We have reconstructed, visualised and quantified the geometry and the fibre and sheet structure in these mouse hearts. These fibre and sheet directions can be mapped throughout the entire heart, and allow the application of fibre tracking algorithms. Thus, the combination of transgenic and DT-MRI methods enables the morphological changes in cardiac structure to be followed and quantified at different stages during the hypertrophic process.

These data add to a family of high-resolution digital models of cardiac geometry and architecture obtained from different species using DT-MRI (3,4), which can be used as platforms on which to run simulations of normal, pathological and pharmacologically-modified electrophysiology (5).

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Supported by the European Union through the BioSim Network of Excellence (LHSB-CT-2004-005137), the Medical Research Council, the British Heart Foundation, and the Multidisciplinary Cardiovascular Research Centre, University of Leeds.

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

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C25

**Maternal undernutrition affects the nutrient transport capacity of the mouse placenta**

P.M. Coan, N. Daw, O.R. Vaughan, S.L. Finn and A.L. Fowden

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Maternal diet during pregnancy is important in determining placental development. In farm animals, maternal undernutrition alters placental nutrient transfer but little is known about the effects of manipulating dietary composition on placental function in any species (1). This study examined transplacental transfer of nutrients transported by facilitated diffusion (14C-methyl-D-glucose) and active transport (14C-methyl aminoisobutyrate, MeAIB) in mice fed diets with varying protein content throughout pregnancy (term = 20 days, d). Group-housed pregnant mice were fed ad libitum either 23% (C23; n=66), 18% (C18; n=83), or 9% (C9; n=70) casein diets made isocalorific by carbohydrate supplementation. At 16d and 19d, mice were anaesthetised (10ul/g fentanyl-fluanisone:midazolam:water, 1:1:2, ip) before in vivo measurement of unidirectional materno-fetal flux of each tracer (2). After maternal cervical dislocation, placentas and fetuses were weighed. Fetuses were decapitated and dissolved in Biosol for scintillation counting. Transplacental tracer clearance was calculated as μl.min-1.g-1 placenta using maternal plasma and accumulated fetal radioactivity (3). All procedures for carried out under the Animal (Scientific Procedures) Act 1986. Results are means ± SE. Statistical significance was assessed by one way ANOVA.

MeAIB clearance in the C9 group matched the C23 group, both having significantly greater clearance than the C18 group at each age (Table 1). However, at E16, glucose clearance was greater in the C9 and C18 groups than the C23 group (Table 1). By E19, glucose clearance was greatest in the C9 group with both C18 and C23 groups transferring significantly less (Table 1).

The results show that maternal dietary composition alters placental nutrient transport capacity with differential effects on MeAIB and glucose transport in late gestation. Upregulation of placental glucose transfer in the mice fed the lowest protein diet may help meet fetal nutrient demands for growth. Variations in the relative proportions of nutrients supplied to the fetus due to dietary-induced adaptations in placental nutrient transport may programme tissue development in utero with consequences long after birth (1).

Transplacental MeAIB and glucose clearance with respect to maternal dietary protein content at days 16 and 19 of mouse pregnancy (n= > 9 litters per group).

| | MeAIB | | Glucose | |
|---|---|---|---|---|
| Isocalorific diets | 16d | 19d | 16d | 19d |
| 23% casein diet | 50±4 a | 147±14 a | 68±10 a | 273±41 a |
| 18% casein diet | 31±3 b | 108±9 b | 148±18 b | 244±49 a |
| 9% casein diet | 55±4 a | 131±11 a,b | 123±19 b | 362±49 b |

Values within columns with different letters (a,b) are significantly different from each other P<0.05

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This study was funded by the BBSRC

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

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C26

**Gene expression changes observed in the placenta from different maternal diets provide evidence for possible candidates for gatekeeper genes in development**

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During pregnancy, the quality of diet a mother consumes is critical for the development of her fetus and a suboptimal diet can have deleterious consequences for the offspring, both short and long term. Studies have shown that a low protein, high fat or a low iron diet results in offspring that develop hypertension and obesity. This has lead to the Gatekeeper hypothesis, which suggests that diverse nutritional stresses affect common

gene(s) or gene pathway(s), as detailed in Figure 1. We are using nutrigenomic approaches to identify these genes or gene pathways.
All animal procedures were approved by the Home Office and methods used were compliant with the UK Animals (Scientific Procedures) Act, 1986. Day 21 gestational placentas from rats fed either a control, low protein, high fat or low iron diet during pregnancy were collected. RNA was prepared for micro array analysis on Affymetrix whole rat genome arrays. The data collected has undergone quality control and normalisation via the NuGO MadMax server. The resultant output produced includes unpaired t-test p-value and mean fold change.
The data was then filtered according to p value of <0.05 and 20% fold change to determine differential gene expression. For each diet a number of genes showed significant changes. These included genes involved in growth regulation and DNA repair and metabolism and a number of ion and metal transporters.
The data has identified several genes and gene pathways that are altered in common to the different diets and may be possible gatekeeper candidates. We are currently using pathway analysis tools to compare directly the different treatments and to test the gatekeeper theory. This approach has also provided evidence of gene expression changes in the placenta during different diet regimes. In addition, this methodology is also of excellent value, providing testable hypotheses for understanding the link between maternal diet and offspring health.
This work was supported by RERAD and the EU (NuGO and EARNEST)

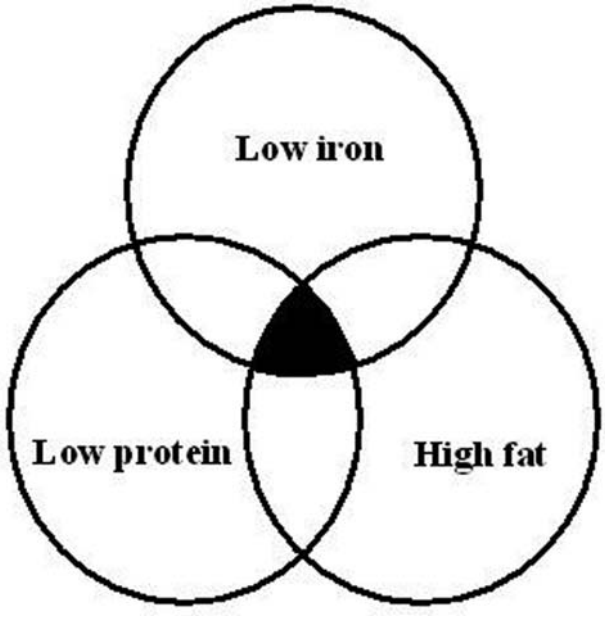


Figure 1: Gatekeeper hypothesis. Comparing the different stresses directly with each other identifies the commonly affected genes. The darkened central point contains these genes in common.

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

C27

**Homocysteine inhibition of system L amino acid transport activity in human placenta**

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BACKGROUND: Elevated plasma levels of homocysteine (Hcy) are associated with vascular-related complications of pregnancy and adverse neonatal outcomes. Fetal plasma Hcy concentration at term is positively correlated with maternal Hcy concentration, suggesting placental transport of Hcy may be an important determinant of fetal plasma Hcy. The mechanisms involved in placental Hcy transport are uncharacterised. We tested the hypothesis that the heterodimeric amino acid transporter system L, comprising CD98 heavy chain linked to either LAT1 or LAT2 light chain, provides one mechanism for placental Hcy transport by measuring the ability of Hcy to inhibit system L activity. System L transports neutral amino acids in a $Na^+$-independent manner, across the microvillous (MVM) and basal plasma membranes of human placenta. Both LAT1 and LAT2 light chains are expressed in the human placenta, with previous studies demonstrating that LAT1 is localised to the MVM, while LAT2 localisation is less clear with functional studies supporting both MVM and BM distribution. Here we focus on MVM, the first plasma membrane barrier to maternofetal transport.
MATERIALS AND METHODS: MVM were isolated from placentas of normal pregnancies at term (n=6). System L activity was measured as $Na^+$-independent $^{35}S$-methionine (0.2μM) uptake into MVM vesicles at 30sec (initial rate) in the absence (control) or presence of varying concentrations of L-Hcy, DL-Hcy or system L model substrates (L-methionine, L-Leucine, 2-amino-2-norbornane-carboxylic acid (BCH)). System A specific substrate methylaminoisobutyric acid (MeAIB) and system $y^+L$ substrate L-arginine were included as negative controls. Gene expression for the components of system L, CD98, LAT1 and LAT2 was examined by real-time quantitative PCR (n=6).
RESULTS: Both L-Hcy and DL-Hcy caused a dose-dependent inhibition of $^{35}S$-methionine uptake into MVM in a comparable manner to system L substrates suggesting similar affinity of system L for Hcy, whereas as predicted, this was not observed for MeAIB and arginine. In all six placentas examined, gene expression for CD98, LAT1 and LAT2 was confirmed.
CONCLUSION: Inhibition of methionine uptake by L-Hcy and DL-Hcy in a $Na^+$-independent manner implies that Hcy can serve as a substrate for system L. Gene expression for LAT1 and LAT2 in human placenta allows for the possibility that either light chain links to CD98 to form a functional complex and mediate Hcy transport across MVM. We speculate that inhibition of placental amino acid uptake by Hcy could impact on fetal growth and development.

Supported by the MRC and Action Research Endowment Fund

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

C28

## Melatonin up-regulates placental expression of catalase and manganese superoxide dismutase under maternal undernutrition but not hypoxic conditions

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The neurohormone melatonin participates in circadian, seasonal and reproductive physiology. Melatonin also acts as a potent endogenous antioxidant by scavenging free radicals and up-regulating endogenous antioxidant pathways (1). The presence of melatonin receptors in the placenta suggests a role in placental function (2) and melatonin protects against oxidative damage induced in rat placenta by ischaemia-reperfusion (3). Complicated pregnancy is often characterised by reductions in oxygen and nutrient delivery, and both hypoxia and undernutrition promote oxidative stress (4). Previously, we reported that melatonin treatment in rats rescued the fall in placental efficiency in undernourished but not hypoxic pregnancy (5). In this study, we have investigated the effects of maternal treatment with melatonin on the expression of placental antioxidant proteins in both hypoxic and undernourished pregnancy. On day 15 of pregnancy, Wistar rats were divided into 6 groups (n=7 per group): control (21% O2), hypoxic (10% O2) and undernourished pregnancy (40% reduced food intake), with and without melatonin treatment (5μg.ml-1 drinking water). Water and food intake were monitored daily. On day 20, the dams underwent euthanasia (0.2 mL, i.p., xylazine and ketamine) and the placentae were snap frozen for subsequent protein isolation. Western blot was used to semi-quantify expression of the antioxidant enzymes catalase, manganese superoxide dismutase (Mn-SOD) and glutathione peroxidase 1 (GPx-1); relative to β-actin levels. Melatonin increased the expression of catalase and Mn-SOD in undernourished pregnancy but had no effect in control or hypoxic pregnancies (Table). The data show that in pregnancy complicated by undernutrition, but not by hypoxia, melatonin may attenuate placental oxidative damage and improve placental efficiency by up-regulating at least two potent antioxidant enzymes.

| Placental enzyme | Pregnancy conditions and treatments | | | | | |
|---|---|---|---|---|---|---|
| | Hypoxic | | Undernourished | | Control | |
| | Vehicle | + Mel | Vehicle | + Mel | Vehicle | + Mel |
| Catalase | 0.70±0.05 | 0.58±0.02 | 0.69±0.03 | 1.01±0.06 * | 0.69±0.03 | 0.63±0.02 |
| Mn-SOD | 0.16±0.05 | 0.18±0.05 | 0.05±0.02 | 0.46±0.10 * | 0.11±0.04 | 0.10±0.02 |
| GPx-1 | 0.30±0.02 | 0.25±0.02 | 0.62±0.18 | 0.39±0.05 | 0.32±0.03 | 0.24±0.04 |

+Mel, melatonin. Values are Mean ± SEM (n=7) for the ratios protein/β-actin obtained by digital band densitometry. *P<0.05 (ANOVA and Tukey's Multiple Comparison Test). Two-tailed Unpaired t-test for control without melatonin versus control with melatonin indicated no action of melatonin per se

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*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

C29

## Effects of undernutrition during mouse pregnancy on tissue glycogen content

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Glycogen is a storage form of glucose found primarily in liver, which is used to provide oxidative substrates and circulating glucose in poor nutritional conditions. Pregnancy increases the glucose demand but its effects on tissue glycogen stores are unknown. This study examined tissue glycogen contents of non-pregnant, pregnant and lactating (L, day I postpartum) mice and of newborn pups in control and undernourished (UN) conditions. Individually housed pregnant (P, n = 44) and non-pregnant (NP, n= 14) C57B1/6J mice were fed a 23% casein diet either ad libitum (control, CT) or at 80% of control intake for 16 or 19 days or until delivery (term 20.5 days). Where possible, placenta and liver samples were collected from adults and neonates after cervical dislocation or decapitation. Glucose levels were measured in blood taken from the severed neck while tissue glycogen was determined enzymatically (1). Results are means ± SE. Statistical significance was assessed by one-way ANOVA or t-test.

In CT adults, hepatic glycogen content was significantly lower at day 19 of pregnancy than at day 16 or in NP mice (Table 1). Undernutrition reduced hepatic glycogen content in NP, P and L adults (Table 1). In contrast, there was no significant difference in mean hepatic glycogen content between litters of CT (27.2 ± 5.3mg/g, n =7) and UN neonates (24.1 ± 6.2mg/g, n =6). Placental glycogen content was significantly lower in UN than CT mice at day 16 (UN 10.5 ± 0.3mg/g, n =7; CT 12.1 ± 0.4mg/g, n =6, P<0.02) but not at day 19 of pregnancy (UN 4.2 ± 0.4mg/g, n = 6; CT 4.4 ± 0.3mg/g, n = 7, P>0.05). Blood glucose levels were lower in control L than P mice and were reduced by undernutrition in NP and day 19 P mice but not in day 16 P or L mice (Table 1). Blood glucose levels were also significantly lower in UN (1.2 ± 0.1mmol/l, n = 6) than CT neonates (2.2 ± 0.1mmol/l, n = 7, P<0.01). The results show that, in controls, the increased glucose demand of late pregnancy and lactation reduces maternal hepatic glycogen content but has little effect on blood glucose levels compared to NP mice. Hepatic glycogen stores were adversely affected in all UN adults but appeared to be protected in UN neonates, despite the low glucose and hepatic glycogen levels of their mothers near term.

Table 1 hepatic glycogen (mg/g) and blood glucose (mmol/l) levels in the four groups of adult CT and UN mice

| | | Non-pregnant | Pregnant Day 16 | Pregnant Day 19 | Lactating |
|---|---|---|---|---|---|
| Glycogen | CT | 39.8±3.4 [ab] | 45.7±3.7 [b] | 20.2±3.8 [c] | 27.0±6.8 [ac] |
| | UN | 2.7±2.2*† | 4.8±3.7* | 1.2±0.4* | 7.0±2.7* |
| Glucose | CT | 9.4±0.8 [ab] | 10.8±0.5 [a] | 11.1±0.7 [a] | 8.3±0.7 [b] |
| | UN | 5.1±0.6 [a]*† | 9.1±1.0 [b] | 4.9±0.5 [a]* | 7.4±1.1 [ab] |

Values within rows with different superscripts are significantly different from each other (P<0.05, ANOVA) * significantly different from corresponding control (P<0.05, t-test). UN for 19 days.

Franko et al., (2007) J. Endocrinol. 191 67-73.

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*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

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C30

**Prenatal diet and adult-onset obesity influence plasma amino acids in adult sheep**

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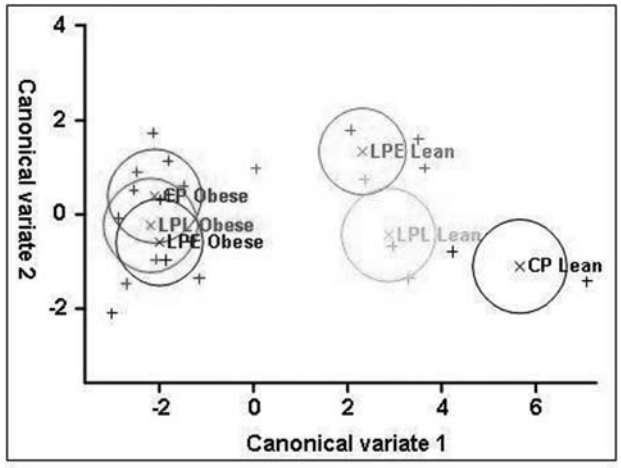
Introduction: Many studies have shown associations between a poor prenatal environment, often leading to low birth weight, and later physiological dysfunction. No study has examined a relationship between prenatal protein nutrition, whole body composition and amino acid status before and after a period of significant weight gain; the aim of the current study.
Methods: 20 sheep were either fed a control protein diet (18% crude protein content) from day 0 (mating date) to term (~145days gestation; Control [CP], n=7) or a low protein (9% crude protein content) diet during early (0-65 days [LPE], n=7) or late gestation (65-145 days [LPL], n=6). At 18 months sheep were blood sampled (5ml, EDTA) and body composition determined by dual-energy X-ray absorptiometry (DXA). Subsequently, for 5 months, sheep were reared in an 'obesogenic environment' to gain weight. Blood sampling and DXA was then repeated. Plasma amino acid concentration (nmol/ml) was determined by GC-MS (EZ:faast, Phenomenex Ltd, CA, USA). Data are presented as estimated marginal means ± S.E.M. and were analysed by Repeated Measures General Linear Model and P<0.05 accepted as statistically significant (SPSS v14).
Results: Birth weight was significantly lower in LPL vs. CP and LPE (4.37±0.19 vs. 5.25±0.19 and 5.12±0.20 kg) but weights at 18months were similar (CP, 57.2±2.1; LPE, 57.4±2.0; LPL, 54.6±2.1 kg). Body composition per se was unaffected by prenatal diet, but an obesogenic environment resulted in significant weight gain (from ~15% to ~30% fat). LPL animals gained significantly less fat than CP or LPE (7.1±0.9 vs. 10.9±0.9 and 10.6±0.9 kg). Overall, plasma amino acids decreased with obesity (lean, 8934±962 vs. obese, 6242±742 μM/ml). Principal components analysis indicated 7 amino acids to account for 95% variation with obesity; glycine, alanine, valine and glutamate all decreased whereas serine, aspartate and glutamine all increased. A representative canonical variate plot (mean, 95% CI) is shown in Fig 1.
Discussion: Prenatal protein restriction during late, but not early, gestation reduced birth weight but had little effect on postnatal growth rate or body composition of adult sheep per se. When coupled with an obesogenic environment, low birth weight reduced the quantity of fat deposited. Resting amino acid metabolism appeared altered by prenatal diet and, when coupled with adult-onset obesity, significantly influenced individual amino acid status.




The British Heart Foundation, the Medical Research Council and the University of Nottingham.

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

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C31

**Cortisol suppresses the anabolic signalling proteins, p-mTOR and p-S6 kinase, in skeletal muscle of fetal sheep near term**

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Glucocorticoids retard growth in utero although the effects of endogenous and exogenous cortisol on metabolic signalling proteins in fetal skeletal muscle are unknown. This study investigated the effects of (a) the developmental change in plasma cortisol concentration near term and (b) a premature elevation in plasma cortisol induced by exogenous hormone infusion on signalling proteins in skeletal muscle of fetal sheep.
After maternal euthanasia (200 mg/kg pentobarbitone iv), hind limb skeletal muscle samples were collected from sheep fetuses at 130 (n=13) and 144 days of gestation (n=7, term 145±2 days). In 11 of the fetuses sampled at 130 days, indwelling catheters were implanted under general anaesthesia (1.5% halothane in O2-N2O) at 115-120 days and the fetuses were infused iv with either saline (0.9% sodium chloride, n=6) or cortisol (2-3 mg/kg/day, n=5) for 5 days from 125 days of gestation. All surgical and experimental procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986. Plasma cortisol concentration was determined by RIA and the

muscle signalling proteins, p-mTOR (mammalian target of rapamyosin), p-S6 kinase and calpastatin, were measured by Western blot.

Between 130 and 144 days of gestation, the prepartum cortisol surge was associated with significant reductions in the protein levels of p-mTOR, p-S6 kinase and calpastatin in fetal skeletal muscle (Table 1). In the cortisol-infused fetuses, p-mTOR and p-S6 kinase levels were significantly lower than those infused with saline (Table 1). When values from all fetuses were considered, significant inverse relationships were observed between plasma cortisol and p-mTOR ($r=-0.46$, $n=20$, $p<0.05$), p-S6 kinase ($r=-0.63$, $n=20$, $p<0.05$) and calpastatin ($r=-0.48$, $n=20$, $p<0.05$). Therefore, in fetal sheep, the growth-retarding actions of cortisol may be mediated, in part, by suppression of anabolic signalling proteins, such as p-mTOR and p-S6 kinase, in skeletal muscle.

Table 1. Mean (±SEM) concentrations of plasma cortisol and muscle signalling proteins in the fetuses of each experimental group. Within each column, values with different superscripts are significantly different from each other ($p<0.05$, one-way ANOVA and Tukey test). Au, arbitrary units.

| Treatment group | Gestational age (days) | Plasma cortisol (ng/ml) | p-mTOR (au) | p-S6 kinase (au) | Calpastatin (au) |
|---|---|---|---|---|---|
| Untreated and saline-infused (n=8) | 130 | $13.2 \pm 1.6^{A}$ | $1.00 \pm 0.09^{A}$ | $1.00 \pm 0.06^{A}$ | $1.00 \pm 0.08^{A}$ |
| Untreated (n=7) | 144 | $86.7 \pm 17.3^{B}$ | $0.47 \pm 0.09^{B}$ | $0.55 \pm 0.11^{B}$ | $0.64 \pm 0.05^{B}$ |
| Cortisol-infused (n=5) | 130 | $91.2 \pm 20.9^{B}$ | $0.61 \pm 0.03^{B}$ | $0.48 \pm 0.04^{B}$ | $0.84 \pm 0.11^{AB}$ |

This study was funded by the BBSRC.

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C32

**Effect of maternal diet and body condition on glucose metabolism and skeletal muscle structure in mature adult sheep offspring**

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Early life nutrition is implicated in the risk of metabolic diseases (e.g. type 2 diabetes) in adulthood. Low birth-weight was associated with defects in the skeletal muscle insulin-signalling pathway of young adult men (1), and insulin resistance was associated with changes in myofibre composition (2). In sheep, maternal undernutrition reduced fetal skeletal muscle myofibre density and composition (3). Recently we reported that lower body condition score (BCS) led to increased fasting glycaemia, mild glucose intolerance and impaired initial insulin secretory response in adult offspring (4). We hypothesised that this would worsen with age, and that altered skeletal muscle structure and insulin signalling pathways are involved.

Ewes were established, by dietary manipulation, at a BCS of 2 (lower (L) n = 10) or >3 (higher (H) n = 14) before and during pregnancy (4). In male offspring at 4.04±0.02 years plasma glucose and insulin concentrations were measured during a glucose tolerance test (0.5 g/kg body weight *i.v.*) and rams were killed by an overdose of barbiturate (*i.v.* 145 mg/kg). We analysed a) insulin-signalling proteins by Western blotting in abdominal fat and vastus muscle (*m.*); b) glucose uptake in isolated strips of vastus and soleus *m.*(5); c) myofibre density and cross-sectional area (CSA) by immunostaining with anti-fast skeletal myosin (3). Data are mean±SE and were analysed by Student's t test.

Glucose tolerance was similar between groups. Basal glucose uptake was similar in L and H group soleus and vastus *m.* isolated strips. However insulin-stimulated uptake tended to be reduced in the soleus *m.* only of L rams (H 1.01±0.06; L 0.84±0.07 pmol.min.mg, $p<0.1$). In vastus, but not soleus, *m.* total myofibre density (H 343±15; L 294±14 fibres/mm$^2$, $p<0.05$) and fast myofibre density (H 226±10; L 194±10 fibres/mm$^2$, $p<0.05$) was lower in L rams. Slow myofibre density tended to be lower in L rams (H 117±7; L 100±6 fibres/mm$^2$, $p<0.1$). Myofibre CSA was unaltered. Protein levels of (i) Akt1 were lower in the vastus *m.* (L=83±7% of H, $p<0.05$), and tended to be lower in abdominal fat (L=71±7% of H, $p<0.1$), of L rams; (ii) GLUT-4 were increased (L=157±6% of H, $p<0.001$), and (iii) IGF-IR tended to be reduced (L=78±12% of H, $p<0.1$), in the vastus *m.* of L rams.

Reduced signalling through Akt1 may therefore mediate the decreased vastus *m.* myofibre density in L rams resulting in reduced glucose tolerance of the young adult offspring (4). However in mature adulthood, glucose tolerance and glucose uptake into vastus *m.* was not altered by maternal BCS, and thus the impact of reduced myofibre density may be offset in part by increased GLUT-4. Such adaptations may lead to complications in metabolic control in an overabundant postnatal nutrient environment.

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NIH, Gerald Kerkut Trust and BHF.

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C33

**A controlled trial evaluating the effectiveness of the Human Patient Simulator as an educational tool for teaching respiratory physiology**

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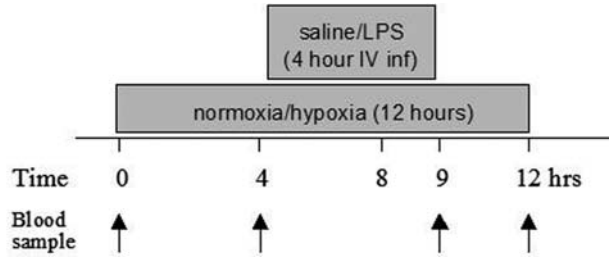
We have previously reported (Lloyd et al., 2006) that use of the Human Patient Simulator (HPS; METI, Florida) can enhance traditional approaches to teaching physiology. HPS scenarios are popular with students (Euliano, 2001) but randomised controlled trials (e.g. Wong et al., 2007) have failed to demonstrate

(101 bpm) groups. There was no difference between the E and HE groups.
WBC and TNF increased in group E and HE. The increase was more pronounced in group HE than in group E with regard to WBC (P<0.05), and more pronounced in group E than in group HE with regard to TNF (P<0.01). IL-6 increased during all types of interventions, albeit more so in groups receiving endotoxin infusion (E and HE) than during hypoxia alone (group H). No difference was found with respect to increase in IL-6 between groups E and HE (Table 1).
These results suggest, that acute hypoxia may modulate certain aspects of the systemic inflammatory response in humans.




| | Intervention | 0 hours | 4 hours | 9 hours | 12 hours |
|---|---|---|---|---|---|
| WBC ($10^9$/l) | E | 5.0 (4.6;5.5) | 6.3 (5.4;7.1) | 9.2 (8.2;10) | 9.5 (8.2;11) |
| | H | 5.2 (4.4;6.0) | 7.2 (6.2;8.2) | 8.0 (7.1;9.0) | 8.4 (7.5;9.2) |
| | HE | 5.9 (5.1;6.7) | 8.7 (6.9;10) | 12 (10;14) | 12 (9.9;13) |
| TNF alpha (pg/ml) | E | 1.2 (0.6;1.8) | 1.1 (0.4;1.7) | 11 (9.0;12) | 3.4 (2.7;4.0) |
| | H | 1.3 (0.7;1.8) | 1.2 (0.7;1.8) | 1.1 (0.5;1.8) | 1.3 (0.6;1.9) |
| | HE | 1.0 (0.7;1.4) | 0.8 (0.6;1.1) | 6.6 (5.0;8.3) | 1.9 (1.6;2.3) |
| IL-6 (pg/ml) | E | 0.5 (0.2;0.8) | 0.9 (0.6;1.2) | 79 (40;118) | 5.0 (2.2;7.7) |
| | H | 1.0 (0.3;1.8) | 1.6 (1.1;2.1) | 2.3 (0.9;3.7) | 2.1 (1.2;2.9) |
| | HE | 0.9 (0.6;1.2) | 1.5 (1.1;1.9) | 44 (20;68) | 3.6 (2.6;4.7) |

Values are mean (lower; upper 95% CI)

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

PC34

**Effect of acclimatization to humid hot environment with transition across five time zones on heart rate variability in elite junior rowers**

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TITLE ONLY

*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

PC35

**Ontogeny of insulin signalling pathways in ovine fetal skeletal muscle during late gestation**

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In man and other animals, the incidence of adult insulin resistance is increased when fetal growth is poor, which suggests that insulin sensitivity is determined, in part, prenatally1. In many species, an increase in fetal glucocorticoid concentrations is responsible for prepartum tissue maturation2. Fetal glucocorticoid levels are also raised earlier in gestation by conditions known to impair fetal growth and adult insulin sensitivity3. Little is known about the ontogeny and developmental control of the insulin signalling pathways in utero. This study determined i) the ontogenic changes in these pathways during late gestation and ii) the glucocorticoid dependence of these changes in sheep.
After maternal and fetal euthanasia (200 mg/kg, Na pentobarbitone), hind limb skeletal muscle was collected from 4 groups of fetal sheep: 1) untreated controls (n=22) at 110, 120, 130 and 140 days (d) of gestation (term ~145d, n≥ 4 per group), 2) adrenalectomised (AX) at 115d and age-matched controls delivered at 145d (n=6 per group), 3) catheterised and infused with cortisol (2-3 mg/kg/day in saline) or saline for 5 days before delivery at 130d (n=6 per group), 4) maternal dexamethasone (2x12 mg in saline im) or saline treatment before delivery at 127d (n=6 per group). All surgical procedures were carried out under halothane anaesthesia (2%, in O2:N2O). After protein extraction and standardization, abundance of the insulin receptor (IR)-b subunit, insulin-like growth factor type I receptor (IGF-1R), protein kinase C zeta (PKC-ζ) and the insulin-sensitive glucose transporter (GLUT4) were measured by SDS-PAGE and Western Blotting using ovine validated antibodies4 (Santa Cruz, CA or Abcam Ltd, UK). Results are mean (± SE) arbitrary units. Statistical significance was assessed by Student's t-Test or ANOVA plus Tukey Test, as appropriate.
From 110d to 120d, there were increases in muscle protein abundance of IGF-1R (10.10±1.30 to 19.15±1.00, P<0.05) and PKC-ζ (5.57±0.20 to 21.25±3.41, P<0.05) but not IRβ in (17.90±3.30 to 27.65±4.69, P>0.05). At 140d, muscle protein abundance of IR-b (13.65±2.58), IGF-1R b (4.47±0.95) and PKC-ζ (7.04±0.76) were significantly less (P<0.05) than the values at 120d but not 110d. Muscle protein GLUT4 abundance was significantly lower at 130 and 140d (18.34±2.75 and 20.63±3.04, respectively) than at 110 and 120d (72.13±6.37 and 73.28±7.32 respectively; P<0.05). Fetal adrenalectomy, intrafetal cortisol and maternal dexamethasone treatment did not significantly alter muscle abundance of any of the proteins, except PKC-ζ, which was higher in AX (10.58±1.24) than control fetuses (7.04±0.76; P<0.05). These data show that ontogenic changes in the insulin-signalling pathway occur in fetal skeletal muscle towards term but that these are unlikely to be glucocorticoid dependent.

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PC36

**Effect of weight and adiposity at conception and gestational dietary intake on pregnancy outcome in young adolescent sheep**

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Adverse pregnancy outcome is common in young adolescent girls. To date studies using adolescent sheep have focussed on the role of maternal diet after conception. However girls become pregnant from diverse nutritional backgrounds which may interact with subsequent gestational intake to influence pregnancy outcome. This aspect has been examined here. Singleton pregnancies to a single sire were established by embryo transfer in two groups of adolescents of identical age but different initial weight and adiposity score (mean ± sem; 47±0.3kg and 2.6±0.02 units vs. 37±0.3kg and 2.1±0.01 units), and classified as good (G) vs. poor (P) body mass index (BMI), respectively. Thereafter, ewes were either offered a moderate intake to maintain maternal adiposity throughout pregnancy (optimally nourished control [C]), undernourished to maintain weight at conception but deplete maternal body reserves (UN, 0.75 x C), or overnourished to promote rapid maternal growth and adiposity (ON, 2.25 x C), resulting in a 2 x 3 factorial, n=15 /group). Blood samples were collected at 28- day intervals to assess metabolic status in relation to pregnancy outcome. Maternal glucose handling and sensitivity to insulin were additionally assessed at the end of the second third of gestation following either i.v. glucose or insulin. Conception rate was 82 % and was independent of initial BMI and gestational intake. For the G-C, P-C, G-UN, P-UN, G-ON and P-ON dams, respectively the average gestational change in external adiposity score was 0, +0.1, -0.8, -0.5, +0.6 and +0.9 units. Length of gestation and colostrum yield at term were independent of initial BMI but influenced by gestational intake (145.1, 146.2, 143.3 days and 362, 309,152 g in C, UN and ON groups respectively, P<0.001, ANOVA). Lamb birth weight was influenced by initial BMI (P<0.03) and gestational intake (P<0.001) and was 5631, 5218, 4942, 4592, 4504 and 3950g for the G-C, P-C, G-UN, P-UN, G-ON and P-ON groups, respectively. Placental and total fetal cotyledon weights were similarly influenced by both initial BMI and gestational intake (P<0.002). At Day 0, maternal insulin, glucose, non-esterified fatty acid (NEFA) and urea concentrations were similar between groups and leptin was higher in G versus P (P<0.05). By Day 28 of gestation, insulin, glucose, NEFA and urea concentrations began to diverge and thereafter directly reflected current gestational intake (ON>C>UN for insulin, glucose, urea; UN>C>ON for NEFA). Initial BMI did not influence glucose or insulin sensitivity but ON dams had higher insulin secretion after glucose administration (P<0.008) and higher glucose concentrations after insulin (P<0.001) compared with the C and UN groups, which were equivalent. Thus, the dominant negative effect on pregnancy outcome was gestational intake with ON>UN compared with C.

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PC37

**Putative role for oestrogen as the missing link between nutrition and feto- placental growth restriction in overnourished adolescent sheep**

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Young pregnant growing adolescents are nutritionally sensitive as competition for nutrients exists between the mother and her developing fetus. This is replicated in the overnourished adolescent sheep, where high (H) dietary intakes to promote rapid maternal growth and adiposity are associated with premature delivery of low birth weight lambs relative to optimally nourished controls (C), where initial adiposity is maintained throughout. In overnourished dams, mid-gestation alterations in placental angiogenesis and uterine blood flow precede late onset placental growth restriction, which in turn limits fetal nutrient supply and growth (1). Exogenous oestrogen influences uterine vascular development and blood flow and may provide the missing link between nutrition and feto-placental growth. Here we investigated the relationship between dietary intake, maternal oestrogen concentrations and feto-placental mass. Singleton pregnancies to a single sire were established in adolescent ewes of equivalent age, weight and adiposity. In Expt.1, oestradiol-17β (E2) concentrations were determined in blood collected from C and H dams prior to necropsy at D50 or 75 of gestation (term=145days), and in ewes switched from H to C-intake at D50 and euthanized on D75 (n=11-13/group/stage). In Expt.2, blood was sampled from C and H dams at 28- day intervals from D0 to 140 and related to pregnancy outcome at term (n=12/group). In Expt.1, total placentome mass was equivalent in C and H at D50 and 75, but was increased by switching H ewes to C (average at D75; 557, 537 and 681g, respectively, P<0.05). Plasma E2 was low and independent of diet at D50. At D75, E2 was decreased in H (P<0.01) and increased in H-C (P<0.05) relative to C (mean ± sem, 1.9±0.16, 3.1±0.17, 2.6±0.15pg/ml, respectively). In Expt.2, gestation length (143±0.6 vs. 145±0.4days), fetal placental mass (279±13 vs. 473±28g) and lamb birth weight (3368±215 vs. 5631±216g) were lower in H than in C groups (P<0.05 to P<0.00001). E2 concentrations diverged by D84 and were 2-3 fold higher in C compared with H at D112 (3.2±0.27 vs. 10.3±1.15pg/ml) and D140 (5.0±0.44 vs. 12.7±1.34pg/ml, P<0.00005). Irrespective of dietary treatment, E2 concentrations at D112 (and D140) were positively correlated with gestation length (r=0.525, P<0.01), fetal placental mass (r=0.695,

P<0.001) and lamb birth weight (r=0.780, P<0.001). Late gestation E2 concentrations were also negatively associated with maternal gestational live weight gain (r=-0.756, P<0.001), and may reflect differences in metabolic clearance rate as well as placental secretion. While these data are commensurate with the hypothesis that nutritionally-mediated suppression of oestrogen may partly underlie impaired feto-placental growth restriction in growing adolescents, definitive proof via E2 supplementation is required.

1. Wallace et al. (2006). Placenta 27, S61-S68.

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PC38

## Morphological adaptations of the mouse placenta with maternal undernutrition

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The placenta is the primary source of nutrients for fetal growth in late gestation. When adverse conditions like undernutrition (UN) compromise the placenta, fetal growth is restricted but often the fetal to placental weight ratio is greater than normal (1). However, the causes of this increased placental efficiency remain unknown. This study examines the effect of gestational undernutrition on the morphological adaptations of the main fetal layers of the mouse placenta; the junctional zone (Jz) and the labyrinthine zone (Lz) responsible for hemotrophic nutrition (2). Singly housed pregnant mice were fed either 23% casein (control, CT, n=56) diet or 80% of CT intake (UN, n=57) during pregnancy. On day (d) 16 and 19 of pregnancy (term=20d) after cervical dislocation, placentas closest to the mean placental weight in each litter were fixed, embedded, sectioned and stained before stereological analysis (CT, n=4-7;UN, n=5-8) (3). Results are means ± SE. Statistical significance of diet was assessed by Student's t-test.

Placental weight and volume were significantly less in UN than CT mice at both ages while UN fetal weight was only lower relative to CT at 19d (Table 1). At 16d placental efficiency measured as f:p weight ratio was significantly greater for UN than CT groups, whereas at 19d, the opposite was found (Table 1). At 16d but not 19d, Jz volume was significantly less in UN than CT placentas. At 19d, undernutrition significantly reduced absolute Lz volume but had no effect on Jz and Lz volumes as percentages of total volume (Table 1).

The results show that, at 16d UN restricts Jz growth, which may explain partly the increased placental efficiency as more maternal resources may reach the fetus. However, by 19d, the UN placenta is less efficient than CT. This may be due to the lack of Lz growth in UN placentas and, hence, reduced fetal nutrient provision. Therefore, placental development can, adapt to compensate for poor nutritional conditions at 16d but these adaptations are insufficient to maintain normal fetal growth to 19d.

Table 1. Fetal and placental weights and placental morphology of UN and CT mice at 16d and 19d.

| | | 16d | | 19d | |
|---|---|---|---|---|---|
| | | CT | UN | CT | UN |
| Weight (mg) | Fetus | 403±8 | 410±16 | 1140±14 | 985±14* |
| | Placenta | 96±2 | 90±2* | 84±3 | 74±1* |
| | f:p ratio | 4.2±0.1 | 4.6±0.1* | 14.2±0.2 | 13.6±0.2* |
| Volume (mm3) | Placenta | 100±5 | 83±4* | 85±3 | 72±2* |
| | Lz | 41±1 | 41±4 | 52±2 | 44±1* |
| | Jz | 43±5 | 27±2* | 20±2 | 17±1 |
| Volume (%) | Lz | 40±1 | 45±2 | 52±1 | 52±1 |
| | Jz | 41±1 | 35±1* | 29±1 | 29±1 |

Values within rows are significantly different from CT *P<0.02.

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PC39

## Tetraethylammonium and 4-aminopyridine-sensitive $K^+$ channels regulate hCG secretion from human term placental villous explants

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Continual renewal of multinucleate syncytiotrophoblast is important for successful pregnancy. Renewal occurs by a process of cell turnover involving proliferation, differentiation and incorporation of cytotrophoblast cells, balanced by apoptotic shedding of syncytiotrophoblast nuclei. Trophoblast turnover is under autocrine/paracrine regulation by human chorionic gonadotropin (hCG), itself produced by differentiated syncytiotrophoblast. In many tissues, cell turnover and endocrine secretion are regulated by $K^+$ channels (O'Grady and Lee, 2005) but the involvement of $K^+$ channels in syncytiotrophoblast renewal has yet to be explored.

Here we use the placental villous fragment explant model to test the hypothesis that $K^+$ channels regulate syncytiotrophoblast renewal. In this model the syncytiotrophoblast is shed over the first 2 days of culture, then regenerates over days 3-6 to form a new syncytiotrophoblast, morphologically similar to fresh placenta, which secretes increasing amounts of hCG (Simán *et al* 2001). Placentas were collected at term, with informed patient consent. Villous fragments were dissected and maintained in culture for 6 days as previously described

(Simán *et al* 2001). Medium was collected daily for analysis of hCG (ELISA: mIU/h/mg protein) and lactate dehydrogenase (LDH), a marker of cellular integrity, (Cytotoxicity Detection Kit: absorbance units/h/mg protein), and explants treated daily with $K^+$ channel modulators, or medium alone, on days 3-6. Data are presented as mean±SE, with n=5 placentas and analysed by 2 way ANOVA with Bonferroni post tests.

hCG secretion increased from day 3 of culture, rising from 1.13±0.14 to 3.63±0.69 at day 6, while LDH release was stable at 0.071±0.02 and 0.066±0.01 respectively, coincident with syncytiotrophoblast regeneration previously reported (Simán *et al* 2001). Tetraethylammonium (TEA: blocker of voltage-gated ($K_V$) and calcium-activated ($K_{Ca}$) $K^+$ channels) prevented the increase in hCG secretion over days 4-6, significantly reducing hCG secretion at 5mM (1.61±0.24) and 10mM (1.11±0.28) on day 6 compared with control ($p<0.05$). 4-aminopyridine (4-AP: blocker of $K_V$ channels) did not alter hCG secretion at 1mM but 5mM significantly inhibited secretion (1.03±0.23 at day 6: $p<0.05$ vs control). In contrast, the $K^+$ channel opener cromakalim (10μM) was without effect (3.41±0.63 at day 6, n=4). None of the $K^+$ channel modulators altered LDH release, indicating that the inhibition of hCG secretion by TEA and 4-AP was not caused by loss of tissue integrity.

We conclude that $K_{Ca}$ and/or $K_V$ channels, sensitive to TEA and 4-AP, modulate hCG secretion by placental trophoblast. Modulation may occur either directly, by altering the secretory mechanism, or indirectly, by influencing the cellular turnover required for syncytiotrophoblast renewal.

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*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

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PC40

## Placental materno-fetal transfer of amino acids by exchangers

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Objectives: The mechanisms mediating amino acid transport across the basal membrane of placental syncytiotrophoblast and into the fetal circulation are not well understood. Our previous data indicate that amino acid exchangers mediate serine (ser) and leucine (leu) transport into the fetoplacental circulation in exchange for specific amino acids (Cleal JK *et al.,* (2007) *J Physiol* 582, 871-882). This study characterises amino acid stimulation of alanine (ala), phenylalanine (phe), tyrosine (tyr), isoleucine (iso), lysine (lys), threonine (thr), and glutamine (gln) transfer into the fetoplacental circulation.

Methods: Human placentas ($n = 5$ per amino acid) 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 of amino acid plus radio-labelled tracer amino acid and 1.8 mM creatinine to determine the rate of paracellular diffusion. Amino acid [12.5 μmol] boluses were administered to the fetal side inflow perfusate. $^{14}C$- and $^{3}H$-labelled amino acids were measured in maternal and fetal (indicating transport) venous samples by liquid scintillation counting. Data (mean ± SEM) were expressed as area under the curve and analysed by one-way ANOVA.

Results: Following fetal arterial boluses of specific amino acids transfer of ala, phe, tyr, iso, lys, thr, and gln increased, indicating that transport by exchange was taking place (Table 1).

Conclusion: This study demonstrates that in the perfused human placenta amino acids are transported into the fetal circulation by exchange mechanisms. The data indicates activity of the exchangers ASC, LAT1 and $y^+$LAT between the placenta and fetal circulation. This provides an important mechanism by which maternal amino acids can be transported to the fetus and thus be available for fetal growth and development.

Table 1: **Amino acid exchange between the placenta and feto-placental circulation**

| | Maternal amino acid (nmol) | | | | | | |
|---|---|---|---|---|---|---|---|
| Fetal bolus | ala | phe | iso | tyr | gln | lys | thr |
| glu | 0.1 ± 0.7 | -14 ± 3 | -8 ± 4 | -7 ± 6 | -7 ± 6 | 3 ± 3 | -7 ± 3 |
| ala | 174 ± 56* | 82 ± 45 | 68 ± 37 | 61 ± 21 | 88 ± 10* | | 76 ± 17* |
| thr | 83 ± 13* | 108 ± 25 | 131 ± 39* | 72 ± 14 | 86 ± 13* | 11 ± 3 | 89 ± 13* |
| ser | 86 ± 25* | 32 ± 14 | 53 ± 15 | 57 ± 27 | 79 ± 4* | 25 ± 6 | 65 ± 9* |
| gln | 69 ± 15 | 74 ± 29 | 17 ± 6 | 39 ± 18 | 81 ± 17* | 66 ± 8* | 40 ± 6* |
| lys | 7 ± 4 | 0.6 ± 10 | | | 10 ± 5 | 62± 10* | |
| leu | 20 ± 3 | 277 ± 69* | 181 ± 32* | 172 ± 36* | 78.8 ± 16* | 81 ± 15 | 31± 7* |
| trp | | 197 ± 30* | 164 ± 29* | 191 ± 24* | 12 ± 5 | -38 ± 4* | 36 ± 19* |
| bch | 5 ± 3 | 140 ± 50* | 115 ± 19* | 144 ± 25* | 14 ± 5 | 15 ± 7 | 6 ± 7 |

*$P < 0.05$, significantly different to glutamate (glu). 2-aminobicyclo- (2,2,1)-heptane-2-carboxylic acid (bch), glycine (gly), tryptophan (trp).

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*Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.*

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PC41

## Renal actions of urotensin II in anaesthetised young rats

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Urotensin II (UII) has been implicated in transepithelial sodium transport in fish [1] and in mammalian renal function [2]. We have shown that infusion of exogenous rat UII in the adult Sprague-Dawley (SD) rat, at a dose with minimal effect on the renal vasculature, decreased renal tubular sodium reabsorption [3]. As urinary concentrating ability develops over the first few post-natal weeks in the rat, the aim of this study was to