CALL FOR PAPERS | Fetal Physiological Programming

Programming of adult cardiovascular function after early maternal undernutrition in sheep

G. S. Gopalakrishnan,¹ D. S. Gardner,² S. M. Rhind,³ M. T. Rae,⁴ C. E. Kyle,⁵ A. N. Brooks,⁶ R. M. Walker,⁶ M. M. Ramsay,⁴ D. H. Keisler,⁵ T. Stephenson,⁶ and M. E. Symonds¹

¹Centre for Reproduction and Early Life, Institute of Clinical Research, University Hospital, Nottingham NG7 2UH; ²School of Biosciences, University of Nottingham, Sutton Bonington Campus, Nottingham LE12 5RD; ³Macaulay Institute, Craigiebuckler, Aberdeen AB15 8QH; ⁴AstraZeneca, Alderly Park, Cheshire SK10 4TF, United Kingdom; and ⁵Department of Animal Sciences, University of Missouri, Columbia, Missouri 65211

Submitted 1 December 2003; accepted in final form 10 February 2004

HYPERTENSION is a major risk factor for ischemic heart disease (IHD). An age-specific increase of 20 (10) mmHg systolic (diastolic) blood pressure above average more than doubles the risk of IHD (42). Hypertension is therefore a common risk factor for death in the population over the age of 50 (40, 52). Hypertension and IHD are both multifactorial in their etiology and, among other predisposing factors (e.g., high fat and salt intake, smoking, and obesity), a suboptimal intrauterine environment has been shown to be of particular importance in determining potential risk status (4). Although poor in utero development is often marked by low birth weight, the association with elevated adult blood pressure is not necessarily dependent on a reduction in birth weight per se but is perhaps due to complex interactions between the pre- and postnatal environment (20). The methodologies and inferences implied by the “developmental origins of adult disease hypothesis” have been criticized (33, 43) but appear robust when subjected to meta-analyses (32, 40). Furthermore, through the use of animal models, which are largely free from methodological bias and socioeconomic confounding, there is sufficient evidence to suggest the hypothesis is biologically plausible and of major importance in terms of public health (6, 31, 39).

To date, however, physiological programming of adult cardiovascular risk has only been demonstrated repeatedly in small animal models such as the rat (38, 51). Rats, being a litter-bearing species, are particularly nutritionally sensitive to either specific (protein; 38) or global undernutrition (51) or high-dose glucocorticoid exposure (5) during pregnancy, owing to the high rate of fetal growth and thus protein accretion relative to maternal size (47). In contrast, the sheep has a similar rate of pre- and postnatal growth to the human and usually produces only one or two offspring weighing between 3 and 6 kg, not unlike humans. However, in this species any observed programmed changes in cardiovascular control after nutritional restriction have been limited to the late gestation fetus or young lamb (18, 26). Programming of adult cardiovascular function in the sheep has only ever been observed after high-dose glucocorticoid therapy for 2 days in early gestation (14–16); however, this study does not address the potential for undernutrition during this period to program adult cardiovascular function, as suggested by retrospective human data (56, 58).

On the basis of the aforementioned rodent studies, the elevation in resting blood pressure of the adult, after maternal undernutrition, appears related in part to programmed elements of the renin-angiotensin system (49, 59, 60), sympathoadrenal function (41), and a tendency to deposit more fat as an adult (65). Interrelationships between these factors exist: for exam-
ple, increased circulating and local angiotensin II action has been shown to stimulate leptin release from adipocytes (10) and prenatal nutrient restriction reduces intrarenal renin (71), which, combined, are predicted to result in reduced circulating angiotensin II concentrations and thus upregulation of tissue angiotensin II receptor densities. Indeed, this has been shown, although the response may be species and tissue specific and time dependent, i.e., type I angiotensin II receptor densities are increased in the adrenal, kidney, and liver of neonatal sheep (67) but decreased in the kidney of adult rats (49) after maternal undernutrition. In addition, circulating leptin, which reflects body adiposity, has clear effects on autonomic, in particular, sympathetic function (28). Early undernutrition of pregnant sheep has been shown to result in fatter term fetuses (7).

In the present study we extended these findings into adulthood in the sheep. In addition to investigating the effects of global nutrient restriction during early to midgestation on body composition of the resultant male offspring at 3 yr of age, we undertook an in vivo analysis of basal and stimulated cardiovascular and endocrine function. As an index of cardiovascular function, we measured resting systolic, diastolic and mean arterial blood pressure, heart rate, and pressor responses to exogenous infusion of the potent vasopressor hormones angiotensin II and norepinephrine, given the observed programming of neonatal angiotensin II receptor densities using this model (67) and of sympathoadrenal activity in others (41) and to the physiological pressor stimulus of feeding in sheep. During the short-term pressor challenges, we also made an assessment of baroreflex function through the parallel changes in blood pressure and heart rate. Operation of the cardiovascular baroreflex is key to maintaining central pressure during ambulatory changes in blood pressure. If inadequate, then risk of later hypertension is increased (17, 24, 50). Alongside these cardiovascular indexes, the current study assesses the resting plasma concentration of leptin as an indicator of body fat mass and the change in leptin concentration before and after angiotensin II or norepinephrine infusion to indicate the endocrine sensitivity of adipose tissue (9, 10, 30). In addition, the resting plasma concentration of glucose and cortisol was measured to provide indexes of resting metabolic and stress status, respectively.

MATERIALS AND METHODS

Animals. All procedures were performed under the United Kingdom’s Animals (Scientific Procedures) Act, 1986. Seventeen mature Scottish Blackface male-bearing ewes of similar age, live weight, and body condition score were mated during a synchronized estrus and randomly allocated to receive either a control (C; n = 8) or nutrient-restricted (NR; n = 9) diet from day 1 of pregnancy. Ewes were group housed under natural day length conditions with unlimited access to water. During the first 95 days of gestation, control ewes were fed 100% metabolizable energy (ME) requirements for live weight maintenance (8.0 MJ/day) as defined by the Agricultural and Food Research Council (1), whereas NR sheep were fed to 50% of that amount (4.0 MJ/day). Thereafter, rations were increased according to litter weight gains to sustain a normal lactation (1). The basal diet consisted of 250 g hay per day (1.0 MJ/day) and 650 g dried grass pellets per day (7 MJ/day; Green Keil, North Eastern Farmers, Aberdeen, UK). The NR diet consisted of a similar proportion of hay (250 g; 1.0 MJ/day) but reduced pellet intake (280 g; 3 MJ/day). The diets contained an adequate amount of vitamins (Vit A, 8,121 mg/kg; D, 2,005 mg/kg, and E, 50 mg/kg diet) with minerals provided as blocks (“Baby Rockies,” Tithebarn, Winsford, Cheshire; containing magnesium, 1 g/kg; iron 200 mg/kg; manganese, 100 mg/kg; iodine 50 mg/kg; zinc 120 mg/kg; cobalt 100 mg/kg; selenium 20 mg/kg, and sodium 38%). All ewes were weighed and body condition scored at 21-day intervals. At term, lambs were delivered naturally with no intervention and birth weights were recorded. The offspring were ewe reared until weaning at 16 wk of age and thereafter grass fed at the Meat and Livestock Institute Glenshaugh Research Station, Aberdeen, until 3 yr of age.

Experimental protocols. At 4 mo before surgery, all uncastrated male sheep were transported to the Sutton Bonington Campus, University of Nottingham, and group housed indoors. For 24 h before surgery, all food, but not water, was withdrawn from the animals. Anesthesia was induced with thiopentone sodium (20 mg/kg iv Intralav Sodium; Rhone Merieux, Dublin, Ireland) and maintained with 1–2% halothane in 50:50 O2-2N2O. Right carotid and jugular catheters were inserted into each sheep and secured and the neck incision was closed. Catheters emerging from the neck were coiled and protected within a 10-in. bandage. All sheep received a dose of long-acting antibiotic (15 mg/kg im amoxicillin; “Dulphamox”; Fort Dodge Animal Health, Southampton, UK) and algesia (1 mg/kg butorphanol; “Fimadyl”; Sherlock’s, Kenilworth, UK) postoperatively. Catheter patency was maintained by daily flushing with heparinized saline (50 IU heparin/ml). All sheep were housed in individual pens but together in a room with controlled lighting (12 h on 12 h off; 8:00 AM-8:00 PM), had established normal feeding patterns within 1–2 h after surgery, and showed no visible signs of discomfort for the duration of the experiment. A period of 2–3 days postoperative recovery was allowed before any experiment being performed, and the investigator was blinded to the dietary origin of the sheep. For experimental protocols, catheters were connected to precalibrated pressure transducers (SensorNor 840; S 4925) attached at heart level and linked to a data-acquisition system (Po-Ne-Mah; Version 3, Gould Instrument Systems). Over a 7–to 10-day period, cardiovascular variables were recorded from all sheep on four separate occasions and days, with ad libitum hay and water available. Each experiment was begun between 9:00 and 10:00 AM. After a baseline period of 1 h, analog signals for real-time systolic, diastolic, mean arterial pressure, and heart rate were recorded second-by-second during an additional 30-min period and were digitized and stored on an Excel spreadsheet for further analysis. From these basal data, pulse pressure (systolic-diastolic) and the rate-pressure product [mean arterial blood pressure (mmHg) × heart rate (beats/min)/103], an index of myocardial work and thus oxygen consumption, were derived. This 30-min recording period was considered the baseline for daily cardiovascular variables. The following protocols were conducted in a random order on separate days and for angiotensin II, norepinephrine, and captopril in all sheep. For the cardiovascular response to feeding, data for n = 5 controls and n = 8 NR only are available.

Feeding. Sheep were not fed concentrate for a period of 24 h. The following morning, after a baseline period of 10 min, the sheep were fed hay and concentrate with ad libitum access to water. Cardiovascular variables were recorded for a 10-min period during which all concentrate feed was consumed and for an additional 10-min postprandial period.

Angiotensin II. After a baseline period of 10 min, stepwise intravenous increases in angiotensin II (0, 1, 2, 4, 8, 16, 32 ng·kg−1·min−1) were administered every 10 min, followed by a 30-min recovery period in which cardiovascular variables returned to baseline.

Norepinephrine. After a baseline period of 10 min, stepwise intravenous increases in norepinephrine (0, 2, 4, 8, 16, 32, and 48 ng·kg−1·min−1) were administered every 10 min, followed by a 10-min recovery period in which cardiovascular variables returned to baseline.
Captopril. After a baseline period of 30 min, the potent ACE inhibitor captopril was infused for 30 min at a dose of 0.12 mg·kg⁻¹·h⁻¹. This dose was previously validated to be the lowest effective dose to completely block the pressor effect of 0.5 µg angiotensin I (61). Indeed, in our own hands, doubling the dose rate of captopril had no greater hypotensive effect (23). After infusion, cardiovascular variables were recorded for an additional 20-min recovery period or until blood pressure had returned to baseline.

Blood samples. On each day of an experiment, a blood sample (5 ml) was taken before the experiment and subsequently immediately after infusion of the highest dose given. In addition, on a separate day after animals had previously been fasted for 12 h, blood samples (2 ml) were taken during baseline (30 min before feeding) and every 30 min thereafter for a total period of 6 h. The blood was drawn into heparinized syringes, placed in chilled blood tubes, and centrifuged at 3,500 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. After all experimental protocols, sheep were killed with a lethal dose of pentobarbitone sodium (Euthatal; 100 mg/kg), and tissue weights were recorded.

Hormone analysis. Plasma concentrations of glucose were measured enzymatically (Trinder; glucose oxidase) as described by Symonds et al. (64). Plasma concentrations of leptin were assayed using a double-antibody RIA, validated for use with ovine plasma as previously described in detail (13). 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/ml with intra- and interassay coefficients of variation 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, Caernarfon, UK) validated for use with ovine plasma (7). The minimum detection limit for the assay was 0.5 ng/ml, and the intra- and interassay (n = 5) coefficients of variation were 4 and 11%, respectively.

Statistical analyses. All data are expressed as means ± SE unless otherwise stated. The data were first tested for normality of distribution, and appropriate parametric or nonparametric tests were used. The data for birth weight and current weight were continuous and analyzed by one-way ANOVA. Cardiovascular variables (blood pressures, heart rate, rate-pressure product) were analyzed by two-way ANOVA with repeated measures for effects of group, e.g., control vs. NR; time, e.g., before, during, and after feeding/pressor challenges and any interaction between group×time. Post hoc statistics were run where indicated using Tukey’s t-test. Hormone data were analyzed by two-way ANOVA with repeated measures. Area under the curve (AUC) for heart rate was calculated using a custom-designed Excel spreadsheet according to Eq. 1:

\[ \text{AUC} = 0.5(a + z) + \frac{2}{b} \sum_{i=1}^{n} \frac{y_i - a}{b} \]

where \( a \) is the first data point, \( z \) is the last data point, and \( b \) are the data points enclosed by the curve. For example, during the 60 min of norepinephrine infusion; \( a \) is the AUC for the trapezium describing minute 1 of infusion, \( b \) is the AUC for minute 60, and \( b \) represent minutes 2–59. Average values were then compared by one-way ANOVA. For a comparison between the slopes of linear regression curves for mean arterial blood pressure and heart rate obtained during norepinephrine infusion, the analyses was conducted using analysis of covariance according to Armitage and Berry (3). All statistical comparisons were conducted using SPSS 11.1 (SPSS, Chicago, IL). For all comparisons, statistical significance was accepted when \( P < 0.05 \).

RESULTS

Offspring weights. Lamb weights were similar between maternal dietary groups (control, 4.4 ± 0.2; NR, 4.0 ± 0.3 kg) and were appropriate given the size of the ewe and plane of nutrition during pregnancy, according to recommended guidelines for the nutrition of housed, pregnant ewes (1). At 3 yr of age there was no difference in body weight between the two groups of sheep (control, 75.6 ± 2.8; NR, 75.0 ± 2.6 kg).

Basal cardiovascular status. Baseline cardiovascular variables were assessed before, during, and after feeding and/or...
Each experimental protocol. In the morning, before feeding of concentrate, values for systolic (control, 99 ± 2 vs. NR, 110 ± 2 mmHg), diastolic (65 ± 3 vs. 74 ± 2 mmHg) and mean arterial pressure (79 ± 2 vs. 89 ± 3 mmHg), heart rate (83 ± 5 vs. 102 ± 4 beats/min), and the rate-pressure product (6.54 ± 0.37 vs. 9.11 ± 0.41 beats·min⁻¹·mmHg⁻¹/10³) were significantly elevated (all P < 0.05) in NR sheep. However, after feeding, these values in NR sheep were not significantly different from controls (Fig. 1). On subsequent days, cardiovascular variables were recorded after the morning feed and averaged values for individual sheep over two to three baseline recording periods were not significantly different between dietary groups (systolic, 109 ± 3 vs. 113 ± 2 mmHg; diastolic, 74 ± 3 vs. 76 ± 2 mmHg; mean arterial pressure, 87 ± 3 vs. 91 ± 2 mmHg; heart rate, 87 ± 5 vs. 92 ± 5 beats/min), in control and NR sheep, respectively.

Cardiovascular responses to angiotensin II infusion. In both control and NR sheep, angiotensin II infusion resulted in dose-dependent increments in arterial blood pressure and decrements in heart rate (Fig. 2, A and B). The magnitude of the increase in pressure and decrement in heart rate were not significantly different between dietary groups (Fig. 2).

Cardiovascular responses to norepinephrine infusion. In both control and NR sheep, norepinephrine infusion resulted in dose-dependent increments in arterial blood pressure (Fig. 3, A and B). In NR sheep, the increase in arterial blood pressure was similar, but heart rate failed to decline significantly (Fig. 3D). Consequently, the AUC for delta heart rate during the challenge was significantly lower in NR relative to control sheep (98 ± 58 vs. −163 ± 46 AUC units, respectively; P = 0.004).

Furthermore, when plotted as a linear relationship between individual data points for mean arterial blood pressure and heart rate (baroreflex sensitivity), the intercept, but not the slope, was significantly elevated in NR (y = −0.23x + 116) sheep relative to controls (y = −0.35x + 114, t = 4.92; P < 0.001), as illustrated in Fig. 4.

Cardiovascular responses to captopril infusion. In control and NR sheep, captopril infusion resulted in a decrease in systolic, diastolic, and mean arterial blood pressure by an average of 4.6 ± 1.6 and 6.2 ± 1.7 mmHg (systolic pressure), respectively, without change in heart rate. There were no significant differences between groups in the response to captopril infusion.

Basal glucose, cortisol, and leptin status. Plasma concentrations for glucose, cortisol, and leptin did not vary over the course of the study period (Fig. 5), and mean values were not significantly different in control and NR sheep (glucose, 5.38 ± 0.30 vs. 5.06 ± 0.72 mmol/l; cortisol, 29.0 ± 5.3 vs. 23.6 ± 1.6 mmol/l; leptin, 3.14 ± 1.03 vs. 4.37 ± 1.15 ng/ml, for control and NR sheep, respectively). However, during both norepinephrine and angiotensin II infusion, the delta change in plasma leptin was significant (P < 0.05) in NR but not in control offspring (controls, before infusion 3.22 ± 1.7 and 2.73 ± 0.96, after infusion 3.16 ± 1.7 and 3.13 ± 1.10 ng/ml for before and after norepinephrine and angiotensin II infusion, respectively; NR, before infusion 3.47 ± 0.9 and 3.50 ± 1.46, after infusion, 4.5 ± 1.3 and 5.30 ± 2.27 ng/ml, respectively). Captopril infusion had no effect on plasma leptin in either dietary group (controls, 3.75 ± 2.65 and 4.16 ± 2.44 ng/ml; NR, 3.47 ± 1.3 and 5.30 ± 2.27 ng/ml, respectively).
NR, 3.81 ± 2.45 and 3.55 ± 2.45 ng/ml before and after infusion, respectively. The delta change in plasma leptin during each pressor challenge is illustrated in Fig. 6B.

Sheep biometry at 3 yr of age. There were no differences between the two dietary groups in the weights of any organ measured with the exception of the liver, which was significantly smaller in NR relative to control sheep, when expressed in absolute (Table 1) or relative (NR; 15.0 ± 0.5 vs. control; 16.8 ± 0.6 g/kg; P = 0.01) terms. Whereas the lower weight of the gonads in NR approached significance in absolute terms (Table 1), this effect was weakened when expressed relative to body weight (P = 0.09). The degree of fatness of the sheep regardless of dietary group (g fat/kg empty carcass) correlated well with plasma leptin (r² = 0.47, P = 0.007, Fig. 6A).

DISCUSSION

We showed for the first time in adult sheep that maternal nutritional restriction from conception to the end of the period of maximal placental growth results in programmed alterations to cardiovascular function in the 3-yr-old offspring. Specifically, irrespective of weight at birth, NR sheep had higher blood pressure before, but not after, feeding and altered baroreflex responses to stepwise norepinephrine infusion. These cardiovascular changes were not associated with altered glucose or glucocorticoid profiles. As adults, NR sheep were of similar body weight to controls, but this masked a significant reduction in hepatic weight. Plasma leptin correlated significantly with the ratio of fat/fat-free mass in both groups and increased after norepinephrine and angiotensin II infusion in NR, but not control, sheep.

The developmental origins of adult disease hypothesis has been subject to methodological and interpretive criticism (32, 43), but stands up when meta-analyses of the human data (33, 40) and evidence from animal models are considered (6, 31, 34, 39). From very early on in the studies of the nutritional regulation of fetal growth, it was clear that the “timing,” severity, and duration of nutrient restriction with respect to later postnatal outcome was important (47, 53, 68, 69). These
“programming” influences may be reconciled with the nature of the growth process. When the episode of nutrient restriction coincided with, or persisted beyond, a period of hyperplastic vs. hypertrophic organ development, its structure and/or function can be permanently and irretrievably altered (47, 68, 69). In the context of the developmental origins of adult disease hypothesis, such alterations lead to a profound limitation in organ function such that the maximal functional capacity is achieved earlier and more readily, creating premature pathophysiological sequelae (34). Perhaps one of the most demonstrated examples in animal models is a permanent reduction in nephron number, as a result of an early insult, and increased incidence of hypertension (66, 70, 71).

Epidemiological data from the “Dutch Hunger Winter Famine” of World War II highlighted early gestation as a particularly sensitive period for the programming of subsequent cardiovascular development (57). This is not surprising given the early priority for establishing systems necessary to support adequate nutrient delivery for tissue growth, e.g., the placenta and fetal cardiovascular system. In the human, a heart beat is detectable from as early as 22 days of an ~276-day gestation period, whereas in the sheep, placental growth is most rapid during days 30–80 of an ~150-day gestation (19). For this reason we chose to restrict maternal nutrient intake over a period encompassing both embryonic and maximal placental growth. To date, many different studies in rats have shown nutritional programming of hypertension (for review, see Refs. 6, 39). The rat, however, may be particularly vulnerable to any nutritional imbalance during gestation given the exceptional rate of protein accretion during prenatal development [estimated at 23-fold that of the human fetus (48)] and the sum weight of the products of conception relative to maternal weight (25–35% vs. 6–10% in the sheep and 3–5% in humans).

The programming of any outcome measure, such as blood pressure, for example, may therefore be amplified in the rat; hence the 20–40 mmHg difference in systolic blood pressure after prenatal protein restriction (39). To date, the one group to have shown prenatal programming of hypertension in sheep, Fig. 5. Plasma concentrations of glucose (A), cortisol (B), and leptin (C) in control and NR sheep. Values are means ± SE for control (○) and NR (●) sheep for a 6-h period with blood sampling every 30 min. Data for glucose are control, n = 8 and NR, n = 9; cortisol, n = 6 and NR, n = 7; leptin, n = 7 and NR, n = 8. Arrow indicates the time of feeding of concentrate to the sheep.
by targeted high-dose glucocorticoid therapy, observed group differences of only 5–10 mmHg (16).

The present study is the first to show that a physiological challenge (undernutrition) during gestation can program resting blood pressure (~7–10 mmHg higher in NR before feeding) and influence cardiovascular control (altered baroreflex responses during norepinephrine infusion) in the adult offspring of a species with similar pre- and postnatal growth rates to humans, which were followed longitudinally from birth. Clearly, however, despite the degree of undernutrition (50%) reflecting the difference between upper and lower quartiles for energy intake in a representative human population (8) and being sufficient to significantly alter maternal metabolism (12), it had no effect on birth weight or postnatal growth rate and only resulted in a moderate increase in offspring blood pressure as the adult sheep ages. Nevertheless, cardiovascular programming is evident in these sheep. The lack of any difference in peripheral responses to vasopressor infusion, coupled with an effect on baroreflex function, may suggest a centrally orientated, rather than peripheral origin, for the programmed change in physiological function. On the other hand, greater increases in plasma leptin concentration after norepinephrine/angiotensin II infusion in NR may indicate persistent programming of the adipocyte, because angiotensin II stimulates leptin release from fat cells (10). However, long-term changes in adipocyte function and angiotensin II activity in NR may well also reflect programming at the level of the brain, given the effects centrally acting leptin and angiotensin II can have on autonomic function (10, 21, 28). Furthermore, the data suggest that the increase in tissue-specific angiotensin II receptor densities evident at birth (67) persists into later life; however, this has yet to be clarified with further molecular studies in adipose tissue and other important sites for angiotensin II action, such as the kidney, brain, and peripheral vascular tissue.

It has long been known that baroreflex function attenuates norepinephrine-mediated increase in blood pressure (for review, see Ref. 55). The mechanism is thought to reflect a combination of decreased sympathetic withdrawal together with less parasympathetic outflow from the area postrema, mediated by angiotensin II stimulation of catecholaminergic neurons, rather than a direct effect on the heart or baroreceptors themselves (55). With respect to the current study, the attenuated fall in heart rate during norepinephrine infusion in NR sheep suggests a weaker vagal dominance during increases in arterial pressure. Further in vivo studies using atropine to antagonize cardiac vagal tone during norepinephrine infusion together with molecular characterization of cardiac adrenergic receptor densities may well clarify this suggestion. A similar alteration to baroreflex function has recently been observed in NR sheep at 1 yr of age, although, at this time, no change in basal blood pressure was observed (22). Thus given that early gestational undernutrition of sheep lowers the resting blood pressure of the fetus (27), has only a marginal effect by 1 yr of age, but increases prefeeding systolic pressure at 3 yr of age suggests that programming of cardiovascular dysfunction in sheep develops gradually with age, reflecting the ontogenesis of essential hypertension in humans (62), in contrast to the overt hypertension observed in the offspring of rats soon after weaning (38).

Taken together, past and present data suggest that the adipocyte is sensitive to the programming influence of maternal undernutrition in terms of size and/or number and function (7, 54, 63, 67). Greater relative fat mass and, in particular, leptin production, is thought to influence central blood pressure control and autonomic outflow and is often associated with increased blood pressure (25, 44, 45). In particular, excess weight (fat) gain appears to specifically increase renal sympathetic nerve activity, as renal denervation attenuates obesity-related hypertension (35). Leptin correlates well with relative fat:fat-free mass, as in the current study; however, it is becoming increasingly clear that leptin is more than the signaling

Table 1. Sheep biometry at 3 yr of age

<table>
<thead>
<tr>
<th>Table 1. Sheep biometry at 3 yr of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Body wt, kg</td>
</tr>
<tr>
<td>Heart wt, g</td>
</tr>
<tr>
<td>Liver wt, g</td>
</tr>
<tr>
<td>Kidney wt, g</td>
</tr>
<tr>
<td>Adrenal wt, g</td>
</tr>
<tr>
<td>Spleen wt, g</td>
</tr>
<tr>
<td>Lung wt, g</td>
</tr>
<tr>
<td>Pancreas wt, g</td>
</tr>
<tr>
<td>Gonad wt, g</td>
</tr>
<tr>
<td>Total adipose tissue wt, g</td>
</tr>
<tr>
<td>Adipose:carcass wt, g/kg</td>
</tr>
</tbody>
</table>

Values are means ± SE. NR, nutrient restricted; NS, not significant.

AJP-Regul Integr Comp Physiol • VOL 287 • JULY 2004 • www.ajpregu.org
molecule thought to mediate “lipostatic” control of body weight (36). Rather, leptin, in part, regulates overall energy balance by reducing food intake but increasing sympathetic activity and thus energy expenditure (45, 46). Leptin infusion has been shown to increase sympathetic, in particular, renal sympathetic nerve activity (28), and blood pressure (11). Indeed transgenic mice overexpressing leptin have increased blood pressure despite being lean and of lower body weight, whereas leptin-deficient obese ob/ob mice have lower arterial blood pressure that is restored to levels observed in wild-type controls when leptin is replaced (2). Ganglionic blockade ameliorates the hypertensive state in mice overexpressing leptin, suggesting mediation by sympathetic nerve activity (2). Thus functional hypothalamic leptin receptors and an intact sympathetic reflex arc appear a prerequisite for expression of obesity-related hypertension.

One putative hypothalamic target site is the melanocortin-type 4 receptor (MC4-R) as intracerebroventricular administration of leptin had been shown to activate the melanocortin system, resulting in increased renal sympathetic nerve activity (29). Chronic blockade of the MC4-R produces gross obesity but has no effect on blood pressure (37). Thus in the context of the present study in which NR sheep show differing leptin responses to “stress hormones” (Fig. 6B), elevated heart rate at any given mean arterial pressure [perhaps indicating increased sympathetic activity (Fig. 4)] and increased prefeeding blood pressure (Fig. 1), it seems reasonable to presume that over a number of years a tendency to store more fat in NR offspring will lead to chronic, yet mild, increases in plasma leptin and thus sympathetic, in particular, renal activity and energy expenditure (45, 46). Leptin infusion will lead to chronic, yet mild, increases in plasma leptin and cortisol, and thus sympathetic nerve activity (2). Thus functional hypothalamic leptin receptors and an intact sympathetic reflex arc appear a prerequisite for expression of obesity-related hypertension.

In conclusion, the present study has shown for the first time that global undernutrition during the periods of embryonic and placental development can program adult physiology in the sheep. Increased prefeeding blood pressure, elevated heart rate at a given mean arterial pressure, and increased leptin responses to catecholamines and angiotensin II were all observed at a given mean arterial pressure, and increased leptin regulation) to a drive for increased weight gain, either by restricting physical activity and/or increasing postnatal food intake. Any in vivo physiological measures can thus be correlated with changes to putative hypothalamic receptors such as leptin and MC4-R.

REFERENCES