https://doi.org/10.1079/BJN20051559 Published online by Cambridge University Press

British Journal of Nutrition (2005), **94**, 938–947
DOI: 10.1079/BJN20051559
© The Authors 2005

Influence of maternal pre-pregnancy body composition and diet during early–mid pregnancy on cardiovascular function and nephron number in juvenile sheep

G. S. Gopalakrishnan[1], D. S. Gardner[1]*, J. Dandrea[1], S. C. Langley-Evans[2], S. Pearce[1], L. O. Kurlak[1], R. M. Walker[2], I. W. Seetho[1], D. H. Keisler[3], M. M. Ramsay[1], T. Stephenson[1] and M. E. Symonds[1]

[1]*Centre for Reproduction and Early Life, Institute of Clinical Research, University Hospital, Nottingham NG7 2UH, UK*
[2]*School of Biosciences, University of Nottingham, Sutton Bonington Campus, Nottingham, UK*
[3]*Department of Animal Sciences, University of Missouri, Columbia, MO 65201, USA*

(Received 15 February 2005 – Revised 16 June 2005 – Accepted 21 June 2005)

The prenatal diet can program an individual's cardiovascular system towards later higher resting blood pressure and kidney dysfunction, but the extent to which these programmed responses are directly determined by the timing of maternal nutritional manipulation is unknown. In the present study we examined whether maternal nutrient restriction targeted over the period of maximal placental growth, i.e. days 28–80 of gestation, resulted in altered blood pressure or kidney development in the juvenile offspring. This was undertaken in 6-month-old sheep born to mothers fed control (100–150 % of the recommended metabolisable energy (ME) intake for that stage of gestation) or nutrient-restricted (NR; 50 % ME; *n* 6) diets between days 28 and 80 of gestation. Controls were additionally grouped according to normal (>3, *n* 7) or low body condition score (LBCS; <2, *n* 6), thereby enabling us to examine the effect of maternal body composition on later cardiovascular function. From day 80 to term (approximately 147 d) all sheep were fed to 100 % ME. Offspring were weaned at 12 weeks and pasture-reared until 6 months of age when cardiovascular function was determined. Both LBCS and NR sheep tended to have lower resting systolic (control, 85 (SE 2); LBCS, 77 (SE 3); NR, 77 (SE 3) mmHg) and diastolic blood pressure relative to controls. Total nephron count was markedly lower in both LBCS and NR relative to controls (LBCS, 59 (SE 6); NR, 56 (SE 12) %). Our data suggest that maternal body composition around conception is as important as the level of nutrient intake during early pregnancy in programming later cardiovascular health.

Programming: Nutrition: Nephron: Blood pressure

The developmental origins of adult disease hypothesis (Barker, 2001) has stimulated a worldwide research effort, not only precipitating major advances in our knowledge of fetal and neonatal biology *per se*, but also potentially accounting for a proportion of the variation in disease status of adult individuals. A role for the nutritional status of the mother as she enters pregnancy and throughout the periods of gestation and lactation is clearly implicated in the programming of later disease risk (Barker *et al.* 1993). However, the precise interactions between maternal body composition and macronutrient intake during pregnancy, birth weight, postnatal growth rate and postnatal dietary exposure are less well defined in terms of programmable endpoints such as blood pressure. Clearly each has a role, but their relative importance has yet to be described.

Through the use of animal models, cardiovascular programming in the offspring has been shown to be associated with both gross maternal nutritional imbalance in terms of macronutrients (Langley & Jackson, 1994) and energy (Ozaki *et al.* 2001; Gardner *et al.* 2004*b*; Gopalakrishnan *et al.* 2004*b*), as well as with the deficiency of single amino acids such as glycine (Jackson *et al.* 2002). In large animals such as sheep global energy restriction targeted over the first 30 or 95 d gestation has limited effect on resting blood pressure *per se*, but does result in a leftward shift of their baroreceptor curve coupled with a reduced bradycardia during hypertensive challenges (Gardner *et al.* 2004*b*; Gopalakrishnan *et al.* 2004*b*). Taken together these findings suggest that these individuals may be at an increased risk of developing later hypertension and CHD as is recognised in man (Eckberg, 1979; Ookuwa *et al.* 1987). The extent to which cardiovascular function may be reset in earlier life remains to be established but may potentially be significant given the strong influence of placental:fetal weight ratio on later hypertension (Barker *et al.* 1990). In this regard it has been established that maternal nutrient restriction targeted over the period of maximal placental growth initially restricts placental mass (Clarke *et al.* 1998) but subsequently results in a disproportionately larger placenta at term (Heasman *et al.* 1998), adaptations that may be predicted to contribute to later cardiovascular dysfunction.

Maternal anthropometrics account for approximately 50 % of the variation in intra-uterine growth, which is mediated in part

Abbreviations: BCS, body condition score; 11β-HSD2, 11β-hydroxysteroid dehydrogenase type 2; LBCS, low body condition score; ME, metabolisable energy; NR, nutrient-restricted.

* **Corresponding author:** Dr David S. Gardner, fax +44 115 970 9382, email david.gardner@nottingham.ac.uk

https://doi.org/10.1079/BJN20051559 Published online by Cambridge University Press

through the mother's ability to maintain placental growth, which is in turn related to maternal body mass and fat stores prior to conception (Robinson *et al.* 1994). For example, sheep that are light at conception and then go on to lose body condition through gestation produce growth-retarded offspring with reduced fat stores (Clarke *et al.* 1997). These adverse fetal adaptations occur even when maternal food intake is maintained through late gestation. In addition, while a low plane of nutrition throughout gestation results in reduced birth weight, so too does a high plane given to young growing sheep (Wallace *et al.* 1996). Therefore, it would appear that regardless of maternal intake, it is the actual fetal nutritional exposure that is of utmost importance. This may be modulated by the condition of the mother prior to, and food intake during, pregnancy. For this reason the present study has established two control groups, well-fed ewes in normal or low body condition as assessed by palpation of their lumbar region (Russel *et al.* 1969), in order to establish whether the long-term cardiovascular outcomes were comparable to those observed following a targeted period of maternal nutrient restriction between early and mid gestation.

In search of a mechanistic basis for the programming of later cardiovascular dysfunction emphasis has been placed on the kidney as it may underpin a majority of the programmable adaptations within the cardiovascular system. To this extent total nephron number, which is determined prior to term in most species except the litter-bearing rat and pig, is one factor mediating early-life programming of cardiovascular dysfunction (Mackenzie & Brenner, 1995). Indeed, 48 h exposure to dexamethasone in sheep only programs adult hypertension when given during early differentiation of the metanephros (days 28–30; Dodic *et al.* 2002). A maternal low-protein diet programs increased apoptosis of the metanephros in rats (Welham *et al.* 2002) and hypertension after this dietary regimen tends to be associated with the nephron complement in the offspring rather than the glucocorticoid status of the mother (McMullen & Langley-Evans, 2004). Thus in the present study we have focused our attention upon cardiovascular and renal function and the extent to which these control mechanisms may be programmed by either a defined period of maternal nutrient restriction between days 28 and 80 of gestation or a low maternal body weight and condition score at conception. As an index of cardiovascular function we have measured resting blood pressure, heart rate and pressor responses to exogenous infusion of the potent vasopressor hormones angiotensin II and noradrenaline (Gardner *et al.* 2004*b*). In addition, during the short-term pressor challenges, we have also assessed baroreflex function through the parallel changes in blood pressure and heart rate. For an index of renal function we have measured nephron number and as measures of overall renal metabolic activity, the relative abundance of cytochrome C and voltage-dependent anion channels. The current study also assesses the resting plasma concentration of leptin as an indicator of body fat mass, together with glucose and cortisol in order to provide indices of resting metabolic and stress status, respectively.

Materials and methods

Animals

All procedures were performed under the UK Animals (Scientific Procedures) Act, 1986. Nineteen mature Welsh Mountain ewes of similar age (2–3 years), parity (second or third pregnancy), live weight (42·8 (SE 0·9) kg) and body condition score (BCS; 2·3 (SE 0·1) arbitrary units) were kept on grass at the University of Nottingham's animal facility at Sutton Bonington for 1 year prior to mating. At mating a single ram was used for all ewes, which were then allocated to receive either a control (*n* 13; seven bearing singletons, six bearing twins) or nutrient-restricted (NR: *n* 6; all singleton-bearing) diet from day 28 to 80 of gestation (term approximately 147 d gestation). Allocation of ewes into control or NR groups was influenced by their BCS – a manual assessment of fat depth in the lumbar region (Russel *et al.* 1969). Ewes with a BCS of ≤ 2 (scale of 0, very thin to 5, obese) were allocated to the control diet to comply with Home Office legislation. Thus the ewes fed control diet formed two groups: those with: (i) BCS ≥ 3 (*n* 7; three singleton-bearing and four twin-bearing ewes); (ii) BCS ≤ 2 (low BCS (LBCS): *n* 6; four singleton-bearing and two twin-bearing ewes). NR ewes had a BCS of ≥ 3 units. The period of nutrient restriction was specifically chosen to target the duration of maximal placental growth and has previously been shown to program many aspects of feto-placental, neonatal and adult development/function (Heasman *et al.* 1998; Whorwood *et al.* 2001; Bispham *et al.* 2003; Gopalakrishnan *et al.* 2004*b*). From day 28 to day 80 of gestation ewes were singly housed under the prevailing day length conditions with unlimited access to water; controls were fed to appetite, equating to 150 % of the metabolisable energy (ME) requirements for live weight maintenance as well as meeting the additional requirements for growth of the conceptus (10–12 MJ/d) as defined by the Agricultural and Food Research Council (1993), while NR sheep were fed to 50 % estimated requirement (4–5 MJ/d). After day 80, all ewes received a diet calculated to meet 100 % of the Agricultural and Food Research Council requirements (8–10 MJ/d). The diet comprised chopped hay with a ME content of 7·91 MJ/kg DM and crude protein content (N × 6·25) of 69 g/kg DM and a barley-based concentrate that had a ME content of 11·6 MJ/kg DM and a crude protein content of 162 g/kg DM (Mostyn *et al.* 2003). The proportion of hay to concentrate fed was approximately 3:1 with regard to dry weight. All diets contained adequate minerals and vitamins.

For all ewes the level of feed offered during gestation was based upon fetal number, i.e. those bearing twins received a higher allowance, and the changing demands associated with increasing conceptus weight as gestation progresses (Agricultural Research Council, 1980). All ewes were weighed and scored for body condition at 14 d intervals. At term, lambs (Males: control, 2; LBCS, 3; NR 2; females: control, 5; LBCS 3; NR 4) were delivered naturally with no intervention and birth weights recorded. All offspring were ewe-reared as singletons (one twin lamb from any twin litter was randomly selected for humane killing at term) until weaning at 12 weeks of age and thereafter grass-fed at Sutton Bonington until 6 months of age. Not all data were available for all animals in each group, and therefore a corresponding *n* has been ascribed to each data set.

Experimental protocols

Surgery. At 6 months of age and at approximately 1 week prior to surgery all sheep were group-housed indoors. For 24 h prior to surgery all food, but not water, was withdrawn from the animals. Anaesthesia was induced with sodium thiopentone (20 mg/kg intravenously; Intraval Sodium®, Rhone Mérieux, Dublin, Republic of Ireland) and maintained with 1–2 % halothane in

https://doi.org/10.1079/BJN20051559 Published online by Cambridge University Press

50:50 O_2/N_2O. Left carotid and jugular catheters (Fecalon universal polyvinyl tubing; 1·2 mm inner diameter, 1·8 mm outer diameter) were inserted into each sheep, secured and the neck incision closed. Catheters emerging from the neck were coiled and protected within a 10 inch bandage. All sheep received a dose of long-acting antibiotic (amoxicillin, 15 mg/kg intramuscularly; Duphamox®, Fort Dodge Animal Health Ltd, Southampton, UK) and analgesia (flunixin meglumine, 1 mg/kg; Finadyne®, Shering-Plough, Kenilworth, UK) post-operatively. Catheter patency was maintained by daily flushing with heparinised saline (50 IU heparin/ml). Catheterised sheep were housed individually, but within sight and touch of other sheep in a highly ventilated air-conditioned building with controlled lighting (12 h on/12 h off; 08.00–20.00 hours). Sheep were feeding 1 h after surgery and showed no visible signs of discomfort for the duration of the experimental period. A period of 2–3 d post-operative recovery was allowed prior to any experiment being performed and the investigator was blinded to the dietary origin of the sheep.

In vivo *experiments*. In total four experiments were performed over a 5–7 d period, the order of which was randomised. All experiments were begun between 09.00 and 10.00 hours and, at the end of all experiments, the animals were humanely killed with a lethal overdose of. sodium pentobarbitone (170 mg/kg intravenously; Dolethal®, Vétoquinol, Bicester, UK). For cardiovascular recording the carotid catheter was connected to a precalibrated pressure transducer (SensorNor 840; S 4925), attached at heart level, and linked to a data-acquisition system (Po-Ne-Mah Version 3; Gould Instrument Systems Inc., Valley View, OH, USA). Hay and water were available at all times. Analogue signals for real-time systolic, diastolic, mean arterial pressure and heart rate were recorded second-by-second for a 1 h baseline period and subsequently during the experimental challenge. All data were immediately digitised and downloaded to an Excel spreadsheet for further analysis. From these data, pulse pressure (systolic minus diastolic) was derived. For experiments 1–3, a 2 ml blood sample was taken before (30 min) and then immediately after (5 min) infusion of the highest dose of pressor agent.

(1) Expt 1: cardiovascular response to angiotensin II infusion. After a baseline period of 10 min, stepwise increases in angiotensin II (0, 1, 2, 4, 8, 16 and 32 ng/kg per min) were administered intravenously every 10 min, followed by a 30 min recovery period in which cardiovascular variables returned to baseline.
(2) Expt 2: cardiovascular response to noradrenaline infusion. After a baseline period of 10 min, stepwise increases in noradrenaline (0, 2, 4, 8, 16, 32 and 48 ng/kg per min) were administered intravenously every 10 min, followed by a 10 min recovery period in which cardiovascular variables returned to baseline.
(3) Expt 3: cardiovascular responses to captopril infusion. After a baseline period of 30 min captopril was infused for 30 min at a dose of 0·12 mg/kg per h. This dose has been previously validated to be the lowest effective dose to completely block the pressor effect of 0·5 μg angiotensin I (Smith *et al.* 1997). After infusion, cardiovascular variables were recorded for a further 20 min recovery period or until blood pressure had returned to baseline.
(4) Expt 4: basal endocrine status. Blood samples (2 ml) were taken every 30 min for a total period of 6 h. The blood was drawn into heparinised (lithium heparin) syringes, placed in chilled blood tubes and centrifuged at 3500 rpm (800 ***g***), 4°C for 5 min, and the resultant plasma stored at −20°C for later analysis of glucose, cortisol and leptin concentration.

Hormone analysis

Plasma concentrations of glucose were measured enzymatically (Trinder; glucose oxidase) as described by Symonds *et al.* (1986). Plasma concentrations of leptin were assayed using a double antibody RIA, validated for use with ovine plasma as previously described in detail (Delavaud *et al.* 2000). Samples were assayed in duplicate (200 μl) using a rabbit anti-ovine leptin primary antibody, iodinated ovine leptin and sheep anti-rabbit secondary antibody. The leptin assay has a sensitivity of 0·10 ng/m1 with intra- and inter-assay CV of 4 and 11 % (n 5), respectively. Total cortisol was measured using a commercially available coated-tube RIA kit (Coat-a-Count cortisol; Diagnostic Products Corp., Ltd, Caernarfon, UK) validated for use with ovine plasma (Bispham *et al.* 2003). The minimum detection limit for the assay was 0·5 ng/m and the intra- and inter-assay (n 5) CV were 6 and 9 %, respectively.

Nephron counts

Determination of the total renal nephron complement was conducted in all control (n 7), LBCS (n 6) and NR (n 6) sheep using an adaptation of a mild acid hydrolysis method (Welham *et al.* 2002) as recently described for use in sheep (Brennan *et al.* 2005). In brief, whole frozen kidneys were cut horizontally through the hilum and from one section two 1 g sliced sections of renal tissue were derived. These sections were covered in 1 M-HCl and incubated for 30 min at 37°C. Acid was then removed and replaced with a known volume (20 ml) of 50 mM-PBS (pH 7·4). The tissue was homogenised using a bench-top homogeniser (Yellowline disperser; IKA Works Inc., Wilmington, NC, USA) and a 20 μl sample subsequently taken and placed on a slide and overlaid with a coverslip. Using a 10 × objective lens, the number of glomeruli in the aliquot was counted in triplicate for each of the two kidney sections. The six results were averaged and used to determine the total number of glomeruli in the sample and therefore the whole kidney. The intra- and inter-assay CV were 11 and 16 %, respectively.

This method for analysis of nephron number in large adult kidneys was validated to give a representative value for whole kidney nephron number as follows: in a whole kidney from ten sheep, two 1 g hilar sections were removed as earlier. The remaining kidney tissue (18–31 g, comprising 94–97 % of the total organ weight) was acid-digested as described earlier for 30 min and then homogenised in 200 ml PBS, using a Waring blender. Glomeruli and thus nephrons were then counted in quadruplicate 20 μl aliquots from each preparation.

There was very close agreement in the estimated total nephron number between these two methods that were strongly correlated, i.e. r 0·967 ($P<0·001$), indicating there is 93·5 % agreement between the two methods. Values are appropriate for the species (Wintour *et al.* 2003) and indicate that, in large adult sheep kidneys, a representative 1 g portion from the hilar region appears a valid approach to determining nephron number. This procedure has the further benefit of preserving the rest of the organ for additional histological and/or molecular analyses.

https://doi.org/10.1079/BJN20051559 Published online by Cambridge University Press

Tissue cytochrome C and voltage-dependent anion channel abundance

Mitochondria were prepared from frozen renal tissue from control (*n* 6) and NR (*n* 6) animals as described by Symonds *et al.* (1992). No data were available from LBCS animals for tissue cytochrome C and voltage-dependent anion channels. Abundance of cytochrome C was determined on 10 μg mitochondrial protein using an antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) at a dilution of 1:1000. Voltage-dependent anion channel abundance was determined using an ovine-specific antibody prepared in-house as previously described by Mostyn *et al.* (2003) and used at a dilution of 1:2000.

11β-Hydroxysteroid dehydrogenase type 2 activity

Renal 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) enzyme activity was determined by measuring the rate of conversion of cortisol to cortisone according to Yang *et al.* (1994). In brief, the homogenate was diluted and protein estimated by the Lowry method before measurement of 11β-HSD2 activity, using NAD as the cofactor. Each assay contained [^{3}H]cortisol (45 000 dpm, specific activity 2·14 MBq/mmol; Amersham Pharmacia Biotech, Amersham, Bucks, UK), unlabelled cortisol (0·1 μM; Sigma, Poole, Dorset, UK) and NAD (400 μM; Sigma) in 0·4 ml Krebs–Henseleit buffer (pH 7·4). The mixture of substrate and cofactor was warmed at 37°C for 10 min before 100 μl tissue homogenate containing 200 μg protein was added. After incubation for 10 min (reaction rate was linear from 5 to 40 min), the reaction was stopped by addition of ice-cold ethyl acetate (5 ml) containing 20 μg cold cortisol and 20 μg cold cortisone (Sigma) as internal carriers for the chromatography. After extraction of the steroids, the extracts were dried under an air stream at 37°C, re-dissolved in 120 μl ethanol and spotted onto TLC plates (Silica gel 150 Å; Whatman, Clifton, NJ, USA). The plates were developed using a mixture of chloroform and methanol (9:1, v/v). The bands containing cortisol and cortisone were visualised under UV light and excised into scintillation vials. Liquid scintillation cocktail (Optiphase II, Hisafe; Wallac Oy, Turku, Finland) was added and the resulting counts corrected for quenching. The conversion of cortisol to cortisone was expressed as the percentage conversion of recovered ^{3}H counts in the cortisone band. Using the specific activity of the cortisol and the percentage conversion to cortisone, the conversion rate of cortisol to cortisone was calculated and expressed as the amount of cortisone (pmol) synthesised per min. The assay was performed in duplicate and included blanks that contained no tissue homogenate to allow correction for non-enzymatic oxidation. The results are expressed as pmol/min per mg protein.

Statistical analyses

All data are expressed as means with their standard errors unless otherwise stated. Cardiovascular variables (blood pressures, heart rate, rate × pressure product) were first grouped into summary measures, i.e. before, during and after a pressor challenge (Matthews *et al.* 1990), and mean values analysed by two-way ANOVA with repeated measures for effects of group (e.g. control *v.* LBCS and NR), time (e.g. prior to, during and after pressor challenge) and any interaction between group × time using SPSS 11.5.2 (SPSS Inc., Chicago, IL, USA). Sex of the offspring and fetal number were included as covariates in the analysis. Where indicated, *post hoc* statistics were run with Bonferroni correction. For glucose and hormone data, values were analysed by either two-way repeated-measures ANOVA (Expt 4) or the paired *t* test (before and after noradrenaline infusion). Total nephron counts and 11β-HSD2 activity were not normally distributed and were analysed by the Mann–Whitney *U* test. For all statistical comparisons significance was accepted when $P<0·05$.

Results

Maternal data and lamb characteristics

At the start of the study, despite all ewes sharing similar grazing and nutritional regimens during a 1-year acclimatisation after arriving at the University of Nottingham's farm at Sutton Bonington, a subgroup of ewes did not gain weight or BCS (group LBCS). Consequently, these ewes were significantly ($P<0·05$) lighter and in poorer condition at conception compared with their contemporaneous controls and NR group (Table 1) despite being indistinguishable in all other respects, e.g. daily behaviour, appetite and food intake throughout pregnancy and conception.

The energy intake of ewes in all groups was similar, except that during the period of feed restriction group NR ate significantly less ME (Table 1). During early–mid gestation all ewes gained weight when measured at birth (minus the products of conception), although the magnitude of gain was greater in adequately fed relative to nutrient-restricted sheep (LCBS, increase of 8·83 (SE 2·22); control, 4·85 (SE 0·80); NR, 1·83 (SE 1·13)

Table 1. Maternal body weight and body condition score (BCS) at the start of the study and at conception and energy intake during gestation

(Values are means with their standard errors)

	At start of study				At conception (1 year later)				Energy intake (MJ/d)			
	Weight (kg)		BCS (units)		Weight (kg)		BCS (units)		Early–mid (day 28–80)		Late (day 81–term)	
Group	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control (*n* 7)	42·0	0·4	2·3	2·3	48·5	1·5	3·0	0·2	12·2	0·6	10·2	0·6
LBCS (*n* 6)	42·7	2·0	2·3	2·3	42·5*	1·2	1·5	0·2*	10·4	0·9	9·7	1·0
NR (*n* 6)	43·8	2·3	2·2	2·2	49·3	1·3	3·5	0·2	4·6***	0·2	8·4	0·4

LBCS, low body condition score; NR, nutrient-restricted.

Mean values were significantly different between groups: *$P<0·05$ (control *v.* LBCS and NR), ***$P<0·001$ (NR *v.* control and LBCS). For details of animals and procedures, see p. 939.

https://doi.org/10.1079/BJN20051559 Published online by Cambridge University Press

kg; $P=0{\cdot}04$, NR *v.* control; $P=0{\cdot}01$, NR *v.* LBCS). The reduction in BCS usually observed over pregnancy (reflecting fat mobilisation to sustain fetal growth) was apparent in control and NR ewes (control, from 3·0 (SE 0·2) to 1·5 (SE 0·2); NR, 3·5 (SE 0·2) to 1·5 (SE 0·1) units; $P=0{\cdot}005$ both cases, paired *t* test) but remained unchanged from 1·5 (SE 0·2) units in LCBS, despite being fed well above (days 28–80) and to (day 80–term) requirements during their pregnancy. Lamb birth weight was similar in adequately fed control ewes (3·48 (SE 0·32); LBCS, 3·76 (SE 0·78) kg) and was similar between singletons and twins within these groups, but was increased in NR relative to controls (4·68 (SE 0·14) kg). Over the first two 3-month periods after birth, the rates of growth were similar in all groups of sheep (0–3 months: control, 265 (SE 13); LBCS, 240 (SE 20); NR, 257 (SE 21) g/d; 3–6 months: control, 65 (SE 13); LBCS, 41 (SE 8); NR, 36 (SE 5) g/d). Growth in NR tended ($P=0{\cdot}06$) to be slower from 3 to 6 months as compared with controls. However, at 6 months of age, there was no difference in body weight between the three groups of sheep (control 33·5 (SE 1·2); LBCS, 29·4 (SE 0·1); NR, 31·4 (SE 1·8) kg).

Cardiophysiology of offspring

Basal status. Resting blood pressure was similar in LBCS and NR, but both groups exhibited a trend toward lower ($P=0{\cdot}057$) pressures relative to controls (Table 2). Values for heart rate were similar between all groups.

Pressor responses to angiotensin II infusion. In all groups of sheep, angiotensin II infusion resulted in a dose-dependent increase in systolic blood pressure, the magnitude of which was similar between groups (Fig. 1). At the peak increase in arterial blood pressure (approximately 50 min), heart rate had decreased by −15 (SE 5) and −17 (SE 3) beats/min in controls and LBCS but remained unaltered in NR (+3 (SE 8) beats/min).

Pressor responses to noradrenaline infusion. In all groups of sheep, noradrenaline infusion resulted in a dose-dependent increase in systolic blood pressure, the magnitude of which was similar between groups (Fig. 2). At the peak increase in arterial blood pressure (approximately 65 min), heart rate had decreased by −16 (SE 8), −15 (SE 7) and −6 (SE 11) beats/min in control, LBCS and NR groups, respectively.

Pressor responses to captopril infusion. In all offspring 30 min of captopril infusion resulted in a decrease in systolic and diastolic arterial blood pressure that was similar in all groups (e.g. decrease in systolic blood pressure: control, −8·0 (SE 4·0); NR, −5·0 (SE 5·5) mmHg) and was not accompanied by any change in heart rate, which remained at baseline values (Table 2).

Basal glucose, cortisol and leptin status. Resting plasma concentrations of glucose did not differ between study days or over the 6 h study period, and therefore an average value was calculated for each individual and group. For all resting humoral data, values for controls and LBCS were not different and were therefore combined and compared *v.* NR. Resting plasma glucose concentration was lower in NR offspring relative to controls (3·46 (SE 0·27) *v.* 4·20 (SE 0·21) mmol/l; $P=0{\cdot}04$, NR *v.* controls and LBCS combined). In addition, plasma glucose concentration was measured after infusion of the highest dose of noradrenaline in order to assess the sympathetically mediated glycaemic response. Plasma glucose increased in response to noradrenaline infusion in all groups (control and LBCS combined: from 3·78 (SE 0·26) to 5·31 (SE 0·34) mmol/l; NR, from 3·82 (SE 0·40) to 6·55 (SE 1·00) mmol/l). Values for cortisol (nmol/l) and leptin (ng/ml) were similar in all study groups (control, 15·4 (SE 2·3) and 3·72 (SE 0·85); LBCS, 16·0 (SE 1·8) and 4·25 (SE 1·25); NR, 18·2 (SE 1·2) and 3·20 (SE 0·98) for cortisol and leptin, respectively).

Renal histology and molecular biology in offspring at 6 months of age

The total nephron count was similar in kidneys from LBCS and NR, but each group had a lower complement of nephrons relative to control sheep (LBCS, 59 (SE 6); NR, 56 (SE 12) % relative to control; $P=0{\cdot}01$ both cases; Fig. 3). The relative abundance of renal cytochrome C was significantly higher in NR relative to controls (118 (SE 14) *v.* 86 (SE 5) arbitrary units; $P<0{\cdot}01$). This effect was specific to cytochrome C as there was no difference in relative voltage-dependent anion channel abundance between groups (83 (SE 6) *v.* 81 (SE 6) arbitrary units). In addition, the activity of renal 11β-HSD2 was similar between groups (median (interquartile range): control, 1·20 (0·26–1·29); LBCS, 1·11 (0·31–1·28); NR, 1·11 (0·86–1·16) pmol/min per mg protein).

Sheep biometry 6 months of age

When expressed relative to body weight, the spleen was significantly ($P<0{\cdot}01$) lighter in NR and perirenal fat significantly

Table 2. Resting cardiovascular status of 6-month-old offspring

(Values are means with their standard errors for the average value obtained from second-by-second recorded data over a 1 h period measured on three separate days)

	Group					
	Control (*n* 7)		LBCS (*n* 6)		NR (*n* 6)	
	Mean	SE	Mean	SE	Mean	SE
Systolic blood pressure (mmHg)	85	2	77	3	77	3
Diastolic blood pressure (mmHg)	63	2	59	2	56	3
Pulse pressure (mmHg)*	23	3	18	2	21	3
Mean blood pressure (mmHg)	74	4	67	2	66	3
Heart rate (beats/min)	96	3	101	7	93	9

LBCS, low body condition score; NR, nutrient-restricted.
*Pulse pressure was calculated as systolic minus diastolic pressure (mmHg).
For details of animals and procedures, see p. 939.

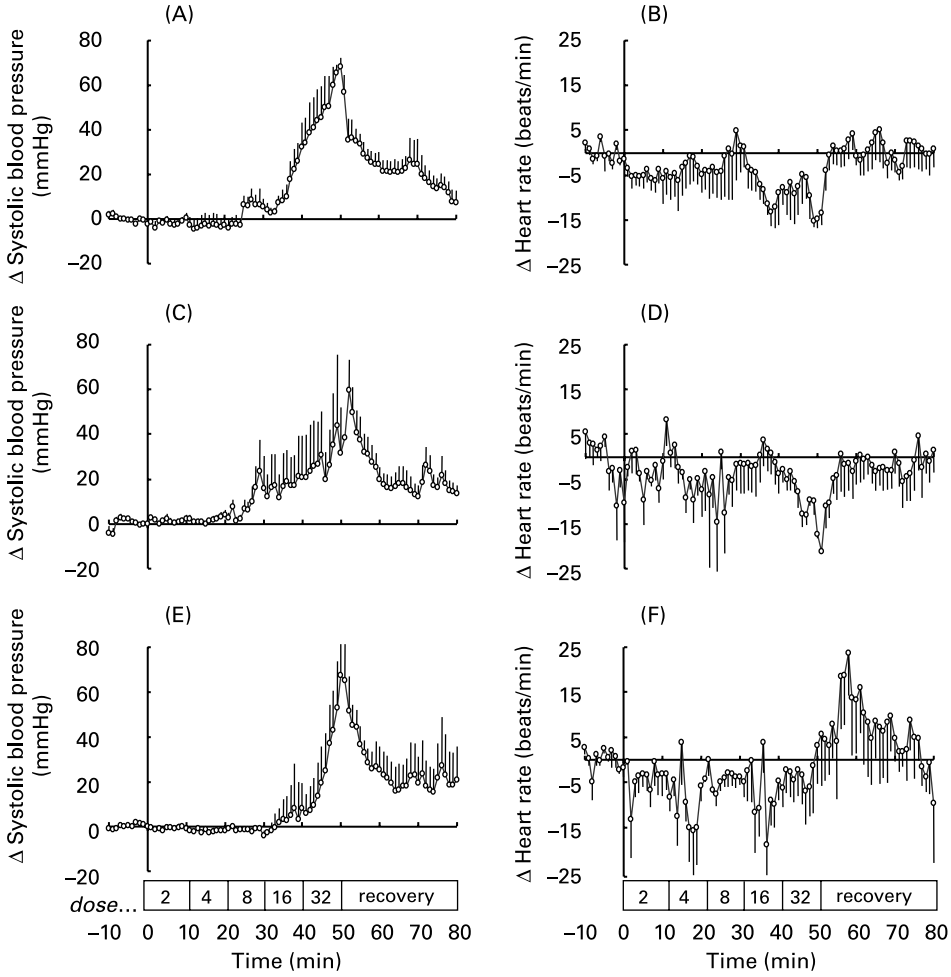

Fig. 1. Mean arterial blood pressure and heart rate responses to incremental stepwise infusion of angiotensin II in control (A and B), low body condition score (LBCS; C and D) and nutrient-restricted (NR; E and F) sheep. Values are 1 min means with their standard errors shown by vertical bars for control (n 5), LBCS controls (n 6) and NR (n 4) sheep for a baseline period (10 min) and 50 min of angiotensin II infusion (stepwise dose increments of 2–32 ng/kg per min every 10 min) followed by 30 min of recovery. Box indicates the period of infusion. For details of animals and procedures, see p. 939.

($P=0{\cdot}04$) reduced in LBCS (Table 3), relative to all other groups. Weights for all other organs were similar between all groups.

Discussion

We have shown that resting blood pressure in offspring born to mothers nutrient-restricted between days 28 and 80 of gestation is not raised but tends to be lower compared with offspring born to contemporaneous controls. These findings are therefore in contrast to previous studies that have generally used the rat as an animal model of the developmental programming of adult hypertension, together with tail-cuff plethysmography, in which the expression of the hypertensive state in previously NR offspring often, but not always (Crowe *et al.* 1995; Gambling *et al.* 2003), appears demonstrable from a very early age (Langley-Evans, 2001). Indeed, initially lower then later higher blood pressure has been noted in rodent models of Fe restriction in pregnancy (Crowe *et al.* 1995; Gambling *et al.* 2003) while one study in a large animal species (sheep) also demonstrated lower fetal blood pressures after early nutrient restriction, but later higher blood pressure as young lambs (Hawkins *et al.* 2000). Our data are in accord with these results and extend findings from our previous studies conducted in offspring at later ages, which have now shown that the offspring of NR sheep tend to have lower resting arterial pressure as juveniles (current study), the same blood pressure as controls by 1 year of age (Gardner *et al.* 2004*b*) and a higher pressure at 3 years of age (Gopalakrishnan *et al.* 2004*b*). It is acknowledged that the breed of sheep is different between these studies but we have found no evidence that this has a significant effect on cardiovascular function (DS Gardner and ME Symonds, unpublished results).

The results from the present study potentially contrast with those recently published in juvenile offspring that were exposed to an identical period of maternal nutrient restriction, in which raised mean arterial blood pressure was reported (Gilbert *et al.* 2005). In this other report, however, the interpretation of the effects of nutrient restriction is potentially confounded by the large number of twins in the control compared with the NR group. Indeed, comparison of mean arterial blood pressure between twin and singleton offspring in that study indicates as large a difference of fetal number (twins 76 (SE 3); singletons 83 (SE 5) mmHg; $P<0{\cdot}05$) as that assigned to maternal diet (control 73 (SE 2); NR 89 (SE 7) mmHg). A potential confounding influence of fetal number is present in the current study in

https://doi.org/10.1079/BJN20051559 Published online by Cambridge University Press

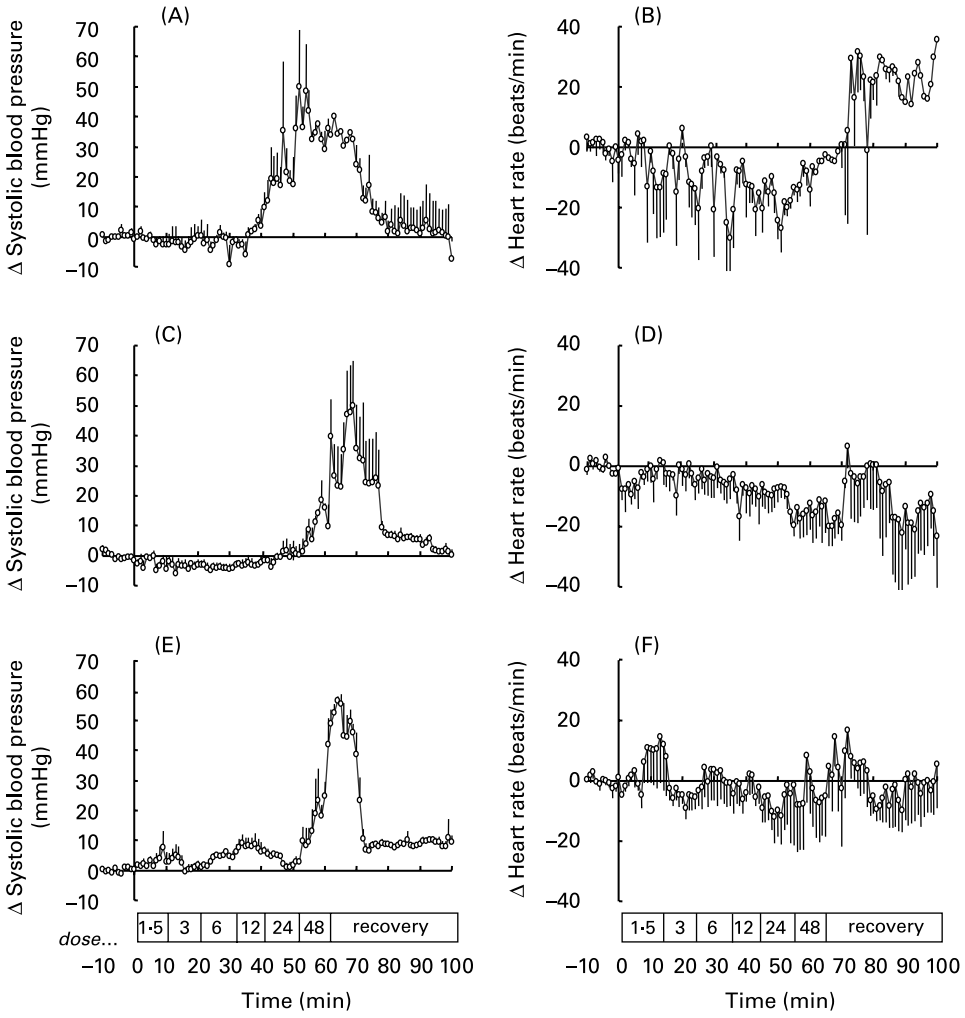

Fig. 2. Mean arterial blood pressure and heart rate responses to incremental stepwise infusion of noradrenaline in control (A and B), low body condition score (LBCS; C and D) and nutrient-restricted (NR; E and F) sheep. Values are 1 min means with their standard errors shown by vertical bars for controls (*n* 5), LBCS controls (*n* 6) and NR (*n* 4) sheep for a baseline period (10 min) and 1 h of noradrenaline infusion (stepwise dose increments of 1·5–48 ng/kg per min every 10 min) followed by 40 min of recovery. Box indicates the period of infusion. For details of animals and procedures, see p. 939.

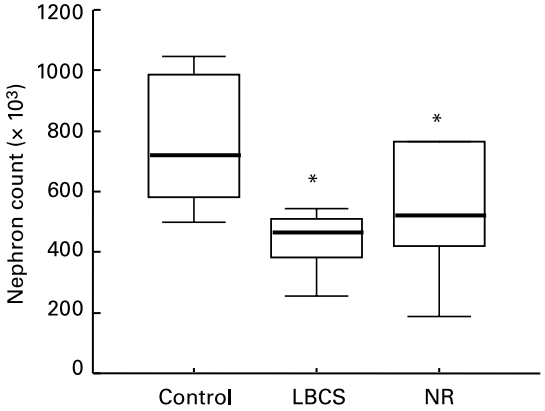

Fig. 3. Total kidney nephron number in control and nutrient-restricted (*NR*) sheep. Data are represented as a box and whisker plot showing median (– within box), 25th and 75th interquartile range (bottom and top of box, respectively) and minimum and maximum values (lower and upper whiskers, respectively) for control (*n* 7), low body condition score controls (LBCS; *n* 6) and NR (*n* 6) sheep. Values were significantly different between groups (control *v.* LBCS and NR): **P*<0·01. For details of animals and procedures, see p. 939.

which there is a proportion of twins in the nutritional control groups but not in the NR group. Comparison of mean systolic (and diastolic) blood pressure between twin and singleton offspring in our study reveals very similar blood pressure with regard to fetal number (e.g. control twins 84 (SE 3); control singletons 87 (SE 3) mmHg). One explanation for the substantial difference in the effect of fetal number between these two studies may relate to the experimental protocols, in that the study of Gilbert *et al.* (2005) did not take into account the additional ME requirements of twin compared with singleton pregnancies. In addition, in the present study each mother was allowed to raise only a single offspring whereas in the study of Gilbert *et al.* (2005) each mother reared both twins.

A number of publications have shown that the prenatal endocrinological development of twins is different to that of singletons, primarily reflecting a specific adaptation to their intra-uterine environment (Schwartz & Rose, 1998; Edwards & McMillen, 2002; Gardner *et al.* 2004*a*). However, both in prenatal and adult life the available evidence would suggest no overt differences in cardiophysiology between twins and singletons

https://doi.org/10.1079/BJN20051559 Published online by Cambridge University Press

Table 3. Body composition of 6-month-old offspring
(Values are means with their standard errors)

	Group					
	Control (*n* 7)		LBCS (*n* 6)		NR (*n* 6)	
	Mean	SE	Mean	SE	Mean	SE
Body weight (kg)	33·5	1·2	29·4	2·6	31·4	1·8
Organ weights (g/kg)						
Kidney	2·99	0·16	2·86	0·21	2·91	0·12
Liver	15·3	0·5	16·3	0·9	15·1	0·45
Spleen	4·10	0·40	3·00	0·30	2·40*	0·30
Heart	4·54	0·26	4·46	0·29	4·57	0·17
Brain	2·35	0·12	2·71	0·28	2·58	0·22
Lungs	9·57	1·10	10·01	0·74	9·32	0·99
Fat mass (g/kg)						
Perirenal	7·84	1·01	5·02*	0·68	8·34	1·83
Omental	11·83	2·08	11·99	3·10	11·81	4·15
g total fat/kg body weight	21·1	2·6	19·0	3·2	21·6	6·1

LBCS, low body condition score; NR, nutrient-restricted.
Mean values were significantly different between groups: *$P<0·05$ (LBCS and/or NR *v.* control). For details of animals and procedures, see p. 939.

when the mother is fed according to fetal number and twins then reared as singletons (Gardner *et al.* 2004*b*). Indeed in this regard our findings are in accord with those of Gilbert *et al.* (2005), in which there was no effect of litter size on total nephron count but this was specifically reduced in NR offspring by a similar magnitude as we report here. Taken together these findings raise the question as to what age an impairment in nephron number may act to contribute to CHD. Interestingly, we found no effect of the maternal environment on 11β-HSD2 activity in the kidney, indicating the reduction that is seen at birth following maternal nutrient restriction between days 28 and 80 of gestation is only transient (Whorwood *et al.* 2001).

The potential divergence between programmed cardiovascular and kidney outcomes that are dependent on the *in utero* environment is further highlighted by our findings in offspring born to LCBS mothers. These LBCS mothers and their metabolic response to overfeeding may reflect the overnourished adolescent ewe model of Wallace (2000), in which the ewe experiences competing metabolic demands for the high nutrient intake to support her own growth and that of her developing conceptus. Ultimately this acts to the detriment of the placenta and thus the fetus, which is markedly growth-retarded as a result at term. In the present study LBCS sheep put on an extra 4 kg body weight compared with controls of good body condition at the same time as maintaining LBCS and producing normal-sized offspring. These adaptations suggest that LBCS mothers are protecting/establishing their body reserves during gestation in preparation for the greater demands of lactation. In this respect they have succeeded, as postnatal growth was similar between all nutritional groups. However, the juvenile offspring have a trend for lower blood pressure and reduced nephron number, in similarity to NR offspring. In addition their fat deposition was specifically reduced in the perirenal but not omental depots, suggesting that nutrient partitioning within these offspring was different. However, as total fat mass was unaffected it was not unexpected that plasma leptin was similar between groups. Overall this raises an important question: are the fetuses from both groups effectively undernourished during early gestation? This seems difficult to reconcile in LBCS ewes that were eating 150 % ME requirements up to 80 d gestation. In overnourished adolescent ewes placental glucose transfer is proportionate between experimental and control groups (Wallace *et al.* 2003). However, perhaps the disturbance to endogenous metabolic cycles and endocrine milieu concerned with assimilating gross energy intake is similar. Preliminary data from our laboratory indicate that a high energy intake during early gestation is counterproductive to fetal growth in the sheep (DS Gardner and ME Symonds, unpublished results), and would itself place specific demands on the mother in order to handle the excess energy.

To date, when considering all of our own studies on young/adult NR offspring, we find two defining characteristics in all NR sheep: (i) an altered pressure–heart rate relationship, i.e. reduction in the bradycardia observed during elevations in pressure; (ii) a reduced nephron complement (median (interquartile range), current study and at 3 years of age: controls, 998 (807–1088) *v.* NR, 350 (271–372) $\times 10^3$ nephrons/kidney; Gopalakrishnan *et al.* 2004*a*). The former observation is not apparent in LBCS but the latter is. Without allowing an LBCS group to grow to maturity we cannot say conclusively whether one of these observations, or an interaction between the two, is a prerequisite for development of hypertension after maternal nutrient restriction. The models established within the present study now provide a framework under which to examine these potential mechanisms. A further hypothesis is that the initial stimulus for later cardiovascular programming is altered apoptosis, particularly in the kidney. The widely reported association of programmed hypertension with a deficit in nephron number in rats (Langley-Evans *et al.* 1999; Woods *et al.* 2001) and sheep (Wintour *et al.* 2003) is pre-empted by increased apoptosis of mesenchymal metanephroi (in rats at least), therefore reducing the adult mature nephron complement (Welham *et al.* 2002) and resulting in glomerular hypertrophy. Consequently, renal functional reserve is more limited in NR. Increased cytochrome C abundance in the kidney of NR animals, which is perhaps indicative of elevated renal metabolic work, supports this contention.

In conclusion, nutrient restriction between early and mid gestation does not increase resting blood pressure but reduces nephron number as juveniles, relative to controls. However, ewes that ate to or above the estimated requirement for ME throughout gestation, but who were in low body condition as they entered pregnancy, similarly produce offspring that have the same resting blood pressure and nephron counts as NR offspring. In some respects, however, there appears to be a divergence in response that may indicate differences in the mechanism of programming: while NR offspring failed to show a depressor effect on heart rate with elevations in blood pressure, juveniles from LBCS ewes did. Thus, maternal body composition around conception appears to be as important as the level of nutrient intake during early pregnancy in programming later cardiovascular health of the offspring.

Acknowledgements

The authors wish to acknowledge the staff of the Joint Animals Breeding Unit for the routine care of the animals used in this study. This work was supported by the British Heart Foundation and The Nutricia Foundation.

https://doi.org/10.1079/BJN20051559 Published online by Cambridge University Press

https://doi.org/10.1079/BJN20051559 Published online by Cambridge University Press

References

Agricultural and Food Research Council (1993) *Technical Committee on Responses to Nutrients. Report no*. 9, pp. 812–815. Wallingford, UK: CAB International.

Agricultural Research Council (1980) Requirements for energy. In *The Nutritional Requirements of Ruminant Livestock*, pp. 115–119. Slough, UK: Commonwealth Agricultural Bureau.

Barker DJP (2001) The malnourished baby and infant. *Br Med Bull* **60**, 69–88.

Barker DJP, Bull AR, Osmond C & Simmonds SJ (1990) Fetal and placental size and risk of hypertension in adult life. *Br Med J* **301**, 259–262.

Barker DJP, Gluckman PD, Godfrey KM, Harding JE, Owens JA & Robinson JS (1993) Fetal nutrition and cardiovascular disease in later life. *Lancet* **341**, 938–941.

Bispham J, Gopalakrishnan GS, Dandrea J, Wilson V, Budge H, Keisler DH, Broughton Pipkin F, Stephenson T & Symonds ME (2003) Maternal endocrine adaptation throughout pregnancy to nutritional manipulation: consequences for maternal plasma leptin and cortisol and the programming of fetal adipose tissue development. *Endocrinology* **144**, 3575–3585.

Brennan KA, Gopalakrishnan GS, Kurlak L, Rhind SM, Brooks AN, Rae MT, Olson DM, Stephenson T & Symonds ME (2005) Impact of maternal undernutrition and fetal number on glucocorticoid, growth hormone and insulin-like growth factor receptor mRNA abundance in the ovine fetal kidney. *Reproduction* **129**, 151–159.

Clarke L, Heasman L, Juniper DT & Symonds ME (1998) Maternal nutrition in early–mid gestation and placental size in sheep. *Br J Nutr* **79**, 359–364.

Clarke L, Yakubu DP & Symonds ME (1997) Influence of maternal bodyweight on size, conformation and survival of newborn lambs. *Reprod Fertil Dev* **9**, 509–514.

Crowe C, Dandekar P, Fox M, Dhingra K, Bennet L & Hanson MA (1995) The effects of anaemia on heart, placenta and body weight, and blood pressure in fetal and neonatal rats. *J Physiol* **488**, 515–519.

Delavaud C, Bocquier F, Chilliard Y, Keisler DH, Gertler A & Kann G (2000) Effect of sheep nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA. *J Endocrinol* **165**, 519–526.

Dodic M, Hantzis V, Duncan J, Rees S, Koukoulas I, Johnson K, Wintour EM & Moritz K (2002) Programming effects of short prenatal exposure to cortisol. *FASEB J* **16**, 1017–1026.

Eckberg DL (1979) Carotid baroreflex function in young men with borderline blood pressure elevation. *Circulation* **59**, 632–636.

Edwards LJ & McMillen IC (2002) Impact of maternal undernutrition during the periconceptional period, fetal number, and fetal sex on the development of the hypothalamo-pituitary adrenal axis in sheep during late gestation. *Biol Reprod* **66**, 1562–1569.

Gambling L, Dunford S, Wallace DI, Zuur G, Solanky N, Srai KS & McArdle HJ (2003) Iron deficiency during pregnancy affects postnatal blood pressure in the rat. *J Physiol* **552**, 603–610.

Gardner DS, Jamall E, Fletcher AJW, Fowden AL & Giussani DA (2004*a*) Adrenocortical responsiveness is blunted in twin relative to singleton ovine fetuses. *J Physiol* **557**, 1021–1032.

Gardner DS, Pearce S, Dandrea J, Walker RM, Ramsey MM, Stephenson T & Symonds ME (2004*b*) Peri-implantation undernutrition programs blunted angiotensin II evoked baroreflex responses in young adult sheep. *Hypertension* **43**, 1–7.

Gilbert JS, Lang AL, Grant AR & Nijland MJ (2005) Maternal nutrient restriction in sheep: hypertension, decreased nephron number in offspring at 9 months of age. *J Physiol* **565**, 137–148.

Gopalakrishnan GS, Gardner DS, Kurlak L, Langley-Evans SC, Rhind SM, Rae MT, Kyle CE, Stephenson T, Symonds ME & Budge H (2004*a*) Programming of nephron number in adult sheep by maternal nutrient restriction in early gestation. *Proc Physiol Soc* **560**P, C18.

Gopalakrishnan G, Gardner DS, Rhind SM, *et al.* (2004*b*) Programming of adult cardiovascular function after early maternal undernutrition in sheep. *Am J Physiol* **287**, R12–R20.

Hawkins P, Steyn C, McGarrigle HG, Calder NA, Saito T, Stratford LL, Noakes DE & Hanson MA (2000) Cardiovascular and hypothalamic–pituitary–adrenal axis development in late gestation fetal sheep and young lambs following modest maternal nutrient restriction in early gestation. *Reprod Fertil Dev* **12**, 443–456.

Heasman L, Clarke L, Firth K, Stephenson T & Symonds ME (1998) Influence of restricted maternal nutrition in early to mid gestation on placental and fetal development at term in sheep. *Pediatr Res* **44**, 546–551.

Jackson AA, Dunn RL, Marchand MC & Langley-Evans SC (2002) Increased systolic blood pressure in rats induced by a maternal low-protein diet is reversed by dietary supplementation with glycine. *Clin Sci* **103**, 633–639.

Langley SC & Jackson AA (1994) Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diet. *Clin Sci* **86**, 217–222.

Langley-Evans SC (2001) Fetal programming of cardiovascular function through exposure to maternal undernutrition. *Proc Nutr Soc* **60**, 505–513.

Langley-Evans SC, Welham SJM & Jackson AA (1999) Fetal exposure to a maternal low protein diet impairs nephrogenesis and promotes hypertension in the rat. *Life Sci* **64**, 965–974.

Mackenzie HS & Brenner BM (1995) Fewer nephrons at birth: a missing link in the etiology of essential hypertension? *Am J Kidney Dis* **91**, 98.

McMullen S & Langley-Evans SC (2004) Maternal low protein diet in rat pregnancy programmes blood pressure through sex-specific mechanisms. *Am J Physiol* **288**, R85–R90.

Matthews JN, Altman DG, Campbell MJ & Royston P (1990) Analysis of serial measurements in medical research. *Br Med J* **300**, 230–235.

Mostyn A, Wilson V, Dandrea J, Yakubu DP, Budge H, Alves-Guerra MC, Pecqueur C, Miroux B, Symonds ME & Stephenson T (2003) Ontogeny and nutritional manipulation of mitochondrial protein abundance in adipose tissue and the lungs of postnatal sheep. *Br J Nutr* **90**, 323–328.

Ookuwa H, Takata S, Ogawa J, Iwase N, Ikeda T & Hattori N (1987) Abnormal cardiopulmonary baroreflexes in normotensive young subjects with a family history of essential hypertension. *J Clin Hypertens* **3**, 596–604.

Ozaki T, Nishina H, Hanson MA & Poston L (2001) Dietary restriction in pregnant rats causes gender-related hypertension and vascular dysfunction in offspring. *J Physiol* **530**, 141–152.

Robinson JS, Owens JA, DeBarro T, Lok F & Chidzanja S (1994) Maternal nutrition and fetal growth. In *Early Fetal Growth and Development*, pp. 317–329 [RHT Ward, SK Smith and D Donnai, editors]. London: RCOG Press.

Russel AJF, Doney JM & Gunn RG (1969) Subjective assessment of body fat in live sheep. *J Agric Sci (Cambridge)* **72**, 451–454.

Schwartz J & Rose JC (1998) Development of the pituitary adrenal axis in fetal sheep twins. *Am J Physiol* **274**, R1–R8.

Smith FG, Chan S & De Wildt SN (1997) Effects of renal denervation on cardiovascular and renal responses to ACE inhibition in conscious lambs. *J Appl Physiol* **83**, 414–419.

Symonds ME, Bryant MJ, Clarke L, Darby CJ & Lomax MA (1992) Effect of maternal cold exposure on brown adipose tissue and thermogenesis in the neonatal lamb. *J Physiol* **455**, 487–502.

Symonds ME, Bryant MJ & Lomax MA (1986) The effect of shearing on the energy metabolism of the pregnant ewe. *Br J Nutr* **56**, 635–643.

Wallace JM (2000) Nutrient partitioning during pregnancy: adverse gestational outcome in overnourished adolescent dams. *Proc Nutr Soc* **59**, 107–117.

Wallace JM, Aitken RP & Cheyne MA (1996) Nutrient partitioning and fetal growth in rapidly growing adolescent ewes. *J Reprod Fertil* **107**, 183–190.

Wallace JM, Bourke DA, Aitken RP, Milne JS & Hay WW (2003) Placental glucose transport in growth-restricted pregnancies induced by overnourishing adolescent sheep. *J Physiol* **547**, 85–94.

Welham SJM, Wade A & Woolf AS (2002) Protein restriction in pregnancy is associated with increased apoptosis of mesenchymal cells at the start of rat metanephrogenesis. *Kidney Int* **61**, 1231–1242.

Whorwood CB, Firth KM, Budge H & Symonds ME (2001) Maternal undernutrition during early- to mid-gestation programmes tissue-specific alterations in the expression of the glucocorticoid receptor, 11β-hydroxysteroid dehydrogenase isoforms and type 1 angiotensin II receptor in neonatal sheep. *Endocrinology* **142**, 2854–2864.

Wintour EM, Moritz KM, Johnson K, Ricardo S, Samuel CS & Dodic M (2003) Reduced nephron number in adult sheep, hypertensive as a result of prenatal glucocorticoid treatment. *J Physiol* **549**, 929–935.

Woods LL, Ingelfinger JR, Nyengaard JR & Rasch R (2001) Maternal protein restriction suppresses the newborn rennin–angiotensin system and programs adult hypertension in rats. *Pediatr Res* **49**, 460–467.

Yang K, Berdusco ET & Challis JR (1994) Opposite effects of glucocorticoid on hepatic 11 × -hydroxysteroid dehydrogenase mRNA and activity in fetal and adult sheep. *J Endocrinol* **143**, 121–126.

https://doi.org/10.1079/BJN20051559 Published online by Cambridge University Press