C1

Regulation of Electrophysiology and Pharmacology of Voltage-Gated Potassium Channels by Ancillary Subunits Found in the Pregnant Uterus

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The electrical profile of the myometrium undergoes dramatic changes during pregnancy, as the myometrial smooth muscle changes from being largely quiescent to exhibiting well coordinated excitation-contraction coupling. Voltage-gated potassium channel currents contribute to repolarization, and therefore play a key role in determining myometrial excitability. KCNE1 is a small protein (130 amino acids) which forms a single transmembrane alpha helix. The expression of KCNE1 in the uterus is strongly and rapidly mediated by estrogen. KCNE1 does not form a functional channel by itself, but acts as an ancillary subunit to the KCNQ1 voltage-gated ion channel. KCNQ1 (KvLQT1 or Kv7.1) in the uterus is thought to contribute to repolarization of the myometrial action potential. Changes in the biophysical and pharmacological behavior of KCNQ1 by KCNE1 will likely contribute to changes in the electrical and pharmacological profile during pregnancy.

We used two-electrode voltage clamp to study the effect of KCNE1 on KCNQ1 when cloned KCNQ1 (P51787) and KCNE1 (NP_000210) were heterologously expressed in Xenopus oocytes. Oocytes were injected with 50 ng mRNA for KCNQ1, and co-injected with KCNE1 as noted, in a 1:1 ratio. We used the KCNQ1-specific open channel blocker chromanol 293B and the sodium/potassium channel blocker quinidine as pharmacological probes.

KCNE1 significantly slows KCNQ1 activation, resulting in a sigmoidal onset of activation. The KCNQ1/KCNE1 current continues to increase, even at the end of a 3 s depolarizing pulse. KCNE1 also slows deactivation, and removes voltage-dependent inactivation. When a 500 ms depolarizing pulse from -90 to +50 mV was applied with a 500 ms inter-pulse interval there was a potentiation of KCNQ1/KCNE1 current. The increase was well described by two exponentials, τ_{fast} = 1.07 ± 0.04 s, and τ_{slow} = 7.66 ± 0.43 s (n = 4). The fast time constant dominates: A_{fast}/A_{slow} = 4.78 ± 0.16 (n = 4).

There was a ~4 fold increase in the pharmacological sensitivity of KCNQ1 to Chromanol 293B when it was co-expressed with KCNE1. IC_{50} for KCNQ1 alone was 65.4 ± 1.7 μM in contrast to the IC_{50} for KCNQ1/KCNE1, which was only 15.1 ± 3.3 μM. KCNE1 had a similar effect on KCNQ1 affinity for quinidine. Application of 400 μM Quinidine reduced KCNQ1 current by 8.0 ± 4.5 % (n = 5), whereas KCNQ1/KCNE1 was reduced by 30.3 ± 4.0 % (n = 4).

These data suggest that the estrogen-dependent KCNE1 ancillary subunit strongly modulates voltage-gated KCNQ1 ion channel physiology and pharmacology. Understanding the contribution of KCNQ1 to the uterine electrical and pharmacological profile therefore requires an understanding of how KCNE1 subunits modulate KCNQ1.

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

C2

Myogenic regulation to intravascular pressure changes of uterine arteries isolated from non-pregnant rats

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Myogenic reactivity is an important regulatory mechanism for maintaining arterial diameter in the face of changing physical stresses. Although well-studied in many vascular beds, surprisingly little is known as to the nature of myogenicity in arteries serving the uterus, which is particularly important given the substantial haemodynamic changes occurring in this organ during pregnancy. Therefore, the aim of this study was to investigate myogenic responsiveness of pressurised uterine arteries, isolated from rats, to changes in intravascular pressure (IVP).

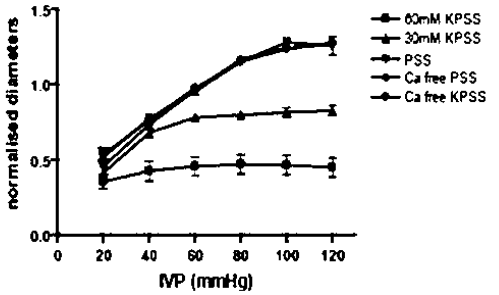
Virgin adult Sprague Dawley rats (150-250g) were humanely killed by stunning and cervical dislocation. Third order uterine arteries were dissected and mounted on an arteriograph and pressurised to 60mmHg. Arteries were superfused at 37°C with physiological salt solution (PSS), gassed with 95% air/5% CO2 and left for 90 minutes to determine the extent of myogenic tone development. The subsequent influence of stepwise (5 min) IVP changes (20-120mmHg) in 20mmHg increments was investigated in arteries with and without pre-constriction to KPSS (30mM or 60mM) or AVP at a concentration determined to produce 50% of maximal constriction to AVP. Endothelium integrity was tested by addition of 10μM Carbachol. Experiments were repeated in calcium-free PSS or KPSS.

Mean arterial lumen diameters (at 60mmHg) were 152.4± 4.74μm and 156.4 ± 4.45μm (mean ± SEM) in calcium-containing and calcium-free PSS respectively. Development of myogenic tone was not observed. 60mM KPSS produced a sustained narrowing of arteries (diameter change 76.4 ± 8.5μm, n=5), whilst 30mM KPSS produced a constriction approximately half of this (diameter change 28.4 ± 5.9μm, n=5). The constriction produced by AVP (0.08-0.55 μM) was similar to that produced by 30mM KPSS (35.6 ± 4.8μm, n=8).

In calcium-free PSS and KPSS increases in IVP produced sequential increases in diameter of all arteries. The responses of arteries in calcium-containing PSS (minus constrictor) were similar. However, arteries pre-constricted with KPSS (30 or 60mM) or AVP exhibited active regulation in response to increases in IVP such that stable diameters were maintained (over 40-120mmHg) (See figure).

These results indicate that isolated uterine arteries from the non-pregnant rat, when held at an in vitro pressure of 60mmHg, do not develop spontaneous myogenic tone. Nonetheless, under the influence of pre-constrictory stimuli of varying magnitude, such vessels do exhibit prominent myogenic responsiveness to IVP changes. These observations have important implications for understanding local autoregulation in the uterine circulation.

Supported by the BHF.



The response of isolated pressurised arteries from non-pregnant rats to changes in IVP.

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C3

Prokineticin-1 upregulates interleukin-8 expression in placenta

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Background: Prokineticin-1 (PK1) is a pleiotrophic peptide whose functions include tissue specific angiogenesis[1], vascular permeability[2] and haemopoiesis[3]. Although expressed in third trimester placenta, the cellular localisations of PK1 and prokineticin receptor 1 (PKR1) and signalling pathway of PK1 are not known. In addition, there is a paucity of data regarding gene upregulation by PK-1.
Aim: To characterise PK1 and PKR1 immunolocalisation, expression and signalling in human placentae.
Methods: Placentae (n=20) were collected after elective caesarean section at term (>37 weeks gestation) from women with uncomplicated pregnancies. PK1 and PKR1 were immunolocalised by standard immunohistochemical techniques. Extracellular regulated signal kinase -1/2 (ERK1/2) phosphorlyation was detected by Western blotting with signalling pathways being dissected using various inhibitors including YM25480, PP2 and AG1478, specific inhibitors of Gq, c-src and epidermal growth factor receptor (EGFR) kinase, respectively. Placental explants (n=6) were treated with 40nM PK-1 and interleukin-8 (IL-8) mRNA expression detected by taqman PCR. PK1 or PKR1 and IL-8 were colocalised using immunofluorescence and confocal microscopy.
Results: PK1 was immunolocalised to endothelium and macrophages in fetal vessels and Hofbauer cells in placental villi. In contrast, PKR1 was predominately localised in syncytial sprouts. ERK-1/2 phosphorylation in placenta was significantly upregulated (5-fold increase; $p<0.05$) following treatment with PK1 for 30 minutes. Dissection of the upstream signalling pathway by the chemical inhibitors demonstrated that PK1 induced phosphorylation of ERK-1/2 was mediated via c-src and EGFR transactivation. Treatment of placenta with PK1 for 4 hours induced a significant increase in IL-8 expression (3.26+0.45 fold increase above control; $p<0.05$). IL-8 expression in response to treatment with PK1 was inhibited following coincubation of the tissue with inhibitors of Gq, c-src, EGFR kinase or ERK1/2. Using double immunofluorescence, co-localisation/co-expression of PK-1 or PKR1 and IL-8 was demonstrated in various cellular compartments within the placenta including trophoblast and macrophages.
Conclusions: The cellular immunolocalisation of PK1 and PKR1 within placenta and upregulation of IL-8 by PK1 is supportive of PK1 being involved in placental vascular physiology. In addition, expression of PKR1 in syncytial sprouts, which characterise areas of hypoxia and immature villous formation, suggest that another role of PK1 may in mediating trophoblast differentiation in response to hypoxia. More studies are required to establish the role of PK1 in normal placental physiology and in hypoxic pre-eclamptic placentae which are characterised by increased syncytial sprouts and vaso-occlusive lesions.

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C4

Production of Hydrogen Sulphide in Intrauterine Tissues

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Hydrogen sulphide (H_2S) is a gasotransmitter which is produced endogenously from L-cysteine via the enzymes cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (Zhao et al., 2003). The possible role of hydrogen sulphide in reproduction has not yet been fully investigated. Sidhu et al. (2001) previously demonstrated that H_2S relaxed uterine smooth muscle in vitro. The aim of the present study was to investigate the endogenous production of H_2S in rat and human intrauterine tissues in vitro. The expression of CBS and CSE was also investigated in rat and human intrauterine tissues, using Western blotting. Basal production rate of H_2S was measured in rat and human intrauterine tissue homogenates using a standard methylene blue assay technique, involving the trapping of yielded H_2S as zinc sulphide (Zhao et al., 2003). The effects of nitric oxide (NO) and hypoxia on the endogenous production rates of H_2S were also investigated. The order of H_2S production rates (mean ± SD, n = 4) for rat tissue was: rat liver (positive control) (777 ± 165 mmol/min/g) > rat uterus (168 ± 102 mmol/min/g) > rat fetal membranes (22.3 ± 15.2 mmol/min/g) > rat placenta (11.1 ± 4.7 mmol/min/g), compared to human placenta (200 ± 103 mmol/min/g). NO significantly increased H_2S production in rat

fetal membranes (P<0.05). Under hypoxic conditions the production of H_2S was significantly elevated in human placenta, rat liver, uterus and fetal membranes (P<0.05). Western blotting (n = 4) detected the expression of CBS and CSE in all rat intrauterine tissues, and in human placenta, myometrium, amnion and chorion. Rat and human intrauterine tissues produce H_2S in vitro possibly via CBS and CSE enzymes. NO increased the production of H_2S in rat fetal membranes. The augmentation of H_2S production in human intrauterine tissues by hypoxia could have a pathophysiology role.

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Zhao, W., Ndisang, J. F. and Wang, R. (2003) Modulation of endogenous production of H2S in rat tissues. Canadian Journal of Physiology and Pharmacology 81, 848-853

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

C5

Pre-natal stress in the pig: effects on the behavioural and neurophysiological development of piglets

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Evidence in humans indicates that experiences throughout the pre-natal period affect long-term development. Babies born at term with a low birthweight are more likely to develop conditions such as diabetes type 2, hypertension and depression in adulthood. The aim of this study was to investigate the effect of pre-natal stress (social mixing) on the behavioural and neurophysiological development of piglets. Thirty-six primiparous sows were divided into two control (C) groups, two groups that were mixed during the second (Mix2) and two groups that were mixed during the third (Mix3) trimester of pregnancy. In the sows, mixing increased lesion scores (2nd trimester means (s.e.): C = 1.6 (0.1), Mix2 = 2.9 (0.3), Mix3= 1.8 (0.1) F=10.4 (anova), p=0.05. 3rd trimester means (s.e.): C=1.8 (0.1), Mix2= 1.6 (0.1), Mix3= 3.2 (0.1), F=22.7 (anova), p=0.02) and change in salivary cortisol levels (2nd trimester means (ng/ml) (s.e.): C = 0.09 (0.07), Mix2 = 2.40 (0.71), Mix3= -0.05 (0.20), F=22.3 (anova), p=0.02. 3rd trimester means (s.e.): C=0.36 (0.15), Mix2= 0.43 (0.32), Mix3= 3.47 (0.58), F=12.0, p=0.04); birthweight of their piglets was unaffected. At 60 days of age, 48 daughters were selected from the three treatments and half were challenged using a restraint test. All were culled and brain tissue collected and analysed using in situ hybridisation. We found increased CRH mRNA expression in the PVN of unrestrained Mix2 daughters (Mean CRH Cell count x grain density (se) in unrestrained piglets: C = 9.1 (3.6), Mix2= 37.3 (9.4), Mix3= 17.9 (4.5), W=6.5, p=0.04), and in the amygdala of Mix2 and Mix3 daughters (Mean CRH Cell count x grain density (se) in unrestrained piglets: C = 13.9 (2.8), Mix2= 20.7 (3.1), Mix3= 24.0 (4.4), and in restrained piglets C = 20.2 (3.7), Mix2= 33.9 (4.7), Mix3= 31.0 (5.9), W=7.6, p=0.02). At 67 days of age, 24 further daughters (8 from each treatment) were mixed, and Mix2 and Mix3 daughters showed a greater and longer salivary cortisol response than controls. At parturition Mix2 and Mix3 daughters were more restless and more responsive to piglets that approached the head of the sow, and Mix2 daughters tended to bite at their piglets more (Mean bites/hour (se): C= 0.2 (0.1), Mix2= 2.1 (0.7), Mix3= 0.1 (0.1), F=7.0, p=0.07), traits which are a component of poor maternal behaviour. Overall the results indicate that stress experienced by the mother during pregnancy can affect the behavioural and neurophysiological development of piglets resulting in more stress-reactive offspring. The effects appear to be strongest when the pre-natal stressor is applied during the second trimester of pregnancy which coincides with the development of the pig foetal HPA axis. The effects of the pre-natal stress on the maternal behaviour of the daughters could have implications for transmission of altered stress reactivity across generations.

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

C6

The CRH concentration before parturition and spontaneous contractile activity in twin pregnancies

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TITLE ONLY

C7

Differences in serum selenium concentrations between normal and pre-eclamptic human pregnancies

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Pre-eclampsia (PE) is a pregnancy specific condition that affects 2-3% of women. The main pathological features are impaired placentation, with inadequate invasion of the spiral arteries by syncytiotrophoblast, and systemic endothelial damage. This not only contributes to increased neonatal and maternal morbidity and mortality but may have life time consequences for the fetus in terms of greater predisposition to adult cardiovascular disease. Selenium (Se), acting through selenoproteins, such as glutathione peroxidase (GPx), has a critical role in angiogenesis, and in regulating antioxidant status [1]. Recent reports strongly implicate poor maternal selenium status as a nutritional factor predisposing the mother to PE [2] but the fetus and placenta

have not been studied in tandem. We have now measured maternal and umbilical venous serum Se concentrations in Caucasian PE women and matched normotensive controls (NC) by graphite furnace atomic absorption spectroscopy (GFAAS) [3]. The study was approved by the Queen's Medical Centre Ethical Committee; informed, written consent was obtained from all subjects. In 22 maternal NC samples, mean ± s.e.m. [Se] was 58.3 ± 3.8 μg/L compared to 36.8 ± 2.6 μg/L in 21 maternal PE samples ($P < 0.001$). Mean umbilical venous concentrations in 21 NC babies were 39.8 ± 2.4 μg/L compared to 29.3 ± 2.5 μg/L in 16 PE samples ($P = 0.005$). There was a significant association between maternal and umbilical serum [Se] in PE samples ($r2 = 0.182$; $P = 0.035$) but not in NC samples ($r2 = 0.028$; $P > 0.2$). Body mass index (BMI) and age have been reported to be correlated with serum [Se] outside pregnancy. However, analysis of variance (ANOVA) showed no such effect in these women (BMI, $P>0.7$; age, $P>0.2$). There was no effect of gestation age on [Se] in maternal ($P>0.2$) or umbilical ($P>0.1$) serum. A low dietary intake of Se might predispose to PE, as it does to other forms of cardiovascular disease, if selenoprotein synthesis were reduced. Conversely, enhanced tissue synthesis of GPx, as has been reported for PE decidua, might lower serum [Se] by utilising such Se as is available. Tissue selenoprotein concentration response to supplementation is known to vary with tissue in the same individual. We are currently analysing the family of GPx proteins and specific factors influencing synthesis in serum and placental tissue to try to determine their primary or secondary role in PE

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

C8

Influence of glucocorticoids on nutritional manipulation of regional adipose tissue distribution

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Introduction: Glucocorticoids (GCs) have an important role in adipogenesis (1). The effects of GCs are mediated by the enzymes 11β-hydroxysteroid dehydrogenase type 1 and 2 (11βHSD1 and -2). 11βHSD1 activates cortisone into cortisol while 11βHSD2 has an opposite effect. The action of GCs is also dependent on the abundance of the glucocorticoid receptor (GR). In sheep the distribution of visceral adipose tissue changes dramatically from mainly perirenal to mainly omental fat in the first 6 months of life. In animals which were formula fed in the first 3 months of life this change is less pronounced (2). The aim of this study was to investigate the influence of GCs on these changes in fat distribution and the nutritional regulation of this effect.

Methods: Twenty-three sheep offspring were randomly allocated to either a lean control (LC, n=8), obese control (OC, n=7) or obese formula fed (OFF, n=8) group. The LC and OC groups were kept with the mother throughout lactation, the OFF group received formula feeding. All animals were weaned at 8-10 weeks into a field (LC; unrestricted activity) or barn (OC and OFF; restricted activity). After one year each sheep was humanely euthanased and adipose tissue from omental, perirenal and subcutaneous depots was collected. RNA was extracted and mRNA abundance for GR, 11βHSD1 and 11βHSD2 was measured using real time PCR and normalised against 18S rRNA. Data were first analysed with REML modelling followed by appropriate parametric or non-parametric post hoc tests (ANOVA / Kruskal Wallis) using SPSS v14.0 for Windows. A significance level of $p = 0.0166$ was used in all post hoc test.

Figure 1: Omental GR expression

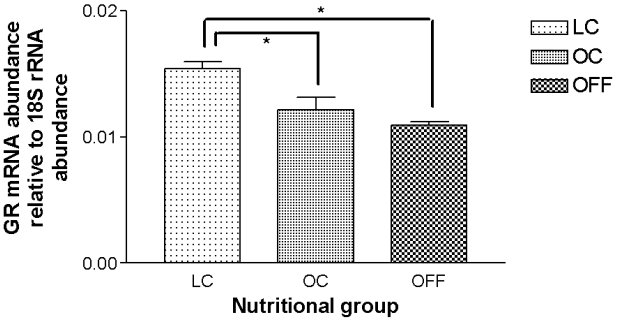
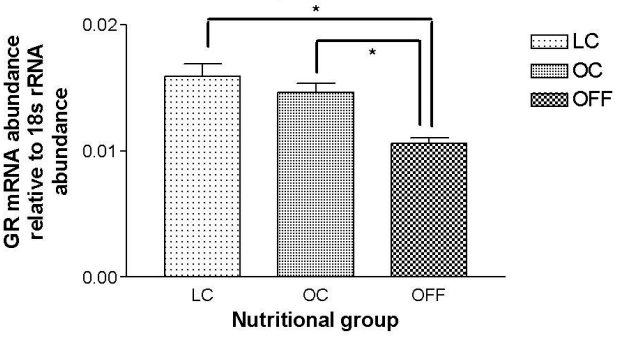


Figure 2: Subcutaneous GR expression



Results: Sheep in the OC and OFF group were significantly heavier than sheep in the LC group. The regional deposition of visceral fat was different in the OFF group compared to the other groups (48±2% omental and 47±2% perirenal fat in OFF vs. 58±3% and 36±3% respectively in LC sheep).

Both GR and 11βHSD1, but not 11βHSD2, were affected by a combination of nutritional group and fat region. In the omental region the GR expression was higher in the LC group compared to the OC and OFF groups ($p=0.007$ LC vs. OC and $p<0.001$ LC vs. OFF, figure 1). In the perirenal region there was no difference between groups and in the subcutaneous region the LC and OC group were increased compared to the OFF group ($p<0.001$ LC vs. OFF and $p=0.002$ OC vs. OFF, figure 2). 11βHSD1 mRNA abundance was higher in the LC group compared to the OC and OFF groups in the omental ($p=0.001$ LC vs. OC and $p=0.001$ LC vs. OFF) and perirenal region ($p=0.015$ LC vs. OC and $p=0.012$ LC vs. OFF). In the subcutaneous region the LC group was only increased compared to the OFF group ($p=0.015$).

Discussion: The relatively decreased omental fat mass in obese formula fed sheep is paralleled by decreased GR and 11βHSD1 expression, indicating nutritional regulation of adiposity in early life.

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

PC1

Anxiety in pregnancy: antenatal influences and perinatal outcome in the hospital and community setting

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Objectives: To investigate factors affecting maternal anxiety during pregnancy using the State Trait Anxiety Inventory (STAI); to assess whether STAI is valid for measuring anxiety during pregnancy and to identify women suitable for a study on stress hormone action during pregnancy.
Methods: 182 women aged 31.2 (6.3) yrs, with a singleton pregnancy, were recruited from "high" and "low" risk antenatal settings. Participants completed the STAI questionnaire, were asked an open-ended question about their feelings regarding the pregnancy and if they would be willing to participate in a further research study during this pregnancy. Maternal and neonatal demographics were recorded. Ethical approval and written informed consent were obtained. Results presented are mean +/- sd.
Results: Mean state (35.6(10.1)) and trait (36.7(8.7)) anxiety scores were correlated ($r=0.48$, $p<0.0001$). Women attending "high risk" clinics had no difference in trait anxiety (36vs.37, p=ns), but higher state anxiety scores (37vs.32,p=0.008) than women attending "low risk" clinics. Lower anxiety scores were associated with increased parity (state $r=-0.20$, $p<0.05$),) and maternal age (trait 0.18, p=0.02). Higher anxiety scores were found in women reporting a negative response to their pregnancy (state 42vs.35, p=0.02; trait 42vs.36, p=0.03), making 'worried' comments (state 38vs.33, p=0.006; trait 39vs.35, p=0.007) and preferring not to participate in further research studies (state 38vs.34, p=0.03). Birthweight was lower in smokers (2938 vs.3264g, p=0.05) but was not associated with maternal STAI score.
Conclusions: Many factors including location of antenatal assessment, parity, age and opinions about research affect STAI score. There need to be taken into account when undertaking research about the effects of anxiety on pregnancy.

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Psychologists Press

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

PC2

Fetal skeletal muscle capillary density is reduced by both early and late gestation maternal undernutrition in sheep

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In humans, 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, along with a reduction in capillary density (Marin *et al.* 1994). Previously we have shown in sheep that an early or late gestation maternal nutrition restriction reduces myofibre number and alters fibre type composition in the fetal triceps brachii (Costello *et al.* 2006) but not in the slow-twitch soleus muscle (unpublished observations). The aim of the study was therefore to investigate the effect of an early or late gestation nutrient restriction on muscle capillary density in the 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), 40% from 1-31 days gestation (dGA) (ER, n=8) or 50% from 104 dGA until post mortem (LR, n=7), with 100% requirements at all other times. All fetuses were singletons and groups contained equal numbers of males and females. At 127 ± 1 dGA (term ~147 dGA) ewes were killed by an overdose of barbiturate (i.v., 145 mg/kg), and the fetal triceps brachii and soleus muscles were removed and immersed in freezing isopentane. 10 μm sections of muscle were cut, stained with anti-human von Willebrand factor and five random fields (magnification x40) were captured. From these fields the capillary density and capillary:muscle fibre ratio were measured, averaged and analysed by ANOVA with Bonferroni post-hoc tests. Data are expressed as mean ± SEM.
The density of capillaries in the triceps brachii was reduced in both the ER ($p<0.01$) and LR ($p<0.05$) fetuses as compared to C (C, 1874 ± 57; ER, 1480 ± 65; LR, 1389 ± 98 capillaries/mm^2). Capillary:muscle fibre ratios were reduced in both the ER ($p<0.01$) and LR ($p<0.01$) as compared to C fetuses (C, 1.80 ± 0.09; ER, 1.48 ± 0.06; LR, 1.44 ± 0.12 capillaries:muscle fibre). No differences were seen in the soleus muscle.
Our findings of decreased capillary density following reduced maternal nutrition in either the peri-implantation period or in late gestation is muscle bed dependent and parallels the effect on myofibre density previously observed (Costello *et al.* 2006). Moreover, the reduction in capillary:muscle fibre ratio in the triceps brachii indicates that the reduction in capillary density was greater than that in the myofibres, i.e. that each fibre would be supplied by fewer vessels. These data suggest a link between blood flow and skeletal muscle growth, and the reductions in both capillary and myofibre density may have long-term implications for skeletal muscle function.

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

PC3

The effect of a moderate early gestation undernutrition on kidney glomerular number in fetal sheep

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Poor *in utero* nutrition is implicated in the association between low birth weight and the development of hypertension in adulthood (Barker *et al.*, 1989). It has been proposed that this association may result from impaired prenatal kidney growth and lower nephron endowment induced by suboptimal *in utero* nutrition (Brenner *et al.*, 1988). In addition thinner mothers, which may indicate relatively poorer nutrition, tend to have fetuses that have kidneys that are relatively narrow for their length (Mukherjee *et al.*, 2005). A 50% nutrient restriction from 1 to 31 days gestation (dGA, term ~147 dGA) alters renal and cardiovascular control in adult male sheep offspring (Cleal *et al.*, 2004). This challenge is associated with altered blood pressure (BP) responses to angiotensin II but no changes in kidney size or function in late gestation fetuses (Braddick *et al.*, 2006). The aim of the current study was to determine if fetal kidney structure is altered by early gestation undernutrition.

Pregnant ewes were housed individually and fed 100% (C, n = 8) or 40% (R, n = 8) of their total nutrient requirements between 1 and 31 dGA, and 100% thereafter. All fetuses were singletons, the sex ratio was 1:1 in C and 5:3 in R groups. At post-mortem (127 dGA) the left kidney was perfusion fixed with formalin. Each kidney was cut in half and cut into 5 mm slices. Systematic sampling was used to select every 10th piece which was embedded in glycomethacylate resin. The selected blocks were sectioned at 2 μm, and every 200th and 210th pair were collected and stained with toluidine blue. The physical dissector technique was used to count the number of glomeruli in the cortex using by light microscopy. Data are mean ± SEM and were analysed by Student's t-test.

Fetal body (C = 2.91 ± 0.08; R = 2.92 ± 0.12 kg) and kidney weight (Right; C = 9.66 ± 0.61; R = 9.80 ± 0.40 g; Left; C = 11.65 ± 0.56; R = 11.33 ± 0.56 g) were similar in both groups. There was no significant difference in fetal glomerular number in the left kidney in R compared to C fetuses (C = 326,100 ± 40,850; R = 457,300 ± 118,700).

Early gestation nutrition restriction did not alter fetal or organ growth or kidney development in late gestation sheep. Therefore altered renal and cardiovascular control in adult sheep following early gestation nutrient restriction is not due to altered glomerular number.

Barker *et al* 1989 *BMJ* **298**, 564-7

Brenner *et al* 1988 *Am J Hypertension* **1**, 335-347

Mukherjee *et al* 2005 *BJOG* **113**, 866

Cleal *et al* 2004 *J Physiol (Lond)* **555P**, 69

Braddick *et al* 2006 *JSGI* **13**, 207A

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

PC4

Hepatic glucocorticoid receptor expression in adult sheep following early gestational and/or postnatal undernutrition.

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In humans and animals environmental constraints during pre- and postnatal life result in phenotypic changes that can be associated with altered cardiovascular and metabolic disease risk in later life (Barker *et al.*, 2002, Poore *et al.*, 2006). The liver is a key organ in glucose and lipid metabolism and altered maternal diet or body composition are associated with changes in fetal liver blood flow (Haugen *et al.*, 2005). Intrauterine challenges such as hypoxia alter blood flow to left and right lobes of the liver differentially. Maternal gestational low protein diet in rats alters adult hepatic gene expression with different effects between liver lobes (Zhang & Byrne, 2000). The glucocorticoid receptor (GR) is a gene associated with disturbances in cardiovascular and metabolic control. However the interaction between pre- and postnatal environments on hepatic GR is unknown. We investigated the effect of reduced early gestation maternal nutrition and/or early postnatal life undernutrition on the expression of GR in adult sheep liver.

Welsh Mountain ewes received 100% (C, n=36) or 50 % of total nutrient requirements (U, n=39) from 1-31 days of gestation, and 100 % thereafter. Offspring were fed *ad libitum* (CC, n=20; UC, n=19) or to reduce body weight to 85 % of individual target weight from 12 to 25 weeks postnatal age and *ad libitum* thereafter (CU, n=17; UU, n=21). Each group contained approximately equal numbers of males and females and the ratio of twins to singletons was ~2:1. Offspring were sacrificed at 2.5 years, the livers were harvested and segments from the left and right lobes were frozen in liquid nitrogen. GR mRNA levels were measured by semi quantitative RT-PCR and normalized using the mean of three housekeeping genes (RPL19, βActin and GAPDH) selected by use of the geNorm$_{TM}$ normalizing kit. All data were analysed by ANOVA.

Early life nutrition had no effect on GR mRNA expression. GR mRNA expression was significantly higher in males than females ($P<0.001$). In both males and females the right liver lobe had higher GR mRNA expression than the left ($P<0.001$). In females, twins had a higher GR mRNA expression than singletons ($P<0.05$).

We conclude that differences in GR expression between left and right lobes of the liver exist in adult sheep, although early gestation and/or postnatal nutrition did not affect these levels. Our

finding of increased GR mRNA expression in males suggests that they may be more sensitive to the adverse effects of excess glucocorticoids.

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Haugen G *et al.*, (2005). Circ Res **96**, 12-14.

Poore KR *et al.*, (2006). Am J Physiol Endocrinol Metab. (In press).

Zhang J & Byrne CD, (2000). Am J Physiol Gastrointest Liver Physiol **278**, G128-G136.

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PC5

Characterisation and functional significance of SK channels in the pregnant rat uterus

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SK channels are small conductance Ca^{2+} – activated K^+ channels which may play an important in maintaining uterine quiescence, as the outward movement of potassium ions serve to re-polarise or hyperpolarize the myometrium. As the relative quiescence of the myometrium changes throughout gestation, the aim of this study is to determine the expression of all three isoforms of SK channels (SK1-3) and their contribution to normal rhythmic contractility throughout pregnancy in the rat.

Whole uterine horns were dissected from nonpregnant (NP) Wistar rats and at different stages of pregnancy:- 10d, 16d, 21d, labouring (L; term 22d). The expression of SK 1-3 was characterised at each stage using RT-PCR, immunohistochemistry on grouped samples in tissue microarrays, and quantitative western blotting.

Measurement of spontaneous phasic contractions were made on strips of longitudinal myometrium at each stage and the role of SK channels was determined by comparing contractions over a 20 min control period to those during a 20 min application of apamin (100nM), a constituent of bee venom that inhibits all three isoforms of SK channels [1].

Consistent expression of all SK isoform transcripts was seen in all samples.

In NP and at all stages of pregnancy, clear staining of SK 1-3 was seen in the myometrial cell layer. No staining was seen in epithelial cells. As the immunostaining of SK2 and SK3 appeared to change in density on the tissue microarrays in late pregnancy, expression of SK2 and SK3 was quantified by western blotting. Both proteins were clearly seen in all samples. There was no change in the expression of SK3 throughout pregnancy, but compared to β actin controls, expression of SK2 protein was significantly decreased in 16d and 21d compared with 10d and NP rats, and then increased in L tissue.

Apamin had no effect on the frequency of contractions but significantly increased the amplitude of spontaneous myometrial contractions in all stages of rat pregnancy (table 1).

These data clearly show that SK 1-3 channels are expressed in rat myometrium and limit spontaneous contraction in native, myometrial strips. SK channels appear to inhibit uterine contractions to a lesser extent than in the bladder, where the effect of 100nM apamin on contaction amplitude was ten fold greater than in the present study [1]. These data also show that SK2 is downregulated during late pregnancy which is in agreement with Mazzone & Buxton who found that SK2 is downregulated in human myometrium at term, [2]. This gestation-dependant regulation of SK2, merits further study.

Table 1

	NP	10d	16d	21d	L
Amplitude of apamin contractions cf control (100%)	* 107 ± 1.0%	* 109 ± 1.9%	* 107 ± 1.9%	* 114 ± 2.0%	* 104 ± 2.0%
	n=6	n=4	n=4	n=7	n=6

* Different from control ($p < 0.05$), paired t test.

Herrera G & Neson M (2002), J Physiol. **541**, 483 – 492

Mazzone J & Buxton IL (2003), Proc. West. Pharmacol. Soc. **46**, 74 – 77

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PC6

Effects of diabetes and insulin resistance in pregnant rats on ex vivo vascular reaction to magnesium

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TITLE ONLY

PC7

The effect of aqueous extract of *magnifera indica* (mango) leaves on female reproductive functions in Sprague-Dawley rats

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The effect of aqueous extract of Magnifera indica (500mg/kg-body weight, orally) on the oestrous cycle, serum level of the gonadotropins and the female sex hormones, pregnancy, and body weight were investigated in adult female Sprague Dawley rats.

Daily administration of extract at a dose of 500mg/kg (treated rats) and distilled water (control) were done during the 4 weeks period of determination of the length of cycle. Separate rats were used for the pregnancy study. Pregnancy was induced by allowing the female rats to mate freely at timed intervals with adult male rats. Number of implantation sites, resorption sites, viable fetuses and fetal weight were measured. Weights of rats were monitored during pregnancy.

Extract treated rats had disrupted uterus cycle, with the occurrence of oestrous phase being significantly reduced($P<0.05$), 1.800 +/- 0.416 days in the treated group and the control was 6.400 +/- 0.036days. Diestrous phase was significantly increased, $P<0.05$, treated 19.400 ± 0.653 days, control 13.100 ± 0.379 days.

Serum FSH level was significantly reduced, P<0.05 in treated rats, the extract showed no significant effect on other hormones which were assayed. There was no significant effect on the number of implantation sites and viable fetuses but fetal weight was significantly reduced, P<0.05, treated 1.939 ± 0.021g, control 3.073 ± 0.031g. There was a significant reduction in weight gain of treated rats when compared with control, by weeks 3 and 4. All these results revealed that the aqueous extract of Magnifera indica may cause disruption of the oestrous cycle inhibiting ovulation, while reducing weight gain during pregnancy and reducing the fetal weight However,it has no significant effect on implantation activities.

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

PC8

Maternal micronutrient deficiency (Cu or Zn or Vit-E) in mice associated with abnormalities in placental IGF signaling and hyperleptinemeia in offspring's

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TITLE ONLY

PC9

Attenuated responsiveness of the adrenal medulla to stress in pregnancy

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Pregnancy changes physiological responses to stress, including attenuation of the hypothalamo-pituitary-adrenal (HPA) axis. This results, at least in part, from opioid inhibition of the central noradrenergic input to the HPA axis1,2, and during the last trimester may protect the foetus from adverse effects of glucocorticoids and stabilise metabolism of the mother and foetus. Although sympathetic and adrenal medulla responses to stress are important for the fight or flight response and immediately elevate glucose availability, their responses during pregnancy are poorly understood. We studied the hypothesis that sympathetic responses are attenuated in late pregnancy. During stress enkephalin is synthesised and secreted from chromaffin cells into blood and may mediate neuroendocrine, cardiovascular and immune functions; so we also measured adrenal medulla enkephalin responses. Virgin and 21 day pregnant rats were blood sampled via a previously implanted jugular cannula before and after exposure to airpuff startle stress3 in their home cages (n=8,6, respectively); control groups were not stressed (n=7,6, respectively). Plasma samples were analysed for ACTH, adrenaline and noradrenaline by RIA. Rats were killed at 90 min and the adrenal medulla was assayed for enkephalin content4. Data are mean±S.E.M.

Airpuff startle increased ACTH secretion at 10 min in virgin but not pregnant rats as expected. Airpuff also significantly increased adrenaline secretion at 2.5 min in virgin but not pregnant rats (to 3031±933 and 1684±205 pg/ml, respectively; 3way ANOVA, interaction across treatment, group and time p<0.05); controls did not significantly change. Delta noradrenaline secretion was 2146±722 pg/ml in stressed virgins and was not significantly different in stressed pregnant rats (1816±834 pg/ml). This indicates that although adrenaline secretory responses are attenuated, peripheral noradrenergic responses remain intact.

Adrenal gland weight was not significantly altered during pregnancy (37.5±2.1 vs 38.3±1.3 mg in virgins) or by the stress exposure. Adrenal medulla enkephalin content was not significantly altered in pregnancy (7.5±1.1 vs virgins 4.9±0.5 pmol/g). Airpuff startle increased enkephalin content in virgin rats (to 11.0±1.8 pmol/g) but not in pregnant rats (to 5.5±0.6 pmol/g, 2 way ANOVA interaction group x treatment p<0.01), suggesting that enkephalin peptide processing increases in response to stress in virgin but not in pregnant rats.

In conclusion, the data show that adrenaline and medulla responses to stress are attenuated in late pregnancy but peripheral noradrenaline responses, reflecting sympathetic activity, are not. Both mechanisms may help protect the pregnancy: attenuated adrenaline responses preventing sudden fluctuations in glucose availability and heart rate and the retained sympathetic responses allowing the mother to respond appropriately to threat.

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PC10

Dynamic region-dependent changes in oxytocin receptor expression in the rat brain at parturition

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Centrally oxytocin plays a pivotal role in parturition, facilitating its own release centrally and peripherally and effecting maternal behaviour. Since quality of social and maternal behaviours depends upon pattern of oxytocin receptor (OTR) distribution1, we investigated the expression and activation of oxytocin-receptive neurones perinatally. OTR mRNA expression was quantified in dioestrus virgin, 21 and 22 day pregnant (pre-parturition), parturient and postpartum (<12h) rats (n=10,8,7,9,9,

respectively) by in situ hybridisation using a 35S-labelled riboprobe; data are mean±S.E.M and each brain region was analysed by 1 way ANOVA and posthoc tests. The highest OTR mRNA expression was in the supraoptic (SON) and paraventricular (PVN) nuclei. Pre-parturition OTR mRNA expression was increased only in the SON compared to virgins (43333±3889 vs. 30556±1111 pixels per cell, $p<0.01$), suggesting oxytocin control of the input from the uterus during labour. During parturition peak OTR mRNA expression was observed in the SON, A2/C2 and A1/C1 brainstem regions, medial preoptic area (MPOA), bed nucleus of the stria terminalis (BNST), olfactory bulbs and medial amygdala (increase above virgin=45,82,68,119,55,79 & 46% respectively; all $p<0.05$). Parturition increased OTR mRNA expression vs. pre-parturition only in the olfactory bulb and amygdala (increase=34 & 21% respectively, $p<0.05$), reflecting a rapid response to birth stimuli. Within 12h postpartum, OTR mRNA expression decreased and was not significantly different from virgins in all regions. OTR mRNA expression in the PVN and lateral septum did not alter perinatally.

Further virgin, 21day pregnant and parturient (n=6,6,8, respectively) rats were perfused-fixed and their brains processed by double immunocytochemistry for Fos and OTR. The number of Fos-positive OTR neurones was significantly increased during parturition but not before, especially in the SON (63.5±8.8 vs virgin 9.8±3.2 cells per region); for A2/C2 and A1/C1 brainstem regions, MPOA, BNST and medial amygdala Fos-positive OTR neurones per region were 3.3,12.0,11.4,15.3 & >22 fold above virgins, respectively ($p<0.05$). Fos and OTR co-expression in the parvocellular PVN and lateral septum was not significantly changed during parturition. So, selected OTR expressing neurones in selected brain regions are activated during birth and play a role in mediating behaviour.

Thus, as in the uterus, there are dynamic changes in oxytocin receptor expressing cells at parturition. Responses are region-dependent, altering the pattern of oxytocin receptor distribution in the brain perinatally. Increased expression and activation of OTR neurones reflects the crucial role they play in orchestrating birth and maternal behaviour; altered patterns may shape quality of behaviour.

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PC11

Fetal iron status regulates maternal iron metabolism during pregnancy in the rat

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Iron metabolism during pregnancy is heavily biased towards maintaining the fetal supply, even at the extent of inducing severe anaemia in the mother. In this study we examined the effect of iron (Fe) deficiency and supplementation on the hierarchy of Fe supply and the gene expression of proteins involved in Fe metabolism.

Female Hooded Lister rats were fed a control diet for 2 weeks following weaning, then a diet with control (50mg/kg) or deficient (7.5mg/kg) Fe content. Four weeks later, they were mated with males of the same strain. Following mating, the dams continued on the deficient diet or were given an Fe supplemented (150mg/kg) diet during either half of pregnancy. A control group were maintained on a normal Fe diet throughout the experiment. The dams were killed by exsanguination under terminal anaesthesia, at either day (D) 0.5, 12.5 or 21.5 of gestation and tissues and blood samples collected. Samples were also collected from fetuses, killed by Schedule 1 method, at D21.5. All animal procedures were conducted in accordance with the UK animals (Scientific Procedures) act. All data are expressed as mean ± s.e.m, n=8 per time point and treatment.

Maternal liver Fe levels were already lower in deficient animals at the start of pregnancy, and never recovered, irrespective of treatment. Haematocrit (Hct; 39±0.5%) was maintained in control and deficient dams until D12.5 but dropped to 28.6±0.4% ($p<0.05$) in the deficient dams by D21.5. In the fetus, in contrast, fetal liver Fe was returned to normal (1.3±0.06 mg/g dry wt) by supplementation in the second half of pregnancy, and Hct (35±0.6%) followed the same pattern. The data show, therefore, that fetal Hct and liver stores are restored at the expense of both maternal Hct and liver stores.

Placental transferrin receptor (TfR) expression was higher in deficient animals and in those supplemented in the first half of gestation only. As expected, levels correlated closely with fetal liver Fe levels ($p<0.0001$). The data suggest that hepcidin from the fetal liver mediates this signalling, since there is significant correlation between the two parameters ($p<0.001$). There was a linear relationship between maternal liver Fe levels, maternal liver TfR and hepcidin mRNA. However, there was a significantly greater interaction between them and fetal liver Fe. This was best described for TfR by a "broken stick" mode ($p<0.001$), with the break occurring at about 1.2mg/g dry wt. This is particularly exciting, since it suggests that the fetal liver is communicating Fe status to the maternal liver, and regulating metabolism through some, as yet unidentified, mechanism. In summary, the data show that the fetus has a remarkable capacity to accumulate Fe at the expense of the mother, and does so by manipulating Fe stores, haematocrit and the genes of Fe metabolism.

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PC12

Regulation of KCNQ and KCNE Gene Expression in Non-pregnant Mouse Myometrium During the Oestrous Cycle

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Background: KCNQ genes encode for the pore forming α subunits of K_v channels. The KCNQ gene family comprises 5 mem-

bers (KCNQ1-5), with KCNQ1 expressed predominantly in cardiomyocytes and KCNQ2-5 localised to neurones where they contribute to the resting membrane conductance. The expression products of KCNQ genes exhibit a range of phenotypes due to the formation of heteromultimers within each family and the association with auxiliary (β) subunits encoded by the KCNE gene family, which modulate channel function and pharmacology. There are only a few reports of ion channels encoded by KCNQ in smooth muscle cells and a paucity of information concerning their role in uterine smooth muscle (myometrium). The aim of the present study is to determine the expression profiles of KCNQ and KCNE genes in myometrial tissue from non pregnant mice during the oestrous cycle.

Methods: The oestrous cycle of c57/BL6 mice was monitored by daily vaginal smearing and uterine horns dissected at the different stages of the cycle: diestrous (n=5), proestrous (n=5), oestrous (n=5), metestrous (n=5). Total RNA was extracted using Trizol (Invitrogen) and cDNA synthesised with Superscript III (Invitrogen). RT-PCR and qRT-PCR for KCNQ1-5 and KCNE1-5 were performed using tRNA from myometrium and heart/brain (positive controls). qRT-PCR data was quantified using a standard curve and expressed relative to β-Actin. Non-pregnant mouse myometrial strips were used for preliminary in vitro tension measurement (n=3).

Results: All of the KCNQ and KCNE isoforms studied were detected in mouse myometrium by RT-PCR but expression levels appeared to vary during the oestrous cycle. qRT-PCR confirmed that KCNE3 was significantly down-regulated in the metestrous group (n= 5, p=0.01), whereas KCNE4 was significantly up-regulated in proestrous group (n = 5, p=0.01), compared to the diestrous group (n =5). Tension studies in vitro indicated that XE991 (1-3 mM, KCNQ channel inhibitor) and retigabine (10μM, KCNQ channel opener) enhanced and attenuated mouse myometrial contractility respectively.

Conclusions: We have comprehensively demonstrated the presence of KCNQ and KCNE isoforms in mouse myometrium, which coupled with the preliminary in vitro tension data, suggests a role for the KCNQ channels in the control of uterine contractility. KCNE mRNA expression appears to be regulated in preference to that of KCNQ. The loss of KCNE3, the presence of which promotes KCNQ1 opening, and an increase in KCNE4 mRNA, which inhibits KCNQ1, suggests that regulation of these accessory subunits is an important mechanism for regulating uterine contractility (and hence receptivity) during different stages of the oestrous cycle.

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PC13

Hypertension in offspring of iron deficient rats, is the kidney involved? - gene expression study

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Inappropriate nutrition during gestation can result in increased risk of diseases such as hypertension in the adult. In our rat model, the possibility that the kidney may play an important role is given credence by the fact that the pups born to Fe deficient mothers, as well as developing hypertension, have smaller kidneys at birth (Gambling et al., 2003; Gambling et al., 2004). In order to test whether the two phenomena are linked, we have examined the expression of genes involved in kidney development and function, specifically vasculogenesis/angiogenesis, apoptosis and cell proliferation.

Female Rowett Hooded Lister rats were fed diets with 2 different Fe contents (50 mg Fe/kg and 7.5 mg Fe/kg diet) before and during pregnancy. The dams were killed by exsanguination under terminal anaesthesia at either day 21.5 (D21)of gestation or within 12 hours of giving birth. Fetuses were delivered by caesarean section and killed by a schedule 1 method. Neonates were killed within 12 hours of birth by decapitation. Fetal and neonatal kidneys were collected and processed for mRNA extraction. All experiments were approved by Home office and carried out according to UK Animals (Scientific Procedure) Act, 1986. Using quantitative real time RT-PCR and SYBR Green, we found no difference in gene expression of the markers of vasculogenesis, angiopoietin 1 and 2, vascular endothelial growth factor A and C or haeme oxygenase 1 and 2. Markers of apoptosis, Bcl2 and Bax, showed no differences in expression in either fetal or neonatal kidneys. Cell proliferation markers, p21 and p27, also were not significantly different between the two dietary groups or either time point. In contrast, at D21, renin gene expression was increased in pups from deficient mothers (relative expression. mRNA/18s mRNA; Fe deficient 43.11 ± 3.9, control 30 ± 2.33, p=0.02). This difference disappeared at birth. Expression of other components of the renin-angiotensin system was not affected by maternal diet either in fetal or neonatal samples. In rats, kidney development continues after birth, but little is known concerning the period where it is most vulnerable to nutritional stress. Changes in expression of renin in our model suggest that there could be a window earlier in kidney organogenesis which is more sensitive to maternal influence. Clearly, this requires further investigation.

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PC14

Comparison of the effects of dose-dependent zinc supplementation of pregnant rats on cognitive behavior and memory function in their offsprings

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TITLE ONLY

PC15

Allopregnanolone acts centrally to restrain oxytocin responses to interleukin-1β in pregnancy

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In the late pregnant rat stimulated oxytocin secretion is inhibited, preserving the expanded neurohypophysial oxytocin stores for birth and minimising the risk of preterm labour. In virgin, but not pregnant rats, systemic administration of the cytokine interleukin-1β (IL-1β), which mimics infection, increases oxytocin secretion and the firing rate of supraoptic nucleus (SON) oxytocin neurons (1). Blood and brain levels of the neuroactive steroid allopregnanolone (AP; progesterone metabolite) increase during pregnancy and AP acts on $GABA_A$ receptors on oxytocin neurons, to enhance inhibitory transmission (2). Here we tested whether AP is the pregnancy-related factor responsible for restraining oxytocin responses to IL-1β in pregnant rats, and whether its actions are central. Jugular vein cannulae were implanted under halothane anaesthesia 5 days prior to the experiments. Oxytocin secretion following IL-1β (500ng/kg i.v.) was measured (by radioimmunoassay) in plasma from: a) virgin and pregnant (day 21) Sprague Dawley rats treated with vehicle (oil) or finasteride (FIN, 25mg/kg s.c.; 5α-reductase inhibitor to block AP production) 20h and 2h before IL-1β. b) Pregnant rats given FIN and AP/oil; and virgins given AP (3mg & 1mg/kg s.c.) or oil pre-treatment (20h and 2h before IL-1β). To investigate central SON oxytocin neuron responses, perfuse-fixed brain sections from pregnant rats treated with oil/FIN + vehicle/IL-1β, and virgin rats treated with oil/AP + vehicle/IL-1β, were processed for Fos (indicator of neuronal activation) and oxytocin immunoreactivity. Results: IL-1β significantly increased oxytocin secretion (3.4-fold; $p<0.001$, two-way repeated measures ANOVA; n=7) and Fos expression in SON oxytocin neurons (4.4-fold; $p<0.001$, two-way ANOVA; n=8) in virgin, but not pregnant rats (1.2-fold for secretion and Fos; n.s., n=7). FIN had no further effect on oxytocin secretion in virgins, but significantly restored an oxytocin response to IL-1β in the pregnant rats (3.3-fold; $p<0.001$, two-way repeated measures ANOVA; n=6), while AP significantly reduced the oxytocin response to IL-1β in virgins by 50% ($p<0.001$, two-way repeated measures ANOVA; n=6) and reversed the effect of FIN in pregnant rats. Consistent with the secretion data, FIN significantly increased IL-1β-induced Fos expression in identified SON oxytocin neurones of pregnant rats (2.4-fold; $p<0.005$, two-way ANOVA; n=6), while AP significantly reduced the number of SON oxytocin neurones activated by IL-1β by 48% ($p<0.05$, two-way ANOVA; n=6). Thus, AP acts centrally to restrain oxytocin secretory responses to IL-1β in pregnancy. This mechanism will serve to reduce the likelihood of preterm labour and prevent depletion of posterior pituitary oxytocin stores, required for parturition.

Brunton PJ *et al* (2006). *Eur J Neurosci* **23**, 1241-1247.

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

PC16

Liver-placental signalling - development of an in vitro model

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Iron (Fe) is one of the key nutrients transported by the placenta during gestation, required for many biological processes. Iron deficiency is disturbingly common and is even more prevalent in pregnant women, as the requirements for Fe increase due to the demands of the growing baby.

Iron is absorbed mainly through the duodenum and is stored in the liver until required. We have shown, using dietary approaches, that the fetus accumulates Fe at the expense of the mother, maintaining normal iron levels even when she is seriously anaemic, but how this is accomplished is not well understood. The fetal requirements for Fe are regulated through the fetal liver and the placenta, and in this presentation, we demonstrate a model that we are using to investigate how the interaction takes place. The model may also be of value to others investigating the interactions between the fetal liver and the placenta.

The approach involves co-culturing BeWo (b30 subclone) cells with HepG2 cells. The BeWo cells are grown on polycarbonate 0.4 μm pore inserts with HepG2 cells on the plate itself. The HepG2 cells on the base represent fetal liver cells and the basolateral side of the placenta while the compartment on top of the b30 cells represents the apical side of the placenta. The cells were cultured together for 7 days and during this time the transepithelial resistance (TEER) of the b30 cells was measured daily. During this time, TEER levels increased to about $175 \pm 3\ \Omega$ (n= 7). There was no significant difference between plates with or without HepG2 cells in the basal layer

At day 7, ^{59}Fe-Tf (1 μM, 0.5 $\mu Ci.ml^{-1}$) was added to the apical side of the placental cells. At increasing time intervals up to 8 h, 0.25 mL aliquots were removed from the basolateral medium, and were replaced by new medium. The aliquots removed were counted for the appearance of ^{59}Fe. Active transport was demonstrated by comparing the differences between cells incubated at 4 °C and 37 °C. Transfer of ^{59}Fe across the BeWo cell layer was markedly greater in plates grown without HepG2 cells (3.86 ± 0.6 pmol/min/filter) than those with (1.56 ± 0.19 pmol/min/filter, $p = 0.004$, $n = 6$). The difference could not be accounted for by accumulation of ^{59}Fe in the HepG2 cells but suggest that the presence of the liver cells in the basal layer inhibits the uptake and transfer of ^{59}Fe by the BeWo cells. At this stage, there are many possible explanations, but we are testing the hypothesis that the liver cells signal their normal iron status, to the placental cells, and are indicating a reduced Fe requirement. This is a very exciting possibility, since, if correct, it suggests that we can study liver and placental interactions in vitro in a model which is relatively easy to manipulate when compared to in vivo studies in animals.

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

PC17

Short-term Growth Hormone administration improves Respiratory Function in an unusual catabolic condition

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This study examined whether six days recombinant human growth hormone (rhGH) administration, 0.058 IU/kg/day, in an abstinent anabolic-androgenic steroid (AAS) using group had any respiratory, cardiovascular and biochemical effects compared with an abstinent AAS control group.

Impairment in respiratory function in adult-onset growth hormone deficiency (AO-GHD) is consequential to a reduction of respiratory muscle strength, responding to replacement therapy with rhGH (Merola et al., 1996).

RhGH significantly improves exercise tolerance in cystic fibrosis (CF) (Hutler et al., 2002) and significantly improves respiratory function in major surgery, and is more beneficial when given pre- and post-operatively than post-operatively alone (Barry et al., 1999).

Male subjects (n=48) were randomly divided, using a double blind procedure into two groups: (1): exercise control group (n=24, mean ± SD, age 32 ± 11 years; height 1.8 ± 0.06 metres); (2): rhGH using group (n=24, mean ± SD, age 32 ± 9 years; height 1.8 ± 0.07 metres). Anthropometry, peak oxygen uptake and respiratory muscle function were investigated. Respiratory measurements examined, were forced expiratory volume in one second, forced vital capacity (FEV1/FVC), MIP and maximum expiratory pressure (MEP). Cardiovascular measurements were blood pressure (BP), heart rate (HR) and rate pressure product (RPP). Biochemical analysis included; glucose, sodium, urea, creatinine, total protein, albumin, testosterone and insulin like growth factor-I (IGF-I).

FEV1/FVC (85 ± 6 vs. 82 ± 5, %), MIP (144 ± 24 vs. 129 ± 28, L), MEP (179 ± 35 vs. 157 ± 32, L), resting HR, (78 ± 11 vs. 67 ± 16, bpm) resting RPP (97 ± 14 vs. 84 ± 24, bpm.mmHg X 10-2) and IGF-I (323 ± 93 vs. 169 ± 50, ng/ml) significantly increased compared with the control group (all $P<0.05$).

Body mass index (27.7 ± 3.1 vs. 27.5 ± 3, kg.m-2), fat-free mass index (22.3 ± 1.9 vs. 21.9 ± 1.9, kg.m-2), peak oxygen uptake (45.4 ± 9.9 vs. 41.8 ± 9.8, ml.kg-1.min-1), MIP (144 ± 24 vs. 131 ± 30, L), MEP (179 ± 35 vs. 165 ± 36, L), IGF-I (323 ± 93 vs. 159 ± 54, ng/ml) and serum sodium (141.8 ± 2.5 vs. 140.6 ± 2.6, mmol/L) significantly increased, whilst body fat (19 ± 6 vs. 20 ± 6, %), total protein (73.1 ± 4.5 vs. 75.7 ± 4.9, mmol/L) and albumin (42.5 ± 4 vs. 44.4 ± 4, mmol/L), significantly decreased within the GH group (all $P<0.017$).

The findings of this study indicated that short term high dose rhGH increased aerobic performance and respiratory muscle strength in former AAS users, but may have an adverse effect on the cardiovascular system, as evidenced by the increase in resting rate pressure product.

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Hutler M, et al. Effect of growth hormone on exercise tolerance in children with cystic fibrosis. Med Sci Sports Exerc. 2002;34:567-72.

Barry MC, et al. Nutritional, respiratory, and psychological effects of recombinant human growth hormone in patients undergoing abdominal aortic aneurysm repair. JPEN J Parenter Enteral Nutr 1999;23:128-35.

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PC18

Effect of maternal low protein and folic acid supplementation on interscapular fat mass and uncoupling protein (UCP)-1 expression in brown adipose tissue of resultant offspring

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Folic acid supplementation to the mother during pregnancy can prevent significant congenital malformations. However, the extent to which it can impact upon other metabolic processes is unknown. Previous studies, using a rat model, have demonstrated that consuming a maternal low protein (MLP) diet plus/minus folic acid supplementation has differential effects upon resting blood pressure (Torrens et al., 2006). However, the extent to which this diet may affect offspring adiposity after weaning is currently unknown. The present study, therefore, aimed to examine the effects of a MLP diet with/without a folate supplementation on interscapular (comprising of brown (BAT) and white (WAT)) fat mass and UCP-1 abundance in BAT.

Fifteen virgin female Wistar rats (180-220g) were mated and randomly assigned to one of four feeding groups: control diet (CP: containing180g casein/kg, 1mg folic acid/kg; n=4), low protein (MLP: containing 90g casein/kg diet, 1mg folic acid/kg; n=3), control protein with folate supplementation (CPF: 180g casein/kg, 5mg folic acid/kg; n=4), or low protein with folate supplementation (MLPF: containing 90g casein/kg diet, 5mg folic acid/kg; n=4). All offspring were weaned at 21 days of age onto standard laboratory chow. One male and one female pup from each dam were culled at 13 weeks of age, and interscapular adipose tissue weight recorded. All procedures accorded with current UK legislation. Mitochondrial fractions were prepared from BAT and UCP-1 abundance determined. Results are given as means ± SEM and expressed as a percentage of a reference sample. Statistical differences ($p<0.05$) were determined using a General Linear Model test.

Neither MLP nor folate supplementation affected offspring body weight at 13 weeks of age. However, females were lighter than males in all four diet groups (Figure 1; $p<0.05$) and possessed slightly more BAT relative to body weight ($p=0.06$) but only in the folate supplemented group. Folic acid supplementation significantly increased relative interscapular fat mass (CP: 2.6±0.3,

CPF: 3.98±0.3, MLP: 2.43±0.3, MLPF: 3.31±0.2; p<0.01). UCP-1 abundance was significantly increased in female, but not male, offspring born to MLP dams (Figure 2; p<0.05). Furthermore, UCP-1 expression was increased in female offspring (p<0.001) irrespective of maternal diet.

Despite an increase in relative interscapular fat mass in folate supplemented offspring, there is no corresponding effect upon UCP-1 expression suggesting that its WAT component may be increased. Consuming a MLP diet significantly increased UCP-1 abundance in females suggesting that sex steroids may be involved in the regulation of UCP-1 expression.

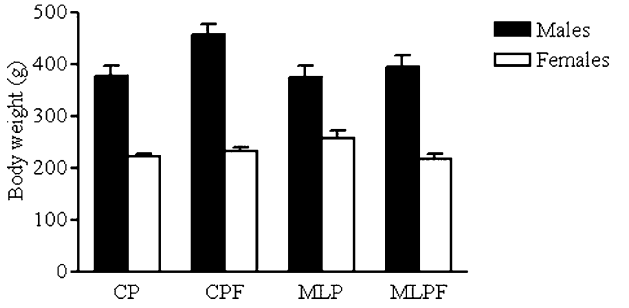

Figure 1: Body weight at dissection (13 weeks)

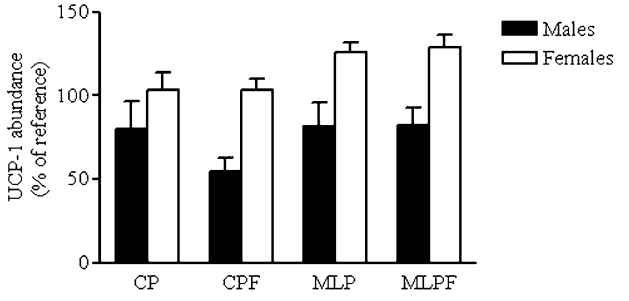

Figure 2: UCP-1 expression in BAT at 13 weeks of age

Torrens C, Brawley L, Anthony FW, Dance CS, Dunn R, Jackson AA, Poston L & Hanson MA (2006). Hypertension 47, 982-987.

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PC19

Effects of cobalt/vitaminB12 status of embryo donor and embryo recipient ewes on neonatal lamb behaviour

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Earlier reports indicate that lambs born to ewes that experienced sub-clinical cobalt/vitamin B12 deficiency during pregnancy are slow to stand and suck and have increased morbidity and mortality. These problems were not prevented by cobalt supplementation during mid to late pregnancy (Fisher & MacPherson, 1991), suggesting that they may originate during early development. The aim of this study was to distinguish between the effects of cobalt/vitamin B12 status of ova and of the ewe uterine environment on lamb behaviour using reciprocal embryo transfer. Scottish Blackface ewes from cobalt-deficient farms were either untreated (Co-, n=82) or were given an intra-ruminal cobalt-containing bolus ~30 days before embryo transfer (Co+, n=82). Day 6 embryos were recovered from 33 superovulated Co- or Co+ ewes and transferred singly to Co- or Co+ recipient ewes. Lamb behaviour was recorded by focal observation at birth and during the first 3 days of life by scan sampling at 2-hourly intervals. Data were analysed by linear mixed models using natural log transformations of behaviour data that were not normally distributed. Circulating concentrations of vitamin B12 on the day of ovum recovery were higher in Co+ than Co- donors (P<0.001). There was no effect of treatment on the proportion of recipients that became pregnant. The number of lambs studied in each of the 4 treatment groups was between 10 and 14. Concentrations of vitamin B12 were lower in Co- compared to Co+ ewes during pregnancy (P<0.001) and in lambs born to Co- compared to Co+ ewes at birth (Co- = 543 pmol/l, Co+=1805 pmol/l s.e.d.=92.1, P<0.001). There was no effect of donor or recipient cobalt/vitamin B12 status on lamb birth weight. There were no consistent effects of donor or recipient cobalt/vitamin B12 status on lamb behaviours immediately after birth. However, lambs born to Co+ recipient ewes were slower to get to their knees and to attempt to stand than lambs from Co- recipients (median time (mins with interquartile ranges): Co+ recipient=12.23 (6.35-13.83), Co-recipient=8.14 (4.98-11.99), P<0.05). Donor and recipient cobalt/vitamin B12 status had no effect on mean ewe-lamb distance during the first 3 days of life, or on any aspect of ewe behaviour. Lambs from Co+ donors were more active than lambs from Co- donors; they stood more frequently (% observations active: Co+ donor = 29.63, Co- donor = 21.99, s.e.d. = 2.43, P<0.005), were more frequently observed interacting with their mother (% observations: Co+ donor = 6.30, Co-donor = 3.11, s.e.d.=1.22, P<0.01) and spent more time exploring their environment (% observations: Co+ donor = 16.55, Co- donor = 6.85, s.e.d.=2.30, P<0.001). These data indicate that nutrient status prior to mating and during the early cleavage stages of embryo development, as well as during later pregnancy, affects lamb behaviours.

Fisher GEJ & MacPherson A (1991). Res Vet Sci 50, 319-327.

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PC20

Pre and postnatal manipulation of growth in the sheep

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Epidemiological studies provide evidence that mechanisms defining weight and body composition during development impact upon obesity in latter life. Impairments of energy supply during the pre and post-natal periods could indeed impact on energy partitioning, modifying intrauterine and postnatal growth performances. As part of the EARNEST European research programme, this study aimed to modify pre and post natal growth

in the sheep model for further analysis of the developmental origins of metabolic diseases.
30 twin-bearing ewes entered the study. During the last third of pregnancy (110 day to term), sheep were divided into two groups: 1) Undernourished (UN, n= 20) receiving 60 % of energy requirements and 2) Controls (C, n= 10) receiving 100 % of requirements. Sheep gave birth naturally at term (147 days). UN offspring were separated in two groups according to maternal diet. 20 were reared as twins (UN-T) and 10 as singletons (UN-S). C offspring (n=10) were reared as singletons (C-S). Body weights from birth to weaning (3 months old) were weekly measured during the first month and on a 2 weeks basis thereafter. Body weight differences and fractional growth rate (FGR) were analysed using SPSS software. All experimental protocols had the required Home Office approval as designated by the Animals Act (1986).
Compared to controls, UN offspring were lighter at birth ($p<0.01$) and remained lighter throughout the entire lactation ($p<0.05$). Although UN-S were smaller than C-S, they grew relatively faster. Indeed, FGR showed that weight at weaning was 6.5 +/- 0.32 and 5.6 +/- 0.24 times the birth weight respectively for UN-S and C-S ($p<0.01$). As compared to UN-S, UN-T had a lower growth rate ($p<0.05$). UN-T females weight during the entire lactation did not differ from UN-S females whereas male twins were lighter than UN-S males at mid lactation ($p<0.05$) and weaning ($p<0.01$). Finally, despite no differences at birth, at the end of weaning, both UN-S and C-S females were lighter than males ($p<0.05$). This is not true for UN-T.
A 60 % caloric reduction during late pregnancy impacted intrauterine growth and produced small birth weight offspring. Interestingly, compared to C-S, UN-S showed higher growth performances in the early postnatal period suggesting a long lasting effect of intra uterine growth retardation. As expected, female singletons were lighter than males. Nevertheless, twin males were not heavier than females suggesting a growth impairment dependent upon gender. Taken together, these data showed perturbations of pre and post-natal growth due to foetal restriction and/or postnatal environment which are predicted to have further long-term effects on adipose tissue development and metabolic control. These extended studies are currently underway

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PC21

Maternal undernutrition in pregnancy retards offspring physical and behavioural development in the mouse

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Prenatal undernutrition is known to have a significant impact on offspring organ development and a long term programming effect on subsequent health of the offspring. Although the brain is relatively protected from the effects of undernutrition, there are significant impacts on brain development (e.g. Feoli et al. 2006). In this study we investigated 1) whether prenatal undernutrition had a functional impact on the neonatal behavioural development in mice; and 2) whether the offspring of mouse lines selected for different growth traits would be disproportionately affected. Mice (n=54) of three lines: fast growing, fat (FF), fast growing, lean (FL) and normal growth, lean (NL) were assigned to a nutritional treatment on day 1 of gestation and were fed either ad libitum (C) or pair-fed to a weight-matched C dam at 80% of ad libitum (R). From birth onwards all dams were fed ad libitum, and three pups from each line were cross-fostered onto each dam within treatment. One male and one female pup from each line per litter (n=324) were assessed daily for physical development (pinnae detachment, tooth eruption, eye opening) and the development of reflex responses and motor skills until weaning at d16. The effects of genetic line, foster dam line and treatment were analysed by linear mixed models. R dams gave birth to smaller litters than C dams (C=16.8, R=15.4 pups, s.e.d.=0.53, $P<0.05$), and to lighter pups (C=1.74 g, R=1.44 g, s.e.d=0.05, $P<0.001$). Pups of FL dams were disproportionately lighter in comparison to other lines (26.1% reduction vs. 16.2% in FF litters, and 7.5% in NL litters, $P<0.01$). Pup physical development was significantly delayed in R pups for all measures (e.g. day of teeth eruption: C=10.30, R=10.69, s.e.d.=0.08, $P<0.001$). Forepaw grasp and placing reflexes were delayed in R pups, but these reflexes were accelerated in hindpaws in R compared to C pups. The appearance of other reflexes (vibrissae placing, righting, cliff drop response, negative geotaxis and climbing) were all delayed by approximately 0.5d in R pups compared to C (e.g. day maximal righting first seen: C=5.90, R=6.58, s.e.d.=0.19, $P<0.001$). In addition R pups tended to be slower to start crawling ($P=0.08$) and were slower to walk (day walking first observed: C=10.12, R=10.64, s.e.d.=0.14, $P<0.001$) than C pups. There were no significant interactions between pup line and treatment, or effects of foster dam on neonatal behavioural development. The data suggest that prenatal undernutrition causes a significant developmental delay in pup physical and neurological development, which is not affected by neonatal nutritional rehabilitation. Although mouse lines selected for fast lean tissue growth appeared less able to buffer foetal growth from the effects of gestational undernutrition, this did not affect neonatal behavioural development.

Feoli AM et al. (2006) Nutrition 22, 160-165

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PC22

Intra-litter variation in placental efficiency: the lightest placenta is the most efficient

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Fetal growth depends on the mother's nutrient supply, which, in turn, depends on the size and nutrient transfer capacity of

the placenta (1). The mouse placenta transfers nutrients by simple and facilitated diffusion and by active transport across the labyrinthine interhaemal membrane (LIM)(2,3). From formation of the placenta at E9 until E16, both the mouse fetus and placenta grow continually. Thereafter, the fetus continues to grow but placental weight plateaus. In normal litters near term, fetal and placental weights vary widely and often relate poorly to one another suggesting that the efficiency of placental nutrient transfer varies within litters. Placental efficiency, estimated as the feto-placental weight (fp) ratio, can be manipulated experimentally by altering gene expression or nutrient intake during pregnancy (4,5). For example, the small placenta of mice deficient in placental-specific *Igf2* is more efficient and transfers more glucose and amino acids than the normally sized wild-type placenta in late gestation (4). Hence, this study investigated the morphology and nutrient transfer capacity of the lightest and heaviest placentas within normal mouse litters at E16 and E19, (term= E20.5). Unidirectional materno-fetal transfer of radioactively labelled, non-metabolisable molecules was measured in vivo (4). Stereology was used to quantify placental compartment volumes and blood vessel volumes. Furthermore, LIM surface area and thickness were measured to determine a theoretical diffusion capacity (TDC)(3). The fp ratio was greater in the lightest placentas at both E16 and E19. Fetal body weight was significantly less with a light than heavy placenta at E16 but was similar in the two placenta groups at E19. Transport of C14-methyl-aminoisobutyric acid (MeAIB) per gram of placenta was greater in the lightest than heaviest placentas at both gestational ages. In contrast, passive and facilitated diffusion, measured using C14-inulin and C14-methyl-glucose respectively, were similar in the lightest and heaviest placentas at E16 and E19, when values were expressed per gram placenta.

At E16 per unit volume of placenta, the lightest placentas consisted of a greater % of Lz than the heaviest placentas at E16 but not at E19. At E16 and E19, surface area and thickness of the LIM was equal in the lightest and heaviest placentas. Hence, the lightest placentas have the same TDC as the heaviest placentas at both gestational ages. These data suggest that the small normal placenta may respond to the growth demands of the fetus and upregulate its nutrient transport capacity by increasing the surface area for nutrient exchange and/or the abundance of amino acid transporters per unit volume of Lz. Thus, the lightest placentas are more efficient and can support a similar fetal mass as the biggest placentas in a litter.

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PC23

The in-vitro functional properties of detrusor smooth muscle from newborn children with bladder exstrophy

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Bladder exstrophy occurs in 1 in 30000 live births where the abnormal bladder opens onto the lower abdominal wall. The neonates need immediate surgery to close the bladder but often need multiple reconstructive surgery during childhood to become continent of urine. The anomaly occurs during the 1st trimester and is thought to be due to premature rupture of the cloacal membrane. There is no information concerning the contractile and cell properties of detrusor smooth muscle from neonatal exstrophy bladders to determine if any such changes contribute to the altered bladder function. The aim of this study was to examine these properties and compare them to those with normal bladder function (controls). Following ethical approval and patient consent full thickness bladder samples were obtained from patients. Detrusor strips were superfused at 37°C with HCO3-/CO2 buffered physiological solution. Nerve-mediated responses were elicited by electrical field stimulation. Muscle-mediated responses (mN.mm-2) were generated by carbachol and a,bmethylene-ATP (ABMA). Detrusor cells were isolated using collagenase and loaded with a Ca2+ indicator (fura-2). The 340/380 ratio, a direct measure of intracellular calcium [Ca2+]i changes was measured following addition of carbachol, ABMA, and KCl. Data are mean±s.d. Significance of differences ($p<0.05$) between means were examined by Student's t-test. Nerve-mediated and muscle-mediated contractions were significantly less in samples with neonatal exstrophy compared to controls. The estimated Tmax of the force-frequency relation were 0.8±0.3 and 2.9±4.7 mN.mm-2, n=5,18. The frequency required to generate half maximum tension was not significantly different. Atropine-resistant contractions were recorded in all preparations, but they were a greater proportion of total nerve-mediated responses in the control group. For carbachol the estimated Tmax values at high concentrations from dose-response curves were 5.4±3.6 vs 30.9±22.2 mN.mm-2 respectively. The carbachol EC50 was significantly reduced in the neonatal exstrophy group (EC50s; 1.8±0.5 vs 3.2±1.2 μM respectively). Responses to 1 μM ABMA were 0.9±0.4 vs 11.2±8.3 mN.mm-2 respectively. Following addition of carbachol, ABMA, and high-KCl to isolated cells, [Ca2+]i transients were observed. The 340/380 ratios were not statistically significantly different between the two groups. The data shows that detrusor from patients with neonatal exstrophy exhibited reduced contractility. However, the detrusor cell function appears normal. Altered muscle-matrix interaction content and/or altered contractile protein sensitivity could play a role in this hypocontractility.

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PC24

Placental materno-fetal transfer of leucine by amino acid exchangers and by non-exchange mechanisms

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Objectives: The mechanisms mediating amino acid transport across the basal membrane and out of the placental syncytiotrophoblast into the fetal circulation are not well understood. Our previous data indicate that amino acid exchangers mediate serine transport into the fetoplacental circulation in exchange for alanine, serine, leucine, threonine, tryptophan and glutamine but not for glutamate. This study characterises amino acid stimulation of leucine transfer into the fetoplacental circulation.
Methods: Human placentas (n = 5) were collected within 30 minutes of delivery and an intact cotyledon was perfused with a modified Earl's bicarbonate buffer. The maternal arterial circulation was perfused with 50 μmol/l L-leucine & glycine, 0.6 μmol/l ^{14}C-leucine and 20 μmol/l ^{3}H-glycine. Amino acid [12.5 μmol] boluses were administered to the fetal side inflow perfusate. ^{14}C-leucine 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.
Results: In the absence of amino acids in the fetal circulation (which are required for exchange) leucine, but not glycine, was transferred to the fetal circulation. Following fetal arterial boluses of specific amino acids transfer of leucine, but not glycine, increased, indicating that transport by exchange was taking place. Leucine exchanged for 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH; a System L substrate), leucine or tryptophan but not serine, glycine, threonine, glutamate or lysine.
Conclusion: This study demonstrated that in the perfused human placenta leucine was transported into the fetal circulation by exchange and non-exchange mechanisms and glycine was not actively transported into the fetal circulation. None of the known amino acid transporters are thought to mediate leucine efflux across the basal membrane except for exchangers. It is therefore unclear what is mediating the non-exchange mediated transport.

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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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The Influence of the Pre- and Postnatal Hypercholesterolemia on the Development of Cardiovascular Dysfunction in Adult Mouse Offspring

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A high fat diet leads to hypercholesterolemia and predisposes the individual to developing cardiovascular disease (CVD). We hypothesised that the mother's diet before and during pregnancy and lactation can also influence predisposition to CVD in offspring fed a hypercholesterolemic diet. We therefore examined the effects of feeding a high fat-high cholesterol diet on cardiovascular function in female mouse offspring from mothers fed a hypercholesterolemic diet during pregnancy and lactation.
Female C57BL/6 mice were fed either a high fat-high cholesterol diet (HF; 45% kcal fat) or standard chow (C; 21% kcal fat) from weaning through pregnancy and lactation. Weaned female offspring from each group were then fed either a HF or C diets to adulthood. Body weight, blood pressure, plasma cholesterol and C-reactive protein levels (a marker of CVD) were measured at 36 weeks post-weaning. Histology of the liver was also performed. Data were expressed as mean ± SEM and analysed by ANOVA followed by post-hoc test.
At 36 weeks post weaning the offspring from high fat fed mothers that were then fed a high fat diet (HF-HF) or a chow diet (HF-C), and offspring from chow fed mothers fed a high fat diet (C-HF) had significantly elevated bodyweight (gm; HF-HF 34.7±0.3; C-HF 34.6±0.4; HF-C 29.6±0.2 vs. C-C 20.6±0.1; $p<0.001$), systolic BP (mmHg; HF-HF 139±0.1; C-HF 151.6±2.0; HF-C 146.2±2.3 vs. C-C 104.7±0.1; $p<0.001$), plasma cholesterol (mml/L; HF-HF 3.2± 0.4; C-HF 3.0± 0.3; HF-C 2.7±0.2 vs. C-C 1.8±0.1; $p<0.001$) and plasma CRP levels (mml/L; HF-HF 11.9±0.2; C-HF 12.8±0.4 vs. C-C 3.8±0.1; $p<0.001$) compared to C-C offspring. Liver histology also showed lipid vacuoles within hepatocytes in the HF-HF, HF-C & C-HF but not the C-C offspring.
We conclude that as expected feeding a HF diet induces CVD risk factors. For blood pressure feeding the dam a HF diet was not protective, as previously reported in a rat model (Khan et al. 2004). Interestingly, blood pressure and cholesterol were also elevated in offspring of the HF-fed dams even when fed C. Our results may have implications for understanding the effects of the 'nutritional transition' to higher dietary intake of energy and fat which lead to increased cardiovascular disease in many societies.
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