitable $_L$-serine uptake was inhibited by 1 mM $_L$-alanine, $_L$-threonine and $_L$-asparagine, which are substrates of LAT 2, but not LAT 1 or LAT 3, suggesting that the uptake is mediated by the LAT 2 isoform of system L.
Uptake kinetics were determined for $_L$-serine and glycine (see figure 1) and analysed using SimFit ver. 5.5 (www.simfit.man.ac.uk). Uptake of $_L$-serine into MVM vesicles had a V_{max} of 2.47 ± 0.20 nmol/mg/min and had a K_m of 290 ± 20 μM (mean ± SEM, n=3). Uptake of glycine into MVM vesicles had a V_{max} of 0.69 ± 0.24 nmol/mg/min and a K_m of 210 ± 32 μM (mean ± SEM, n=4). This indicates that MVM vesicles have a higher uptake capacity for $_L$-serine than for glycine.
This study indicates that MVM placental uptake capacity for $_L$-serine is substantially greater than for glycine, and that this serine uptake is primarily mediated by system L and system A. If placental transfer, as well as uptake, of $_L$-serine is also greater than for glycine then fetal conversion of serine to glycine may be a significant source of fetal glycine.

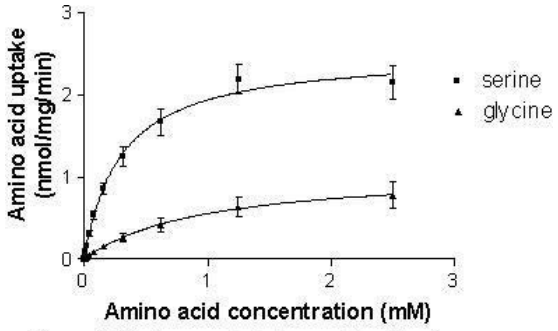

Figure 1, Serine and glycine uptake Kinetics into MVM vesicles. Data is Mean and SEM.

Widdowson EM, Southgate DAT, Hey NT. (1979). In: Visser HKA, (ed). Nutrition and metabolism of the fetus. The Hauge: Matinus Nigoff, 169-177.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C161

Gene expression and activity of System β in human placenta over gestation

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System β is a Na^+-dependent amino acid transporter specific for taurine and other β amino acids. It is encoded by the TauT gene which has been cloned from human placenta[1] and identified at protein level in the syncytiotrophoblast[2]. System β is functionally active in the placenta[3] and is responsible for transport of taurine from mother to fetus. Taurine is regarded as an essential amino acid during fetal growth because the human fetus has poor synthesis capabilities[4]. As pregnancy advances and growth of the fetus accelerates, demand for nutrient supply increases. Here we test the hypothesis that placental System β gene expression and activity increases over gestation in relation to the rising fetal requirement.

First trimester (6-13 weeks) and term (38-40 weeks) placentas were collected within 30min of delivery. In order to measure TauT gene expression, placental tissue was homogenised and total RNA extracted to generate the cDNA template for QPCR. TauT mRNA was measured using the intercalating dye SYBR Green 1, quantified from a Human Reference Total RNA standard curve and normalised to a term placental calibrator sample. System β activity was measured by incubating fresh villous tissue (2-3mm^3)at 37°C in Tyrodes solution (+/-Na^+;choline replaced Na^+) containing 30nM ^{3}H-taurine. After specified time periods, uptake was terminated by washing in ice cold Tyrodes. Fragments were lysed in distilled water and the lysate counted for radioactivity. Fragments were then dissolved in NaOH and assayed for protein content. To distinguish transport of taurine into the tissue by system β from non-specific accumulation, the difference between uptake in the presence and absence of Na^+ was calculated. This represents the Na^+-dependent component of taurine uptake by system β and is expressed in fmol per mg protein.

These results show that mRNA expression and Na^+-dependent activity of the System β amino acid transporter is present in first trimester placenta and is not different to that at term.

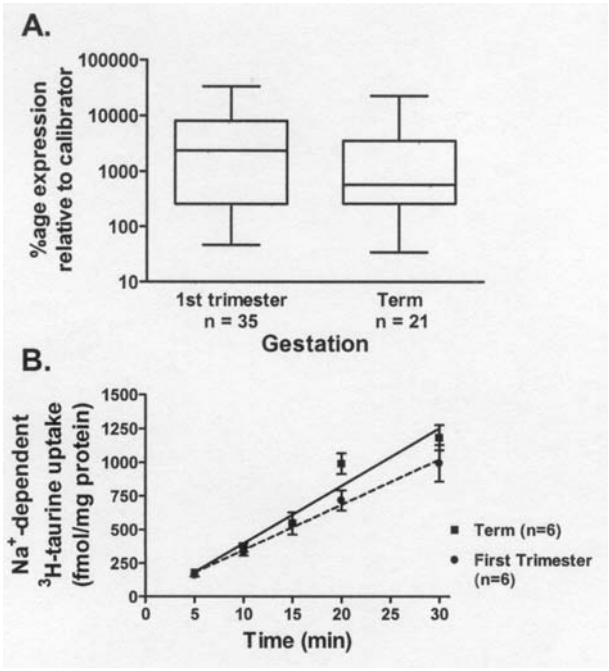

Figure 1 n = no of placentas A: TauT mRNA expression in first trimester and term placenta relative to the calibrator. Expression does not change significantly over gestation (2-tailed Mann Whitney test). B: Na+-dependent 3H-taurine uptake (mean ± SEM) is linear over 30min in first trimester (r2=0.69) and term fragments (r2=0.85) p<0.0001, least squares linear regression. The slopes of the two lines are not significantly different from each other indicating the rate of uptake does not change over gestation.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C162

Effect of maternal nutrient restriction from conception up to 95 days gestation on hepatic GH and PRL receptors and SOCS-3 mRNA abundance in the adult sheep

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Signalling through cytokine receptors, growth hormone (GHR) and prolactin receptors (PRLR), are dependent upon the activation of the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway. Suppressor of cytokine signalling-3 (SOCS-3) inhibits such cytokine signalling via a feedback loop. GHR and PRLR are nutritionally regulated *in utero* however; it is not known whether downstream signalling genes such as SOCS-3 are affected and what the long term consequences may be. The aim of this present study was to determine whether maternal undernutrition up to 95 days gestation compromises liver growth, GHR, PRLR and SOCS-3 mRNA levels in the resulting adult.

Eighteen singleton-male-bearing Scottish Blackface ewes of similar weight and body condition score were individually housed from day of mating. Eight control (C) ewes were fed 100% of total metabolisable energy requirements (ME) whilst ten nutrient restricted (NR) ewes consumed 50% of ME requirements up to 95 days of gestation. All ewes then consumed 100% of ME requirements up to term. Rams were humanely euthanased (100 mg kg^{-1} pentobarbital sodium: Euthatal, i.v) at 3 years of age, to enable liver tissue sampling. The abundance of GHR, PRLR and SOCS-3 to 18S rRNA was analysed by RT-PCR. Results are given as means and SEM in arbitrary units (a.u.), and expressed as a percentage of a reference sample. Statistical nutritional differences were analysed using either a T-test or Mann-Whitney U test ($p<0.05$).

There were no differences in growth, birth or body weights between C and NR offspring up to 3 years of age. However, NR livers were significantly smaller than controls (C 1.26 ± 0.04; NR 1.18 ± 0.03 kg ($p=0.016$)). GHR, PRL and SOCS-3 mRNA abundance were similarly reduced in NR offspring (C 22.2 ±5.2; NR 11.2 ± 2.5 a.u. ($p<0.05$); C 71.3 ± 16.8; NR 24.6 ± 8.0 a.u. ($p<0.01$); C 119.2 ± 8.3; NR 101.2 ± 2.3 a.u. ($p<0.001$)), respectively.

Maternal nutrient restriction from 0-95 days of gestation results in long term morphological and molecular adaptations in the liver of the adult male offspring. The persistence of hepatic programming of GH/PRL signalling into early adulthood emphasises the importance of adequate maternal nutrition in early fetal organogenesis.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C163

Effects of maternal protein deficiency at different stages of pregnancy on hepatic gluconeogenic enzyme activities in rat fetuses at term

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In rats, protein deprivation during pregnancy alters hepatic glucose handling in the adult offspring, in part by changing the activity of key enzymes involved in glucose metabolism (1). Our preliminary data suggest that these changes arise in utero in response to undernutrition (2). Since responsiveness of the hepatic glucogenic pathway to undernutrition increases towards term in fetal sheep (3), this study examined the effect of feeding rats a low protein (LP) diet for different periods of pregnancy on fetal hepatic activities of the rate-limiting glucogenic enzymes, glucose-6-phosphatase (G6Pase) and phosphenolpyruvate carboxykinase (PEPCK).

After mating (0 days), female Wistar rats were fed ad libitum either normal chow (20% protein, n=6) or a low protein diet (8% protein) for set periods from 0-10 days (n=6), 10-20 days (n=7), or 0-20 days (n=6). On day 20 (term 21 days), the fetuses were delivered for tissue collection under terminal anaesthesia. Fetuses and placentae were weighed and fetal livers frozen in liquid nitrogen for determination of G6Pase and PEPCK activities (4). Data are presented as mean (± SE) of litter means for each animal.

Maternal protein deprivation from 0-10 days of pregnancy had no significant effect on fetal or placental weights, or on hepatic G6Pase and PEPCK activities (Table 1). In contrast, fetuses of mothers fed LP from 10-20 days or 0-20 days had lower body weights and higher G6Pase and PEPCK activities than controls (Table 1). Neither hepatic protein content nor litter size varied significantly with dietary treatment.

These results show that undernutrition during the second half of gestation increases hepatic G6Pase and PEPCK activities in the fetal rat. This suggests that the critical period for prenatal nutritional programming of hepatic glucose metabolism may be the last 10 days of gestation in the rat.

Table 1: Morphometry and hepatic gluconeogenic enzyme activities of rat fetuses from mothers fed control or LP diets for different periods of gestation.

	Treatment Period	Weight		Activity (U/g wet weight)	
Diet	(Gestational days)	Fetus (g)	Placenta (mg)	G6Pase	PEPCK
Normal	0-20	3.83 ± 0.07^{a}	781 ± 48^{a}	1.21 ± 0.10^{a}	0.27 ± 0.02^{a}
LP	0-10	3.67 ± 0.08^{ab}	768 ± 48^{a}	1.48 ± 0.15^{ab}	0.31 ± 0.02^{ab}
	10-20	3.54 ± 0.05^{bc}	659 ± 28^{ab}	1.72 ± 0.05^{b}	0.35 ± 0.02^{c}
	0-20	3.35 ± 0.04^{c}	574 ± 22^{b}	1.76 ± 0.15^{b}	0.32 ± 0.01^{bc}

Within each column, values with different letters are significantly different from each other (One Way ANOVA, $p<0.05$).

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C164

Maternal dietary restriction during pregnancy in the rat alters hepatic gene expression in the offspring by a promoter methylation-dependent mechanism

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Nutritional constraints during gestation result in persistent phenotypic changes to the offspring which are associated with increased risk of chronic non-communicable disease (Bateson et al. 2004; Gluckman & Hanson 2004). The molecular mechanism(s) underlying this process is not known. We investigated the effect of reduced maternal protein intake during pregnancy on the regulation of hepatic transcription factors that control lipid metabolism in the offspring.

Female Wistar rats (n = 5 / group) were fed diets containing 18%(w/w) or 9%(w/w) protein (both with 1mg/kg folic acid), or 9%(w/w) protein + 6mg/kg folic acid (9%F) from conception until delivery, and chow throughout lactation. Pups were weaned at 28 days and humanely killed at 34 days. One liver was analysed per litter. The methylation status of the peroxisomal proliferator activated receptor (PPAR) α and γ promoters was determined by real time PCR after digestion with Aci1 (Pham et al. 2003). PPARα, γ and acyl-CoA oxidase (AOX), a PPARα target gene, mRNA expression was determined by RTPCR (Burdge et al. 2004).

The 9% protein diet decreased hepatic PPARα promoter methylation (33.7%, $p<0.001$) and increased PPARα mRNA expression (854%, $p<0.0001$) in the offspring compared to controls. AOX expression was increased (317%, $p<0.001$) in the 9% group. There were no significant differences between the 18% group and the 9%F group. The methylation status of PPARγ1 and PPARγ mRNA expression did not differ between groups.

These results show that maternal protein restriction during pregnancy altered the regulation of PPARα expression by decreasing the methylation of promoter sequence. The increase in AOX expression is consistent with up-regulation of PPARα activity, and suggests greater peroxisomal β-oxidation and dysregulation of fatty acid metabolism. The absence of changes to PPARγ expression suggests the effects of prenatal under-nutrition are gene-specific. Prevention of these effects by folic acid shows that altered 1-carbon metabolism is central to the phenotypic changes associated with the restricted diet.

These observations provide one causal mechanism linking maternal nutrition during pregnancy, regulation of gene expression and altered phenotype in the offspring.

Bateson P et al. (2004) Nature 430, 419-421.

Gluckman PD and Hanson M.A. (2004) Science 305, 1733-1736.

Pham TD et al. (2003) Am J Physiol Regul Integr Comp Physiol 285, R962-R970.

Burdge GC et al. (2004) Nutr Res 24, 639-646.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C165

Developmental programming of aortic dysfunction by maternal fat-feeding does not persist to the second generation.

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We previously reported reduced aortic elasticity and endothelium dependent vasodilatation in six month old offspring of rats fed a diet rich in animal fat in pregnancy and suckling (Armitage *et. al.*, 2004). Here we examine aortic function in second-generation offspring to assess whether deficits are transgenerational. Sprague Dawley rats were fed a fat rich diet (20% animal lard and 5% corn oil w/w) or a control diet (5% corn oil w/w) for 10 days prior to mating, pregnancy and during suckling. Offspring (from control OC, and fat fed dams, OHF) were weaned at 21 days of age and fed a standard rat chow (RM1, SDS, UK) to 110 days of age then OC and OHF were fed a standard breeding diet (RM3, SDS, UK) for 10 days prior to mating, during pregnancy and suckling. Second generation offspring (OC2 and OHF2, n=14 per group, equal ♂and ♀) were weaned at 21 days of age and fed RM1 until six months of age then humanely sacrificed (rising CO_2 concentration). Thoracic aorta was dissected, cut into rings of 2.5mm length and mounted on an organ bath in physiological salt solution (PSS) at 37°C with a basal force of 5mN. Vessels were subjected to a run-up procedure, and dose response curves to phenylephrine ($3x10^{-9} – 10^{-5}$ M), ACh ($3x10^{-9} – 10^{-5}$ M, with and without 10^{-3} M L-NAME), and nitric oxide ($10^{-8} – 10^{-4}$ M) were conducted. PSS was then replaced with Ca free PSS and vessels subjected to a length- force curve (cumulative 500, 200, 50 μm). Dose response curves were analysed by RM ANOVA and fitted EC_{50} values by ANOVA. For all analyses, gender was incorporated as an independent variable in the initial model but removed if not significant.

Bodyweights did not differ between OC2 and OHF2 groups, however brain and kidney mass were increased in OHF2 compared with OC2 animals ($P<0.02$). There was no difference in aortic contractility to phenylephrine (RMANOVA, $P<0.45$, EC_{50} OC2 -7.9±0.1 vs OHF2 -7.3l±0.1, $P<0.09$). ACh response was not different between groups (RMANOVA, $P<0.1$, EC_{50} OC2 -7.5±0.1 vs OHF2 -7.6±0.1, $P<0.2$). Nitric oxide responses did not differ between groups (RMANOVA, $P< 0.09$, EC_{50} -6.7±0.2 vs -6.6±0.3, $P<0.9$). Length-force curves did not differ between groups (RMANOVA, $P<0.68$).

There were significant differences in some organ weights but otherwise second-generation offspring off fat fed dams did not show any alteration in aortic function. This lack of significance was not due to insufficient power and suggests that the vascular deficits seen in the first generation offspring (Khan *et. al.*, 2003) are not transmitted across further generations.

Armitage JA *et.al.* (2004) *J Soc Gynecol Invest.* **11**,183.

Khan IY *et.al.* (2003) *Hypertension* **43**,168-173.

British Heart Foundation, Tommy's the Baby Charity

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC173

Potassium channel gene expression over gestation in human placenta

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The syncytiotrophoblast facilitiates exchange of ions between the maternal and fetal blood and expresses K^+ channels at the mRNA and protein level[1]. One of the functions of K^+ channels is to set syncytiotrophoblast membrane potential and cell excitability which affects electrochemical gradients and electrogenic nutrient transport across the placenta. Over the course of pregnancy, the syncytiotrophoblast microvillous membrane potential changes, being more negative in first trimester than at term[2]. At around 10-12 weeks maternal blood flow in the placenta is established, resulting in increased oxygenation, which may affect oxygen sensitive K^+ channels. This study, therefore, examined how the expression of six K^+ channels (TASK1, TASK2, BKCaα, BKCaβ, Kv1.5, Kir2.1), some with known oxygen sensitivity, alters with placental development across gestation.

First trimester placentas (6-9 and 10-13 weeks) were collected with local ethical approval following written informed consent, from medical and surgical terminations performed for socioeconomic reasons, and placentas from normal vaginal and Cesarean section deliveries at term. Tissue sampling, total RNA isolation and cDNA production were prepared as described previously[3]. K^+ channel expression across gestation was quantified using Sybr Green 1 quantitative PCR. The six K^+ channels and β actin sequences were derived from GenBank with reference to BLAST assessment and primers designed with Beacon Designer software. 1μl of each placental sample was run as triplicate reactions with a passive reference dye. To quantify expression sample cycle threshold values were used to calculate initial input amounts using a standard curve constructed from cDNA generated from quantitative human reference RNA and normalised to a human reference cDNA calibrator sample. Gene expression is presented as percentage expression relative to calibrator. Of the genes examined only TASK2 and BKCaβ transcription are gestation dependent, showing upregulated transcription in late first trimester in relation to term (Figure 1). These data therefore provide the basis for functional studies on these channels in relation to placental development.

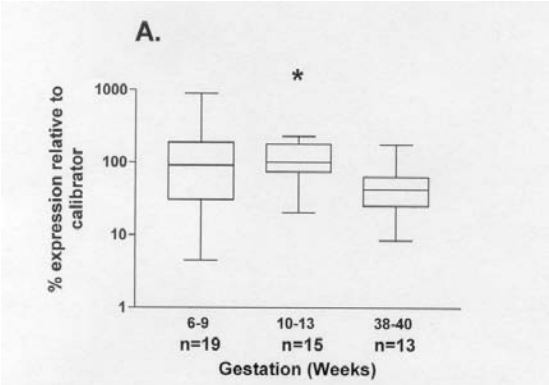
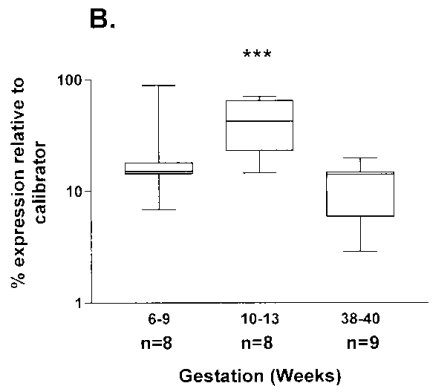


Figure 1 A. TASK2 relative mRNA expression in first trimester and term placenta. Expression is significantly higher at 10 -13 weeks compared with term placentas (Kruskal -Wallis, with Dunn's post hoc test * P<0.05).



B. BKCaβ relative mRNA expression in first trimester and term placenta. Expression is significantly higher at 10 -13 weeks compared with term placenta (Kruskal -Wallis, with Dunn's post hoc test *** P<0.001).

Bai *et al* 2004 *J Soc Gynecol Investig* (In press).

Birdsey *et al* 1997 *Am J Physiol.*; **273**: R1519-28.

Lacey *et al* 2004 Placenta (In press)

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC174

The expression of rnd3, a constitutively active GTP-binding rho family protein, in myometria isolated from non-pregnant and pregnant humans.

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The rho family of small G-proteins are postulated to be involved in the generation of uterine contractions associated with successful parturition. For instance, pharmacological inhibition of rho-associated kinase (ROK), a downstream effector of rhoA, reduces agonist-induced Ca^{2+}-sensitisation and contractility of myometrial strips *in vitro* (Oh *et al.* 2003; Moran *et al.* 2002). Recent reports suggest that constitutively active members of the rho family, termed rnd1 or rnd3, may impede Ca^{2+}-sensitisation during pregnancy and that rnd3 protein, and rnd1 mRNA, are up-regulated at mid- to late gestations in rabbit and rat myometria respectively (Kim *et al.* 2003; Cario-Toumaniantz *et al.* 2003). However, the expressions of rnd proteins in human myometria have yet to be determined. We, therefore, examined the content of rnd3 protein in myometrial biopsies isolated, following written informed consent, from women undergoing hysterectomy (non-pregnant; NP) or Caesarean section at term (37-39 weeks) not in labour (PNL), in spontaneous labour (SL) or in oxytocin-stimulated labour (OT). Such biopsies were processed for SDS-PAGE/Western blotting with anti-rnd3 monoclonal antibody (1:500; Upstate) and anti-α-actin monoclonal antbody (1:100,000; Sigma) and ECL signals quantified by densitometry. Myometrial α-actin content remained invariant during late pregnancy and labour compared to NP. In contrast, rnd3 expression, when correlated to α-actin, increased significantly near to term: the rnd3:α-actin ratio for myometria from PNL women was 1.89±0.35-fold that of corresponding values from NP tissues ($P\langle 0.05$, unpaired t-test; mean ± S.E.M., n=4). Rnd3 levels were elevated further in myometria from labouring women: rnd3:α-actin ratios were raised in tissues from SL and OT to 2.07±0.09-fold and 1.93±0.30-fold, respectively, of myometria from PNL women (n=4). This data suggests that late pregnancy, and labour onset, is associated with elevated myometrial expression of rnd3. This appears counter-intuitive unless rnd3 has a functional role(s) that (i) is in addition to that suggested previously of limiting Ca^{2+}-sensitisation of contraction and/or (ii) is different in human myometrium than so far proposed for rnd proteins in rat (Kim *et al.* 2003) or rabbit (Cario-Toumaniantz *et al.* 2003) myometrium.

Oh JH *et al.* (2003). *J Vet Med Sci* **65**, 43-50.

Moran CJ *et al.* (2002). *Mol Hum Reprod* **8**,196-200.

Kim Y-S *et al.* (2003). *Biochem Biophys Res Commun* **311**, 972-978.

Cario-Toumaniantz C *et al.* (2003). *J Physiol* **552**, 403-413.

Supported by Tommy's, the Baby Charity [No. 1060508].

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC175

Calcium Channels from Syncytiotrophoblast Basal Membrane

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As in other transport epithelia, Ca^{++} plays multiple roles in human placental syncytiotrophoblast, regulating diverse cellular processes and at the same time being transported from mother to fetus. Epithelial Ca^{++} transport and membrane signalling functions rely, among others, on action of transporters and ion channels; functional expression of the latter has been scarcely studied in syncytiotrophoblast owing to difficulties in membrane access with electrophysiological techniques. Thus, our laboratory has worked on the characterization of Ca^{++}-permeable ion channels through reconstitution of purified membranes in giant liposomes suitable for patch-clamp. Having formerly detected a non-specific cation channel allowing Ca^{++} permeation in the apical membrane of healthy, term human placentas, we have presently sought to characterize calcium currents present in the basal membrane. Highly purified membranes were obtained through a double protocol of apical and basal membrane isolation, which includes differential centrifugations, basal membrane precipitation with $MgCl_2$ and band separation through sucrose step gradients. Purified basal membranes were reconstituted through a dehydration-rehydration cycle in 10-50 μm liposomes, which were subjected to electrophysiological recordings. Western blots of apical and basal purified membrane fractions using polyclonal antibodies against three different calcium channels were performed to support our electrophysiological findings.

In 5 of 13 experiments using symmetric conditions (140 mM KCl or K gluconate), we detected the presence of channels with high permeability to Ca^{++} (PCa^{++}/PK^{+} up to 99.5). We proceeded to work in barium gradients (40 mM $BaCl_2$ pipette, 0.1 mM $BaCl_2$ bath), detecting barium-conducing channels in all of the experiments (n = 30). Barium total patch currents were consistently blocked by addition of $NiCl_2$, Nifedipine or Ruthenium Red to the bath, with approximate EC50 concentrations of 500 μM (n=8), 1 μM (n=5), and 0.5 μM (n=3), respectively. Western blots using polyclonal antibodies against the α subunit of voltage-gated calcium channels, and against TRPV5 and TRPV6 show the presence of these proteins in both basal and apical membrane fractions. These results are consistent with those of other laboratories, which have detected the expression of both TRPV calcium channels (blocked by Ruthenium Red) and L-type voltage-gated calcium channels (blocked by Nifedipine) in human placental tissue and cell lines. These basal membrane calcium channels would not be directly involved in mother-to-fetus directed calcium transport, but could participate in diverse other relevant trophoblast processes, such as exocytosis and calcium transport regulation.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC176

NITRIC OXIDE (NO) ROLE IN PULMONARY CIRCULATION DURING HYPOXAEMIA IN HIGHLAND AND LOWLAND NEONATAL LLAMA

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NO is an important mediator in the decrease of the pulmonary vascular resistance (PVR) at birth. Since chronic hypoxia modifies the expression and activity of the endothelial nitric oxide synthase, we hypothesise that NO plays a vital role in the control of the pulmonary circulation in high altitude newborn llamas (HA), a highland adapted species. We instrumented 7 low altitude llamas (LA) (10-12 days old; 15 ± 1 kg, 580 m) and 6 HA (8-11 days old; 11 ± 1 kg, 4,600 m). Under general anaesthesia (ketamine 10 mg/kg I.M.), we placed polyvinyl catheters into femoral artery and vein and a Swan Ganz catheter into the pulmonary artery. The studies commenced 3 days after surgery. All experiments were based on a 3 h protocol divided in four periods: 45 min of basal (B), 15 min of B + L-NAME (B+I), 1 h of hypoxaemia (H+I) (PO2: 33 ± 2 mmHg) and 1 h of recovery (R). The L-NAME infusion (20 mg x kg-1 bolus + 0.5 mg x kg-1 x min-1 infusion, I.V.) started 15min before hypoxaemia (N+I) until the end of H+I. We performed vehicle studies in which we injected 0.9% NaCl with no significant changes due to the infusion. We measured pH, PO2, PCO2, % saturation of haemoglobin (Hb) and Hb concentration in descending aorta and pulmonary artery. Systemic arterial pressure (SAP), heart rate (HR), pulmonary arterial pressure (PAP) and cardiac output (CO, by thermodilution) were measured. Systemic vascular resistance (SVR) and PVR were calculated. Data was expressed as means + SE and analysed by two-way ANOVA and Newman-Keuls test (*p<0.05).

Basally, HA had a lower PO2 (52±3* *vs* 95±4 mmHg), PCO2 and %sat Hb than LA. In the LA and HA, HR and CO decreased during B+I. CO remained low during H+I and R. Furthermore, an increase in SAP and SVR was observed during B+I, H+I and R in both groups. In contrast, PAP (17±2 *vs* 23±2* mmHg) and PVR increased in B+I only in HA. In addition both PAP and PVR increased importantly during H+I and returned to B+I values in R in both groups.

The major circulatory changes observed in highland neonatal llama after L-NAME, during basal and acute hypoxaemic conditions, suggest that NO plays a vital role in the control of the pulmonary and systemic circulation in chronic hypoxaemic newborns. In contrast, in the lowland neonatal llama NO has only a role in the pulmonary circulation during hypoxaemia.

Animal procedures approved by the University of Chile Ethical Committee.

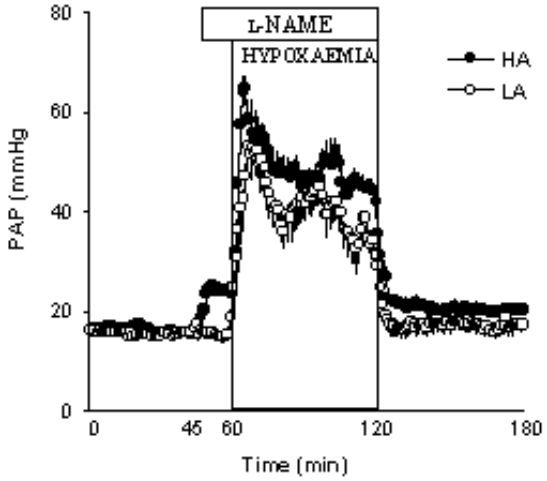

Fig 1: PAP during hypoxaemia in highland (HA) and lowland (LA) newborn llama. p<0.05: * HA vs LA; x HA vs basal; xx LA vs basal.

FONDECYT 1010636 and The Wellcome Trust CRIG 072256.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC177

ADRENOCORTICOMEDULLAR RESPONSE TO ACUTE HYPOXEMIA IN CHRONIC HYPOXEMIC NEWBORN LAMBS

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Acute hypoxaemia increases cortisol and catecholamine plasma concentration in fetal sheep. Chronic hypoxia of pregnancy at high altitude blunts fetal cortisol response to an ACTH challenge (Harvey LM et al. 1993) and increases the noradrenergic contribution compared to lowland fetal sheep. However, it is unknown the effect of chronic hypoxaemia of pregnancy on these responses in highland newborn lambs. The aim of this study was to determine ACTH, cortisol and catecholamine plasma concentration during basal and acute hypoxaemic conditions in lambs which have undergone gestation at high and at low altitude.

Under general anesthesia (ketamine 10 mg/kg i.m.), 5 lambs born at low altitude (8±1 days old; 6±1 kg, 580 m ; LA) and 6 at high altitude (12±2 days old; 5±1 kg 3,580 m; HA), were chronically catheterized with femoral vascular catheters. Three days after surgery, the lambs were submitted to experiments based on a 3h protocol: 1h basal (B), 1h of acute hypoxemia (H; PaO2 32±1

mmHg) and 1h of recovery (R). Blood samples were obtained to measure pH, PO2, PCO2 values and ACTH, cortisol (RIA), and catecholamines plasma concentrations (HPLC). Data are expressed as means±SEM and differences were tested using two way ANOVA for repeated measures followed by the Newman-Keuls test (*P<0.05).

The results showed that HA lambs have a marked decrease in plasma cortisol (ng ml^{-1}) basally and during acute hypoxemia (B 18±3*; H 32±8*; R 24±7) compared to LA (B 64±12; H 107±36; R 29±7). Furthermore, there was a lack of correlation between ACTH and cortisol plasma concentrations in HA, correlation that was significant in LA. Noradrenaline (pg ml^{-1}) showed a substantial increase in HA (B 2849±499*; H 4926±1270*; R 4334±1091*) compared to LA (B 801±80; H 1176±407; R 953±172). In contrast, plasma adrenaline did not show any differences between HA and LA

These data suggest that chronic hypoxaemia during fetal and neonatal life blunts adrenocortical response in highland lambs. Moreover, the pronounced stimulation of the noradrenergic system observed in chronic hypoxaemia not only in fetal but also in postnatal life may have consequences for cardiovascular health in later life.

All animal procedures approved by the Ethical Committee, Faculty of Medicine, University of Chile.

Harvey LM et al. (1993). Am J Physiol 264:E741-E747

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC178

ELECTROCORTICOGRAM, CAROTID BLOOD FLOW AND BRAIN TEMPERATURE DURING 24H OF PROLONGED HYPOXAEMIA IN THE LLAMA FETUS

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The llama (Lama glama) is adapted to live in the low oxygen milieu of the Andean altiplano. The ability of the llama fetus to grow and develop successfully in this adverse environment suggest very efficient adaptive mechanisms to withstand hypoxia. The llama fetus responds to acute hypoxaemia with a reduced increase of brain blood flow and a substantial reduction of the brain O2 consumption (Llanos et al, 2003). These observations are consistent with a marked cerebral hypometabolism in the hypoxaemic fetal llama as a strategy to preserve the CNS integrity. We hypothesise that the llama fetus brain responds to prolonged hypoxaemia with some degree of hypometabolism, decreasing the Electrocorticographic activity (ECoG)and Brain Temperature (BT). Therefore, we studied the brain ECoG, the BT and carotid blow flow (CBF) in llama fetuses subjected to a prolonged hypoxaemic period of 24h.

Eight llama fetuses (5 normoxaemic (NG; maternal FiO_2 0.21), 3 hypoxaemic (HG; maternal FiO_2 0.1)), between 0.6—0.8 gestation, were chronically instrumented under maternal and fetal general anaesthesia (sodium thiopentone 10—12 mg kg-1 i.v.; 1% inhalatory halotane). Approval was obtained from the local Ethics Committee. In the fetuses, we placed a transonic flowmeter in the common carotid artery, bipolar electrodes in the parietal duramater, and a thermistor in the brain parietal cortex and central arterial and venous catheters. Arterial blood samples were obtained every hour; CBF, ECoG and BT were registered continuously during 24h in hypoxaemic and normoxaemic fetuses. The PO2 in the HG decreased when compared with the NG (13.2±0.8 vs. 18.6±0.8 mmHg, $p< 0.05$) with no changes in pH or PCO2. The CBF increased after 8h of the experimental protocol in the HG (29±5 vs. 14±1.0 ml min-1 Kg-1, $p< 0.05$), while BT tended to decrease progressively in this group The fetal ECoG showed a tendency to increase the percent of time in high voltage-low frequency state during hypoxaemia, state which has a lower brain O2 consumption.

The increase in carotid blood flow in the hypoxaemic fetuses, could be the result of a rise in the brain-stem blood flow, whilst the brain cortex hypometabolism could be suspected by the tendency to drop in brain temperature and to increase in the percent of time in high voltage-low frequency state, even when differences did not reach significance. These findings suggest that the brain of the llama fetus adapts very effectively to a prolonged episode of hypoxaemia.

Llanos et al. (2003). High Alt Med Biol 4:193-202.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC179

Reliable Detection of β–Actin Transcript from Human RNA Samples Contaminated with Genomic DNA

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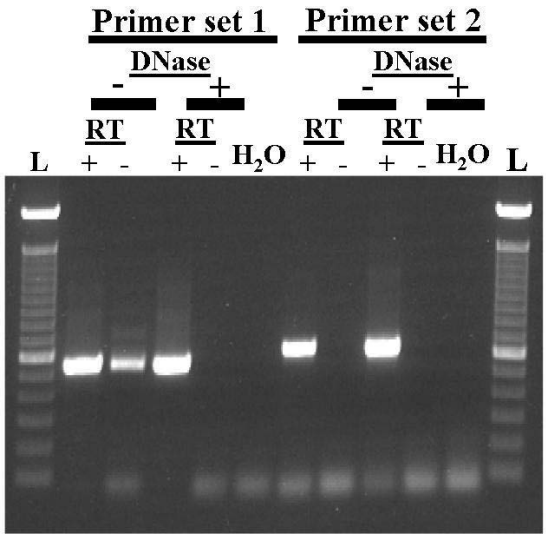
β–actin is often favoured as a reference gene in transcript expression studies. However, when using human samples, e.g. placental or uterine biopsies, the small tissue mass tends to give low total RNA yields that are further reduced by DNaseI treatment to remove genomic DNA. A further problem if not DNaseI–treating such samples is the possible co–amplification of processed pseudogenes from genomic DNA. Therefore, in RT–PCR analysis of small human uterine smooth muscle samples, we have compared the efficacy of traditional exon–intron spanning primers for β–actin (Primer set 1: forward 5′–ggg acc tga ctg act acc tc–3′ and reverse 5′–act cgt cat act cct gct tg–3′; Dye *et al.* 2004), with and without DNaseI treatment, to primers suggested to only recognise reverse transcribed β–actin and not pseudogenes (Primer set 2: forward 5′–cct cgc ctt tgc cga tcc–3′ and reverse 5′–gga tct tca tga ggt agt cag tc–3′; Raff *et al.* 1997). Total RNA was extracted from human myometrial biopsies, obtained at Caesarean section following written informed consent, using Trizol. RNA samples (n=3) were either digested with DNaseI or left untreated. cDNA synthesis was performed in the presence (RT+) and absence (RT−) of reverse transcriptase and PCR performed using both sets of β–actin primers.

The exon–intron spanning primers of Dye *et al.* (2004) produced signals of similar size in both the (RT+) and (RT–) lanes in DNaseI–untreated samples indicative of the amplification of pseudogenes (Fig 1). DNaseI–treated samples only produced signals in (RT+) lanes indicating successful removal of genomic DNA from the total RNA. In contrast, the β—actin primers of Raff *et al.* (1997) only produced signals in (RT+) lanes even in those samples contaminated with genomic DNA (Fig. 1). This confirms the usefulness of these primers in only detecting reverse transcribed β–actin product, and not any coamplified pseudogene product, from human uteri. This is of particular use when analysing β–actin RNA from small amounts of precious starting material that one doesn^{t} wish to exhaust with additional purification procedures. However, in such DNaseI–untreated human samples it will remain important, for detecting other genes of interest containing more than one exon, to design primers that span exon–intron junctions.

Fig. 1. Comparison of RT–PCR with 2 β–actin primer sets for human myometrial RNA samples ±DNaseI treatment. RT =reverse transcriptase, L = DNA 100–2000 bp Ladders. –ve control = H_20.



Raff T *et al.* (1997). Biotechniques **23**, 456-460.

Dye JF *et al.* (2004). FASEB J. **18**, 125-127.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC180

Capacitative calcium entry currents are enhanced by interleukin-1β in cultured human myometrial smooth muscle cells

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Pro-inflammatory cytokines are implicated in the cascade of events leading to human parturition. We hypothesise that cytokines induce changes in key calcium homeostatic mechanisms, which in turn augments myometrial contractility prior to labour. We have recently demonstrated using fura-2 and fluorescent digital imaging that capacitative calcium entry (CCE) is present in primary cultured human myometrial smooth muscle (HMSM) cells, and is amplified by treatment with interleukin-1β (Tribe et al., 2003). The aim of the present study was to characterize the channel currents associated with CCE in these cells. HMSM cells were isolated from myometrial biopsies obtained from women undergoing caesarean section at term (non-labour, 38-41 weeks gestation). Cells were maintained in primary culture until confluence was reached (in DMEM with 5% fetal calf serum) and then either serum-deprived (0.5% fetal calf serum) for 48 h (controls) or serum-deprived for 24 h and treated with IL-1β (10 ng/ml) for a further 24 h. Treated and control cells were isolated using a cell dissociation solution containing EDTA and CCE currents recorded using the whole cell voltage clamp technique. The membrane potential was clamped at −70mV and currents activated by cyclopiazonic acid and thapsigargin were continuously recorded using a CsCl2 (mM 135 CsCl2, 10 HEPES, 3ATP, 1MgCl2, 5TEA, 10 EGTA, 2CaCl2, 14-AP) pipette solution. Neither K+ nor L-type calcium channels were active under these conditions.

In control HMSM cells, inhibition of SERCA pumps activated a noisy inward current at −70mV in 25% of cells, which was abolished by replacing extracellular sodium with NMDG. In IL-1β treated cultured human myometrial smooth muscle cells, on the other hand, the current was detected in the majority of cells examined and Na+ removal led to only a 50% decrease in current amplitude instead of a complete block. Lanthanum rapidly inhibited this current in both control and IL-1β-treated cells. These results suggest that IL-1β alters the functional expression of CCE currents to promote calcium influx in HMSM cells, and that this pathway may contribute to the preparation of the uterus for labour.

Tribe R M et al. (2003), Biology of Reproduction 68, 1842-1849

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC181

Immunohistochemical follow-up and functional correlation of human trophoblast microvillous membrane transplantation to the plasma membrane of *Xenopus laevis* oocytes.

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Foetal growth and development is dependent on transport functions of syncytiotrophoblast. The placental transfer involves specific transport mechanisms through both apical (maternal-facing) and basal (foetal-facing) plasma membranes.

We have developed strategies to investigate the biophysical characteristics of ionic channels involved in transplacental transport by electrophysiological methods. A useful approach is the transplantation of purified human trophoblast plasma membranes to the membrane of *Xenopus* oocytes, allowing the study of macroscopic currents. Our previous and present results indicate

that injection of apical membrane vesicles bearing ionic channels into *Xenopus* oocytes results in their functional incorporation. However, we do not have information about the temporal and spatial distribution of proteins after injection. The aim of this study was to explore the structural integration of placental proteins into the oocyte plasma membrane. We correlate oocyte macroscopic currents with immunofluorescent staining with anti human placental alkaline phosphatase monoclonal antibody (anti-hPLAP).

Xenopus laevis oocytes were injected with purified apical membrane obtained by differential centrifugations and sucrose gradients. Injected oocytes were fixed in absolute methanol (-20oC) at different times after injection (0-24 h). Non injected and oocytes injected with buffer were used as controls. Paraffin embedded 15µm sections were used for immunofluorescence technique with anti-hPLAP. Control oocytes did not show, neither in plasma membrane nor cytoplasm, a specific labeling. In contrast, an evident peripheral distribution was observed at 16 h post injection in the oocyte plasma membrane domain. Oocytes at initial times (0-8 h) after injection had a cytoplasmatic labeling near the injection site. Currents from injected and non-injected oocytes were monitored by a two-electrode voltage clamp system within 16-24 h post injection. Similar to our previous results (Ivorra et. al, 2002), the elicited macroscopic currents from injected oocytes become larger than those of controls.

These results confirm a correlation between electrophysiological findings of placental channels in the oocyte membrane and immunohistochemical follow-up of a specific placental membrane protein. The conclusion from our study is that the channels, already assembled in the syncytiotrophoblast membranes, can be successfully transplanted to the oocyte membrane. This method allows access to the electrophysiological study of human normal and pathological placental transport.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC182

Maxi chloride channel from human placenta: Is it a voltage dependent anion channel family member?

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For over a decade we have been characterizing a Maxi Cl^- channel from human placental syncytiotrophoblast apical membrane with distinct biophysical properties: conductance over 200 pS, multiple substates, voltage dependent open probability, and permeation to anionic amino acids. The large unitary conductance and bell-shaped voltage dependence are similar to those of the voltage-dependent anion channel (VDAC) expressed in outer mitochondrial membrane. A growing body of evidence pointing to the expression of VDAC isoforms in the plasma membrane, has suggested that VDAC is the molecular correlate of the Maxi Cl^- channels in many cell types, such as rat astrocytes, PC12 cells and C1300 cells. Recently a Maxi Cl^- channel in C127i cells has also been described to resemble mitochondrial VDAC with respect its dual ATP-conducting and ATP-open channel blocking properties.

The aim of this study was to investigate if the Maxi Cl^- channel of human syncytiotrophoblast apical membrane belongs to the VDAC family.

The presence of VDAC was demonstrated by confocal microscopy immunocolocalization in apical syncytiotrophoblast membrane of villous trophoblast tissue sections and by Western blotting in purified apical membrane from human placenta. A functional relationship between the apical Maxi Cl^- channel and VDAC was demonstrated using the excised patch electrophysiological technique. We demonstrated that the apical membrane Maxi Cl^- channel, reconstituted in giant liposomes suitable for patch clamp methods, was permeable to ATP ($P_{ATP}/P_{Cl} = 0.05 \pm 0.006$, mean ± S.E.M.; n=4) and that the chloride current was also blocked by ATP with a Kd of 11.4 ± 1.8 (mean ± S.E.M.; n=3), results similar to those previously reported in C127i cells. In purified apical membranes reconstituted with and without (control) anti-VDAC antibody, Maxi Cl^-channel activity was detected in 83% of patches in the control condition, but only in 7.7% of patches from liposomes incubated with monoclonal antibodies.

Finally, we analysed a human placental cDNA sequence highly homologous to a voltage-dependent anion channel, recently published by NCBI, to search for open reading frames (ORF) in a 5′ sequence upstream of the protein sequence. We identified an ORF that translates a leader sequence peptide determining a secretion pathway for the protein, like that previously described for a mouse VDAC gene. We demonstrated that the human trophoblast expresses a plasmalemmal VDAC isoform by RT-PCR using primers that hybridize to a VDAC sequence coding for an N-terminal leader peptide required for its plasma membrane sorting.

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PC183

Na,K-ATPase and voltage-gated Na channels after 24 h hypoxaemia in the fetal llama brain

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Neuronal ion gradient is ATP dependent and hypoxia leads to rapid brain ATP depletion, depolarization and damage. The llama (Lama glama) is an animal tolerant to hypoxia. The fetal llama (FLL) brain responds to acute hypoxaemia with a little vasodilatation and a reduction of O2 consumption without developing seizures (Llanos, 2003). These observations suggest a brain

hypometabolism in the hypoxaemic FLL as a strategy to preserve neurone integrity. We studied if FLL brain responds with a co-ordinated reduction of Na,K-ATPase and voltage-gated Na (NaVg) channels as a neuroprotective strategy upon 24 h hypoxia. Eight FLL at 70±3% of gestation were chronically instrumented (with Ethics Committee approval) under general anaesthesia (i.v. 10 mg kg-1 sodium thiopentone, inhaled 50% N2O-1% halotane). Polyvinyl catheters were placed into the fetal abdominal aorta, inferior vena cava and amniotic cavity. Experiments began after 3 days of post-operative recovery. Three FLL were submitted to 1 h normoxia, followed by 24 h hypoxia (HF). Fetal hypoxia was obtained by reducing maternal FiO2 with a N2/CO2/air mixture. Five FLL were studied in normoxia (CF). pH and blood gases were monitored. Caesarean delivery and killing of the FLL was done immediately after the end of recording. Brain cortex was used for Na,K-ATPase activity as paranitrophenol formation and ouabain binding. Immunoblot and RT-PCR measurements for poly-ADP-ribose-polymerase (PARP) and NaVg channels were performed, respectively.

Fetal PO2 (13.2±0.1mmHg vs 18.2±0.3mmHg), Hb saturation (25.0±0.2% vs 42.7±1.0%) and O2 content (3.4±0.2 ml dl-1 vs 7.0±0.2 ml dl-1) obtained from descending aorta were significantly lower in the HF than the CF ($P<0.05$, Student's unpaired t test). We also measured lower Na,K-ATPase activity (0.078±0.001 μmol mg-1 min-1 vs 0.113±0.006 μmol mg-1 min-1) and ouabain BMAX (33.0±2.3 pmol mg-1 vs 48.8±4.5 pmol mg-1) in the H group than the C group ($P<0.05$). Hypoxia did not affect ouabain KD, NaVg channel protein and transcript level, or the ratio between intact and apoptosis-induced PARP fragment.

The lack of increase of apoptosis-induced PARP fragment together with the drop of Na,K-ATPase activity and density suggest a metabolic arrest neuropreservation strategy upon 24 h hypoxia in the FLL brain cortex. The absence of changes in NaVg channels expression does not exclude hypoxia-induced post-translational changes to reduce Na conductances as a co-ordinated response to the observed decrease in Na pump.

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