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Functional development of pancreatic β cells in the newborn horse

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To survive the nutritional transition at birth, the neonate must have functional pancreatic β cells capable of secreting insulin in response to glucose (Fowden *et al.* 1982). In newborn horses, regulation of glycaemia is poor compared to other species (Madigan, 1991). Hence, this study examined insulin and proinsulin secretion in newborn foals during the first 10 days after birth when enteral nutrition is being established.

Over a 48 h period at 3 time intervals after birth (Days 1–2, 5–6 and 9–10 *post partum*), pancreatic β cell responses to glucose (0.5 g/kg, iv, 40 % glucose, Days 1, 5 & 9) or saline (20 ml 0.9 % (w/v) NaCl, Days 2, 6 and 10) were measured in 7 Pony foals. Blood samples were taken from the jugular vein via an indwelling catheter (inserted under local anaesthesia) at 5–15 min intervals for 30 before and 60 min after administration. Foals were muzzled throughout this period. Plasma glucose concentrations were determined enzymatically. Plasma insulin and total proinsulin concentrations were measured by immunoassay validated for equine plasma (Hemmila *et al.* 1984). Statistical analyses were made by ANOVA and paired and unpaired *t* tests. Mean ± S.E.M. are presented throughout.

Basal plasma glucose and insulin concentrations did not alter significantly between Days 1 and 10, whereas basal proinsulin levels increased significantly from 7.0 ± 1.1 pmol/l on Day 1 to 14.9 ± 3.3 pmo/l on day 5 ($P < 0.05$, $n = 7$) and then remained stable. Glucose administration evoked β cell responses at all ages studied. The maximum increment in plasma insulin in response to glucose did not vary with age. In contrast, the maximum proinsulin increment increased significantly from 5.7 ± pmol/l on Day 1 to 12.4 ± 2.9 pmol/l on Day 9 ($P < 0.05$, $n = 7$). The maximum increment in plasma glucose was similar at the 3 ages but the half time for glucose disappearance was greater on Day 1 than at older ages ($P < 0.004$). Proinsulin and glucose, but not insulin, levels fell significantly in response to saline administration at all 3 ages. When the data from each time period were combined, there were significant linear correlations between the plasma insulin and proinsulin concentrations (Days 1–2 y = 0.043x + 3.679; Days 5–6 y = 0.081x + 2.562; Days 9–10 y = 0.079x + 5.177). The slope of this relationship was significantly shallower on Days 1–2 than at Days 5–6 or Days 9–10 ($P < 0.05$).

These results demonstrate that pancreatic β cells of the neonatal foal undergo developmental changes during the first 10 days of life as suckling is established. The ontogenic increase in proinsulin secretion suggests that there are either changes in insulin processing or an increased demand for insulin with postnatal age.

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All procedures accord with current UK legislation

C91

The role of CGRP in the glycaemic and haemodynamic responses to acute hypoxaemia in the ovine fetus

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The fetal defence to hypoxaemia involves metabolic and cardiovascular responses. These include hyperglycaemia and redistribution of the cardiac output towards the brain and away from peripheral circulations, such as the femoral vascular bed (Giussani *et al.* 1994). The glycaemic response is mediated by sympathetic pathways and increased catecholamines (Jones *et al.* 1980). Fetal peripheral vasoconstriction is triggered by a chemoreflex via the sympathetic chain, maintained by catecholamines, and offset by enhanced NO activity (Morrison *et al.* 2003). The role of calcitonin gene related peptide (CGRP) in fetal cardiovascular and metabolic physiology is unknown, but is gaining interest as it can act both as an autonomic neurotransmitter and as a vasodilator. This study determined the role of CGRP in the glycaemic and peripheral haemodynamic responses to acute hypoxaemia in the ovine fetus during late gestation.

Under halothane anaesthesia, 5 sheep fetuses were prepared with catheters and a Transonic probe around a femoral artery at 0.8 gestation (term ≈ 145 days). Five days later, animals underwent a 2.5 h protocol: 1 h normoxia, 0.5 h hypoxaemia and 1 h recovery, during either saline infusion or treatment with the CGRP antagonist ($CGRP_{8–37}$, 50 μg kg^{-1} I.A. bolus + 20 μg min^{-1} I.V. infusion). Hypoxaemia during saline or antagonist treatment occurred on separate days in randomised order. Treatment started 30 min before hypoxaemia and ran until the end of the challenge. Arterial samples were collected to determine blood gas and metabolic status. At the end of the experiment, animals were humanely killed with pentobarbitone (200 mg kg^{-1} I.V.).

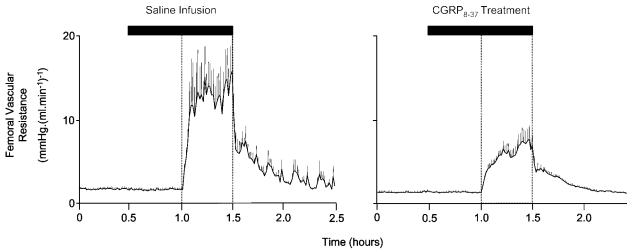

Figure 1. Values are mean ± S.E.M. calculated every minute for femoral vascular resistance during 1 hour of normoxia, 0.5 hour of hypoxaemia (box) and 1 hour of recovery for fetuses during saline infusion ($n = 5$) or during treatment with the CGRP antagonist ($n = 5$).

Fetal treatment with the CGRP antagonist did not alter basal blood gases, metabolic status or cardiovascular variables. During hypoxaemia, similar falls in P_aO_2 occurred in fetuses during either saline (20.2 ± 0.9 to 10.6 ± 0.7) or antagonist (20.2 ± 0.9 to 10.4 ± 1.1 mmHg) treatment, without alterations in P_aCO_2. Hypoxaemia induced hyperglycaemia, hypertension, bradycardia and femoral vasoconstriction in all fetuses. However, the increments in blood glucose (0.8 ± 0.2 *vs.* 0.3 ± 0.1 mM) and femoral vascular resistance (Figure) during hypoxaemia were significantly smaller during treatment with the CGRP antagonist ($P < 0.05$).

These data suggest a role for CGRP in the sympathetic activation of the glycaemic and peripheral vasoconstrictor responses to acute hypoxaemia in the late gestation ovine fetus.

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All procedures accord with current UK legislation

C92

The renin-angiotensin system in young 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. In this study we investigated the effects of early gestation and postnatal nutrient restriction on RAS function in adulthood.

Welsh Mountain ewes 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 wk growth trajectory) from 12 to 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 ~10 months of age carotid artery loops were created under general anaesthesia (3 % halothane/O_2). At ~17 months of age catheters were inserted into the carotid artery and jugular vein under general anaesthesia (3 % halothane/O_2). RAS function was assessed using a frusemide challenge (5 mg kg^{-1} body weight; I.V.) and heart rate (HR), mean arterial blood pressure (MAP), diastolic blood pressure (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. Plasma angiotensin converting enzyme (ACE) concentrations were measured by an enzyme assay as previously described (Forhead *et al.* 1998) and expressed as nmol/ml/min.

We have previously shown, in males, but not females, MAP and SBP AUC were greater in CU compared to the CC group ($P < 0.05$) (Cleal *et al.* 2003). This effect of postnatal undernutrition was not seen in males exposed to early gestation nutrient restriction (UU). In males, basal plasma ACE concentrations were significantly lower ($P = 0.0001$) in the early gestation nutrient restricted groups (UC, 14.65 ± 0.51; UU, 13.73 ± 1.03) compared to the control groups (CC, 17.11 ± 0.51; CU, 17.98 ± 0.98). The elevated blood pressure response to frusemide in postnatal nutrient-restricted sheep was blunted by exposure to a prior early gestation nutrient restriction: this effect was associated with reduced plasma ACE concentrations.

This study suggests that the RAS is affected by a sex specific interaction between periconceptual and postnatal nutrition, which could have consequences for renal/cardiovascular function in later life.

Cleal JK *et al.* (2003). *Pediatric Research* **53**, 18A.
Forhead AL *et al.* (1998). *Reprod Fertil Dev* **10**, 393–398.

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All procedures accord with current UK legislation

C93

Undernutrition from 1–30 days gestation in sheep blunts the angiotensin II evoked baroreflex response in the offspring at one year of age

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Periconceptual undernutrition of sheep has been shown to program a number of cardiovascular and endocrinological perturbations mostly in late gestation fetuses but also in young sheep. In general it would appear that undernutrition during early development reduces fetal blood pressure and baroreflex sensitivity but increases the rate of rise in arterial blood pressure postnatally. The mechanisms mediating this effect are as yet unclear and no study has yet followed these previously undernourished lambs into later life. In addition, there are marked differences in baroreflex responses evoked by either phenylephrine or angiotensin II infusion. Thus the present study has examined cardiovascular function, in particular baroreflex control, in periconceptually-undernourished lambs at one year of age.

Twenty Mule ewes were randomly assigned into two groups to receive either a control diet (Control, $n = 12$) providing adequate energy and protein throughout gestation to term (term ~147 days gestation, dGA) or a nutrient restricted diet (a 50 % reduction in calculated requirement) from day 1–30 dGA (NR, $n = 8$). Ewes were individually housed from day 1 to term whereupon they lambed naturally. All lambs were ewe-reared as singletons to weaning and grass-fed thereafter. At one year of age the lambs were surgically instrumented under 1–2 % halothane (50:50 O2/N2O) with carotid and jugular cannulae. After at least 2 days recovery the lambs were walked into metabolic crates for cardiovascular recording at one-second intervals using pre-calibrated pressure transducers (SensorNor 840; S 4925) attached at heart level linked to a data acquisition system (Po-Ne-Mah; Version 3, Gould Instrument Systems Inc). Baroreflex curves were constructed from the parallel changes in diastolic blood pressure and heart rate induced by phenylephrine (PE; 75 μg kg^{-1} bolus), sodium nitroprusside (SNP; 2.5 μg kg^{-1} min^{-1} infusion for 5 mins) and angiotensin II (stepwise increments every 10 mins from 0–60 μg kg^{-1} min^{-1}). At the end of all experiments the lambs were humanely euthased with a lethal dose of sodium pentobarbitone. Basal data are Means ± S.E.M. and were analysed by one-way ANOVA (for group). Baroreflex curves were analysed by multiple linear regression. All statistical analysis was conducted using SPSS 11.1.

Basal systolic blood pressure; 100 ± 4 *vs.* 99 ± 3 mmHg, diastolic blood pressure; 77 ± 4 *vs.* 70 ± 3 mmHg and heart rate; 106 ± 5 *vs.* 100 ± 4 beats min^{-1} were similar in control and NR lambs, respectively. However, pulse pressure (systolic-diastolic; 23 ± 1 *vs.* 28 ± 2 mmHg, $P = 0.06$) tended to be higher and the rate pressure product (heart rate × mean arterial pressure; 9.3 ± 0.7 *vs.* 7.9 ± 0.5 (beats min^{-1} mmHg) × 103, $P = 0.005$) significantly lower in NR relative to control fetuses.

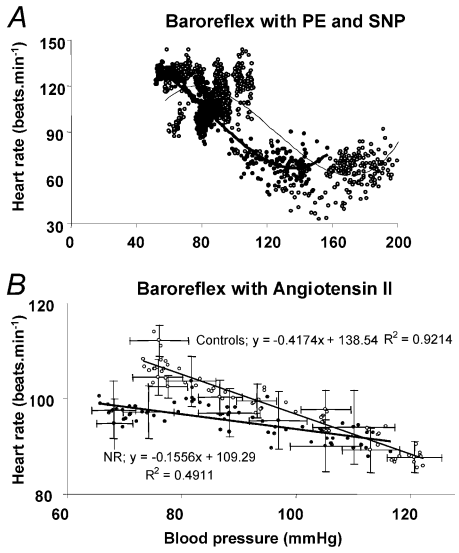

Figure 1. *A*, mean data for control and NR lambs during PE and SNP infusion. Error bars have been omitted for clarity. *B*, mean ± S.E.M. data during angiotensin II infusion.

An analysis of baroreflex function with PE and SNP indicated a reduced set point but similar sensitivity in NR lambs (Fig. 1, left panel). However with angiotensin II, despite similar increments in pressure the reduction in heart rate was markedly blunted in NR lambs relative to controls (Fig. 1, right panel). Analysis of baroreflex function was markedly blunted per se when using angiotensin II rather than PE.

The data illustrate that periconceptual undernutrition has little effect on peripheral vascular sensitivity to angiotensin II per se, but does program angiotensin II induced baroreflex control of the heart. The mechanisms for this effect may well relate to the developmental programming of angiotensin II receptor expression, in particular, in the cardiovascular control regions within the brain stem.

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All procedures accord with current UK legislation

C94

Differential effects of refeeding following maternal undernutrition during early to mid gestation on glucocorticoid receptors (GCR) in the ovine fetal liver

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Maternal undernutrition during early to mid gestation (28–77 days (d)) followed by adequate feeding up to term determines fetal tissue development in the absence of any effect on fetal body or tissue weight. GCR mRNA abundance is nutritionally upregulated in the adrenal, kidney, liver, lung, and perirenal adipose tissue of resultant neonatal offspring (Whorwood *et al.* 2001). However the extent to which hepatic GCR gene expression is programmed by nutrient availability to the fetus before birth remains to be determined.

Eighteen singleton-bearing ewes of similar body weight and parity were randomly allocated to one of two feeding groups from 28 d gestation (Dandrea, 2001). Nine nutrient restricted (NR) ewes consumed 60 % of their total metabolisable energy (ME) requirements from 28 to 80 d gestation, whilst control (C) ewes consumed 150 % of ME requirements. At 80 d gestation, four NR and five C ewes were fed 150 % of ME requirements until term (term = 147 d). Ewes were humanely euthanased (100 mg kg $^{-1}$ pentobarbital sodium: Euthatal, I.V.) at either 80 d or 140 d gestation, to enable fetal and liver tissue sampling. The relative abundance of hepatic GCR to 18S rRNA was analysed by RT-PCR. Results are given as means and standard errors in arbitrary units (a.u.) as a percentage of a reference sample present on all gels. Statistical differences between nutritional groups were analysed using an unpaired student *t* test ($P < 0.05$).

Maternal nutrient restriction between 28–80 d gestation had no effect upon fetal body or liver weights. GCR mRNA abundance was comparable in 80 d NR fetuses (C 50.3 ± 14.3; NR 47.0 ± 2.5 a.u.). However, following 60 d of nutritional rehabilitation to 150 % of ME requirement GCR mRNA abundance in 140 d late gestation fetal livers was significantly decreased (C 98.3 ± 12.9; NR 40.1 ± 6.3 a.u. ($P = 0.01$). This result differs to that seen by Whorwood *et al.* 2001 where NR offspring when re-fed to 100 % ME requirements and had increased hepatic GCR levels.

In conclusion, GCR mRNA abundance in late-gestation fetuses is more susceptible to levels of refeeding following maternal undernutrition than by nutrient availability in early gestation. That is NR fetuses failed to undergo the normal gestational rise in hepatic GCR mRNA.

Dandrea J *et al.* (2001). *Exp Physiol* **87**, 353–359.

Whorwood CB *et al.* (2001). *Endocrinology* **142**, 2854–2864.

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All procedures accord with current UK legislation

C95

The effect of chronic umbilical cord occlusion on uncoupling protein-2 (UCP2), voltage-dependent anion channel (VDAC) and cytochrome c protein abundance in the late gestation ovine fetal lung

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Umbilical cord occlusion (UO) sufficient to restrict fetal blood supply by 30 % results in a range of fetal endocrine adaptations, including increased fetal plasma cortisol and premature maturation of mitochondria within brown adipose tissue (Gnanalingham *et al.* 2002). This study aimed to determine if UO results in changes in UCP2, VDAC and cytochrome c abundance within the fetal lung.

Nine ewes were entered into the study, which were all chronically instrumented under halothane anaesthesia with fetal vascular catheters. Five fetuses were then subjected to 3 days UO beginning at 125 days gestation by automated compression of the umbilical cord, with the remaining 4 acting as controls (C). At 137 ± 2 days gestation, all animals were humanely killed and fetal lungs dissected and frozen in liquid nitrogen. Total lung RNA was extracted, reverse transcribed and UCP2 mRNA abundance

measured by RT-PCR using oligonucleotide primers designed specifically to ovine UCP2. The abundance of VDAC and cytochrome c mitochondrial proteins was determined by differential centrifugation and immunoblotting. The mRNA results are given as means and S.E.M. in arbitrary units, as a ratio of 18S rRNA and are expressed as a percentage of a reference sample, whilst the mitochondrial protein data are represented as a percentage of a reference sample. Statistical differences between groups were analysed by an unpaired Mann-Whitney U test ($P < 0.05$).

There were no significant differences in body (C 2.85 ± 0.23; UO 2.49 ± 0.10 kg) or lung (C 74.8 ± 2.0; UO 63.1 ± 6.0 g) weights between groups, although UO lungs had a tendency to be smaller. UO resulted in enhanced abundance of UCP2 mRNA (C 72.3 ± 3.0; UO 114.8 ± 5.4, $P < 0.01$), and mitochondrial VDAC (C 25.8 ± 0.9; UO 35.8 ± 1.7, $P < 0.05$), and cytochrome c (C 5.4 ± 0.7; UO 8.2 ± 0.4, $P < 0.01$) proteins.

In conclusion, chronic UO results in precocious maturation of lung mitochondria. These changes may be important in promoting gaseous exchange within the growth-restricted fetal lung, and may reflect an underlying susceptibility to infection in this group.

Gnanalingham MG *et al.* (2002). *Society for Endocrinology* **4**, OC2.

All procedures accord with current UK legislation

C96

Determinants of body fat depth in young adult sheep following moderate postconceptional undernutrition and undernutrition in early postnatal life

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Small size at birth due to perturbations of growth and development in early life confers an increased risk of adverse health outcomes in adulthood such as obesity, cardiovascular and metabolic disorders (Breier *et al.* 2001). In addition, poor growth in early infancy, regardless of birth size, is associated with an additional risk of coronary artery disease, which is further confounded by rapid weight gain after 1 year of age (Eriksson *et al.* 2001). We are interested in the interaction between poor fetal development and adverse postnatal conditions, as mediated by undernutrition, on adiposity in later life. Body fat was therefore examined in young adult sheep whose mothers were exposed to moderate undernutrition (UN) in the immediate postconceptual period and who themselves faced a subsequent postnatal UN challenge.

Welsh Mountain ewes received either 100% (*ad libitum*; group C, $n = 38$) or 50% of global nutritional requirements (group U, $n = 40$) from conception to 30 days of gestation, and *ad libitum* thereafter. Offspring were fed either *ad libitum* (CC, $n = 21$ and UC, $n = 19$) or at an intake level that reduced body weight to 85% of target weight (predicted from their individual growth trajectory, 0 −12 weeks) from weaning (12 weeks) to 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. Body weights were measured at birth, at 12 weeks of age and every week thereafter. At 17 months of age, back fat depth was measured by ultrasound and corrected for current body weight. Data (mean ± S.E.M.) were analysed by unpaired Student's *t* test and linear regression.

In females, when considering all treatment groups together, increased fat depth at 17 months was predicted by poor growth rate during the postnatal UN challenge (12 −25 weeks; $R^2 = -0.15$, $P < 0.05$) and high 'catch-up' growth after the challenge, as assessed by the fractional growth rate from 25 weeks to the time of fat measurement (kg gained/day/starting weight; $R^2 = +0.26$, $P < 0.005$). Postnatal UN had no effect on mean fat depth in females that received control nutrition in the postconceptual period. However, corrected fat depth in females that received both pre-and postnatal UN challenges (UU; 0.16 ± 0.01) was significantly ($P < 0.05$) greater than those that were exposed to prenatal UN alone (CU; 0.12 ± 0.01). In males, fat depth tracked with current weight and was unaffected by UN treatment group.

These findings are the first in sheep to show that, in common with observations in humans, a combination of poor growth in early postnatal life followed by a high subsequent growth rate is associated with increased adult fat deposition. In addition, the predisposition to increase body fat following perturbed prenatal nutrition may not be revealed until well after a subsequent period of UN in early postnatal life when nutrition is restored to 'normal'. These effects were only observed in females. The long-term effects of altered patterns of early life growth on the changes in body fatness with age, and on metabolic and cardiovascular homeostasis, remain to be determined.

Breier BH *et al.* (2001). *Mol Cell Endo* **185**, 73–79.
Eriksson JG *et al.* (2001). *BMJ* **322**, 949–953.

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All procedures accord with current UK legislation

C97

The effect of feeding on leptin concentration and regional adipose tissue deposition in singleton and twin sheep at one year of age

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Previous studies indicate cardiovascular and endocrinological differences between singleton and twin ovine fetuses (Edwards & McMillen 2002) although no study has examined any differences when these fetuses become adults. Twins are generally of lower birth weight than singleton fetuses and low birth weight has been inversely correlated with metabolic and cardiovascular anomalies that aggregate to form the metabolic syndrome. The present study has examined cardiovascular function, fat deposition and leptin concentration in single and twin sheep at one year of age during the fed and fasted state.

Twelve Mule ewes, half bearing singletons and half bearing twins, were individually housed and fed to requirement from day 1 to term whereupon they lambed naturally. All offspring were ewe-reared as singletons to weaning and grass-fed thereafter. At one year of age they were surgically instrumented under 1–2% halothane (50:50 O2/N2O) with carotid and jugular cannulae. At least 2 days later cardiovascular recordings and blood samples were taken pre and post feeding. Leptin concentration was measured by RIA. Each animal was then euthanased with a lethal dose of sodium pentobarbitone and all major organs and tissues weighed. Data are Means ± S.E.M. and were analysed by two-way

ANOVA (for group i.e. singles *vs.* twins and treatment i.e. fed *vs.* fasted) using SPSS 11.1.

There were no differences in basal cardiovascular parameters between singles and twins (e.g. systolic blood pressure; Singletons 102 ± 3; Twins 99 ± 5 mmHg) with the exception of pulse pressure which tended to be higher in singletons (Singletons, 26 ± 1; Twins, 21 ± 1 mmHg; $P = 0.009$). These values were unchanged during the fed or fasted state. During the fed state, plasma leptin concentrations were similar between groups (Singletons, 2.7 ± 0.6; Twins, 1.9 ± 0.5 ng ml^{-1}). However, while fasting significantly reduced plasma leptin concentration in singleton lambs (by 62 % to 1.0 ± 0.1 ng ml^{-1}) it had little effect in twins (by 25 % to 1.5 ± 0.3 ng ml^{-1}). Twins had significantly more regional fat than singletons (Twins *vs.* Singletons, perirenal depot, 196 ± 28 *vs.* 111 ± 22 g; cardiac, 54 ± 5 *vs.* 32 ± 3 g; subcutaneous, 20 ± 3 *vs.* 10 ± 2 g). These significant differences were maintained when expressed relative to current weight.

The data confirm the little effect that the constrained late-gestational intrauterine environment that twins experience has upon later basal cardiovascular variables. However, the results of this study do suggest that more subtle effects on metabolism may be indicated in twins as reflected in a greater propensity for fat deposition and insensitivity of leptin concentration to variations in food intake.

Edwards LJ & McMillen IC (2002). *Biol Reprod* **66**, 1562–1569.

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All procedures accord with current UK legislation

C98

The effect of maternal parity on leptin mRNA expression and perirenal adipose tissue deposition over the first month of postnatal life in sheep

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Organ weight at birth can have an important influence of postnatal morbidity and mortality. One factor that can influence size at birth is maternal parity for which birth weight may be expected to increase, even when maternal body weight has changed very little between first and second pregnancies. The extent to which maternal parity can have a differential effect on adipose tissue deposition and its leptin synthetic capacity is not known.

Ten twin bearing sheep of similar body weight were entered into the study of which 5 were primiparous (P) and 5 multiparous (M). All ewes were fed 100 % of total metabolisable energy (ME) requirements throughout gestation and all ewes delivered normally at term. An intravenous overdose of sodium pentabarbitone was used to humanely euthanase one lamb at one day of age and the other at one month of age ($n = 5$ per group). Tissue sampling was performed and samples immediately snap frozen in liquid nitrogen and stored at −80 °C until analysis. Total RNA was extracted and RT-PCR analysis was performed using oligonucleotide primers specific to ovine leptin (forward 5'-CAC CAA AAC CCT CAT CAA GAC G-3' and reverse 5'-ACA TTT CTG GAA GGC AGA CTGG-3', amplicon size 192bp) (Bispham *et al.* 2003). Results are expressed in arbitrary units (a.u.; mean plus/minus s.e.m) as a ratio of an 18S rRNA internal control. Statistical differences were analysed using Kruskal-Wallis and Mann-Whitney U tests.

At one day of postnatal age lambs born to multiparous ewes had significantly lower expression levels of leptin than there primiparous counterparts (P: 88.9 ± 11.3; M: 47.7 ± 10.1 a.u. ($P < 0.05$)). This difference between groups persisted up to one month of age (P: 75.3 ± 17.5; M: 30.5 ± 7.3 a.u. ($P < 0.05$)) when the offspring of the primiparous mothers possessed more fat (P 153 ± 10: M 108 ± 14 g ($P < 0.01$). There was no difference in body weights between groups at either 1 or 30 days of age.

Maternal parity is a major factor determining fat deposition and leptin synthetic capacity over the first month of postnatal life. The extent to which the enhanced fat mass and greater potential leptin production may contribute to altered fat deposition in later life remains to be established.

Bispham J *et al.* (2003). *Endocrinology* **144**, 3575–85.

All procedures accord with current UK legislation

C99

The influence of birth weight and endocrine profile on adipose tissue (AT) and skeletal muscle (SM) uncoupling protein 3 (UCP3) expression in neonatal pigs

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Epidemiological studies have shown that infants of low birth weight show poor neonatal growth and increased susceptibility to adult diseases such as diabetes in later life. Pigs provide an ideal model to examine the influence of size at birth due to the natural variance in piglet weight within a litter. This study examined whether birth weight influences the expression of UCP3 in fat and muscle and its endocrine regulation in neonatal pigs.

Piglets from 11 litters were ranked according to body weight at birth and 3 animals from each were assigned to small (SFD $n = 11$), normal (NFD $n = 11$) or large for dates (LFD $n = 11$) groups. Body weight was recorded on days 7 and 14 of postnatal life when a venous blood sample was also taken. Piglets were humanely euthanased with an overdose of barbiturate (100 mg kg^{-1} pentobarbital sodium: Euthatal) on days 7 ($n = 15$) and 14 ($n = 18$) to obtain AT and SM. Plasma leptin, insulin like growth factor (IGF)-1 and insulin were measured using radioimmuno and enzymatic assays. UCP3 expression in AT and muscle was measured using RT-PCR as described previously (Mostyn *et al.* 2002). GLM and Spearman analysis were carried out to investigate statistical differences; results are presented as means ± standard errors.

The SFD group weighed less than the LFD group throughout the study. In AT, UCP3 expression on day 7 was found to be significantly lower in the SFD group (SFD, 34.4 ± 10.9; NFD, 69.48 ± 12.8; LFD 77.64 ± 9.0 % of reference ($P < 0.05$)) a similar trend was observed, but did not reach significance, on day 14 (Mostyn *et al.* 2003). UCP3 expression in SM was significantly higher than that of AT, but was similar between groups on both days. Leptin and insulin were significantly higher in the SFD group on day 7 only. IGF-1 was significantly higher in the LFD group on day 14.

In conclusion, low birth weight is associated with reduced expression of UCP3 in subcutaneous AT but not SM. UCP3 expression is differentially regulated by IGF-1, leptin and insulin

(tradional regulators of body weight) and also size at birth. It remains to be established if these differences represent a 'global' effect on adipose tissue transcript expression or perpetuate into later life.

Mostyn A *et al.* (2002). *Endocrinology Abstracts* **4**, OC3.
Mostyn A *et al.* (2003). *Endocrine Abstracts* (in press).

All procedures accord with current UK legislation

C100

Protein supplementation at specific stages of gestation decreases mitochondrial protein abundance in ovine fetal thymus

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Mitochondria's regulation of apoptosis and cellular energy metabolism is influenced by the voltage-dependent anion channel (VDAC) and cytochrome c (Cyt C). The abundance of VDAC is up regulated by maternal nutrient restriction (Mostyn *et al.* 2003) whilst thymus mass is decreased by maternal under nutrition (Osgerby *et al.* 2002). This study aimed to determine whether protein supplementation of the maternal diet at defined stages of gestation promoted the growth, development and mitochondrial protein abundance of fetal thymus tissue.

Twenty-nine twin bearing ewes of similar body weight and parity were randomly allocated into four groups from 10 d gestation. Controls were fed a standard diet of chopped hay and barley based concentrate that was increased with gestation. Supplemented groups were randomly allocated to be provided with additional protein in the form of fishmeal (66 % crude protein plus an equal amount of molasses to aid palatability) between either 10–40 d gestation, 40–70 d gestation or from 110 days gestation. All the ewes were humanely euthanised with an overdose of barbiturate (100 mg kg^{-1} pentobarbital sodium: Euthanal) at 140 d gestation to enable sampling of fetal thymus. Mitochondrial fractions were prepared and VDAC and Cyt C abundance determined by immunoblotting using a polyclonal antibody raised to ovine VDAC and Cyt C. Results are given as means with their standard errors. Statistically significant differences with respect to nutritional supplementation and protein abundance were determined using a General Linear Model (GLM) and Tukey test.

Period of supplementation	Fetal body weight (kg)		Thymus weight (g)		Tot. Mito Pr Conc. (mg/ml)		VDAC abundance % ref		Cyt C abundance % ref	
	Mean	SEM	Mean*	SEM	Mean	SEM	Mean*	SEM	Mean	SEM
40-70 d	5.75	0.25	8.68	0.63	11.3	1.7	551	71	5.1	2.5
Control	5.03	0.56	6.17	0.81	7.0	0.7	984	119	8.3	1.8

*P<0.05

Protein supplementation during mid gestation resulted in larger fetuses with heavier thymuses. The specific mitochondrial VDAC and Cyt C abundance of these thymuses were reduced despite total mitochondrial protein concentration increasing. In conclusion protein supplementation of the maternal diet during the period of maximal placental growth increases thymus growth. This is associated with a decrease in mitochondrial proteins known to regulate energy metabolism. The effects of this on organ function, postpartum remains to be determined.

Mostyn A *et al.* (2003). *British Journal of Nutrition* **90**, 323–328.
Osgerby JC *et al.* (2002). *Journal of Endocrinolgy* **173**, 131–141.

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All procedures accord with current UK legislation

C102

Cold exposure during mid to late gestation down regulates the gene expression of insulin-like growth factors 1 and 2 in the postnatal ovine liver

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Insulin-like growth factors 1 (IGF-1) and 2 are key components in fetal development, play a large role in energy balance and are primarily synthesized by the liver. Cold exposure by winter shearing has shown to promote growth and development, dependant on maternal nutrition; however the effects on IGF-1 and 2 have not been determined.

Twenty-six multiparous twin-bearing ewes of similar body weight were entered into the study, thirteen were shorn (S) during mid gestation and thirteen left unshorn (US). Six out of each group were nutrient restricted (NR; fed 50 % of total energy requirements) over the final month of gestation and seven controls (C) were fed to 100 % of requirements until term. All lambs were born spontaneously and one lamb from each ewe were then humanely euthanased (100 mg kg^{-1} pentobarbital sodium: Euthatal, i.v) at 1 day of age and one at 30 days of age. Tissue samples were stored at −80 °C until molecular analysis. The relative abundance of IGF-1, IGF-2 and their receptors mRNA to 18S rRNA were determined using RT-PCR.

Sampling age	Body weight (kg)		IGF-1 (a.u)		IGF-2 (a.u)	
	Mean	SEM	Mean	SEM	Mean	SEM
1 Day SC	5.5	0.3	53	9	75*	7
SNR	4.1	0.2	5**	0.8	86*	10
30 Days SC	17.3	0.7	100	14	80*	8
SNR			7**	0.9	74*	10
1 Day USC	4.6	0.3	121	33	191	27
USNR	4.3	0.2	45	8	147	31
30 Days USC	16	0.8	151	36	142	32
USNR	14.8	0.6	97	22	157	32

Significantly different from control at * P<0.05, ** P<0.001 level, using Mann-Whitney and T-Test were applicable

Maternal cold exposure resulted in significantly heavier total body weight at one day in SC as compared to the US group and down regulated IGF-1 and 2 mRNA abundance, with no effect on receptor expression (data not shown).

In conclusion maternal cold exposure promotes fetal growth and at the same time compromising hepatic gene expression of IGF-1 and 2, and this effect is increased in nutrient restriction with IGF-1.

This work was supported by the BBSRC

All procedures accord with current UK legislation

C103

Expression of IGF-1 splice variants in levator ani muscle of women following vaginal delivery: Can we quantify vaginal delivery trauma?

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Initial studies in animals have shown that in skeletal muscle the repair mechanisms following injury involve expression of two different insulin-like growth factor-1 (IGF-1) splice variants: IGF-1Ea and IGF-1Ec (MGF). These are known to play different roles in muscle repair (Goldspink 1999, Hill and Goldspink 2003). Both up regulate protein synthesis and MGF replenishes the muscle satellite (stem) cell pool that is important for muscle repair. The levator ani (LA) muscle is the main muscle responsible for providing functional support for the different pelvic organs in women. It is also the main muscle that is directly affected by increased intra-abdominal pressure during pregnancy and the strains of vaginal delivery. Damage to the LA muscle following vaginal delivery often results, in the short and long term pelvic floor dysfunction resulting in genitourinary prolapse and incontinence (Allen *et al.* 1990, MacClennan *et al.* 2000

This work was given ethical approval by the LREC at The Royal Free Hospital. Fourteen nulliparous women were recruited following vaginal delivery. Biopsies were conducted vaginally on the pubo-visceral component of the LA muscle under epidural analgesia when a tear had occurred or an episiotomy was required to expedite delivery. Ten women attending the Gynaecology Clinic for other reasons than pelvic floor dysfunction were recruited as controls and biopsies obtained under general anaesthetic. Samples ranging from 20 to 40 mg were processed using Real Time Quantitative Polymerase Chain Reaction (RT-PCR). From the delivery group five controls were obtained from the pubo-visceral component of the LA muscle. Part of the biopsy sample was incubated in formalin and paraffin-wax embedded sections were stained with an antibody for MGF. RNA was extracted from the rest of the samples using RNAeasy and reverse transcribed into cDNA. This was then analysed by Real Time RT-PCR using Roche Light Cycler technology with oligonucleotide primers that selectively amplify the two IGF-I splice variants (Hameed *et al.* 2003).

Compared with the control population (Mann-Whitney test, statistical significance $P < 0.05$), MGF mRNA levels after delivery showed a marked elevation ranging between 1.81x 10–8 to 8.76x10–6 μg mg-1 ($P < 0.02$). IGF-1Ea was even more up regulated with values of 4.05x10–8 to 3.16x10–3 μg mg-1 post delivery ($P < 0.04$). Statistical analysis of these pilot data (Spearman's correlation test), suggested correlation between the expression of IGF-1 splice variants and length of the second stage. There was no statistical correlation between mother's BMI, baby's weight, and age, IGF-1 splice variants. However, individual variations seen in the population studied may reveal those women showing a poor muscle repair capacity and therefore at risk for pelvic floor dysfunction.

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Hameed M *et al.* (2003). *J Physiol* **541**, 247–254.

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MacClennan AH *et al.* (2000). *Br J Obstet Gynaecol* **72**, 41–6.

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All procedures accord with current local guidelines and the Declaration of Helsinki

C104

Increased placental NAD(P)H oxidase mediated superoxide generation in early pregnancy

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NAD(P)H oxidase enzymes are a major source of superoxide generation in vascular smooth muscle, vascular endothelium and in neutrophils. Superoxide has been implicated in signalling events leading to upregulation of antioxidant genes, angiogenesis, proliferation and matrix remodelling. Recently, it has been demonstrated that NAD(P)H oxidase is expressed in human placenta at term and is a major source of placental superoxide (Matsubara *et al.* 2001; Dechend *et al.* 2003). However it is not known whether the enzyme is active in placenta earlier in pregnancy.

The Institutional Medical Ethical Review Committee approved the study protocol. Superoxide production was assessed using a lucigenin chemiluminescence method in placentas obtained with informed consent from women undergoing termination of pregnancy ($n = 19$; mean gestational age ± S.E.M. is $11^{+6} \pm 1^{+0}$ wks) and in placentas delivered by elective caesarean section after a full term uncomplicated pregnancy ($n = 15$; gestational age $38^{+6} \pm 0^{+4}$ wks). NADPH was added to generate NAD(P)H oxidase mediated superoxide synthesis which was detected by lucigenin. Specificity for NAD(P)H oxidase was assessed using specific inhibitors (end-concentration; target) L-NAME (100 μmol/l; nitric oxide synthase), Rotenone (20 μmol/l; mitochondrial $O_2\cdot$synthesis), allopurinol (100 μmol/l; xanthine oxidase), DPI (10 μmol/l; NAD(P)H oxidase) and TIRON (50 mmol/l; $O_2\cdot$ scavenger). The area under the curve (AUC) was calculated as a measure of total superoxide production ($O_2\cdot_{total}$) and expressed as arbitrary light units (ALU). Gestational differences in superoxide generation were calculated using linear regression with robust standard errors in STATA 8.

Placental superoxide synthesis ($O_2\cdot_{total}$) was higher in early pregnancy placentas compared with those obtained from women with full term pregnancy (geometric mean ± S.E.M.: 81.0 ± 6.4 *versus* 29.4 ± 3.5: $P < 0.0001$). The signal was unaffected by addition of L-NAME, rotenone, or allopurinol, but was totally inhibited by both DPI and TIRON. This indicates specificity of the signal for NAD(P)H oxidase. This study confirms that there is substantial basal NAD(P)H activity in normal human placenta in the third trimester but also shows that activity is higher in early pregnancy. Raised activity early in gestation could regulate expression of redox sensitive genes including VEGF and others, which may contribute to placental growth and trophoblast proliferation. Superoxide generation may also serve to upregulate antioxidant defences in the early placenta. The role of NAD(P)H oxidase in the placenta requires further investigation.

In conclusion we have demonstrated an elevated superoxide production by NAD(P)H oxidase in early human placenta tissue. The results justify further and more detailed investigation of the role of this enzyme in human placental physiology and pathophysiology.

Dechend R *et al.* (2003). *Circulation* **107**, 1632–1639.

Matsubara S & Sato I (2001). *Histochem Cell Biol* **116**, 1–7.

This work was supported by Tommy's the Baby Charity.

All procedures accord with current local guidelines and the Declaration of Helsinki

C105

Localisation of ATA2 during adaptive regulation of amino acid transport system A in the BeWo choriocarcinoma cell line

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Nutritional challenge induces the adaptive regulation of several nutrient transporters including the amino acid transport system A. Depriving the human choriocarcinoma BeWo cell line of system A substrates causes up-regulation of both system A transport and gene expression. This response is believed to be biphasic, consisting of an acute response involving the trafficking of pre-made transporters from intracellular stores to the plasma membrane similar to the process seen in insulin-stimulated translocation of the glucose transporter GLUT4 (Bose *et al.*2002). The chronic response produces up-regulation of gene expression and increased transporter protein synthesis. In order to study this biphasic response we used immunocytochemistry to track changes in the localisation of the system A transporter ATA2 in the BeWo cell line.

Cells were maintained in DMEM supplemented with 10 % fetal calf serum and 2 % penicillin/ streptomycin. Cells were seeded onto glass coverslips and grown overnight to allow them to achieve a flattened morphology. Amino acid deprivation was induced by culturing cells in Deprivation Media (DM, Earles Balanced Salt Solution, pH7.4 supplemented with 1 × MEM essential amino acid solution without non-essential amino acids). Cells exposed to either control or DM media were fixed for immunocytochemistry at 0, 0.5, 1, 2, 4, 6 and 8 h (3 coverslips/treatment at each time point). ATA2 was detected using a human-specific rabbit anti-ATA2 antibody.

Increased staining of the plasma membrane was seen after just 30 min of exposure of the cells to DM accompanied by depletion in intracellular ATA2 stores compared to control. Intracellular stores started to be replenished after 4 h of exposure to DM. These data differ slightly from our Western blot data (Jones HN *et al.* unpublished), where there is no apparent change at 6 h deprivation. This discrepancy is currently being investigated. Staining intensity of the intracellular stores and the plasma membrane increased further with exposure times to DM of 6 and 8 hours.

These results support the hypothesis that adaptive regulation of system A in the BeWo choriocarcinoma cell line is a biphasic process involving the trafficking of ATA2 between cellular compartments.

Bose A *et al.* (2002). *Nature* **420**, 821.

We thank Dr A. L. Schwartz (St. Louis Children's Hospital, St. Louis, Missouri, USA) for the supply of the BeWo cells and Dr P. Sarmientos (PRIMM, Milan, Italy) for production of the ATA2 antibody. This work is funded by SEERAD flexible fund and Framework V QLK-1999-003377.

C106

Acute effects of cortisol on the activity of Na^+/H^+ exchanger (NHE) in the human placental syncytiotrophoblast

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A reduction in the activity of placental 11-β-hydroxysteroid dehydrogenase (11-β-HSD-2), which converts cortisol to cortisone, correlates directly with low birth weight (Seckl 2001). Syncytiotrophoblast NHE activity is reduced in pregnancies that result in offspring that are small for gestational age (Johansson *et al*, 2002). The activity of renal epithelial NHE has been shown to be stimulated by cortisol (Ambuhl *et al*, 1999). In this study we hypothesised that cortisol stimulates the activity of syncytiotrophoblast NHE's in human placenta.

Villous fragments from term human placenta (obtained as approved by Local Ethics Committee) were incubated in Tyrode's (containing in mM; NaCl 135, KCl 5, $CaCl_2$ 1.8, $MgCl_2$ 1, MOPS 10 and glucose 5) with 1μM of the intracellular pH (pH_i) sensitive dye, BCECF (Molecular Probes), for 5 min at 37°C, washed in Tyrode's without BCECF and incubated at 37°C for 5 min. BCECF in the syncytiotrophoblast was excited at 440 and 490 nm wavelengths and emitted light (540 nM), detected using a CoolSnap camera (Roper Scientific), with data acquisition using MetaFluor software (Universal Imaging Corporation). NHE activity was measured as the rate of Na^+-induced recovery of pH_i following an acid load, imposed by prepulsing with 20 mM NH_4Cl, expressed as pH units/30sec, mean ± S.E.M., n = number of placentas. Carbenoxolone (100 μM), an inhibitor of 11-β-HSD-2, and/or cortisol (1 μM) were added for 3 min, in the absence of Na^+ following the NH_4Cl pulse and in Na^+ Tyrode's during the recovery period.

Resting pH_i in the syncytiotrophoblast was 7.34 ± 0.05 (n = 20). Control rate of recovery from an acid load was 0.10 ± 0.02 pH units/30sec (n = 11) and this was Na^+ dependent and inhibited by 500 μM amiloride (to 0.018 ± 0.008; n = 5, $P < 0.05$ Student's t test). Neither carbenoxolone alone (0.12 ± 0.02; n = 9), nor cortisol (0.12 ± 0.02, n = 8) had an effect on basal recovery. However adding cortisol and carbenoxolone together significantly elevated the rate of recovery to 0.31 ± 0.10 ($P < 0.05$, ANOVA followed by Bonferroni's post hoc test, n = 9). This elevated recovery was Na^+ dependent and inhibited by amiloride.

In conclusion the activity of syncytiotrophoblast NHE's was increased by acutely elevated cortisol levels. An altered ability to regulate syncytiotrophoblast pH_i may alter the activity of many transporters which are acid sensitive and important for transfer of nutrients essential for normal fetal growth (Sibley *et al*, 2002).

Ambuhl PM *et al.* (1999). *J Clin Invest* **103**, 429–435.

Johansson M *et al.* (2002). *J Clin Endo Metab* **87**, 5686–5694.

Seckl JR (2001). *Mol Cell Endocrinol* **185**, 61–71.

Sibley CP *et al.* (2002). *Placenta* **23A**, S39–S46.

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All procedures accord with current local guidelines and the Declaration of Helsinki

C108

P2Y2 and P2Y6 purinergic receptor agonists raise $[Ca^{2+}]_i$ in human placental trophoblast

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Activation of purinergic receptors by extracellular nucleotides raises intracellular calcium ($[Ca^{2+}]_i$) (Burnstock, 1997). We have previously shown expression of mRNA for the purinergic P2Y receptor subtypes, P2Y2, 4 and 6 in human placenta (Roberts *et al.* 2001). Both UTP (P2Y2 and P2Y4 selective) and 5BrUTP (P2Y6 selective) stimulate efflux of ^{86}Rb from cytotrophoblast cells (Roberts *et al.* 2003), which is thought to occur via Ca^{2+}-activated K^+ channels. In this study we aimed: (i) to determine the effect of P2Y2, 4 or 6 receptor activation on $[Ca^{2+}]_i$ in cytotrophoblast cells; and (ii) to examine P2Y2 and 4 protein expression in human placental trophoblast.

Cytotrophoblast cells were isolated from term placenta, collected following informed consent and in accordance with local ethical guidelines, as previously described (Greenwood *et al.* 1996). Cells were seeded onto glass cover-slips and maintained in culture for 18hrs. For measurement of $[Ca^{2+}]_i$ cells were loaded with fura 2-AM for 30 min at 37 °C and treated for 2 min with either 10 μM UTP or 500 μM 5BrUTP. Experiments were carried out both in the presence (+Ca; 1.8 mM Ca^{2+}) and absence (0Ca; no added Ca^{2+} + 0.5 mM EGTA) of extracellular Ca^{2+}. The results are expressed as the peak % increase in 340:380 nm ratio above baseline. Expression of P2Y2 and P2Y4 receptor protein was assessed by Western blotting using commercially-available primary antibodies (Alomone). (P2Y6 antibody unavailable).Both UTP and 5BrUTP significantly elevated $[Ca^{2+}]_i$ in cytotrophoblast cells in +Ca and 0Ca (Fig. 1). Western blotting identified P2Y2 protein in first trimester and term placenta, and cytotrophoblast cells. P2Y4 was not detected in any placental tissue or trophoblast cell samples ($n = 3$).The increase in $[Ca^{2+}]_i$ with UTP and 5BrUTP in the absence of extracellular Ca^{2+} suggests the activation of P2Y2, 4 and 6 receptors, which mobilise Ca^{2+} from intracellular stores. We have been unable to detect P2Y4 protein and therefore speculate that extracellular uridine nucleotides activate P2Y2 and possibly P2Y6 receptors in the human placenta.

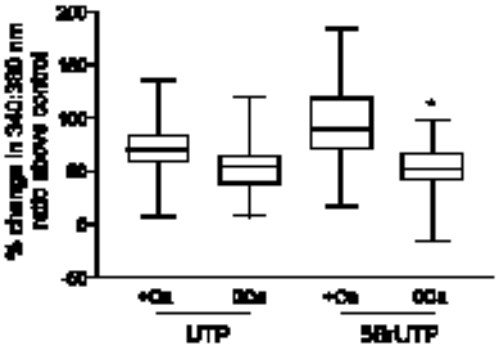

Figure 1. % change in $[Ca^{2+}]_i$ in response to 10mM UTP and 500mM 5BrUTP in +Ca and 0Ca. (34 observations from n=6 placentas). *$P < 0.05$ +Ca v 0Ca 5BrUTP Kruskal-Wallis with a Dunns post test. In every case the peak was significantly elevated above control, $P < 0.0001$ Wilcoxon matched pairs test on raw data.

Burnstock G (1997). *Neuropharmacology* **36**, 1127–1139.

Roberts VHJ *et al.* (2001). *Placenta* **22**, P56.

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All procedures accord with current local guidelines and the Declaration of Helsinki

C109

Effect of oxygen tension on nifedipine-induced relaxation of human placental chorionic plate arteries

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Normal fetal development depends on adequate placenta blood flow. Regulation of fetoplacental blood flow has been examined in the perfused cotyledon *in vitro* and, in this model, lowering oxygen tension raises vascular resistance. The mechanism of regulation of smooth muscle contraction by oxygen in fetoplacental vessels is poorly understood. Here we examine the effects of oxygen on agonist-induced constriction of placental chorionic plate arteries and relaxation to nifedipine, a L-type voltage-gated Ca^{2+} channel blocker.

Term placentae ($n = 18$) were obtained from uncomplicated pregnancies. Chorionic plate arteries (intraluminal diameter 312 ± 15 μm; $n = 76$) were mounted on a wire myograph under standard conditions. Vessels were normalised as described previously (Wareing *et al.* 2002). Constriction profiles were produced to KCl (15–60 mM) or endothelin (ET-1; 10^{-10}–2×10^{-7} M) and then paired vessels were constricted with sub-maximal doses of KCl (45 mM) or ET-1 (1.19×10^{-7} M) and relaxation assessed to incremental doses of nifedipine (10^{-8}–10^{-4} M applied for 2 min) or DMSO (diluent). Relaxation was expressed as a percentage of the time matched controls. Experiments were performed at 21 % (air/5 %CO_2) or 2 % (N_2/5 %CO_2) oxygen.

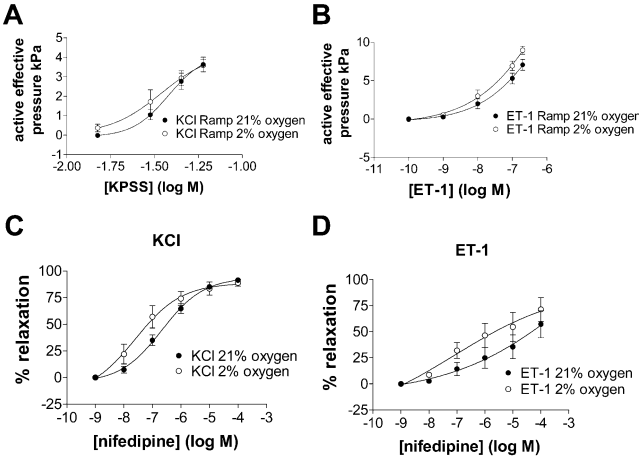

Figure 1. Constriction response profiles for KCl (A) and ET-1 (B). Nifedipine-induced relaxation of chorionic plate arteries preconstricted with KCl (45mM; C) or ET-1 (EC80 concentration; D).

The maximum constriction to KCl was not affected by reducing oxygen from 21 % to 2 % (mean ± S.E.M.: 3.6 ± 0.4kPa and 3.6 ± 0.3kPa respectively). Constriction to 45 mM KCl in 21 % oxygen was almost completely abolished by 10^{-4}M nifedipine (relaxation = 91.3 ± 1.7 %; Fig. 1A) indicating that KCl-induced

constriction is mediated by nifedipine-sensitive Ca^{2+} channels. Although reducing oxygen to 2% did not affect the maximum relaxation with nifedipine (88.9 ± 3.2%), there was a significant increase in sensitivity to nifedipine at the lower oxygen tension ($P = 0.02$ two way ANOVA; Fig. 1A). The maximum constriction to ET-1 was higher in 2% than 21% oxygen (9.0 ± 0.5kPa and 7.1 ± 0.7kPa respectively: $P < 0.05$ unpaired t test). The sub-maximal constriction to ET-1 in 21% oxygen was only partially inhibited by 10^{-4}M nifedipine (relaxation = 57.3 ± 12.9%; Fig. 1B) indicating that nifedipine-sensitive and insensitive mechanisms contribute to constriction with ET-1. Reducing oxygen tension to 2% did not affect the maximum relaxation with nifedipine (71.5 ± 11.3%) but induced a significant increase in nifedipine sensitivity ($P = 0.014$ two way ANOVA; Fig. 1B).

This study demonstrates that nifedipine-sensitive mechanisms have a role in the constriction of chorionic plate small arteries in response to KCl and ET-1. We propose:(1) constriction to ET-1 and nifedipine-induced relaxation is oxygen sensitive and (2) oxygen sensitivity is mediated by L-type voltage-gated Ca^{2+} channels which are activated by low oxygen tension.

Wareing M *et al.* (2002). *Placenta* **23**, 400–409.

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All procedures accord with current national and local guidelines

C110

An image analysis technique for the investigation of human placental morphology in pregnancies complicated by pre-eclampsia and intrauterine growth restriction

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The purpose of this study was to use visual image analysis to observe changes in the morphology and composition of placental villi in pregnancies complicated by pre-eclampsia (PE) and intrauterine growth restriction (IUGR).

Placental biopsies from 9 normal pregnancies, 5 cases of PE, 5 cases of IUGR and 5 cases of PE with IUGR were collected and randomly sampled following written informed consent, according to local ethical committee guidelines. Following formalin-fixation, wax-embedded sections were cut (5 μm) and stained with haematoxylin and eosin. These placental sections were subjected to image analysis using Metamorph software. Monochrome images (Fig. 1*A*) were pseudocoloured and thresholding applied to quantify areas of villous (1*B*), syncytiotrophoblast (1*C*) and syncytial nuclei (1*D*). All additional measurements were obtained from these primary variables.

Fetal weights were reduced in all complicated pregnancies, but only smaller placentae were obtained in cases of IUGR (631 ± 34 g *vs.* 356 ± 53g, mean ± S.E.M. $P < 0.05$ 2-way ANOVA). PE, with and without IUGR, had no effect on total villous area or intervillous space. IUGR alone showed a consistent reduction in villous area (56.0 ± 2.4% *vs.* 43.6 ± 3.3%, $P < 0.05$). The ratio of syncytium to villous was reduced in all cases of PE (0.38 ± 0.03 *vs.* 0.24 ± 0.07, $P < 0.05$), but this remained unchanged in IUGR. Birthweight was positively correlated with both placental size and total villous area occupied ($P < 0.0001$ and $P < 0.05$, Pearson's coefficient). Increasingly positive relationships were recorded between syncytiotrophoblast area and syncytiotrophoblast cytoplasm and birthweight ($P < 0.01$ and $P < 0.001$).

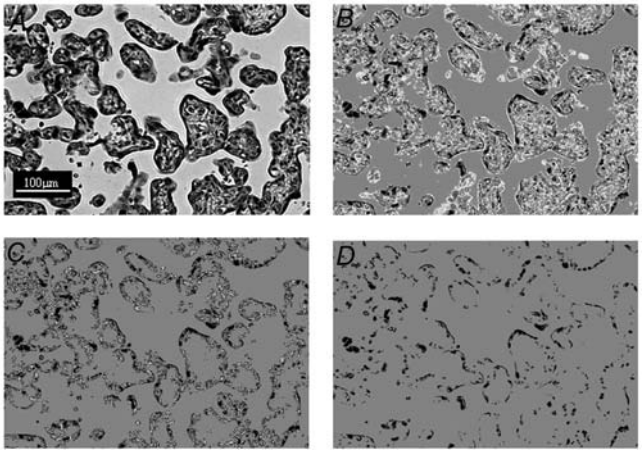

Figure 1

These measurements point to impoverished villous development in idiopathic IUGR. Even correcting for villous anomalies, the placental syncytium appears to be the dominant element in placental efficiency. These observations suggest that idiopathic IUGR and IUGR in PE have a separate aetiology, idiopathic IUGR arising through a reduction in placental weight alone, and IUGR in PE caused by changes in syncytiotrophoblast amounts, more specifically the syncytiotrophoblast cytoplasm.

This work was supported by Tommy's – The Baby Charity

All procedures accord with current local guidelines and the Declaration of Helsinki

PC112

Regulation of $P2X_7$ purinergic receptor protein expression by iron status in trophoblast cells

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Iron deficiency in pregnancy can cause fetal growth restriction, which may be due to altered placental function (Black, 2001). Intracellular Ca^{2+} ($[Ca^{2+}]_i$) regulates many aspects of placental function (Clarson *et al.* 2002). One of the mechanisms by which $[Ca^{2+}]_i$ is maintained is via activation of purinergic receptors such as $P2X_7$ and $P2X_4$ (Roberts & Clarson, 2002). In this study, therefore, we investigated the effect of altered iron status on $P2X_7$ expression in the trophoblast-derived BeWo cell line.

BeWo cells were examined under three conditions: iron depletion (20μM desferioxamine (DFO) for 40h), iron supplementation (5μM iron transferrin (FeTf) for 18h), and iron depletion followed by iron supplementation (DFO +FeTf), and compared to control. Expression of $P2X_7$ and $P2X_4$ was assessed by Western blotting using commercially available antibodies (1:100 dilution, Alomone Laboratories) and normalised to β-actin expression (1:2500 dilution; Sigma). Expression was quantified by denstiometry (ImageJ software).

From Western blotting we identified specific bands for $P2X_4$ and $P2X_7$ at the expected sizes (64 and 120 kDa respectively) in all groups studied. There was no difference in expression of $P2X_4$ between control cells and the 3 conditions investigated (control: 1.0 ± 0.0003, DFO: 0.939 ± 0.265, FeTf: 1.123 ± 0.280, DFO + FeTf: 1.541 ± 0.179, mean ± S.E.M. $n = 5$). Treatment of BeWo cells with FeTf had no effect on $P2X_7$ expression (control: 1.0 ± 0.0001, FeTf: 1.655 ± 0.389, mean ± S.E.M. $n = 6$). By contrast, treatment with DFO caused a significant increase in $P2X_7$ expression (control: 1.0 ± 0.0001, DFO: 2.020 ± 0.274,

mean ± S.E.M. $n = 6$, $P < 0.05$ ANOVA with Bonferroni test). This increase was reversed when DFO treated cells were supplemented with FeTf (DFO: 2.020 ± 0.274, DFO + FeTf: 1.144 ± 0.256, mean ± S.E.M. $n = 6$, $P < 0.05$ ANOVA with Bonferroni test).

From this study we have shown that iron deficiency can alter expression of $P2X_7$ purinergic receptor protein which may have an effect on $[Ca^{2+}]_i$ in the trophoblast cells of human placenta.

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PC113

Adaptive regulation of amino acid transport system A in the human choriocarcinoma BeWo cell line

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Adaptive regulation of amino acid transport system A is the response to the reduction of substrate availability seen in several cell types including adipocytes (Hyde *et al.* 2001). Chronic depletion of amino acids results in increased system A gene expression and *de novo* synthesis of new transporter proteins. We have studied the response of two system A transporters, ATA1 and ATA2, to amino acid deprivation in the human choriocarcinoma cell line BeWo.

The cells were maintained in DMEM supplemented with 10 % fetal calf serum and 2 % penicillin/ streptomycin. Polycarbonate filters were seeded and cells grown to form a confluent monolayer. Amino acid deprivation was induced by culturing cells in Deprivation Media 1 (DM1, Earles Balanced Salt Solution, pH7.4 supplemented with 1 × MEM essential amino acid solution without non-essential amino acids) or DM2 (DM1 with 0.5 × MEM essential amino acid solution) for 6 hours. ^{14}C-MeAIB trans-cellular transport was studied after pre-exposure of the cells to control and DM1 media. Protein and mRNA samples were isolated from control and treated cells and the expression of system A transporters analysed by Western and Northern blotting. Significance was assessed using non-paired t tests.

MeAIB trans-cellular transport was significantly ($P < 0.001$, $n = 9$) up-regulated after exposure of the BeWo cells to DM1 compared to control. ATA2 protein expression remained at control levels after exposure of the cells to DM1. However, ATA2 mRNA levels were significantly ($P < 0.0001$, $n = 12$) increased by 6 h exposure of cells to DM1 and DM2 compared to control. In contrast, ATA1 mRNA expression was decreased ($P < 0.05$, $n = 12$) by incubation of cells in DM1 and returned to control level in DM2. We could not measure ATA1 protein levels.

These results suggest that ATA2 is responsible for the adaptive regulation of system A in the BeWo cell line and that ATA1 may act as a basal transporter.

Hyde R *et al.* (2001). *Biochem J* **355**, 563.

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PC114

LPS-evoked nitric oxide synthesis increases branching morphogenesis and maturation of fetal lungs

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Although inflammation of the fetal membranes often results in premature delivery, recent studies have revealed that elements of the lung immune response can advance crucial aspects of fetal lung maturation. In a previous study, we demonstrated that exposure of fetal rat lung explants to the bacterial endotoxin, lipopolysaccharide (LPS), evoked a spontaneous increase in airway branching morphogenesis over a permissive concentration range (Land and Darakhshan, 2003). Although LPS stimulates a diverse range of cellular responses, recent studies show a positive correlation between the administration of a pharmacological NO-donor ((Z)-1-[2-(2-aminoethyl)-N-(2-ammonioethyl)amino]-diazen-1-ium-1, 2-diolate-NO) and improved airway bifurcation (Young *et al.* 2002), therefore, we hypothesised that the increase in airway branching evoked by this toxin requires the activation of inducible nitric oxide synthase (iNOS) and endogenous NO-release.

Gestation day 14 rats and their fetuses were killed humanely and fetal rat lung explants were cultured for 96 h at fetal (23 mmHg) or ambient (142 mmHg) PO_2. Low concentration of LPS (2 μg/ml) increased the airway surface complexity (ASC = perimeter/square root of area), from 1.66 ± 0.09 to 1.99 ± 0.14 ($P < 0.05$) and 1.54 ± 0.11 to 2.01 ± 0.14 ($P < 0.05$, all values are Mean ± S.E.M.; ANOVA, *post hoc* Tukey's HSD) at fetal and ambient PO_2, respectively ($n = 5$). This concentration of LPS also increased the release of nitrate (a measure of NO release, determined by the Griess assay), from 3.63 ± 0.66μM nitrate/μg protein to 22.07 ± 2.68 ($P < 0.01$) and 11.22 ± 1.89 to 44.25 ± 7.4 ($P < 0.01$) at fetal and ambient PO_2, respectively ($n = 7$). High concentration of LPS (50μg/ml) evoked widespread necrosis in explants and no increase in ASC was observed. Whereas 2 μg/ml LPS increased iNOS protein in the explants, selective inhibition of iNOS by 100 μM L-N6-(1-Iminoethyl)lysine hydrochloride (L-NIL) abrogated LPS-induced nitrate release ($n = 9$) and the resultant increase in ASC ($n = 6$).

LPS stimulates the synthesis and release of transforming growth factor-β (TGF-β), a potent inhibitor of branching morphogenesis and respiratory epithelial cell differentiation in fetal lung (Zeng *et al.* 2001). The dose dependent morphogenic effect we observed with LPS may therefore arise through the TGF-β-mediated suppression of iNOS activity, as noted in other studies (Vicencio *et al.* 2002; Vodovotz *et al.* 1999). LPS (0.5–50 μg/ml) had no effect on the expression of the 3 TGF-β receptor isoforms (TβRI, II and III) at either fetal or ambient PO_2, raising the possibility that LPS may act directly to modulate the release of TGF-β. We conclude that LPS-mediated iNOS activation and endogenous NO release is a potent inducer of branching morphogenesis in fetal rat lung explants. Constitutive expression of all three TGF-β receptor isoforms in the fetal lung highlights the possibility that TGF-β may negatively regulate lung morphogenesis *via* this pathway.

Land SC & Darakhshan F (2003). *Am J Physiol Lung Cell Mol Physiol* (in press).

Vicencio AG *et al.* (2002). *J Appl Physiol* **93**, 1121–1130.

Vodovotz Y *et al.* (1999). *Cancer Res* **59**, 2142–2149.

Young SL *et al.* (2002). *Am J Physiol Lung Cell Mol Physiol* **282**, L379–385.

Zeng X *et al.* (2001). *Dev Dyn* **221**, 289–301.

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All procedures accord with current UK legislation

PC115

Functional studies on human placental explants following retroviral transduction with MDR1(P-glycoprotein)

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We have previously shown that MDR1 P-glycoprotein (P-gp) is expressed on the microvillous membrane (mvm) of the human placenta and that it plays an important role in the capacity of cultured cytotrophoblast cells to efflux xenobiotics (Atkinson *et al.* 2003a). Recently we demonstrated raised expression of P-gp in placental villous tissue in explant culture transduced with MDR1 using a retroviral vector (Atkinson *et al.* 2003b). However after transduction P-gp was distributed diffusely in the explant and not discretely localised to the mvm. Here we test the hypothesis that diffuse P-gp distribution after transduction alters the capacity of the tissue to efflux xenobiotics.

Placentas were collected by procedures approved by the local research ethics committee. Small villous fragments dissected from normal term placentas within 1hour of delivery were cultured for 7 days in CMRL 1066 medium. For retroviral transduction the explants were co-cultured throughout with the retroviral packaging cell line GPAM MDR1. On day 7 the explants were used to measure the time course of ^{3}H-vinblastine accumulation in the presence and absence of cyclosporin A (cycA, an inhibitor of multi-drug resistance proteins).

Time course data for control explants confirms an increased accumulation of 3H-vinblastine in the presence of cycA consistent with inhibition of P-gp and reduced efflux (Fig. 1). Transduced explants show higher accumulation of ^{3}H-vinblastine in the presence of cycA compared to controls as demonstrated by a significantly higher cycA-sensitive accumulation i.e. lower efflux (Fig. 2).

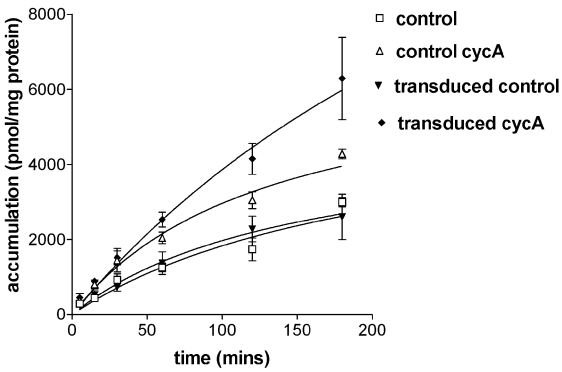

Fig.1 Accumulation of ^{3}H-vinblastine in the presence and absence of 20μM cycA in both control and transduced explants (Mean ± se n=4)

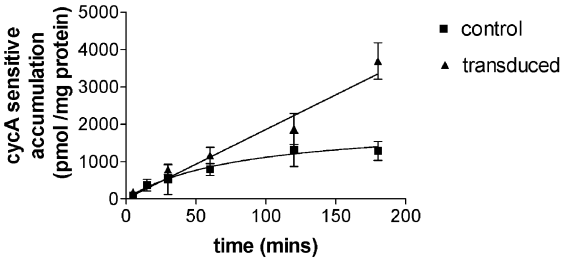

Fig. 2 cycA sensitive accumulation of ^{3}H-vinblastine in control and transduced explants. Transduced tissue shows a significantly higher accumulation than control (p<0.0006 Two way ANOVA, Mean ± se n=4)

These experiments confirm P-gp activity in cultured placental villous explants and suggest a decreased capacity of the tissue to efflux xenobiotics following retroviral transduction, despite an increased overall expression of P-gp. The increased accumulation is likely related to the broader distribution of P-gp following transduction. This study therefore demonstrates the importance of the normal mvm localization of P-gp in xenobiotic efflux from the trophoblast and illustrates the consequences of any disruption to the trafficking of this protein.

Atkinson DE *et al.* (2003*a*). *Am J Physiol Cell Physiol* **285**, C584–91.
Atkinson DE *et al.* (2003*b*). *Placenta* **24**, A.14.

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All procedures accord with current local guidelines and the Declaration of Helsinki

PC116

Localisation of leptin and the leptin receptor in fetal sheep during late gestation

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Leptin is a hormone secreted predominantly from adipose tissue, which plays a major role in energy homeostasis in the adult animal. The leptin receptor has five splice variants (ObRa, b, c, d and e). ObRb is the long form of the receptor, which is mainly responsible for signal transduction. The function of both leptin and its receptors in the developing fetus is less clear. Studies in the mouse have shown expression of leptin and its receptor in a number of fetal tissues including the placenta, bone, hair follicles, lung and brain (Hoggard *et al.* 1997). The aim of this study was to investigate the localisation of leptin and its receptor in the sheep fetus during late gestation.

Tissue samples were obtained from fetal sheep at 130d ($n = 7$) and 144d ($n = 5$) of gestation after maternal administration of a lethal dose of barbiturate. Tissue samples were embedded in paraffin and sections were cut for immunocytochemical analysis. Sections were dewaxed and non-specific binding was blocked by incubation in 5% goat serum for 1h. Tissue sections were incubated overnight at 4°C with a primary anti-body raised either against leptin (1:1000) or the leptin receptor (ObRb, Linco Research Inc., 1:100). Sections were then incubated for 40 min with 4 nm gold-labelled goat anti-rabbit secondary antibody (Jackson Immunoresearch; 1:40). Enhancement of the gold labelling was carried out using IntenSETM Silver Enhancement Reagents (Amersham Biosciences).

In the sheep fetus, leptin and leptin receptor were colocalised in a wide variety of tissues at both gestational ages. These included placenta, adipose, skin, adrenal, kidney and intestinal tissues. In the placenta, both leptin and its receptor were expressed in the trophoblast of the fetal villi. Co-localisation in the skin was specific to the epidermis and hair follicles. In the adrenal gland, leptin and its receptor were expressed predominantly in the cells of the cortex. The kidney showed co-localisation in the tubule epithelia of the nephrons but not the glomeruli. In intestinal tissues, such as the duodenum, expression of both proteins was strongest in the villus epithelial cells and in the longitudinal smooth muscle. No differences in the localisation of the leptin and its receptor were observed with gestational age. The widespread distribution of leptin and the leptin receptor suggests that they may play a significant role in growth and development *in utero*.

Hoggard N *et al.* (1997). *Proc Natl Acad Sci U S A* **94**, 11073–11078.

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All procedures accord with current UK legislation

PC117

Expression of mRNA for tandem-pore domain K^+ channels TASK and TREK in cells cultured from human placenta and choriocarcinoma cell lines

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Transport of charged solutes by the syncytiotrophoblast, which separates maternal and fetal circulations, is driven in part by electrochemical gradients, a determinant of which is membrane potential (Greenwood *et al.* 1996). TASK and TREK are members of the mammalian tandem-pore domain K^+ channel family that play a role in determining resting membrane potential and potentially K^+ homeostasis in non-excitable cells (Lesage & Lazdunski, 2000). Placental K^+ homeostasis is likely to be an important factor in determining net solute and nutrient transfer to the fetus, therefore expression of molecules participating in K^+ movement are of interest. The aim of this study was to determine mRNA expression of TASK and TREK in two trophoblast models: primary cultured cytotrophoblast cells and choriocarcinoma cell lines.

Cytotrophoblast cells were isolated from human term placentas from normal pregnancies (Greenwood *et al.* 1996). Placentas were taken with informed written consent and according to local ethical committees' regulations. Three separate cytotrophoblast cell isolations cultured for 18 (mononucleate) and 66 h (multinucleate), and three different passages of placental choriocarcinoma cell lines (JAr, JEG and BeWo) were used. Total RNA was extracted and quantified using standard techniques. RT-PCR was performed using gene-specific primers for TASK1 to 5 and TREK1 and 2 that amplified products of correct sizes (relative to a molecular ladder) when validated with control cDNA.

TASK1 was detectable in cytotrophoblasts at 18 (2/3 samples) and 66 h (3/3 samples) as was TASK2 (2/3 samples at 18 hours, 3/3 samples at 66 hours). TASK2 was observed in all choriocarcinoma cell lines (3/3 samples per cell line) contrasting with TASK1 whose signal was variable. TASK3 was detected in most samples. TASK4 was detected in all cytotrophoblast samples, JAr and BeWo cells with variable intensity. TASK5 was visible in 18 and 66 h cytotrophoblasts (2/3 samples for each) as well as BeWo cells (3/3 samples). TREK1 was observed in 18 and 66 h cytotrophoblasts (2/3 samples for each), as well as JAr (2/3 samples), JEG (3/3 samples) and BeWo (1/3 samples) cells. TREK2 was detected in all three choriocarcinoma cell lines but not cytotrophoblasts.

These data indicate different patterns of gene expression for TASK and TREK isoforms between cytotrophoblasts and choriocarcinoma cell lines.

Greenwood SL *et al.* (1996). *J Physiol* **492**, 629–640.

Lesage F & Lazdunski M (2000). *Am J Physiol* **279**, F793–802.

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All procedures accord with current local guidelines and the Declaration of Helsinki

PC118

Physiological labour affects cytokine production profile of gestational membranes *in vitro*

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Parturition is an inflammatory process involving leukocyte infiltration of gestational tissues and cytokine release from the gestational membranes (GMs) (Young *et al.* 2002). It is proposed that the inflammatory response of post-labour GMs will differ from their naive pre-labour counterparts. This study examined the effect of labour on GM baseline cytokine release and potential secretory capacity (the difference between lipopolysaccharide (LPS)-stimulated and baseline profiles).

Placentas were obtained from women delivering at term after uncomplicated spontaneous vaginal delivery ($n = 10$) and clinically comparable women undergoing term elective caesarean sections ($n = 10$). Local ethical approval was obtained. Six mm GM discs were excised with a tissue punch, washed in Hank's balanced salts solution (HBSS), and incubated in a 1:1 ratio of Dulbecco's modified Eagle medium (DMEM) and Ham's F12 nutrient mixture supplemented with 5% fetal calf serum in the presence/absence of 10 μg/ml LPS under a humidified 5% CO_2 atmosphere at 37 °C. Serial supernatant samples collected at 0, 3 and 24 h were assayed for tumour necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-6, IL-8 and IL-10 levels by multiplex cytokine immunoassay. Cytokine profiles were expressed as pg/ml/mg wet tissue ($\pm$ S.E.M.) and statistical analysis was performed by Mann-Whitney-U tests.

Baseline IL-6 and IL-10 release was significantly higher from post-labour membranes than their pre-labour counterparts at 3 h (1, 215 ± 86 *vs.* 287 ± 14 and 0.190 ± 0.027 *vs.* 0.001 ± 0.001 pg/ml/mg wet weight, respectively; $P < 0.05$), while baseline levels of IL-8 and IL-10 were higher at 24 h (2, 057 ± 254 *vs.* 1, 417 ± 223 and 0.683 ± 0.120 *vs.* 0.024 ± 0.001 pg/ml/mg wet weight, respectively; $P < 0.05$). IFN-γ release did not differ between both groups (23.38 ± 2.89 *vs.* 13.21 ± 4.87 pg/ml/mg wet weight at 24 h) Post-labour GMs also had a greater TNF-α secretory capacity at 3 h (161.81 ± 21.12 *vs.* 70.62 ± 12.83 pg/ml/mg wet weight; $P < 0.01$), while that of other cytokines was unaffected. Term labour enhanced GM baseline release of IL-6, IL-8 and IL-10, likely as a result of inflammatory priming. Furthermore, labour also enhanced pro-inflammatory TNF-α secretory capacity, probably as a result of leukocyte infiltration of the GMs. These responses differed from published data for isolated amnion and chorion, suggesting that

there is significant paracrine interaction between these layers, and that a representative *in vitro* GM model cannot be inferred from data derived from isolated layers.

Young *et al.* (2002). *Biol Reprod* **66**, 445–9.

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All procedures accord with current local guidelines and the Declaration of Helsinki

PC119

Maternal nutrient restriction during early to mid-gestation and 11 β hydroxysteroid dehydrogenase type 2 (11βHSD2) activity in the kidneys of juvenile offspring in sheep

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Maternal nutrient restriction over the period of rapid placental growth (i.e. 30–80 days) with subsequent adequate nutrition up to term, results in newborn with larger kidneys which exhibit an increased abundance of glucocorticoid receptor mRNA but a decline in the levels of 11βHSD2 mRNA expression and activity (Whorwood *et al.* 2001). The resulting offspring exhibit lower resting blood pressure but an increased responsiveness to noradrenaline (Gopalakrishnan *et al.* 2001). The aim of this study was therefore to determine the longer term consequences beyond the neonatal period of maternal nutrient restriction (NR), between early to mid gestation, on enzyme activity of 11βHSD2 in the kidneys of juvenile sheep.

Ten Welsh Mountain ewes of similar body weight and fat distribution were individually housed from 28 days gestation. Four ewes were NR, these consumed 3.5 MJ of metabolizable energy (ME) per day (~60 % of ME requirements for maintenance and growth of the conceptus) until 80 d gestation, with six controls (C) consuming 6.8–7.5 MJ/day. After 80 d gestation, until term (147 d), all animals consumed 6.8–7.5 MJ/day, sufficient to fully meet their ME requirements. Lambs were delivered spontaneously and each ewe raised a single lamb that was weaned at 6 weeks of age. At 6 months of age all lambs were humanely killed to enable tissue sampling. The kidneys were weighed and snap frozen in liquid nitrogen for subsequent enzyme preparation. Results for weight are expressed as mean values and standard deviations. Enzyme activities were determined in kidney homogenates by means of a radiometric conversion assay which determines the rate of conversion of tritiated cortisol to tritiated cortisone. Enzyme activities were compared between groups using a Mann-Whitney U test.

Kidney weights (g) from juvenile lambs were comparable between groups, mean ± S.D., (C) 46 ± 6.5, (NR) 47.5 ± 4.7). Plasma cortisol concentrations (nmol/l) were also similar (C) 22 ± 4, (NR) 20 ± 5. 11βHSD2 enzyme activity, expressed as median and interquartile range, did not differ significantly between the diet regimes (C) 1.21 (0.12–1.39) pmol/min/mg protein; (NR) 0.69 (0.14–1.26) pmol/min/mg protein. The nutritional programming of attenuated kidney 11βHSD2 expression and activity in the newborn period does not appear to persist into young adulthood suggesting this is not the major mechanism for development of hypertension in adult life. It is possible that other biochemical changes in kidney function are important in this regard.

Gopalakrishnan G *et al.* (2001). *Proc Nutr Soc* **60** OCB, 197A.

Whorwood CB *et al.* (2001). *Endocrinol* **142**, 2854–2864.

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All procedures accord with current UK legislation

PC121

Endogenous nitric oxide effects no change in epithelial Na^+ channel ENaC mRNA levels in the H441 lung epithelial cell line

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The amiloride-inhibitable epithelial Na^+ channel (ENaC) is a critical component of the fluid reabsorption pathway in the lung and so contributes to the natural removal of lumenal fluid during birth and the maintenance of fluid homeostasis in the healthy postnatal lung (Barker *and* Olver. 2002). However, work by others has shown that this delicate balance is disrupted during lung inflammation through the nitric oxide (NO)-dependent inhibition of amiloride-sensitive Na^+ transport (Ding *et al* 1999), an effect that is absent in mice lacking the gene for inducible nitric oxide synthase (iNOS) (Hardiman *et al* 2001). We have previously shown that exposure of H441 lung epithelial cells to moderate hypoxia (PO_2 23 mmHg) significantly evokes the activation of the inducible isoform of nitric oxide synthase (iNOS) and release of NO (Laird *et al* 2002). Importantly, this effect was potentiated during hypoxia by the pro-inflammatory bacterial endotoxin, lipopolysaccharide (LPS). The aim of these studies was to determine if there is a link between hypoxia/LPS-evoked NO synthesis and the transcriptional regulation of αENaC in H441 bronchial epithelial cells.

Competitive reverse transcriptase-polymerase chain reaction (comp RT-PCR) found hypoxia (PO_2 23 mmHg) activated αENaC gene expression 2.06e+07 ± 2.06+06 transcript copies (mean ± S.E.M.; $n = 6$) though remained unchanged from basal (PO_2 142 mmHg) expression (1.42e+07 ± 2.26e+06; $n = 6$). Potent NOS activator, *E. coli* lipopolysaccharide (LPS 50 μg ml^{-1}) was associated with an inducible NO synthase (iNOS)-dependent increase in the lung production of NO but also resulted in unaltered αENaC gene expression at both 142mmHg and 23mmHg from control levels (1.63+07 ± 4.01+06; $n = 5$), (1.46+07 ± 3.23+06; $n = 5$). Inhibition of iNOS by the specific iNOS inhibitor, L-N^6-(1-Iminoethyl)lysine hydrochloride (L-Nil), maintained normal αENaC gene expression at atmospheric and fetal PO_2 (1.25+07 ± 5.26+06; $n = 5$), (1.71+07 ± 5.06+06; $n = 5$). No significant differences were observed between groups.These data suggest that the inhibition of αENaC by hypoxia/LPS evoked NO production is not explained by a parallel change in gene expression.

Barker P M & Olver R E, (2002). *J Appl Physiol* **93**, 1542–1548 .

Ding J W *et al.* (1998). *Am J Physiol* **274**, L378–L387.

Hardiman K M *et al.* (2001). *Am J Physiol Lung Cell Mol Physiol* **281**, L722–L731.

Laird S M *et al.* (2002). *Proceedings of The Physiological Society, Leeds* **140P**, PC96.

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PC122

Effects of maternal dietary protein restriction on body weight and organ growth in rat offspring

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Epidemiological evidence and animal models provide strong evidence that poor early growth is a risk factor for late life cardiovascular disease, stroke, hypertension and type 2 diabetes (Barker, 1995), but the mechanisms involved in the correlation between onset of adult disease and *in utero* nutrition are unclear. A commonly used animal model to study these effects is the offspring of the rats fed a low protein diet during pregnancy and lactation. In this study, we investigated growth of Wistar rats born to mothers fed a low protein (8 %) diet (LPD rats) or a normal protein (20 %) diet (NPD rats) ad libitum beginning 2 weeks prior to mating and continuing throughout gestation and lactation were investigated. Litters were culled at birth to 8 pups per dam and litters weaned by 28 days to the normal protein (20 %) diet. Body weights of offspring were measured weekly up to 24 weeks. Organ weights were taken at 3 or 12 weeks. Rats were anaesthetised I.P. with hypnorm (0.4ml kg^{-1}) and hypnovel (0.4ml kg^{-1}), heparinized (100, 000 U kg^{-1} I.P.) and humanely killed. Organs (brain, heart, lungs, spleen, pancreas, liver, kidney and adrenal glands) were removed and their dry and wet weights were measured at 3 and 12 week of age.

Table 1. Body and organ weight in LPD rats, expressed as a percentage of mean NPD rat weights.

	3 Weeks (n=30)	12 Weeks (n=20)	24 Weeks (n=12)
Whole body	34.5 ± 6.4	75.5 ± 12.7	79.8 ± 13.8
Heart	49.7 ± 0.5	80.0 ± 1.9	n.d.
Lung	64.3 ± 1.7	80.0 ± 1.7	n.d.
Spleen	31.2 ± 0.2	77.2 ± 1.1	n.d
Pancreas	33.2 ± 0.3	60.8 ± 1.0	n.d.
Liver	34.0 ± 2.7	70.3 ± 8.7	n.d.
Kidney	41.2 ± 0.7	78.1 ± 2.6	n.d.
Adrenal	58.7 ± 0.10	66.5 ± 0.42	n.d.
Whole Brain	96.7 ± 1.3	91.3 ± 3.4	n.d.
Cerebellum	102.0 ± 1.6	114.5 ± 3.9	n.d.

Values are mean ± SEM. n.d: Not determined.

Weights of the LPD rats were significantly lower than for NPD rats at 3 and 6 weeks ($P < 0.001$) and some catch up of body weight was observed after weaning to normal protein diet (Table 1). However significant disparity of weights were remained at the adults at 12 weeks and remained at 24 weeks ($P < 0.05$, Table 1). These results suggest that growth of the offspring is consistently retarded and not fully recovered upon feeding with balanced diet after weaning in this model. Deficiencies in growth of all organs was seen when compared to NPD rats, except whole brain and in particular the cerebellum. Most organs demonstrated proportional catch up growth in adulthood including the kidneys, which contrasts with other models. However, two endocrine organs, the pancreas and adrenal gland remained disproportionately small, and to a lesser extent the liver, which has implications for poor glucose tolerance and control of blood pressure in adulthood.

Barker DJP (1995). *Proc Roy Soc. Lond* **262**, 37–43.

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All procedures accord with current UK legislation

PC123

Effect of iron deficiency on duodenal iron uptake in pregnant rats

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Iron deficiency anaemia is the most common nutritional disorder worldwide. Anaemia is especially serious during pregnancy, where Fe deficiency has deleterious consequences for the mother and her developing fetus. The mother has therefore evolved various adaptations to increase dietary Fe absorption during pregnancy. In this study we investigated these adaptations at the primary site of Fe absorption, the duodenal mucosa, in dams and neonates in response to Fe deficiency.

Weanling Female Hooded Lister rats were placed on a control diet for 2 weeks, then on either a normal (50 mg kg^{-1}) or decreased (7.5 mg kg^{-1}) Fe content diet for 4 weeks prior to mating. The rats were maintained on the same diet throughout pregnancy. Duodenal biopsies, taken after stunning and cervical dislocation, were used to determine *in vitro* 59Fe uptake (μg Fe uptake/g wet weight) immediately following birth in dams and pups.

An increase in Fe uptake was observed in the pups ($n = 8$, control 36 ± 6, deficient 114 ± 19 $P = 0.002$), and the dams ($n = 8$, cntl 7.0 ± 0.88, deficient 39.5 ± 8.4 $P = 0.005$) placed on an Fe deficient diet. These changes correlated to liver Fe levels (μg/g dry weight), which were reduced in the Fe deficient pups ($n = 8$, cntl 2640 ± 312, deficient 1713 ± 168 $P = 0.029$). Duodenal mucosa was also collected for mRNA analysis. Expression of Fe uptake, Divalent Metal Transporter 1 (DMT1), and Fe efflux, Ireg1, genes was determined by quantitative real-time PCR. Expression levels were normalised to that of actin for individual samples. In the Fe deficient pups an increase in DMT1 (cntl 2.75 ± 1.11 $n = 9$, deficient 13.1 ± 2.07 $n = 5$ $P = 0.0004$) and Ireg1 (cntl 15.3 ± 6.1 $n = 9$, deficient 44.5 ± 7.93 $n = 5$ $P = 0.017$) was observed. Suprisingly these changes were not reflected in the Fe deficient dams, DMT1 (cntl 1360 ± 460 $n = 6$, deficient 1740 ± 267 $n = 7$ $P = 0.503$ and Ireg1 (cntl 74.0 ± 4.21 $n = 9$, deficient 60.4 ± 8.26 $n = 5$ $P = 0.172$). All values given as mean ± S.E.M., Student's unpaired t test was used to determined significance between two sets of data. $P < 0.05$ were considered significant.Fe deficiency during pregnancy caused a decrease in liver iron levels, and an increase in duodenal Fe uptake in newborn pups, this was a result of increased DMT1 and Ireg1 expression. Fe deficient dams also showed elevated duodenal Fe uptake, although to a lesser extent then that of the pups, also this increase was not a consequence of upregulated DMT1 and Ireg1 expression.

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All procedures accord with current UK legislation

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Characterisation of the neonatal inflammatory response *in vitro*

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A dysfunctional cytokine-mediated inflammatory response is implicated in the pathogenesis of neonatal disease, including periventricular leukomalacia and bronchopulmonary dysplasia (Jones *et al.* 1996; Yoon *et al.* 1997). The aim of this study was to characterise the chronological progress of the neonatal inflammatory response using an *in vitro* model based on lipopolysaccharide (LPS) stimulation of cord blood from term neonates.

Blood samples ($n = 12$) obtained with ethics approval were incubated in RPMI 1640 medium with and without 10 μg/ml LPS under a humidified 5 % CO_2 atmosphere at 37 °C for 24 h. Their white cell count was determined in parallel. The concentrations of tumour necrosis factor (TNF)-α, interleukin (IL)-6, IL-8, interferon (IFN)-γ and IL-10 were determined by multiplex immunoassay at 0, 1, 3, 6 and 24 h of incubation. Potential secretory capacity (mean ± S.E.M.; expressed as pg/million white cells, WC) was defined as the difference between stimulated and control responses. Data were analysed by Kruskal-Wallis tests and post hoc Mann Whitney-U tests.

All cytokine secretory capacities increased significantly within 1 h ($P < 0.001$), which was less pronounced for IL-10 ($P < 0.05$). TNF-α secretory capacity peaked between 3–6 h (1, 581 ± 377 pg/million WC), and declined by 24 h. This pattern was also observed for IFN-γ, which peaked at 3 h, while the secretory capacities for IL-6, IL-8 (peak value 631 ± 75 pg/million WC) and IL-10 (peak value 311 ± 37 pg/million WC) increased throughout the incubation period. Overall IFN-γ secretory capacity was lowest (72 ± 10 pg/million WC) while that for IL-6 was greatest (61, 489 ± 7, 059 pg/million WC).

The neonatal inflammatory response is similar in chronological progress to that determined in adults. However, the comparatively low IFN-γ secretory profile suggests that a degree of immaturity in the neonatal response, likely associated with T-cell naive $CD45RA^+$ phenotype. These results provide insight into the chronological progress of the neonatal inflammatory response, and may prove useful in improving our understanding of the aetiology of various neonatal complications and their treatment.

Yoon *et al.* (1997). *Am J Obstet Gynecol* **177**, 406–411.

Jones *et al.* (1996). *Pediatr Res* **39**, 966–975.

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Acquisition of the progesterone induced $[Ca^{2+}]_i$ signal in capacitating human spermatozoa

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Testicular sperm in mammals are morphologically differentiated but require a period of residence in the female tract to become fertilisation-competent, including the ability to undergo the acrosome reaction (a Ca^{2+}-mediated secretory event that can be induced by progesterone from the cumulus). The physiological changes that occur to sperm during this period are collectively referred to as capacitation. This process can be induced *in vitro* in 'capacitating' media containing bicarbonate and BSA (cholesterol acceptor) and has been shown to include increased protein tyrosine phosphorylation. We investigated the effects of two *in vitro* media and capacitation duration on the progesterone-induced $[Ca^{2+}]_i$ increase in populations of human spermatozoa.

This work was carried out, according to local ethical guidelines, with the Birmingham Women's Hospital (HFEA no. 0119). Donors gave informed consent. Spermatozoa were harvested by direct swim-up into capacitating medium [sEBSS containing $CaCl_2$ (1.8 mM), KCl (5.37 mM), $NaCO_3$ (26.20 mM), NaCl (116 mM), NaH_2PO_4 (1.02 mM), $MgSO_4$ (0.8 mM), sodium lactate (41.75 mM), sodium pyruvate (2.73 mM), glucose (5.55 mM) and 0.3 % BSA] or non-capacitating medium [$CaCl_2$ (2 mM), KCl (5 mM), NaCl (150 mM), $MgCl_2$ (1 mM), glucose (10 mM) and Hepes (10 mM)]. Cells were then labelled with Fura-2 and progesterone-induced $[Ca^{2+}]_i$ increases in the sperm were measured by fluorimetry.

After incubation in capacitating medium for 6 h (required for the cells to undergo progesterone-induced acrosome reaction), the peak amplitude of the P_4-induced $[Ca^{2+}]_i$ response (303.7 ± 45.2;n = 6) was significantly greater than that of cells incubated for an equivalent period in non-capacitating medium (89.3 ± 11.5; $n = 10$; $P < 0.01$; unpaired t). However, comparison of the $[Ca^{2+}]_i$ response to progesterone measured immediately after swim up into sEBSS with that obtained after 6 h incubation showed no significant difference ($P > 0.05$; unpaired t). When sperm incubated for 6 h in the non-capacitating medium were then re-suspended for 17 min in the capacitating medium, the peak amplitude (289.1 ± 25.2; $n = 7$) of the P_4-induced $[Ca^{2+}]_i$ increase was also significantly higher than that of cells in non-capacitating medium ($P < 0.01$; unpaired t). Conversely, if sperm incubated in the sEBSS for 6 h were transferred to non-capacitating medium for 17 min, the peak amplitude of the P_4-induced $[Ca^{2+}]_i$ increase (121.2 ± 13.1; $n = 10$) was significantly lower than that of the cells in sEBSS ($P < 0.01$; unpaired t).

We conclude that the cells can acquire or lose their competence to respond to progesterone with a $[Ca^{2+}]_i$ signal within 17 min of suspension in appropriate medium but that acquisition of competence to undergo AR requires other changes which require longer to develop.

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