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The effect of 4-aminopyridine, a voltage-gated K^+ channel blocker, on the differentiation of human cytotrophoblast cells *in vitro*

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Syncytiotrophoblast, the transport epithelium of the human placenta, is maintained during pregnancy by cellular turnover, involving apoptosis and loss of syncytial nuclei which are replaced by differentiation and fusion of cytotrophoblast cells (1). In non-placental tissues, voltage-gated K^+ (K_v) channels participate in cellular fusion and developmental regulation (2,3). The placenta expresses mRNA for K_v channel subtypes (4) but their role in syncytiotrophoblast development remains to be explored. The aim of this study was to determine the effect of 4-aminopyridine (4-AP), a blocker of K_v channels, on cytotrophoblast differentiation using a well characterised *in vitro* model.

Human cytotrophoblast cells were isolated from 6 normal term placentas and maintained in culture for 66h as previously described (5). At 18h, cells are mononucleate and by 66h they aggregate, fuse and differentiate into multinucleate syncytia. An increase in human chorionic gonadotrophin (hCG) secretion accompanies morphological differentiation (5). Cells were exposed to 0.01-5.0mM 4-AP over 18-66h or left untreated. Culture medium was collected at 18 and 66h, and analysed for hCG and lactate dehydrogenase (LDH) activity, markers of differentiation and cell death respectively. Cell protein was determined at 18 and 66h and cells fixed in methanol for qualitative assessment of aggregation and multinucleation by phase contrast microscopy. Paired 18 and 66h untreated controls were analysed using a Mann Whitney U test. The effects of 4-AP at 66h were expressed as a % of the corresponding 66h control (100%) and the median and interquartile range compared to 100% using a Wilcoxon Signed Rank Test.

In control cultures, mononucleate cells at 18h aggregated and fused to become multinucleated by 66h. This was associated with an increase in hCG secretion from 7 to 150 mIU/ml/mg protein/h (18 vs 66h: $p<0.02$) and a fall in LDH release from 0.7 to 0.2 absorbance units/mg protein/h (18 vs 66h: $p<0.03$). At 0.1 and 0.01mM, 4-AP significantly inhibited hCG secretion to 86% (75-92%; $p<0.04$) and 72% (46-93%; $p<0.02$) of control, without any affect on either cell protein or LDH release; microscopic examination revealed cell aggregation but multinucleation was less evident than with 66hr controls. 5.0mM 4-AP reduced hCG secretion and cell protein at 66h to 0.9% (0-21%; $p<0.02$) and 34% (16-43%; $p<0.02$) of control and simultaneously increased LDH release to 361% (267-761%; $p<0.02$) of control. Extensive cellular necrosis was observed indicating that 4-AP was cytotoxic at 5.0mM.

Inhibition of hCG secretion by 0.01 and 0.1mM 4-AP implicates a role for K_v channels in cytotrophoblast differentiation. Further work is needed to characterise K_v channel participation in maintenance of syncytiotrophoblast.

In summary, we demonstrated that hCG secretion was inhibited by 0.01 and 0.1mM 4-AP, suggesting that Kv channels participate in cytotrophoblast cell differentiation *in vitro*.

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

C77

Placental serine transport into the fetus is mediated by amino acid exchangers

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The mechanisms mediating amino acid transport across the basal membrane of the placental syncytiotrophoblast and into the fetal circulation are not well understood. This study investigates the hypothesis that exchange transporters may mediate the process of amino acid transport in the placenta. Specifically, the mechanisms by which serine and glycine transfer occurs across the isolated perfused human placental cotyledon are investigated.

Placentas (n = 5) were collected within 30 min of delivery and perfusion of an intact cotyledon with a modified Earl's Bicarbonate Buffer established as described previously (Brownbill *et al.* 1995). Perfusion was considered to be successful where there was > 95% recovery of fetal perfusate. The maternal arterial circulation was perfused with 100 μmol/l L-serine, glycine, 0.6 μmol/l ^{14}C-L-serine, 20 μmol/l ^{3}H-glycine and 1.8 mM creatinine. The role of exchangers in the transport of serine and glycine was assessed by giving of glutamate (20 μmol) and alanine (10 and 20 μmol) boluses to the fetal arterial circulation. The rationale was that alanine is a substrate for amino acid exchangers and so should stimulate serine/glycine transfer, while glutamate is not an exchanger substrate and should not stimulate transfer. Appearance of ^{14}C-L-serine and ^{3}H-glycine were measured in maternal and fetal venous samples by dual label liquid scintillation counting. Data (mean ± SEM) were expressed as area under the curve (AUC) and analysed by one-way ANOVA followed by Bonferroni post hoc test.

Under our basal conditions, the proportion of maternal substrate appearing in the fetal venous circulation for serine (8.6 ± 1.0%) and glycine (8.3 ± 0.41%) was similar to that of creatinine (8.2 ± 1.1%), a marker of transfer by paracellular routes. There was no significant increase in transfer of serine or glycine following a fetal arterial bolus of 20 μmol glutamate (serine 0.9 ± 1.1 nmol and glycine 2.5 ± 3.1 nmol). These values were then used as baseline for analysis of the effects of alanine boluses. Following a 10 μmol alanine bolus there was an increase in serine

release (AUC 29.4 ± 5.1 nmol, $p < 0.05$). Serine release following a 20 μmol alanine bolus was greater than baseline and that following the 10 μmol alanine bolus (AUC 61.0 ± 5.8 nmol, $p < 0.05$). Glycine release was not significantly altered following 10 or 20 μmol alanine boluses (AUC 8.4 ± 2.8 and 5.5 ± 2.5 nmol, respectively).

The increased transport of serine into the fetal circulation following increasing doses of alanine demonstrates for the first time that amino acid exchangers mediate serine transfer from the placenta into the fetal circulation in the intact human cotyledon. The same mechanism does not appear to operate for glycine transfer.

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

C78

Copper deficiency during pregnancy affects the protein expression profile of rat placenta

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Copper (Cu) is an essential micronutrient for humans and animals. Moderate Cu deficiency during pregnancy can be serious for the baby, leading to both short and long term consequences such as connective tissue abnormalities, skeletal defects and abnormalities in cardiac ultra structure. During pregnancy, the placenta regulates the transfer of micronutrients to the growing fetus. Little is known about the molecular adaptation in the placenta as a response to Cu deficiency. We compared the protein expression profile from placenta of rats fed diet low in Cu (0.75 mg Cu/kg diet) to that of placenta of rats fed control diet (5 mg Cu/kg diet). 12 Female Rowett Hooded Lister rats were weaned onto control diet. They were then randomly assigned to one of the two dietary treatments: control (n=6) or Cu deficient (n=6) diet for 4 weeks prior to mating and throughout pregnancy up to gestation day 21 where the placentas were isolated. Protein extracts were separated by 2-D gel electrophoresis, analyzed by PDQuest and subsequently by mass spectrometry and sequencing. Principal component analysis of the spot density values revealed that 24% of all variance in the data set was accounted for by the first principal component and an additional 15% was accounted for by the second principal component. There was a clear separation of the spots into two groups corresponding to the two treatments. Spot intensities were analyzed by Student's t test at 5% significance to determine differences in responses between the control and Cu-deficient group. Cu deficiency during pregnancy resulted in differential expression of 60 proteins in the rat placenta. These were identified as proteins involved in, for example, signal transduction, protein synthesis, calcium signaling, iron metabolism and structural proteins, enzymes and chaperones. Using pairwise correlation analysis (Pearson correlation coefficient > 0.6) we identified two clusters of proteins in the data set. One cluster of proteins included acute phase alpha-1 protein, transferrin and serotransferring, liver regeneration related protein, aconitase hydratase, 3-alpha hydroxysteroid dehydrogenase, tropomyosin 1, myosin regulated light chain 2-A, 26 protease regulatory subunit S10B, ribosomal protein RS 40K, voltage-dependent anion-selective protein 1, beta actin, keratin type II, proliferating nuclear antigen and guanine nucleotide-binding protein beta-2 chain. Of these, 7 proteins were over expressed and the rest down regulated in the placenta of Cu deficient animals as compared to controls. The other cluster identified comprised mainly of secreted proteins which were all down regulated. In this study, we have, by using proteomics techniques, provided novel insights into the mechanism by which Cu deficiency during pregnancy may affect the developing fetus.

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Epidermal growth factor reduces apoptosis induced via the intrinsic pathway in human villous trophoblasts

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Pre-eclampsia is a multi-system disorder of pregnancy and is a major cause of maternal and fetal morbidity and mortality. Its aetiology is unclear, although apoptosis is increased in the villous placenta and has a suggested pathogenic role [1]. Both the intrinsic and extrinsic apoptotic pathways have been identified in placental trophoblast. Possible activators of the intrinsic pathway are reactive oxygen metabolites, generated by oxidative stress, as a result of inappropriate placental perfusion. Epidermal growth factor (EGF) suppresses the extrinsic pathway to apoptosis in trophoblasts *in vitro*; although no investigations of oxidative stress-induced intrinsic apoptosis have been performed [2]. In pre-eclampsia, there is a known reduction in circulating levels of EGF. To assess whether apoptosis induced via the intrinsic pathway *in vitro*, by hydroxyl radicals from hydrogen peroxide (H_2O_2), can be inhibited by the addition of exogenous EGF.

Placental villous explants were taken from 3 randomly selected areas of normal term human placentae (n=6) and cultured with increasing concentrations of H_2O_2 (0, 100, 1000μmol/l) and EGF (0, 10, 100ng/ml) for 6 or 48 hours. Tissue was also pre-incubated with EGF for 24 hours prior to the addition of H_2O_2. Human chorionic gonadotrophin (hCG) and lactate dehydrogenase (LDH) were measured in the resulting media, using an enzymatic and radioimmunoassay; assessing cytotrophoblast differentiation and tissue viability, respectively. TUNEL staining for DNA fragmentation, a late event in apoptosis, was performed on paraffinised tissue sections and expressed as an apoptotic index (TUNEL positive nuclei/total nuclei). Tissue was also fixed for electron microscopy. There were no significant effects of H_20_2 and/or EGF on LDH liberation or hCG production after 6 and 48 hours culture. However, the apoptotic index was increased at the highest dose of H_2O_2 at both time points ($p \leq 0.03$, Friedman's statistical test). This apoptotic effect was diminished by the addition of EGF at

either concentration ($p \leq 0.05$). Pre-incubation with EGF significantly reduced apoptosis in the absence of H_2O_2 ($p \leq 0.03$). Electron microscopy confirmed a reduction in cytotrophoblast apoptosis in explants cultured with EGF.
This is the first time EGF has been shown to antagonise the intrinsic pathway of apoptosis in placental villous trophoblasts; a potential primary pathogenic event in pre-eclampsia. This study proposes the replacement of EGF as a therapeutic intervention in pre-eclampsia.

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C115

Differential effects of a low protein diet throughout pregnancy on glucose-6-phosphatase and glycogen content in maternal and fetal rat liver

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Human epidemiological studies have shown that low birth weight is associated with glucose intolerance, insulin resistance, obesity and type 2 diabetes in the adult. In rats, experimental induction of intrauterine growth retardation leads to abnormal glucose metabolism in the adult offspring but whether these abnormalities track from fetal life remains unclear (1). Short-term undernutrition of sheep during late gestation has been shown to alter fetal hepatic gluconeogenic enzyme activities (2). However, little is known about the effects of prolonged dietary manipulation during pregnancy on hepatic glucogenic capacity. Therefore, this study measured hepatic glycogen content and activity of glucose-6-phosphatase (G6Pase), the final enzyme in the glucogenic pathways, in pregnant rats and their fetuses after maternal protein deprivation throughout pregnancy.

Pregnant Wistar rats were fed either an 8% protein diet (LP, n=7), or a standard 20% protein diet (CON, n=4) throughout pregnancy. After terminal anaesthesia on day 20 of a 21 day gestation, maternal and fetal livers were collected and frozen for later analysis of G6Pase activity (3) and glycogen content (4). Blood glucose concentration was measured with a glucometer after decapitation of each pup. Sex of the pups was determined by measuring ano-genital distance. Livers from two males and two females from each litter were analysed. Data were analysed via Students t test. Litter means (± SEM) are reported below.

Fetal body weight was significantly less in LP than CON (LP 3.4 ± 0.1g vs. CON 3.8 ± 0.1g; $p<0.001$) but was not affected by their sex. Fetal plasma glucose did not differ with diet (LP 4.6 ± 0.3mM (n=7) vs. CON 4.5 ± 0.2mM (n=4)) or between sexes (LP male 4.3 ± 0.4 (n=7) vs. LP female 4.7 ± 0.3 (n=7); CON male 4.2 ± 0.1 (n=4) vs. CON female 4.7 ± 0.2 (n=4)). Maternal G6Pase activity and glycogen content, and fetal glycogen content were significantly higher with the LP than CON diet, while fetal hepatic G6Pase did not differ with diet (Table 1). There were no significant differences in fetal hepatic G6Pase activity or glycogen content with pup sex with each diet.

These findings indicate that prolonged protein deprivation during rat pregnancy enhances both the maternal and fetal glucogenic capacity by late gestation. The changes in glucose metabolism observed in adult rats protein-deprived in utero may, therefore, be present much earlier in life.

Table 1: Hepatic G6Pase activity and glycogen content in mothers and fetuses on different diets

	Mother		Fetus	
	CON	LP	CON	LP
G6Pase (U/g)	7.2 ± 1.2	11.8 ± 0.7*	1.1 ± 0.1	1.3 ± 0.1
Glycogen (mg/g)	24.1 ± 0.8	50.5 ± 8.0*	51.1 ± 3.5	100.7 ± 4.2*

*Significant difference from CON (t test, $p<0.05$)

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C116

Fetal skeletal muscle fibre number and type are altered by early and late gestation maternal undernutrition in sheep

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Epidemiological studies have shown that small size at birth is associated with reduced muscle strength (Sayer *et al.* 2004) and type 2 diabetes (Hales *et al.* 1991) in adulthood. In sheep, reduced early gestation maternal nutrient intake reduces fetal skeletal muscle fibre number (Zhu *et al.* 2004) and alters myofibre composition in postnatal life (Fahey *et al.* 2005). Insulin resistance is associated with a shift towards a higher proportion of the relatively insulin-resistant type 2 fast-twitch fibres at the expense of the insulin-sensitive type 1 slow-twitch fibres (Marin *et al.* 1994). In this study we investigated the effect of early or late gestation nutrient restriction on muscle fibre number and type in late gestation fetal sheep.

Pregnant Welsh Mountain ewes of uniform body weight were housed individually, and received either, 100% of total nutrient requirements throughout gestation (C, n=6: female (f)=3, male (m)=3), 40% for first 30 days gestation (dGA) (ER, n=8: f=3, m=5) or 50% from 104 dGA until post mortem (LR, n=7: f=3, m=4), with 100% requirements at all other times. All fetuses were singletons. At ±127 dGA (term ~147 dGA) ewes were killed by an overdose of barbiturate (i.v., 145 mg/kg) and the fetal triceps brachii removed and immersed in freezing isopentane. A 10 µm section was cut, stained with anti-fast skeletal myosin and five random fields (magnification x40) were captured. Fibre density, type and cross-sectional area (CSA) were measured and analysed by ANOVA with Bonferroni post-hoc tests. Data are expressed as mean ± SEM.

The total density of myofibres was reduced in both the ER ($p<0.01$) and LR ($p<0.05$) compared to C fetuses (C, 3869 ± 318; ER, 3013 ± 453; LR, 3075 ± 535.6 fibres mm^{-2}). Slow fibre density was significantly lower in LR ($p<0.05$), but not ER fetuses (C, 603 ± 195; ER 494 ± 99; LR 370 ± 122 fibres mm^{-2}). Fast fibre density tended to be lower in ER ($p=0.07$) but not LR fetuses (C, 3286 ± 402; ER 2680 ± 399; LR 2855 ± 557 fibres mm^{-2}). Fast and slow fibre CSA was similar in all nutritional groups.

These data show that reduced maternal nutrition in either the peri-implantation period or late gestation affects myofibre density, but that late gestation nutrient restriction has particularly profound effects on slow-twitch fibres. These observations may

have important implications for understanding the determinants of muscle strength and glucose tolerance in later life.

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

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Differential effects of maternal nutrient restriction during early fetal development on perirenal adipose tissue adiponectin, leptin and tumour necrosis factor alpha mRNA expression of adult offspring

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Adipose tissue is a major endocrine organ responsible for the release of hormones, termed adipokines, with roles in the inflammatory response and insulin sensitivity. Of these, leptin and tumour necrosis factor (TNF) alpha increase with obesity, whilst adiponectin decreases. Adiponectin has anti-inflammatory properties and reduces insulin resistance. Although maternal nutrient restriction during pregnancy programmes offspring adipose tissue sensitivity and later adiposity, whether it regulates adipokine expression in perirenal adipose tissue (PAT) in the absence of an effect on total fat mass is currently unknown.

Singleton-male-bearing Scottish Blackface ewes of similar weight were individually housed from day of mating. Eight control (C) ewes were fed 100% of total metabolisable energy requirements whilst ten nutrient restricted (NR) ewes consumed 50% of ME requirements up to 95 days of gestation. All ewes then consumed 100% of ME requirements up to term (147 days). Rams were overdosed with barbiturates at ~3 years of age to enable tissue sampling. Adiponectin, leptin and TNF alpha mRNA abundances were quantified by real time qRT-PCR with standard curves generated from isolated gene specific PCR amplicons to ensure equal PCR efficiency. Quantitative gene expression was measured using the 2−deltaCT method with the housekeeping gene 18S as an internal control. Statistical differences between nutritional groups were analysed using a Mann-Whitney U test ($p<0.05$).

Offspring growth rate, body weight and adiposity to 3 years of age were unaffected by maternal nutrient restriction. Although there were no differences between groups in the mRNA abundances of leptin or TNF alpha in PAT, adiponectin mRNA was significantly up-regulated in NR offspring.

In conclusion, although maternal nutrient restriction from 0-95 days gestation had no effect upon body or adipose tissue weight, mRNA abundance for adiponectin was up-regulated in NR adult offspring. As adiponectin is inversely related to fat mass, inflammation and insulin resistance, its up-regulation may reflect a compensatory mechanism for adult offspring following maternal nutrient restriction in early gestation. Such an adaptation in NR offspring may have prevented/ delayed increased adiposity associated with early maternal undernutrition.

Table 1. mRNA abundance of adipokines in perirenal adipose tissue

	Fat mass (g/kg)	Leptin	TNF alpha	Adiponectin
C	16.8 ± 2.6	$3.0 \times 10^{-6} \pm 2.3 \times 10^{-6}$	$1.2 \times 10^{-4} \pm 3.7 \times 10^{-5}$	$5.5 \times 10^{-2} \pm 1.2 \times 10^{-2}$
NR	19.4 ± 3.4	$4.4 \times 10^{-5} \pm 2.8 \times 10^{-5}$	$6.6 \times 10^{-5} \pm 3.3 \times 10^{-5}$	$3.2 \times 10^{-1} \pm 1.2 \times 10^{-1}$*

Values are means and SEM, *$p<0.05$.

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

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Renal mitochondrial dysfunction accompanies glucose intolerance in an ovine model of developmental programming

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In humans abnormal mitochondrial function has been associated with type II diabetes (T2D; Kelley et al. 2002). We have previously demonstrated poor glucose tolerance in the adult offspring of ewes undernourished (0.5 maintenance) over late gestation (Gardner et al. 2005). Therefore, in the present study, we hypothesize that mitochondrial dysfunction in the kidney accompanies glucose intolerance in these sheep.

Blue faced Leicester cross Swaledale sheep were fed a dietary regime as described by Gardner (2005): Control group (C n=7) received 100% metabolizable energy (ME) as defined by the Agricultural and Food Research Council, throughout gestation; early nutrient restricted group (NRE, n=6) 50% ME gestation from days 1-30 with 100% ME thereafter; and late nutrient restricted group (NRL, n=4) 50% ME from days 110-term (~day147) with 100% ME at all other times. Ewes delivered spontaneously and at 3 months, lambs were weaned onto pasture at 10 weeks and remained on grass until 12 months. Renal tissue was then harvested post mortem from the offspring, snap frozen and stored at -80°C. Mitochondrial respiratory chain complexes I to IV and citrate synthase activity were measured spectrophotometrically (Rahman et al. 2001). Respiratory chain enzymes were expressed as a ratio of citrate synthase to compensate for mitochondrial enrichment of the sample. Results were analyzed by ANOVA and expressed as means ± SEM.

Higher mitochondrial activities in linked complex II III assay was observed in NRL vs. other groups, C (0.17±0.014) vs. NRL (0.27±0.051) $P<0.05$ and NRE (0.19±0.018) vs. NRL (0.270±0.051) $P<0.05$.

We conclude that late gestational global under nutrition leads to abnormal renal mitochondrial function that is associated with

glucose intolerance. Our data show a direct correlation between poor glucose control that is symptomatic of T2D in humans with abnormalities in mitochondrial complex activities.

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PC201

Small birth weight in Meishan piglets is linked to low milk leptin and upregulation of glucocorticoid receptor in subcutaneous adipose tissue

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Epidemiological studies provide clear evidence that mechanisms defining weight and body composition at birth impact upon obesity and insulin resistance development in latter life. The fetal programming of weight and body composition is a complex phenomenon resulting from both environmental (i.e. maternal nutrition, infections or placental conditions) and genetic factors (Ong et al. 2004; Symonds et al. 2005). Compared to Large White (commercial), Meishan (an ancient oriental breed) piglets are known to be smaller and fatter at birth. The comparison of those breeds could then represent a 'natural' animal model to discriminate the specific early mechanisms regulating birth weight and body composition. They could be both linked to maternal milk composition and/or fat mass transcriptional activity.

Five Meishan (M) and 5 Large White (LW) sows gave birth naturally at term and the median piglet from each litter was entered into the study. Milk from sows was collected daily during the first 4 days after birth in order to measure leptin concentrations (leptin Linco, Mo, USA). Piglets were weighed daily and their total lean mass was determined by total body electrical conductivity (TOBEC) analysis. At 4 days of age they were overdosed with barbiturate. Subcutaneous adipose tissue was sampled in order to determine insulin-like growth factor I (IGF-I), IGF binding protein (IGF-BP3), peroxysome proliferator activated receptor (PPAR-gamma) and glucocorticoid receptor (GC-R) gene expressions by real-time RT-PCR. Gene expressions were assessed with the 2 – delta Ct method, taking 18S rRNA as a housekeeping gene, and expressed as relative values taken means Large White piglet values as reference (1.00).

Average milk leptin was 2-fold lower ($p<0.05$) in the M than in the LW group. M piglets were lighter and fatter than LW piglets throughout the study ($p<0.05$). No significant differences were observed between the groups for IGF-I, IGF-BP3 and PPAR-gamma gene expressions. GC-R gene expressions were approximately 2.5 times higher in M than in LW subcutaneous adipose tissues ($p< 0.01$). GC-R gene expressions was negatively related to average milk leptin ($r = -0.47$; $p< 0.05$), total lean mass ($r = -0.80$; $p= 0.009$) and birth weight ($r = -0.86$; $p< 0.001$).

Both milk composition and subcutaneous fat mass transcriptional activity differed between M and LW pig breeds. The differences in milk leptin and GC-R transcription may be supporting a stimulation of food intake and fat mass development in Meishan piglets. The comparison of these breeds could represent a useful 'natural' model to study the mechanisms programming appetite control and fat mass development.

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PC202

Gestational changes in expression and localization of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) in human placenta

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There is mounting evidence that multi-drug resistance proteins play an important role in fetal protection from drugs and xenobiotics in maternal plasma. The role of these proteins may be of particular importance early in gestation when fetal susceptibility to teratogens is greatest. Here we examine the expression and localization of two multi-drug resistance proteins, P-gp and BCRP, in human placenta during the first trimester as compared to term.

First trimester (1 each at 6, 8, 9, 11 and 12 weeks) and term placentas (n=4) were collected and villous fragments dissected, fixed in 4% formaldehyde, paraffin embedded and 4μM sections cut. The sections were de-waxed in xylene and endogenous peroxidase activity blocked using 0.5% hydrogen peroxide in methanol. For BCRP, antigen retrieval was then performed using 10mM sodium citrate buffer. Following this all sections were blocked for 10 min using DAKO protein block and incubated with primary antibody: P-gp Clone F4 (1:20) or BCRP BXP-21 (1:50) overnight at 4°C. A biotinylated secondary rabbit anti-mouse antibody was then applied for 1 hour at room temperature (1:200), followed by 0.5μg/ml avidin peroxidase in 0.125M TBS. The sections were then placed in a bath of 0.05% diaminobenzidine tetrahydrochloride dehydrate (DAB) with 0.015% hydrogen peroxide for 5 min. Methyl green (0.25%) was used as counterstain. Negative controls were processed in exactly the same way, but TBS replaced the primary antibody during the 4°C incubation. Sections were viewed using a Leitz Dialux 22 microscope fitted with a Sony DXC930P camera. All samples for each antibody were processed simultaneously to allow comparison of staining intensity at different gestations.

At term both proteins were located predominantly on the microvillous membrane of the syncytiotrophoblast whilst in all first trimester samples a broader distribution throughout the syncytium was observed (Fig. 1). In each first trimester sample staining was markedly heavier when compared with the four term samples, suggesting higher expression of these efflux proteins earlier in gestation. This correlates well with observations of decreasing placental RNA expression for P-gp over gestation (Sun *et al.* 2005) but conflicts with reports of unchanged RNA expression for BCRP (Mathias *et al.* 2005). Further quantitative determinations are required to clarify this.

A higher functional expression of P-gp and BCRP in the first trimester placenta could act to maintain low levels of xenobiotics in the first trimester fetus thereby giving increased protection from xenobiotic insult during this critical period of development.

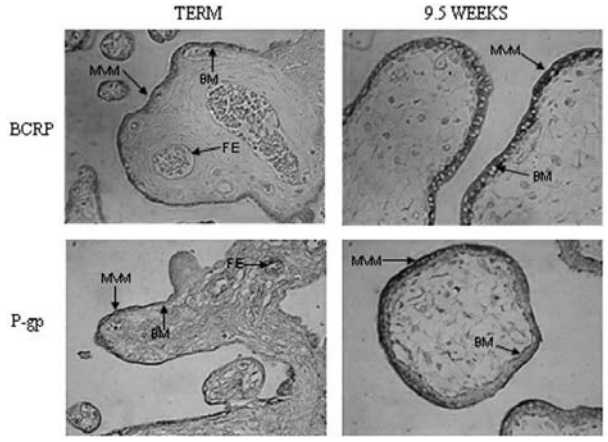

Figure 1. Expression and localization of BCRP (BXP-21) and P-gp (F4) in human placenta

at term (representative of 4 samples) and 9 weeks gestation. A similar distribution is seen in all first trimester samples (magx400, MVM-microvillous membrane, BM-basal membrane, FE-fetal endothelium).

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

PC203

Expression, localisation and function of urotensin II and its cognate receptor in the human placenta

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Urotensin II (U-II) is a highly conserved cyclic peptide that binds to its cognate G protein coupled receptor U2R. The U-II-U2R system is the most potent vasconstrictor known. Pre-eclampsia is characterised by hypertension and high levels of protein in the urine, with the disease manifesting in approximately 5-7% of pregnancies. Here we investigate the roles of U-II and U2R in the physiology of the normal and pre-eclamptic placentae, in particular, focusing in on placental arteries and the syncytiotrophoblast (the cellular interface between the mother and the fetus). We defined pre-eclampsia for this study as a BP >140/100 on two separate occasions with >2+ proteinuria on dipstick. All patients were >37 weeks gestation. Changes in the levels of U-II ligand or receptor would be expected to alter the physiology of the placenta and possibly mediate the hypertensive response characteristic of pre-eclampsia. Previous studies have suggested an increased level of U-II in pre-eclamptic patients although nothing was known about the receptor (Balat et al. 2005). Here we present immunolocalisation studies that indicate the U2R is within the syncytiotrophoblast and fetal arterial vessels of normopregnant and those with pre-eclampsia. Addition of U-II to the BeWo cell line (a recognised syncytiotrophoblast model) led to activation of extracellular signal-regulated kinase 1/2 (ERK). Furthermore contractions of normal placental arteries were measured using wire myographic studies and showed U-II has an EC_{50} of 600±5nM (n=4). Contractile responses of vessels to U-II from pre-eclamptic placentas are to be investigated. Real time PCR studies showed a 1.8-fold increased level (although not significant using two-way Mann Whitney U test) of U2R in patients with pre-eclampsia compared to patients without pre-eclampsia (n=7 for each). We, however, failed to detect U-II mRNA in the placenta suggesting the peptide is synthesised remotely.

We have identified the presence of the U-II receptor in human placenta localised to the syncytiotrophoblast and placental vessels. Administration of U-II peptide vasoconstricted isolated placental vessels, consistent with the known role of U-II in the vasculature. UII stimulation of the human placental BeWo cell activates ERK signalling. Ascribing a function to this will provide a valuable insight into the role of U-II in this tissue and may provide further insights into the pathogenesis of pre-eclampsia.

Balat et al. (2005). Eur J Obstet Gynecol Reprod Biol 120, 33-38.

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

PC204

Copper deficiency increases copper uptake and transporter expression in a placental model

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Copper is involved in a variety of biological processes such as embryo development, connective tissue formation and nerve cell function. Consequently, copper transport at the cell surface and the delivery of copper to intracellular proteins are critical events in normal physiology (1). The copper transport proteins CTR1 and CTR2 have been identified as possible regulators of cellular copper transport in several cell types (2). However, little is known about the regulation of Cu transport mechanisms in the placenta. We have studied the response of copper transport to Cu deficiency in the human choriocarcinoma cell line b30. The cells were maintained in DMEM supplemented with 10% fetal calf serum and 2% penicillin/ streptomycin. Copper deficiency was induced by the addition of 20μM of the copper chelator diamsar to the medium for 18 hours. Copper uptake was studied using real-time microfluorimetry. Briefly, control or copper-deficient cells on coverslips were loaded with 10μM Phen Green (fluorophore) for 1 hour at 37°C, coverslips were mounted onto the microscope stage and perfused with control Tyrode buffer, cells were exposed to increasing levels of exogenous Cu. Fluorescent quenching (ΔF/cell/min) was measured at 495nm in individual cells using a Nikon Eclipse 2000-U fluorescent microscope and Metaflur software. Protein and mRNA samples were isolated from control and copper-deficient cells and the expression of copper transporters (CTR1/2) analysed by Western and Northern blotting. CTR1 localisation in control or Cu-deficient cells was compared using immunocytochemistry. Copper uptake was increased in copper-deficient

cells (Vmax = 128.3±9.96 ΔF/cell/min; mean±SEM)) compared to time-matched controls (48.17±6.05 ΔF/cell/min). However, no difference was seen in Km values. Both CTR1 and CTR2 mRNA levels were significantly (Student's t test, $p<0.05$, n = 12) increased by copper-deficiency. CTR1 protein showed both increased expression ($p<0.01$, n=9) and relocalisation following copper deficiency. We could not measure CTR2 protein levels. These studies demonstrate a compensatory response to Cu deficiency in b30 cells involving increased activity via increased expression of the Cu transporter mechanisms.

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We thank Dr A. L. Schwartz (St Louis Children's Hospital, St Louis, Missouri, USA) for the supply of the b30 cells. This work is funded by International Copper Association.

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PC205

Potassium channel gene expression in the human placental vasculature of normal and growth-restricted pregnancies

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In normal pregnancy (NP), voltage [K_v] and ATP [K_{ATP}]-sensitive potassium (K) channel mRNA is expressed in the placental vasculature [1]. We have shown that K channels are important in the control of placental vascular reactivity in NP [2] and in pregnancies affected by intrauterine growth restriction (IUGR) [3]. However, there are no data on gene expression of K channels in the NP and IUGR placental vasculature. We aimed to determine K channel gene expression in NP and IUGR placental tissue.

Placental homogenate, chorionic plate arteries and veins (NP; N=6: IUGR; N=5) were obtained after vaginal delivery or Caesarean section at term. Tissue sampling, total RNA isolation and cDNA production were performed as described previously [4]. K channel expression was assessed using SYBR Green 1 quantitative PCR. Channel sequences were derived from GenBank with reference to BLAST and primers designed with Beacon designer software. Each sample (1µl) was run as quadruplicate reactions with a passive reference dye. Quantification was performed using sample cycle threshold values to calculate initial input amounts from a standard curve of cDNA generated using a human reference RNA and normalised to a human reference cDNA calibrator. Expression was assessed relative to the calibrator.

In IUGR placental chorionic plate arteries and veins showed similar expression levels of $K_v2.1$; placental homogenate expression was increased compared to arteries only. Similar expression patterns were seen for $K_v9.3$ and $K_{IR}6.1$. In NP a similar pattern of expression was observed with $K_v2.1$; similar arterial and venous expression; raised expression in placental homogenate compared to arteries only. With $K_v9.3$ and $K_{IR}6.1$, expression levels were similar in arteries, veins and placental homogenate. When comparing IUGR and NP gene expression directly, only venous expression of $K_v2.1$ was increased (Fig 1; $P<0.05$ Mann Whitney U test).

We found variable placental K-channel expression in NP and IUGR. Only venous expression of $K_v2.1$ was increased in IUGR versus NP. Aberrant K_v channel expression may contribute to altered placental vascular resistance associated with IUGR pregnancies.

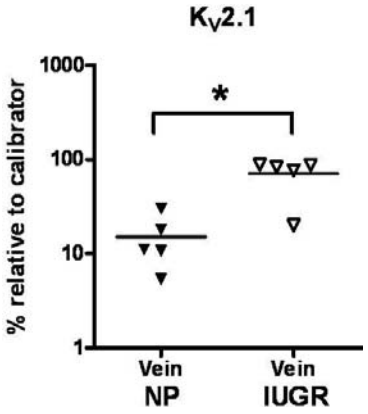

Figure 1

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Supported by the British Heart Foundation and Wellcome Trust.

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

PC206

Regulation of placental iron uptake by maternal hepcidin

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Hepcidin, a small peptide synthesised in liver, regulates iron homeostasis by inhibiting intestinal Fe absorption, iron recycling by macrophages and the release of Fe from stores. Hepcidin reduces the expression of Divalent Metal Iron Transporter 1 (DMT1) necessary for intestinal Fe uptake, and inhibits cel-

lular Fe efflux by binding and inducing the degradation of the Fe exporter Ireg1. Duodenal Fe absorption increases during pregnancy to compensate for the increasing requirements of the placenta and fetus. An inverse relationship between hepcidin and intestinal Fe transporters during pregnancy has been demonstrated by Millard et al. (2004). Placental Fe transfer also increases during pregnancy; however, the relationship between maternal hepcidin expression and placental Fe transfer has yet to be established.

The aim of this study was twofold. Firstly, to determine the relationship between hepcidin expression and duodenal and placental DMT1 and Ireg1 at different stages of pregnancy and secondly, to determine the effect of hepcidin on Fe uptake in BeWo cells (an in vitro model for syncytiotrophoblasts).

mRNA levels of Fe transporters were quantified by real-time PCR in post mortem tissue from pregnant Wistar rats during the 2nd (day 14-15) or the 3rd (day 19-21) trimester (n=6). Changes in mRNA levels were not evident during the 2nd trimester (Table 1). However hepcidin expression was significantly reduced in the 3rd trimester when the expression of duodenal and placental DMT1 was increased.

In the second study BeWo cells were incubated with synthetic hepcidin (10-100μM) for 24h. Cells were then taken to measure Fe uptake from Fe59-Tf and expression of DMT1 and Ireg1 was quantified by real-time PCR. Iron uptake was reduced in cells exposed to hepcidin. This reduction was dose dependent (0μM treatment: 530.82±20.57; 10μM: 183.17±38.58; 100μM: 125.63±28.12 μmol Fe uptake/g/min, n=3, $P<0.001$). Cells incubated with hepcidin also had decreased DMT1 (0μM: 1.00±0.05; 1μM: 0.41±0.05; 10μM: 0.48±0.20; 100μM: 0.57±0.02, $P<0.05$) and Ireg1 (0μM: 1.00±0.05; 1μM: 0.46±0.16; 10μM: 0.70±0.06; 100μM: 0.77±0.04, $P<0.05$) expression.

These data demonstrate that the gestational increase in placental and duodenal iron transporters is linked with a decrease in hepcidin expression. In addition we demonstrate that in vitro hepcidin can regulate placental iron absorption by reducing the uptake of transferrin bound Fe and probably Fe efflux (not determined in this study). This reduction is linked with decreased expression of DMT1 and Ireg1.

All data are presented as mean±SEM. Differences between groups were calculated by ANOVA or Kruskal-Wallis.

Table 1. mRNA expression of iron transporters and regulators during pregnancy

	Non-pregnant	2nd trimester	3rd trimester
Hepcidin	1.00+0.1	1.12+0.37	0.05+0.02*
Duodenal DMT1	1.00+0.38	1.20+0.24	10.12+2.44*
Duodenal Ireg1	1.00+0.27	1.20+1.03	1.92+0.12
Placental DMT1	—	1.00+0.39	3.01+0.94*
Placental Ireg1	—	1.00+0.46	1.52+0.33

*Significant difference in expression from the non-pregnant group ($P<0.05$).

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Gut 53, 655-660.

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

PC207

Prenatal undernutrition with postnatal obesity influences renal function in adult sheep

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Using nuclide renography, obesity was found to independently affect recovery in patients with renal damage (1). This study has revisited the paradigm of prenatal undernutrition in sheep, to include the effect of postnatal obesity on renal function in sheep as assessed with nuclear scintigraphy using Tc-99m DTPA.

At day 23 gestation 37 ewes received either a control (C, 7 MJ/day; n=24) or nutrient restricted diet (NR, 50% C intake ~3.5 MJ/day; n=13) from day 30 to 80 gestation. Thereafter all sheep were fed to 100% requirements to term (12-13 MJ/day near term). Offspring delivered spontaneously and ewe reared as singletons (one twin being humanely put down) to weaning. From weaning to 1yr of age offspring were either barn reared (restricted activity; Obese controls OC, n=8 females; Obese nutrient restricted ONR, n=13(11 females, 2 males)) or pasture grazed (unrestricted activity; Lean controls LC, n=8 females). At this time a proportion of the offspring (LC, n=7; OC, n=3; ONR, n=3; all females) were catheterised for renography. At time zero 100MBq technetium-99 diethylenetriaminepentaacetic acid (Tc-99m DTPA) in 0.5ml NaCl was injected I.V. and left and right kidney renograms produced from analysis of 60 frames over 20min; derived indices of renal function were then calculated. GFR was determined by the 3-sample method (2). Data are means ± S.E.M. and were analysed by one-way ANOVA using SPSS v14.

After weaning, physically inactive lambs (OC & ONR) gained weight rapidly and were 90 ± 2 and 89 ± 1 kg in OC and ONR, respectively vs. LC, 58 ± 4 kg at 12 months of age ($P<0.001$). Body fat at this time was 46 ± 2 and 49 ± 1% in OC and ONR, respectively vs. LC, 24±2% ($P<0.001$) as determined chemically (Direct Laboratories, Wolverhampton). Obese sheep had larger kidneys and a trend for higher uncorrected GFR (OC, 134 ± 17; ONR, 114 ± 17; vs. LC, 93 ± 9 ml min^{-1}, $P=0.07$). However, corrected for kidney weight, ONR tended to have lower GFR (ONR, 68 ± 7; vs. OC, 84 ± 10; vs. LC, 84 ± 10 ml min^{-1} $(100g\ kidney)^{-1}$, $P=0.08$). In both left and right kidneys the upslope (the positive slope of the renogram occurring before the peak and calculated as the X values (cpm) at 5 and 90% of the peak Y value) was significantly slower in obese sheep (right kidney data: OC, 48 ± 7; ONR, 38 ± 3; vs. LC, 130 ± 26 cpm, $P<0.05$) and time to peak tended to be longer (right kidney data: OC, 2.7 ± 0.2; ONR, 3.3 ± 0.4; vs. LC, 2.4 ± 0.2 min, $P=0.05$).

Undernutrition over the period of nephrogenesis in sheep appears to impact upon GFR corrected for kidney weight. Obesity per se appears to markedly influence the renal handling of Tc-99m DTPA indicating increased renal resistance to uptake of nuclide.

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Supported by the British Heart Foundation and the University of Nottingham.

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

PC208

RHOB GTPase mRNA and protein expression in human placental tissues

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RHO GTPases are small monomeric proteins that play a key role in the regulation of the actin cytoskeleton. Transfection of activated RHOA and RHOB GTPases into quiescent fibroblasts induces the formation of actin stress fibres and adhesion complexes [1].

Although RHOB GTPase has over 89% sequence homology to RHOA, it is differentially localised, regulated and prenylated. RHOB is localised to endosomes where it regulates receptor-mediated vesicle trafficking and actin filament assembly through the RHO effector protein mammalian Diaphanous-related formin (DRF) Dia1 [2]. Activated RHOB promotes the formation of thin actin filaments and inhibits vesicle motility by associating endosomes with subcortical F actin filaments [3].

We aim to quantify the presence and expression of RHOA and RHOB GTPases in human placenta. We also aim to determine the expression of RHOB in other intrauterine tissues as it could be an important confounding factor on previous reports.

Whole tissue extracts of myometrium and placenta from five individual non-pregnant and pregnant samples were prepared by mechanical homogenisation and protein expression was determined using immunoblotting and densitometry. mRNA expression of RHOA and RHOB in placental samples relative to pregnant myometrial samples from the same patient samples was determined using quantitative RT-PCR. Immunohistochemical localisation of paraffin-embedded placental tissue sections from five individual patient samples was demonstrated using antibodies against RHOA and RHOB GTPases.

RHOA and RHOB GTPase mRNA were both expressed in the placental tissue samples. RHO GTPase protein expression was high in non-pregnant and pregnant myometrium, decidua basalis and decidua parietalis, fetal blood and placental tissues. Immunohistochemical analysis revealed that RHOB GTPase has a cytoplasmic distribution in myometrial cells, with staining localised to the perinuclear region in endometrial cells. In human placenta, trophoblast and decidua cells stained positive for RHOB GTPase.

To our knowledge, this is the first time RHOB GTPase mRNA and protein expression has been described in human myometrial and placental tissues. We have also demonstrated that both RHOA and RHOB GTPase are expressed in myometrial, decidual and placental tissues samples. This is an important confounding factor on previously reports of RHOB protein expression in human myometrium. We aim to further explore RHOB GTPase function in placenta by identifying possible effectors using immunoprecipitation and immunoblotting.

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

PC209

Relaxation of human chorionic plate arteries by histamine

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Placental chorionic plate arteries (CPAs) exhibit inconsistent responses to endothelial-dependent dilators [1-3], with minimal relaxation in the short term. Here we tested the hypothesis that long term administration of endothelial-dependent agonists would promote significant relaxation of CPAs.

Term placentas (N=11) were obtained post-delivery (vaginal or Caesarean section) from uncomplicated pregnancies. Biopsies were placed into ice-cold HCO_3^--buffered physiological salt solution (PSS). Arteries were dissected from the chorionic plate, mounted onto a wire myograph, normalised at 0.9 of $L_{5.1kPa}$ and equilibrated (37°C; 20 min; 5% O_2/5% CO_2 ~7% O_2). Vasoconstriction was assessed with 120mM potassium solution (KPSS). Histamine hydrochloride (HIS), bradykinin (BK) and acetylcholine (ACh; all 10^{-6}M) were added to pre-contracted vessels (EC_{80} dose of the thromboxane-mimetic U46619 for 30 min) for 60 min. Experiments with HIS were also performed in the presence of indomethacin (I; 10^{-5}M), indomethacin plus N^{ω}-nitro-L-arginine, (IN; both 10^{-5}M) and indomethacin plus N^{ω}-nitro-L-arginine in 25mM KCl (INK). Arterial relaxation to donated nitric oxide (NO) was assessed using sodium nitroprusside (SNP; 10^{-5}M).

Normalised luminal internal diameters were 294±18μm (n=44 arteries). Maximal arterial relaxation was 69±3, 86±6 and 95±3% of constriction to U46619 at EC_{80} concentration with HIS, BK and ACh respectively (Mean +/- SEM; N=5). BK-induced relaxation was transient (100±4% at 60 min) whereas HIS induced a maintained relaxation (74±4% at 60 min; N=5). Maximal arterial relaxation was 68±7%, 76±6%, 79±1%and 86±3% (N=6) of the EC_{80} U46619-induced contraction respectively ($P<0.05$; Friedman's test followed by Dunn's post hoc test) with HIS alone and in the presence of I, IN and INK, respectively. Arterial relaxation at 60 mins was 71±8%, 84±4%, 89±3% and 92±3% (N=6) of the EC_{80} U46619-induced contraction, respectively ($P<0.05$; Friedman's test followed by Dunn's post hoc test) with HIS alone and in the presence of I, IN and INK, respectively.

10^{-6}M HIS but not ACh or BK promotes maintained relaxation of CPAs. This relaxation is partially reduced by blockers of the vascular endothelium. The slower onset of response may relate to ultrastructural features of CPAs, which show well developed endothelial cells, no internal elastic lamina and considerable

extracellular matrix separating circularly oriented smooth muscle cells [4].

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

PC210

Modelling the effects of diffusive coupling on spatio-temporal excitation patterns in the gravid human uterus

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Effective labour requires synchronous uterine contractions. Synchronisation in the electrical excitation in the gravid uterus can be inferred by non-invasive electromyography (Simpson *et al.* 1998) or spatio-temporal magneto-myography (Ramon *et al.* 2005).

We have constructed a 3-D model of the myometrial wall of the third trimester gravid human uterus, with geometry from magnetic resonance image data, with a simplified (FitzHugh-Nagumo) model for excitation. Propagation is modelled by a reaction diffusion equation, with a diffusion coefficient D, dimensions of distance^2 time^{-1}, that describes the diffusive spread of excitation in space (Winfree, 1991). An increase in D simulates the increased inter-cellular coupling produced by upregulation of connexin-43 (Cx-43) in phase 1 of parturition. The increase in Cx-43 can be modelled by a linearly scaled increase in D. A solitary excitation wave has a wavelength λ that is the product of its duration and velocity. The velocity is proportional to $\sqrt{D}$. An irregular spatial distribution in parameters allows spatiotemporal-irregularity in activity, which can produce near synchronous excitation.

For a homogeneous myometrium, near synchronous excitation could be produced by a single, excitation wave, with a $\lambda \sim$ size of the uterus (Fig. 1b), or by repeated excitation by re-entrant waves with λ less than uterine size (Fig. 1c). A pair of such re-entrant waves can be initiated by a localised perturbation occuring in the vulnerable window of a preceeding propagating wave. Smooth, spatial gradients in excitation or coupling parameters produce slow drift of re-entrant waves, which lead to self-termination of re-entrant waves by moving their cores to a boundary (Biktashev & Holden, 1999). Persistent re-entrant excitation can occur if drift leads to a single re-entrant wave pinned at the cervix (Fig. 1d). Wave effects – long wavelength, re-entry, or spatio-temporal irregularity - can all contribute to the spatio-temporal synchronisation of excitation necessary for uterine contractions.

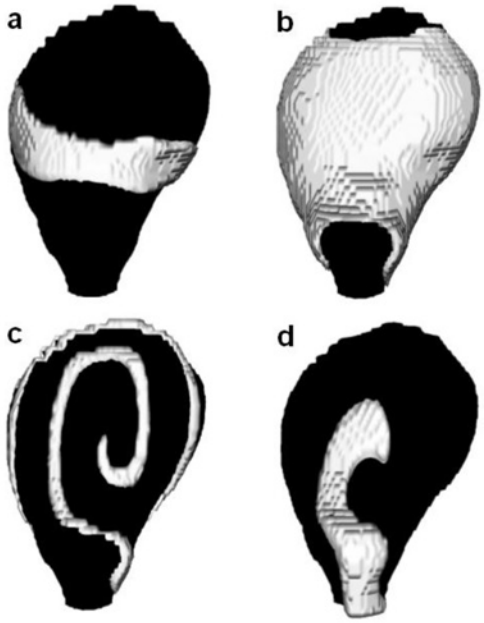

Figure 1. Anterior view of computed excitation wave patterns on gravid uterus, white is excitation. a (D= 0.03), b (D=0.16) solitary circular waves initiated at the fundus; c pair of re-entrant spiral waves (0.01) and d (D=0.05) solitary broken wave pinned to the cervix.

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

PC211

Expression and localisation of 2,3 bisphosphoglycerated mutase in mouse and human placenta

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Bisphosphoglycerate mutase (BPGM) is an enzyme expressed in erythrocytes. It catalyses the formation of 2,3 bisphosphoglycerate (2,3-BPG), which modulates oxygen transport by stabilizing the deoxygenated form of haemoglobin. Without 2,3-BPG, haemoglobin cannot function. Fetal haemoglobin is morphologically different to adult having almost no binding affinity to 2,3-BPG. Therefore if both fetal and adult haemoglobin are in the presence of 2,3-BPG, it will preferentially lower the oxygen

affinity of adult haemoglobin. The fetal haemoglobin, being largely unbound to 2,3-BPG will retain a high affinity for oxygen taking up the oxygen released by the adult haemoglobin. Microarray experiments on placentae, utilising a mouse model of growth restriction highlighted BPGM as a key down-regulated molecule in growth restriction. We hypothesised that this down-regulation might be present in human growth restriction. We therefore examined pre-eclamptic pregnancies (at term), a disease strongly associated with growth restriction.

We report that BPGM is expressed in, and localised to, the mouse labyrinthine trophoblast and human syncytiotrophoblast, as demonstrated by *in situ* localisation and immunohistochemical staining (Pritlove et al. 2005). Insulin-like growth factor II (IGF-II) plays a key role in cellular growth and is paternally expressed in the fetus and placenta. Pregnant Igf-2 knockout (KO) mice exhibit reduced placental growth and deliver growth-restricted pups as a result of insufficient nutrition during gestation (Constancia, 2002). Microarray data showed a 3-fold down-regulation of BPGM expression in the Igf-2 mouse placentae (heterozygote KO IGF-2 male vs. B6CBF1 female) when compared to wild type (n=3) which was confirmed using quantitative real-time PCR (TaqMAN, ABI systems). Real time PCR data also reported that BPGM expression was lower in placentas of 7 pre-eclamptic pregnancies compared to 7 normal (no clinical evidence of growth restriction) suggesting a correlation between low BPGM expression and growth restriction.

The physiological relevance of this was investigated by measuring maternal circulating 2,3-BPG during gestation in both IGF2 KO and WT mice (n=6 at three time points). A statistically significant reduction was noted at E11 and at E15 ($p<0.05$). No difference was noted at E18. We are uncertain as to whether the decreased levels of BPGM seen in both dysfunctional mouse and human pregnancy represent a cause or effect of growth restriction. These results, however, do raise the intriguing possibility that measurement of circulating 2,3-BPG in human pregnancy might also alter in a pattern that would allow identification of IUGR well before clinical symptoms manifest.

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