Peri-Implantation Undernutrition Programs Blunted Angiotensin II Evoked Baroreflex Responses in Young Adult Sheep

David S. Gardner, Sarah Pearce, Jennifer Dandrea, Ronald Walker, Margaret M. Ramsay, Terence Stephenson, Michael E. Symonds

Abstract—An adverse environment around conception and implantation influences later fetal growth and development to term in humans and sheep. Indeed, preimplantation undernutrition of rats elevated the systolic blood pressure of the resultant adult offspring. In this study, adult cardiovascular function is examined in a slower growing, non–litter-bearing species after peri-implantation undernutrition. Eight ewes were fed to 50% equivalent food intake of 12 control ewes from 1 to 30 days (term 147 days) only. Following consumption of an adequate diet to term, natural lambing, and then weaning, resting cardiovascular status and baroreflex function were examined in the resultant young adult offspring. Birth weight and postnatal growth to 1 year of age were unaffected by early undernutrition; however, nutrient-restricted sheep had increased pulse pressure, a reduced rate pressure product, and a leftward shift in their baroreflex function curve. Baroreflex sensitivity during angiotensin II infusion was also blunted in early nutrient-restricted sheep but the tachycardia following a reduction in central blood pressure appeared potentiated, relative to controls. The data suggest that peri-implantation undernutrition may program long-term cardiovascular dysfunction that ultimately increases the risk of hypertension later in life. An increase in regional angiotensin II activity during this critical early phase of development is a likely candidate mechanism for the effects observed. The data have broad implications for the health outcome of those offspring from mothers who were poorly nourished during early, often unknown pregnancy and for embryos artificially manipulated because of infertility treatment. (Hypertension. 2004;43:1290-1296.)

Key Words: sheep ■ angiotensin II ■ blood pressure ■ baroreflex

Hypertension is a major risk factor associated with coronary heart disease and represents a common cause of death in the population more than 50 years old.1 Hypertension and coronary heart disease are both multifactorial in their etiology but epidemiological studies in a number of different human populations have shown that the prenatal environment has an important role in determining the incidence of these diseases.2 Consequently, there has been renewed interest in the effect of poor gestational nutrition on pre- and postnatal growth after the early studies of McCance and Widdowson.3,4 These original hypotheses have been advanced to include changes in physiological function leading to adult pathology—the “developmental origins of adult disease hypothesis.”5,6 The methodologies and inferences of the hypothesis have been criticized7,8 but meta-analyses have illustrated the strength of the epidemiological findings.1,9 Work from the many animal models that now exist strongly supports the hypothesis and suggests that programming of disease risk is both biologically plausible and of major importance in terms of public health.5,10

The majority of studies investigating the developmental origins of adult disease hypothesis have concentrated on exposing the developing fetus to a poor diet throughout gestation. However, transient undernutrition during defined critical periods of fetal development has been shown to have an equal programming influence.11–16 A major restriction of nutrient availability may be expected to influence the development of fetal form and function over late gestation, when the stoichiometry of nutrient supply and demand is close.12 However, while it is known that manipulation of embryos as a result of infertility treatments may alter fetal growth, leading to large offspring syndrome, and perhaps influence later health outcomes,17 a number of studies have now shown that the preconceptual18–21 or even preimplantation15,16,22 nutritional environment may also program later physiological function, a time at which overall fetal demands for energy are remarkably low. In only 2 of these studies, both of which studied protein restriction in the rat21,22 (a species with a remarkable rate of protein accretion and thus sensitivity to protein restriction), were the exposed fetuses followed-up
into adult life. In both, periconceptual21 or preimplantation22 protein restriction elevated the systolic blood pressure of the young adult offspring. The mechanism underlying this effect remains elusive. Whereas the follow-up evidence from the Dutch Hunger Winter Famine suggests that undernutrition confined to early development has the greatest effects on the cardiovascular system,23,24 no study to date has yet followed-up adult cardiovascular function after exposure to undernutrition during the peri-implantation period only in a non–litter-bearing species whose pre- and postnatal growth rates are more comparable to human infants. Hence, the present study investigated the hypothesis that global energy restriction during early gestation programs altered cardiovascular control and increases blood pressure in the resultant offspring as young adults (ie, at 1 year of age). Resting cardiovascular status was assessed by measuring systolic, diastolic, and mean arterial blood pressure and heart rate. Short-term cardiovascular control was assessed through an analysis of the range, sensitivity, and set-point of the baroreceptor reflex using phenylephrine (PE) and sodium nitroprusside (SNP) infusion to raise and lower arterial blood pressure, respectively. In addition, a cursory examination of baroreflex function was made through similar changes in pressure and heart rate during angiotensin II infusion, a protocol known to produce a different baroreflex response to the physiologically purer stimuli of PE and SNP.25 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.26–28 We show that global undernutrition during this period does indeed program cardiovascular dysfunction in the young adult offspring and suggest that the primary triggering mechanism may be related to regional increases in angiotensin II activity, especially in centrally located areas involved in baroreflex function.

Materials and Methods

Animals
All procedures were performed under the UK Animals (Scientific Procedures) Act, 1986. Twenty Blue-faced Leicester × Swaledale ewes were used in the study. All ewes were of similar age (7 to 8 months), body weight, and fat distribution as assessed from physical characteristics in the lumbar spine and loin. Ewes were mated with rams and randomly assigned to one of two groups: Group I ewes were fed to 50% AFRC ME requirements from days 1 to 30 gestation and thereafter grass-fed until aged 1 year. During this time, the weights and growth of individual lambs were recorded monthly.

At 1 year of age, all sheep were group-housed indoors for 1 week prior to surgery. For 24 hours prior to surgery all food, but not water, was withdrawn from the animals. Anesthesia was induced with sodium thiopentone (20 mg · kg⁻¹ IV intravascular sodium; Rhone Mérieux, Dublin, Ireland) and maintained with 1% to 2% halothane in 50:50 O₂/N₂O. Carotid and jugular catheters were inserted into each sheep, and the incision closed. All sheep received a dose of long-acting antibiotic (15 mg · kg⁻¹ IM amoxycillin [Duphamox]; Fort Dodge Animal Health Ltd, Southampton, UK) and analgesia (1 mg · kg⁻¹ flunixin meglumine [Finadyne]; Shering-Plough, Kenilworth, UK) post-operatively. Catheter patency was maintained by daily flushing with heparinized saline (50 IU heparin · mL⁻¹). All sheep had established normal feeding patterns within 1 to 2 hours after surgery and showed no visible signs of discomfort for the duration of the experimental period.

Experimental Protocols
A period of 2 to 4 days post-operative recovery was allowed with the investigator blinded to the dietary origin of the sheep prior to any experiment being performed. Over a 10-day period and on 5 to 6 separate occasions and days, all sheep were placed in metabolism crates with ad libitum hay and water available. After at least 1 hour catheters were connected to precalibrated pressure transducers (SenNor 840; S 4925) attached at heart level linked to a data acquisition system (Po-Ne-Mah; Version 3, Gould Instrument Systems Inc) and a baseline recording was taken during a 30-minute period. Analogue signals for real-time systolic, diastolic, mean arterial pressure, and heart rate were recorded at 1-second intervals, digitized, and stored on an Excel spreadsheet for further analysis. From these data, pulse pressure (systolic–diastolic) and the rate pressure product (mean arterial blood pressure [mm Hg]·heart rate [beats·min⁻¹]; an index of myocardial work and thus oxygen consumption) were derived.

Angiotensin II
Stepwise intravenous increases in angiotensin II (0, 2.5, 5, 10, 20, 40, and 60 ng · kg⁻¹ · min⁻¹) were administered every 10 minutes, followed by a 10-minute recovery period in which cardiovascular variables returned to baseline.

Phenylephrine
Sheep were administered a bolus dose (75 μg · kg⁻¹ · IV given for 2 minutes) of the sympathomimetic (α-adrenergic agonist) phenylephrine hydrochloride (Sigma, Poole, UK), and cardiovascular variables were followed for a 1-hour period.

Sodium Nitroprusside
After cardiovascular variables returned to baseline, the sheep were infused (2.5 μg · kg⁻¹ · min⁻¹ · IV) with the endothelium-dependent vasodilator SNP (Abbott Laboratories, Maidenhead, UK) for 5 minutes after a 5-minute baseline recording period.

Statistical Analyses
All data are expressed as means±SEM unless otherwise stated. The data for maternal feed intake, birth weight, current weight, postnatal growth rates, and cardiovascular variables (blood pressures, heart rate, rate pressure product) were continuous and analyzed by 1-way ANOVA with repeated measures (SPSS Inc). Baroreceptor reflex curves describing the relationship between mean arterial blood pressure and heart rate were analyzed by a 4-parameter logisitc sigmoid function (Sigmaglot; SPSS Inc) using Equation 1:

\[ MAP = \frac{a}{1 + e^{-\left(b \cdot HR - c\right)}} + y \]

where \( a \) is the heart rate (HR) range (ie, max HR–min HR; beats·min⁻¹), \( b \) is a measure of the slope over the linear portion of the curve (1/mm Hg), \( c \) is the mid- or setpoint (mm Hg) for equal pressor and depressor responses, and \( y \) is the minimum HR. The
sigmoidal parameters generated for each individual animal were compared by Student t test. The maximal gain or baroreflex sensitivity was derived by calculus from the first derivative of Equation 1.

\[
\text{Equation 2:} \quad \text{Maximal gain} = \frac{a}{4b} 
\]

Areas under the curve (AUC) were calculated using a custom designed Excel spreadsheet according to Equation 3.

\[
\text{Equation 3:} \quad \text{AUC} = 0.5 \times (a + \frac{1}{2} \times \sum b) 
\]

Where \(a\) is the first data point, \(b\) is the last data point, and \(b - a\) are the data points enclosed by the curve. Average values were then compared by 1-way ANOVA. For a comparison between the slopes of linear regression curves obtained during angiotensin II infusion the data were analyzed by ANCOVA.\(^3\) For all comparisons, statistical significance was accepted when \(P<0.05\).

Results

Nutrition and Pregnancy

Ewe body weight and condition score at the time of conception was not different between dietary groups (controls 46.0±5.5 kg and 2.0±0.5 A.U.; NR 45.9±5.1 kg and 2.5±0.5 A.U., means±1 SD). Control ewes consumed between 7.68±0.24 to 17.7±0.59 MJ/d during weeks 1 and 21 of pregnancy, respectively, and gained 11.6±1.5 kg through the duration of gestation. NR ewes consumed 3.61±0.11 MJ/d for the first 30 days of pregnancy (ie, ~50% control intake) and after 1 week of realimentation, dietary intake was equivalent to control intake (Figure 1). Consequently, NR ewes lost more weight than controls through the first 4 weeks of pregnancy (4.1±0.4 versus 1.5±0.4 kg, respectively; \(P<0.05\)) and their overall weight gain tended to be lower but not statistically different (8.2±1.2 kg).

Birth Weights and Postnatal Growth Rates

There were no differences between singlets and twins of either group for any data; therefore, subsequent values represent the combined data. Lamb weights were similar between maternal dietary groups (control 3.9±0.3; NR 4.2±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, lowland ewes.\(^9\) There was no difference in the growth rate (current weight-birth weight per time; kg/wk) of control or NR lambs during the postnatal period (controls 1.93±0.15, 1.43±0.09, 0.92±0.07; NR 1.80±0.14, 1.43±0.07, 0.92±0.06 kg/wk for growth over 0 to 3, 4 to 6, and 7 to 12 months, respectively) but the rate of growth slowed over time in both groups (\(P<0.001\)). At 1 year of age there was no difference in body weight between the 2 groups of sheep (control 51.5±3.5; NR 51.6±3.0 kg).

Cardiovascular Responses to Angiotensin II Infusion

Baseline cardiovascular status was assessed on different days between 9:00 to 10:00 am on 5 to 6 occasions for each individual and data averaged to give a mean basal value for each sheep. Values for systolic (100±4 versus 98±3 mm Hg), diastolic (77±4 versus 70±3 mm Hg), mean arterial pressure (87±4 versus 81±3 mm Hg), and heart rate (107±5 versus 100±4 beats·min\(^{-1}\)) were similar in control and NR sheep, respectively. However, values for pulse pressure (28±1 versus 23±1 mm Hg, \(P=0.06\)) and the rate pressure product (7.91±0.46 versus 9.36±0.72 [beats·min\(^{-1}\)]·mm Hg/10\(^3\); \(P=0.005\)) tended to be higher and lower in NR relative to control sheep, respectively. In control sheep, angiotensin II infusion resulted in dose-dependent increments in arterial blood pressure and decrements in heart rate (Figure 2A and 2C). In NR sheep, however, the increment in arterial blood pressure was similar, but heart rate failed to decline significantly (Figure 2B and 2D). Consequently, the area under the curve for \(\Delta\) heart rate during the challenge was significantly (4-fold) lower in NR relative to control sheep (~125±120 versus ~841±107 U respectively; \(P=0.004\)). When plotted as a linear relationship between individual data points for mean arterial blood pressure and heart rate (baroreflex sensitivity) the slope was significantly different between dietary groups (controls 1.93±0.15, 1.43±0.09, 0.92±0.07; NR 1.80±0.14, 1.43±0.07, 0.92±0.06 kg/wk for growth over 0 to 3, 4 to 6, and 7 to 12 months, respectively) but the rate of growth slowed over time in both groups (\(P<0.001\)). At 1 year of age there was no difference in body weight between the 2 groups of sheep (control 51.5±3.5; NR 51.6±3.0 kg).
respectively (Figure 4). The slope of the linear relationship between individual data points for mean arterial blood pressure and heart rate during PE (ie, parasympathetic arm of the baroreflex) was similar in control and NR sheep (control $y = -1.00x + 208$, $r^2 = 0.91$; NR $y = -1.02x + 194$, $r^2 = 0.88$). Infusion of SNP significantly lowered arterial pressure, the reduction in diastolic pressure being significantly greater than the reduction in systolic pressure in both dietary groups (diastolic by 29±5 and 24±4; systolic by 19±3 and 15±4 mm Hg, in control and NR sheep, respectively; $P<0.05$). Consequently, pulse pressure significantly increased in control and NR sheep (by 11±3 and 11±4 mm Hg, respectively; $P<0.05$). The reduction in pressure was compensated for by significant increases in heart rate in control and NR sheep (Figure 5), however the mean increment from baseline was greater in NR sheep (by 12±6 and 30±6 beats·min⁻¹, respectively; $P=0.04$). Figure 6 shows the mean individual data points for the relationship between mean arterial pressure and heart rate, during the initial (2 minutes) phases of parasympathetic and sympathetic activation induced by PE and SNP, respectively. Statistical analysis of the curve indicated no significant difference in either the operating range of the baroreflex (a, 70±14 and 65±5 beats·min⁻¹), estimated slope (b, −6.5±2.1 and −6.6±1.5 mm Hg) or derived maximal gain (b, −5.9±2.4 and −3.1±0.5 beats·min⁻¹/mm Hg) and the minimum heart rate achieved (y, 58±4 and 62±5 beats·min⁻¹) in control and NR sheep, respectively. However, the set point (x) was significantly shifted to the left (ie, toward a lower mean pressure) in NR relative to control sheep (86±7 versus 100±3 mm Hg, respectively; $P=0.05$, Figure 6).

Cardiovascular Responses to PE and SNP Infusion

Resting mean arterial blood pressure and heart rate was similar prior to PE and SNP challenges in both groups of sheep (control: PE 92±3 mm Hg and 115±4 beats·min⁻¹; SNP 92±4 mm Hg and 107±5 beats·min⁻¹; NR: PE 89±5 mm Hg and 99±5 beats·min⁻¹; SNP 82±4 mm Hg and 93±4 beats·min⁻¹). Injection of a bolus dose of PE significantly increased mean arterial blood pressure (by 120±11 and 100±13 mm Hg) and decreased heart rate (by 88±4 and 80±4 beats·min⁻¹) in control and NR sheep, respectively (Figure 4). The slope of the linear relationship included with means every 9 minutes only. There was a significant change in the slope of the relationship between mean arterial blood pressure and heart rate values obtained during the 1 hour of angiotensin II infusion. For clarity, SEM has been expressed relative to body weight there were, again, no significant differences between groups. The significance of

Sheep Biometry at 1 Year of Age

There were no differences between the 2 dietary groups in the weights of any organ measured with the exception of the brain, which was significantly ($P=0.03$) smaller in NR relative to control sheep (Table). When organ weights were expressed relative to body weight there were, again, no significant differences between groups. The significance of

![Figure 3](https://example.com/f3.png)

**Figure 3.** Regression of mean arterial blood pressure with heart rate during angiotensin II infusion. Data points are the minute means for control (○, n=12) and NR (●, n=8) for paired mean arterial blood pressure and heart rate values obtained during the 1 hour of angiotensin II infusion. For clarity, SEM has been included with means every 9 minutes only. There was a significant change in the slope of the relationship between mean arterial blood pressure and heart rate in NR sheep ($y = -0.15x + 109$; $r^2=0.49$, n=8) relative to controls ($y = -0.41x + 138$; $r^2=0.92$, $t=10.96$; $P<0.0001$, n=12).

![Figure 4](https://example.com/f4.png)

**Figure 4.** The change in mean arterial blood pressure and heart rate during PE infusion in control and periconceptually nutrient-restricted sheep. Values are minute means±SEM for control (left panel, n=11) and NR (right panel, n=7) sheep for a baseline period (10 minutes) and for 30 minutes after a bolus dose of PE (75 μg·kg⁻¹).
the difference in the weight of the brain was, however, weakened when expressed relative to body weight \((P/0.08; \text{data not shown}).\)

**Discussion**

The present study provides the first documented evidence of programming influences operating prior to and during embryo attachment in a species that, in terms of fetal number, pre- and postnatal growth, is comparable to human infants. Furthermore, the peri-implantation nutritional environment of the embryo is clearly able to program cardiovascular dysfunction in the young adult independently of reductions in birth weight or accelerated postnatal catch-up growth, key processes thought to contribute to the developmental origins of adult cardiovascular disease.\(^\text{33}\) It is known that baroreceptor sensitivity per se is blunted when analyzed under conditions of high, but physiological, infusion doses of angiotensin II, when compared with the purer stimulus produced with the synthetic \(\beta\)-adrenergic agonist PE;\(^\text{25}\) this is reproduced in the current article (witness the differing curve gradients produced by angiotensin II and PE). This may be due, in part, to the differing methodologies used to assess baroreflex function in this study (ie, acute [PE and SNP] versus a more prolonged stimulus with angiotensin II infusion) or a centrally orientated mechanism (eg, angiotensin II-mediated inhibition of efferent parasympathetic outflow from the area postrema), rather than a direct effect on the heart or baroreceptors themselves (for a review, see Reference \(^\text{25}\)). Nevertheless, in this respect, there is no difference between dietary groups. Importantly, whereas the full range and sensitivity of the baroreflex is similar between dietary groups when constructed with PE and SNP treatment, the baroreflex set-point is significantly shifted to the left in NR sheep. We speculate that these data, together with the observation of no greater pressor responses to peripherally infused angiotensin II infusion (Figure 2) but increased blunting of baroreflex sensitivity, suggest a central origin for the resetting of baroreflex function in NR sheep. Possible mechanisms for these data are (1) increased angiotensin II action within the cardiovascular control centers of the brain (ie, the area postrema and/or nucleus of the solitary tract) or (2) a direct chronotrophic action on the heart. The present study cannot differentiate between these 2 possible mechanisms, but nutritional regulation of regional angiotensin II receptor populations (AT\(_1\) and AT\(_2\)) has been demonstrated in term ovine neonates\(^\text{34}\) and adult rat offspring.\(^\text{35,36}\) Interestingly, increased densities of central AT\(_1\) in mice has been shown to influence baroreflex setting and sensitivity.\(^\text{37}\)

The current study has shown that peri-implantation undernutrition has no major effect on resting systolic or diastolic pressures\(^\text{25–27}\) in the resulting young adult sheep. However, at this age, we show evidence of altered baroreflex function and renin-angiotensin system activity. At 3 years of age, male sheep that have undergone a similar nutritional paradigm also have altered baroreflex function but, importantly, have ele-

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**Figure 5.** The change in mean arterial blood pressure and heart rate during SNP infusion in control and periconceptually nutrient-restricted sheep. Values are minute means±SEM for control (left panel, \(n=11\)) and NR (right panel, \(n=7\)) sheep for a baseline period (5 minutes), 5 minutes of SNP infusion (2.5 \(\mu g\cdot kg^{-1}\cdot min^{-1}\)) and 5 minutes of recovery. \(P<0.05\) control vs NR for the mean increment in heart rate during SNP infusion.

**Figure 6.** The range and function of the baroreflex in control and NR sheep. Data points are (A) all individual points for a representative control (\(\bullet\)) and NR (\(\square\)) sheep and (B) for means±SEM (included every 20 seconds for clarity) for control (\(\bullet\), \(n=11\)) and NR (\(\square\), \(n=7\)) for mean arterial blood pressure and heart rate values obtained during PE and SNP infusion. The slope, range, minimum heart rate value, and sensitivity of the relationship between mean arterial blood pressure and heart rate were similar in control and NR sheep. However the set-point was significantly \((P<0.05)\) shifted to the left in NR sheep relative to controls.
Sheep were euthanized at 1 year of age using a lethal dose of sodium pentobarbitone (200 mg·kg⁻¹). Body and all major organ weights were measured.

vated prefeeding blood pressure. We therefore speculate that the results of the current study may provide an early indication of compromised cardiovascular control that eventually leads to a pathophysiological outcome as the individual ages.

**Perspectives**

Global undernutrition, during peri-implantation embryonic development only, programs cardiovascular dysfunction in the resultant young adult offspring. The data suggest 2, possibly interrelated, mechanisms for this phenomena: (1) altered baroreflex control and (2) regional increases in angiotensin II activity. The results of this study have wide-ranging implications with regard to the health outcomes of those offspring from mothers who were poorly nourished during early, often unknown pregnancy and from embryos artificially manipulated because of infertility treatment.

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