0031-3998/07/6204-0422
PEDIATRIC RESEARCH
Vol. 62, No. 4, 2007

The Effect of Maternal Undernutrition in Early Gestation on Gestation Length and Fetal and Postnatal Growth in Sheep

JANE K. CLEAL, KIRSTEN R. POORE, JAMES P. NEWMAN, DAVID E. NOAKES, MARK A. HANSON, AND LUCY R. GREEN

Centre for Developmental Origins of Health and Disease [J.K.C., K.R.P., J.P.N., M.A.H., L.R.G.], University of Southampton, Southampton, SO16 5YA United Kingdom; Department of Veterinary Reproduction [D.E.N.], Royal Veterinary College, London, AL9 7TA United Kingdom

ABSTRACT: *In utero* undernutrition in humans may result in cardiovascular (CV), metabolic, and growth adaptations. In sheep, maternal nutrient restriction during pregnancy, without effects on fetal or birth weight, results in altered CV control in the offspring. Adjustment of gestation length after undernutrition could be a strategy to enhance postnatal health/survival. The aim of this study was to determine in sheep the effect of a 50% reduction in maternal nutrient intake [undernutrition group (U) *versus* 100%, control group (C)] during 1–31 d of gestation (dGA) on gestation length and offspring size. By 28 dGA, U ewes had gained less weight than C, and twin-bearing ewes had gained less weight than singleton-bearing ewes regardless of group ($p < 0.05$). In different-sex twin pairs, maternal undernutrition resulted in longer gestation compared with C (146.5 ± 0.6 *versus* 144.6 ± 0.6 d, $p < 0.05$). Increased weight gain by weaning (20.8 ± 0.8 *versus* 17.9 ± 0.8 kg, $p < 0.05$) was observed in U male twins. These findings suggest that the strategy (*i.e.* growth rate or length of time *in utero*) adopted by the fetus to enhance immediate survival depends on offspring number and sex. This is likely to reflect the degree of constraint imposed on the fetus. **(*Pediatr Res* 62: 422–427, 2007)**

Epidemiologic studies show that the early life environment may have long-term effects on the risk of adult-onset diseases such as hypertension and coronary heart disease (CHD) (1). In studies of the Dutch Hunger Winter, maternal undernutrition during early gestation, when the nutrient demands of the conceptus are minimal, is associated with increased prevalence of cardiovascular disease (CV) disease in adulthood (2). *In utero* undernutrition in humans may result in CV and metabolic and growth adaptations (3–5) aimed at increasing energy supply or reducing energy expenditure but with postnatal consequences. The nature, timing, and intensity of an *in utero* nutritional challenge, as well as maternal age, parity, and offspring number and sex, will determine the degree of constraint (6) imposed on the fetus and are likely to dictate the strategy adopted by it to enhance immediate survival.

One way of reducing fetal energy expenditure is to reduce growth, as shown in several animal species including rat (7) and sheep (8). However, maternal nutrient restriction during pregnancy, in the absence of effects on fetal weight or birth weight, does result in altered CV control in the offspring. In sheep, a 15% reduction in maternal nutrient intake for the first half of gestation produced low fetal arterial blood pressure (9) and impaired small artery function (10) in late-gestation singleton fetuses, but no change in weight. This was followed by increased blood pressure in early postnatal life (11). Altered postnatal CV function has been confirmed in both rats (12) and sheep (13) to be associated with perturbations of the peri-implantation environment. Moreover, the first 30 days of gestational age (dGA) have been identified as a critical window for *in utero* effects on renal development and later CV function (14,15). These observations suggest that net fetal growth can be sustained during maternal nutrient restriction and that CV adaptations may be involved.

An additional adaptive strategy to enhance postnatal health/survival after undernutrition could be adjustment of gestation length. Altered gestation length will depend on the size of the nutritional challenge. In sheep, a 15% reduction in maternal body weight by undernutrition in the peri-implantation period (−60 to 30 dGA) resulted in reduced gestation length mediated by accelerated maturation of the fetal hypothalamopituitary-adrenal (HPA) axis (16). This suggests that the strategy adopted to cope with this level of maternal nutritional constraint is early delivery. In contrast, a 15% reduction in maternal nutrient intake during early gestation reduced pituitary and adrenal responses in late-gestation ovine fetuses (17). This may be expected to prolong gestation, but the effect of such a relatively mild challenge on gestation length is unknown. The aim of this study was therefore to investigate the effect of such a nutritional challenge on gestation length in relation to other constraints on the fetus, which are known to influence gestation length, such as number and sex of fetuses.

METHODS

Animals. All procedures were carried out in accordance with the regulations of the UK Home Office Animals (Scientific Procedures) Act of 1986.

Welsh Mountain ewes in their second or third parity (following on from a pregnancy in the previous season) and of uniform good body condition ($n = 88$) were brought in from pasture and housed on straw in open barns at the

Received December 5, 2006; accepted May 19, 2007.
Correspondence: Jane Cleal, Ph.D., Institute of Developmental Sciences, Mailpoint 887, Southampton General Hospital, Tremona Road, Southampton S016 6YD, UK; e-mail: jcleal@soton.ac.uk
This study was supported by The British Heart Foundation.

Abbreviations: AC, abdominal circumference; **BCS,** body condition score; **C,** control group; **CRL,** crown-rump length; **CV,** cardiovascular; **dGA,** days gestational age; **FL,** femur length; **HPA,** hypothalamopituitary-adrenal; **U,** undernutrition group

Royal Veterinary College, Hertfordshire, UK, where they were acclimatized to a complete pelleted diet (GJW Titmus, Hertfordshire, UK). The diet consisted of barley, wheat, micronized full fat soya, grass meal, molasses, chopped straw, calcium carbonate, dicalcium phosphate salt, and sheep vitamin/mineral supplement. As fed, it provided 9.6 MJ/kg (metabolizable energy) and 14.75 g crude protein. Rations were set according to initial body weight and adjusted according to guidelines for pregnant sheep (AFRC Technical Committee, 1993). Water was provided *ad libitum*.

In the normal breeding season, the estrous cycle of groups of three to four ewes was synchronized by withdrawing vaginal medroxyprogesterone acetate impregnated sponges (Veramix, Upjohn Ltd., Crawley, UK) 12 d after insertion. One of three raddled Welsh Mountain rams was introduced for 3 d, and 0 dGA was taken as the first day on which an obvious raddle mark was observed. Pregnancy was confirmed by measuring plasma progesterone concentrations at 16 dGA using an enzyme immunoassay kit (Ridgeway Science Ltd., Avington, UK) and fetus number was determined by ultrasound scanning at 60 dGA. Ewes were individually housed on straw from 7 d before conception to 37 dGA and group housed thereafter with animals at a similar gestational age.

Before conception, ewes were randomly assigned to a control group or a dietary restricted group. From 1–31 dGA (term = 147 d), ewes received either 100% [control group (C), $n = 41$; 20 singleton (s) and 21 twin (t)] or 50% of global nutritional requirements (undernutrition group (U), $n = 47$; 29s and 18t)] and 100% of requirements for the remainder of gestation. Maternal body weight and body condition score (BCS on scale of 0–5, where 0 is emaciated and 5 is obese) were measured weekly.

Cortisol and adrenocorticotropin (ACTH) were measured by immunoassay (Immulite analyzer, DPC, UK; single measurement) in maternal plasma (ethylenediaminetetraacetic acid treated) collected on 0, 30, and 65 dGA. The sensitivity of the cortisol assay was 0.2 μg/dL and the intra- and interassay coefficients of variation were 4.6% and 7.9%, respectively. The sensitivity of the ACTH assay was 9 pg/mL and the intra- and interassay coefficients of variation were 1.1% and 4.6%, respectively.

At 70 dGA, a subset of pregnant ewes (C, $n = 8$; 5s and 3t and U, $n = 11$; 8s and 3t) was killed by an overdose of barbiturate (pentobarbitone sodium Ph. Eur. 160 mg /kg i.v.; Animalcare Ltd., York, UK) and fetuses were removed (C, $n = 10$; 5s and 5t (loss of one fetus) and U, $n = 14$; 8s and 6t). Fetal and placental weight and fetal crown-rump length (CRL), abdominal circumference (AC), femur length (FL), and biparietal diameter (BPD) were recorded. Fetal organs were removed and weighed.

The remaining ewes delivered and suckled their lambs naturally until weaning at 12 wk of age (C, $n = 51$; 15s and 36t and U, $n = 51$; 21s and 30t lambs). There were approximately equal numbers of males and females in each group. At birth, all lambs were weighed and CRL, AC, FL, and BPD measurements were taken. Lambs were weighed again at 12 wk of age.

Statistics. Data are expressed as mean ± SEM. Biometric data were analyzed using an unpaired *t* test or univariate analysis of variance (ANOVA) full factorial model in which factors were early gestation diet, sex, and twinning (SPSS Inc., Chicago, IL). When analyzing the 70-dGA fetal biometric data, the factor of sex was excluded from the ANOVA, as there were insufficient animal numbers to allow this. Gestation length was also analyzed using Kaplan-Meier survival analysis (SPSS Inc.). Significance was accepted at $p < 0.05$.

RESULTS

Maternal body weight. At conception, there was no difference in maternal body weight (C, 42.8 ± 0.7 kg; U, 41.9 ± 0.7 kg) or BCS (C, 3.0 ± 0.1; U, 2.8 ± 0.1) between the two groups. There was no difference in weight at conception between twin-bearing and singleton-bearing ewes (twin, 43.08 kg *versus* singleton, 41.61 kg). By 28 dGA, U ewes had gained less weight ($p < 0.001$, Fig. 1) and had a lower BCS (−0.04 ± 0.08 *versus* 0.53 ± 0.09, $p < 0.001$) than C ewes. In addition, twin-bearing ewes gained less weight than singleton-bearing ewes by 28 dGA ($p < 0.05$, Fig. 1).

Maternal cortisol and ACTH concentrations. At 0 and 65 dGA, there were no differences in maternal baseline plasma cortisol concentrations between the dietary groups. At 30 dGA, U ewes had lower baseline plasma cortisol than C ewes

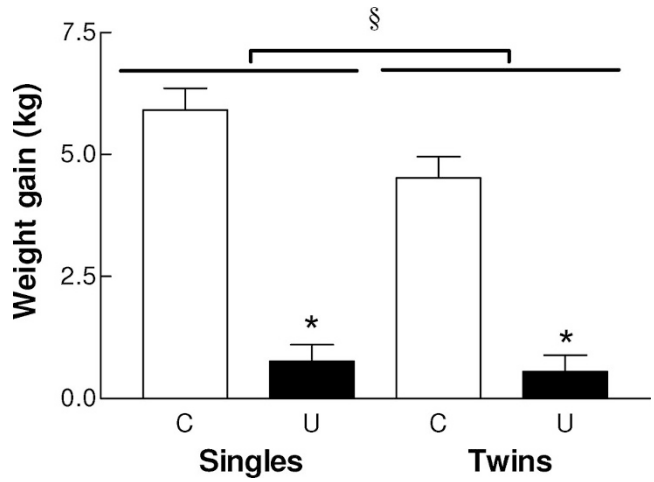

Figure 1. Maternal weight gain between 0 and 28 dGA. C (*open columns*: single $n = 18$ and twin $n = 20$) and U (*solid columns*: single $n = 28$ and twin $n = 18$) groups. *$p < 0.001$, U significantly different from C, two-way ANOVA. §$p < 0.05$, twin-bearing ewes significantly different from singleton-bearing ewes, two-way ANOVA. Data are mean ± SEM.

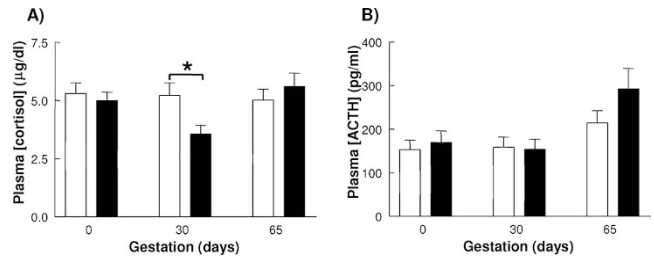

Figure 2. Maternal plasma cortisol (*A*) and ACTH (*B*) on 0, 30, and 65 dGA. C (*open columns*: $n = 41$) and U (*filled columns*: $n = 44$) groups. *$p < 0.05$, U significantly different from C, two-way ANOVA. Data are mean ± SEM.

($p < 0.05$, Fig. 2). At 0, 30, and 65 dGA, there were no differences in maternal baseline plasma ACTH concentrations between the dietary groups (Fig. 2). No differences were found in baseline cortisol and ACTH concentrations between singleton and twin pregnancies.

Fetal and placental biometry. Early gestation undernutrition had no effect on maternal weight, placental weight, placentome number, total conceptus weight, fetus/placenta weight ratio, fetus weight, biometry, or organ weights (except thymus and pancreas, which were increased in U, Table 2) at 70 dGA (Table 1). In twin pregnancies, the total conceptus weight was greater, with fewer placentomes per fetus (41.4 ± 1.0 *versus* 79.5 ± 4.3) and a greater fetus/placenta ratio compared with singleton pregnancies ($p < 0.01$, Table 1). In singleton, but not twin, fetuses at 70 dGA, CRL was less ($p < 0.05$) in U compared with C (Table 1).

Gestation length. There was no difference in gestation length between the two dietary groups (C, 145.3 ± 0.4 d *versus* U, 146.1 ± 0.4 d). Regardless of dietary group, ewes carrying twins delivered earlier than those carrying singleton fetuses ($p < 0.05$, ANOVA and Kaplan-Meier analysis, Fig. 3) and there was a tendency for a shorter gestation length in male same-sex than female same-sex twins ($p = 0.06$, data not shown). In ewes carrying a male plus female twin combination, but not same-sex twins, there was a significant increase

Table 1. *Fetal weight and biometric measurements in C and U sheep fetuses at 70 DGA*

	Singleton		Twin	
	C (n = 5)	U (n = 8)	C (n = 5)	U (n = 6)
Ewe weight, kg	45.3 ± 3.8	44.1 ± 1.6	44.4 ± 1.0	43.5 ± 1.0
Fetal weight, g	160.1 ± 10.2	146.9 ± 3.7	159.7 ± 5.2	164.2 ± 6.5
Placental weight, g	460.1 ± 60.8	461.5 ± 20.6	415.1 ± 49.3	377.5 ± 33.2
Fetal/placental weight*	0.35 ± 0.01	0.34 ± 0.01	0.41 ± 0.05	0.48 ± 0.05
Placentome number*	76.3 ± 6.4	79.7 ± 7.2	42.3 ± 1.5	39.8 ± 1.5
CRL, mm	163.3 ± 2.3	154.5 ± 1.4†	166.8 ± 3.3	167.7 ± 3.4
AC, mm	119.8 ± 4.7	119.9 ± 2.3	128.2 ± 2.9	123.3 ± 2.7
BPD, mm	27.7 ± 0.7	25.8 ± 0.6	27.2 ± 0.7	28.5 ± 0.4
FL, mm	32.8 ± 2.8	28.9 ± 1.0	32.8 ± 3.2	36.7 ± 0.3

Data are mean ± SEM.
* $p < 0.01$, twin fetuses significantly different from singleton fetuses, two-way ANOVA.
† $p < 0.05$, U significantly different from C, two-way ANOVA.

Table 2. *Fetal organ weights as a percentage of body weight (and as absolute weight) in C and U sheep fetuses at 70 DGA*

	Singleton		Twin	
	C (n = 5)	U (n = 8)	C (n = 5)	U (n = 6)
Brain, % (g)	2.79 ± 0.10 (4.41 ± 0.13)	2.91 ± 0.08 (4.28 ± 0.10)	2.86 ± 0.10 (4.54 ± 0.13)	2.82 ± 0.09 (4.61 ± 0.11)
Thymus, % (g)	0.07 ± 0.01* (0.12 ± 0.02)*	0.10 ± 0.01 (0.14 ± 0.02)	0.07 ± 0.01 (0.10 ± 0.02)	0.10 ± 0.01 (0.16 ± 0.02)
Heart, % (g)	0.92 ± 0.04 (1.46 ± 0.07)	0.83 ± 0.03 (1.22 ± 0.06)	0.89 ± 0.04 (1.42 ± 0.07)	0.96 ± 0.04 (1.56 ± 0.07)
Lung, % (g)	4.90 ± 0.26 (7.80 ± 0.50)	4.90 ± 0.20 (7.22 ± 0.40)	4.87 ± 0.26 (7.77 ± 0.50)	4.84 ± 0.24 (7.97 ± 0.46)
Liver, % (g)	6.74 ± 0.28† (10.88 ± 0.70)	6.81 ± 0.22 (10.01 ± 0.55)	6.28 ± 0.28 (10.01 ± 0.70)	6.21 ± 0.25 (10.20 ± 0.63)
Kidney, % (g)	0.46 ± 0.03 (0.73 ± 0.06)	0.51 ± 0.03 (0.74 ± 0.05)	0.48 ± 0.03 (0.76 ± 0.06)	0.54 ± 0.03 (0.78 ± 0.05)
Adrenal, % (g)	0.018 ± 0.002† (0.029 ± 0.004)†	0.018 ± 0.002 (0.026 ± 0.003)	0.022 ± 0.002 (0.036 ± 0.004)	0.023 ± 0.002 (0.038 ± 0.004)
Pancreas, % (g)	0.16 ± 0.02* (0.25 ± 0.03)	0.20 ± 0.01 (0.29 ± 0.02)	0.13 ± 0.02 (0.21 ± 0.03)	0.16 ± 0.02 (0.25 ± 0.03)

Data are mean ± SEM.
* $p < 0.05$, U significantly different from C, three-way ANOVA.
† $p < 0.01$, twin fetuses significantly different from singleton fetuses, two-way ANOVA.

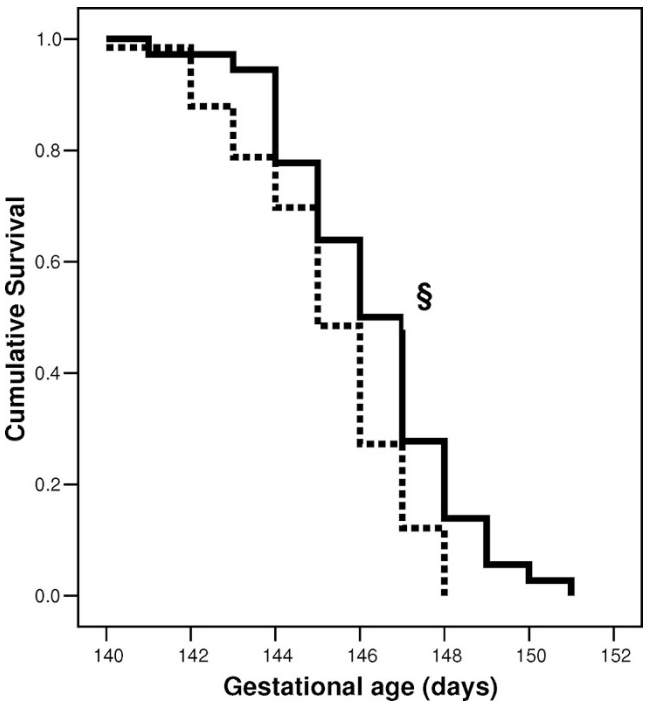

Figure 3. Kaplan-Meier survival analysis of the cumulative incidence of nondelivery in ewes carrying singleton or twin fetuses. §$p < 0.05$, twin-carrying ewes (*broken line*) significantly different from singleton-carrying ewes (*solid line*).

in gestation length in U (146.5 ± 0.6 d, n = 8) compared with group C (144.6 ± 0.6 d, n = 9) ($p < 0.05$, t test and Kaplan-Meier analysis, Fig. 4). In male twin, but not singleton, lambs, gestation length was inversely correlated with maternal weight gain between 0 and 28 dGA ($p < 0.05$, $R^2 = 0.14$).

Birth and postnatal biometry. At birth, regardless of dietary group, twin lambs had smaller ($p < 0.05$) biometric measurements than singletons (Table 3). There was no difference in weight and biometry between male and female lambs (Table 3). In both singleton and twin fetuses (male and female combined), there were no differences between C and U in weight (singleton: C, 3.9 ± 0.2 kg *versus* U, 4.0 ± 0.2 kg; twin: C, 3.7 ± 0.1 kg *versus* U, 3.6 ± 0.1 kg) or biometry (data not shown) at birth. In male twin, but not singleton, lambs, birth weight was inversely correlated with maternal weight gain between 0 and 28 dGA, an effect that was stronger in same-sex male twins (Fig. 5). There was no relationship between birth weight and maternal weight change 0–28 dGA in female fetuses. In all animals together, weight gain between birth and 12 wk old was positively correlated with birth weight in U ($p < 0.05$, $R^2 = 0.10$), but not C ($p = 0.68$, $R^2 = 0.004$). At 12 wk of age, (weaning) twins weighed less ($p < 0.001$) than singletons, and females weighed less ($p < 0.001$) than males (Table 3). In twin, but not singleton, male offspring, weight at 12 wk and weight gain between birth and 12 wk were greater ($p < 0.05$) in U compared with C (Table 3).

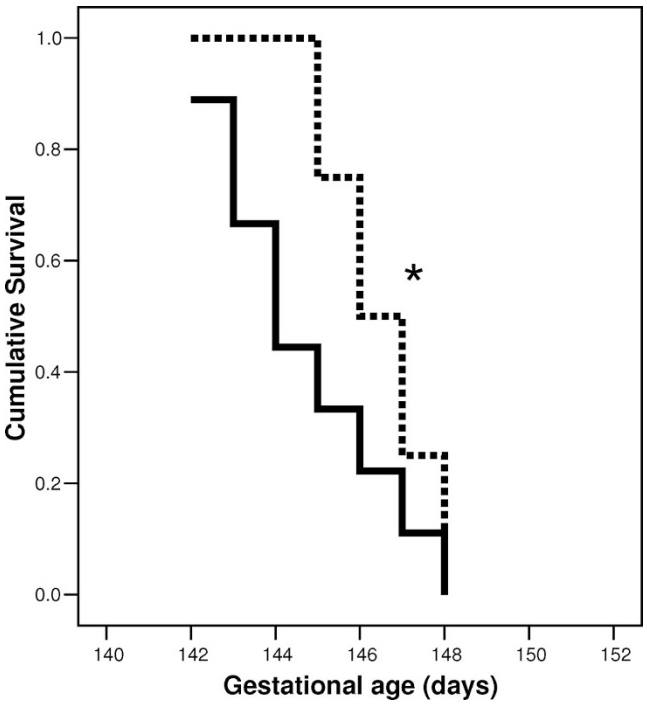

Figure 4. Kaplan-Meier survival analysis of the cumulative incidence of nondelivery in ewes carrying different-sex twin fetuses. *$p < 0.05$, U (*broken line*) significantly different from C (*solid line*).

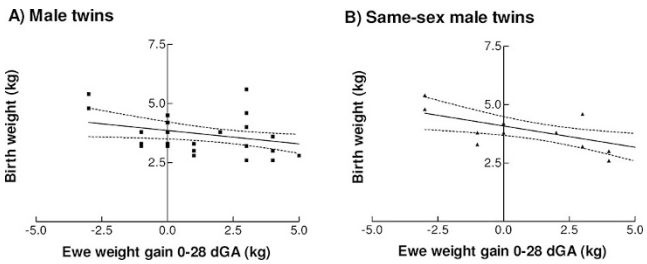

Figure 5. The relationship between birth weight and maternal weight change during 0–28 dGA. (*A*) Male twin lambs $R^2 = 0.15$, $p < 0.05$. (*B*) Same-sex male twins $R^2 = 0.40$, $p < 0.01$.

DISCUSSION

This study has shown that moderate maternal nutrient restriction from 1 to 31 dGA in sheep affects twin pregnancies to a greater extent than singleton pregnancies. Moreover, this nutrient restriction produces a small but significant increase in gestation length in different-sex twins and increased weight gain by weaning in male twins.

Maternal body composition and nutrition as well as age, parity, and offspring number and sex determine the degree of constraint imposed on the fetus and are likely to dictate the strategy it adopts to enhance immediate survival. In this study, the undernutrition given during early gestation may have acted to constrain fetal growth (although no measurements of growth were taken at this time in this study). Male embryos may be affected by undernutrition to a greater extent than females as they grow at a greater rate (18). However there were insufficient numbers in the present study to analyze according to sex. Maternal undernutrition had no effect on placental weight, fetal body weight, or biometry at 70 dGA or weight at birth. Although we cannot rule out a type II error, the undernutrition did increase the thymus and pancreas (as a percentage of body weight) at 70 dGA. Such increased pancreatic weight may result from altered maturation and lead to altered postnatal function. It may be too soon or too difficult to detect small changes in body weight or biometry at 70 dGA, but the effects of the undernutrition may become apparent in later life in terms of catch-up growth.

Constraint on fetal growth and development is likely to be greater in twin than singleton pregnancies, each of which has a distinct fetal growth pattern (19). In this study, twin fetuses were smaller than singletons as evident from biometry at birth but not mid-gestation weight or birth weight. Maternal weight gain during 0–28 dGA was less in twin-carrying compared with singleton-carrying ewes. This suggests that even around the time of implantation, a twin pregnancy has a greater demand on maternal energy expenditure or increases partitioning of nutrients toward the conceptus, each of which could deplete maternal body resources. In this study, the ewes could not increase food intake to cope with such demands as we have rationed their food intake according to 100% or 50% of recommended food intake for singleton pregnancies, as it is not technically feasible to assess fetal number by ultrasonography at the time in gestation that the challenge was carried out.

Twin fetuses may have a greater exposure to the physiologic, neuroendocrine, or metabolic adaptations made by the mother during times of suboptimal nutrition. Alternatively, twins may receive a greater nutritional insult than the singleton fetuses by virtue of a greater fetus-to-placenta weight ratio. The effects of adaptations to undernutrition when combined with twinning may become more apparent later in gestation as growth rate and energy demands increase and *in utero* space becomes more limiting. Such speculation is supported by the small ($R^2 = 0.15$) but significant association between increased birth weight and reduced ewe weight gain from 0 to 28 dGA in male twin, but not singleton, lambs. Interestingly, there was a similar but stronger association ($R^2 = 0.4$) in male-male twins, and in female offspring, no such association was seen. These data suggest that the effects of undernutrition on fetal growth are also influenced by its sex. At 12 wk of postnatal age, twin male lambs, which received early gestation undernutrition, were heavier and had an increased weight gain from birth until 12 wk old compared with controls. Hence, the increased constraint of being a twin and a male embryo (18) in a nutrient-restricted early intrauterine environment induces a phenotype more likely to gain weight once removed from the constrained *in utero* environment and adequate nutrition is restored. Such an increase in weight gain could be detrimental to CV function in later life, as studies in humans have shown that accelerated weight gain during early postnatal life is associated with increased risk of CV disease in adulthood (20,21). The male sheep in this study have indeed shown an association between increased weight at weaning and reduced glucose tolerance at 2.5 y of age (22), a factor associated with the metabolic syndrome.

In different-sex twin pairs only, maternal undernutrition increased gestation length. We speculate that the female fetus

Table 3. *Birth weight and biometric measurements*

Male	Singleton		Twin	
	C (n = 8)	U (n = 15)	C (n = 15)	U (n = 14)
Birth weight, kg	3.8 ± 0.3	4.0 ± 0.3	3.5 ± 0.2	3.8 ± 0.2
CRL, cm*	41.6 ± 1.2	43.8 ± 0.9	37.5 ± 0.8	38.2 ± 0.9
AC, cm*	42.2 ± 1.0	42.5 ± 0.8	38.5 ± 0.7	39.8 ± 0.8
BPD, mm*	57.8 ± 1.2	61.0 ± 0.9	53.4 ± 1.1	54.9 ± 1.3
FL, cm*	12.0 ± 0.4	12.0 ± 0.3	10.8 ± 0.3	11.1 ± 0.3
12-wk weight, kg*†	26.1 ± 1.1	28.1 ± 0.8	21.8 ± 0.8	24.5 ± 0.9‡
Weight gain 0–12 wk, kg*†	22.3 ± 1.0	24.4 ± 0.9	17.9 ± 0.8	20.8 ± 0.8‡
Female	**Singleton**		**Twin**	
	C (n = 7)	U (n = 6)	C (n = 13)	U (n = 16)
Birth weight, kg	3.9 ± 0.3	4.0 ± 0.3	3.8 ± 0.2	3.5 ± 0.2
CRL, cm*	44.7 ± 1.5	43.8 ± 1.5	39.1 ± 0.9	39.1 ± 0.9
AC, cm*	44.6 ± 1.5	42.5 ± 1.5	39.5 ± 0.8	39.9 ± 0.8
BPD, mm*	57.17 ± 1.7	57.0 ± 1.7	56.0 ± 1.0	52.3 ± 1.0
FL, cm*	12.7 ± 0.6	12.4 ± 0.6	11.3 ± 0.3	11.3 ± 0.3
12-wk weight, kg*	23.2 ± 0.7	24.0 ± 0.7	21.7 ± 0.8	19.9 ± 0.8
Weight gain 0–12 wk, kg*	19.3 ± 0.8	20.0 ± 0.8	17.8 ± 0.8	16.4 ± 0.8

Data are mean ± SEM. Male and female offspring at birth.

* $p < 0.01$, twin fetuses significantly different from singleton fetuses, three-way ANOVA.

† $p < 0.01$, male lambs significantly larger than female lambs, three-way ANOVA.

‡ $p < 0.05$, U significantly different from C within singles and twins, unpaired t test.

influences this increased gestation length because different-sex and female same-sex human twins are reported to have longer gestation than male same-sex twins (23). In keeping with this concept, there was a tendency for longer gestation length in female same-sex compared with male same-sex twins in the present study; however, the mechanisms underlying this sex-twin effect are unclear. Interestingly, in male twin, but not singleton, lambs, gestation length was inversely correlated with maternal weight gain between 0 and 28 dGA. These data suggest that, in male twins, undernutrition (reduced maternal weight gain) leads to increased birth weight and increased gestation length. These observations could be a direct consequence of alterations to the preimplantation embryo environment because *in vitro* culture of sheep embryos increases gestation length compared with *in vivo* embryos (24). The increase in the growth rate of ewes in the period after cessation of the nutritional insult may also have contributed to the increased gestation length because in humans (25) and in a high-growth mouse model [resulting from the lack of expression of Socs-2 (see Corvo *et al.* (26)], a greater weight gain during pregnancy is associated with a longer gestation length.

Mechanisms linking altered intrauterine environment to altered gestation length could involve the HPA axis, as the preparturient fetal cortisol surge is essential for the initiation of normal labor (27). Indeed, in the present study, nutrient restriction resulted in reduced maternal plasma cortisol levels at 30 dGA, and previously we demonstrated that mild undernutrition (a 15% reduction in maternal nutrient intake) during early gestation reduced pituitary and adrenal responsiveness in late gestation ovine fetuses (28). This is consistent with our current observation of a small increase in gestation length after a moderate maternal nutritional challenge. In contrast, accelerated maturation of the HPA axis seen with a more severe undernutrition challenge (16) appears to trigger early maturation and delivery of the fetus. Taken together, these findings suggest that the developmental strategy adopted by the fetomaternal unit depends greatly on the timing, duration, and severity of the insult. The increased gestation length, although modest, could thus be part of a strategy for allowing more time *in utero* for maturation and growth or could be *via* changes in HPA axis function, which convey some later advantage.

In summary, this study has shown that moderate early gestation maternal nutrient restriction can increase gestation length without altering mid-gestation fetal size or birth weight and can have additional influences on postnatal growth. However, these findings are influenced by fetal sex and number, indicating that aspects of the maternal diet may be transmitted in a graded manner to the embryo during the very early stages of development. The results of this study will be important for informing public health strategies on diet in pregnancy aimed at reducing the risk of later CV and metabolic disease in humans.

REFERENCES

1. Gluckman PD, Hanson MA 2004 Living with the past: evolution, development, and patterns of disease. Science 305:1733–1736
2. Roseboom TJ, van der Meulen JH, Osmond C, Barker DJ, Ravelli AC, Schroeder-Tanka JM, van Montfrans GA, Michels RP, Bleker OP 2000 Coronary heart disease after prenatal exposure to the Dutch famine, 1944–45. Heart 84:595–598
3. Haugen G, Hanson M, Kiserud T, Crozier S, Inskip H, Godfrey KM 2005 Fetal liver-sparing cardiovascular adaptations linked to mother's slimness and diet. Circ Res 96:12–14
4. Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN, Bleker OP 1998 Glucose tolerance in adults after prenatal exposure to famine. Lancet 351:173–177
5. Roseboom TJ, van der Meulen JH, Ravelli AC, Osmond C, Barker DJ, Bleker OP 2001 Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. Mol Cell Endocrinol 185:93–98
6. Gluckman PD, Hanson MA 2004 Maternal constraint of fetal growth and its consequences. Semin Fetal Neonatal Med 9:419–425
7. Woodall SM, Breier BH, Johnston BM, Gluckman PD 1996 A model of intrauterine growth retardation caused by chronic maternal undernutrition in the rat: effects on the somatotrophic axis and postnatal growth. J Endocrinol 150:231–242

8. Mellor DJ, Murray L 1982 Effects of long-term undernutrition of the ewe on the growth-rates of individual fetuses during late pregnancy. Res Vet Sci 32:177–180
9. Hawkins P, Steyn C, Ozaki T, Saito T, Noakes DE, Hanson MA 2000 Effect of maternal undernutrition in early gestation on ovine fetal blood pressure and cardiovascular reflexes. Am J Physiol Regul Integr Comp Physiol 279:R340–R348
10. Ozaki T, Hawkins P, Nishina H, Steyn C, Poston L, Hanson MA 2000 Effects of undernutrition in early pregnancy on systemic small artery function in late-gestation fetal sheep. Am J Obstet Gynecol 183:1301–1307
11. Hawkins P, Steyn C, McGarrigle HH, 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
12. Kwong WY, Wild AE, Roberts P, Willis AC, Fleming TP 2000 Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. Development 127:4195–4202
13. Gardner DS, Pearce S, Dandrea J, Walker R, Ramsay MM, Stephenson T, Symonds ME 2004 Peri-implantation undernutrition programs blunted angiotensin II evoked baroreflex responses in young adult sheep. Hypertension 43:1290–1296
14. Dodic M, May CN, Wintour EM, Coghlan JP 1998 An early prenatal exposure to excess glucocorticoid leads to hypertensive offspring in sheep. Clin Sci 94:149–155
15. Wintour EM, Moritz KM, Johnson K, Ricardo S, Samuel CS, Dodic A 2003 Reduced nephron number in adult sheep, hypertensive as a result of prenatal glucocorticoid treatment. J Physiol 549:929–935
16. Bloomfield FH, Oliver MH, Hawkins P, Campbell M, Phillips DJ, Gluckman PD, Challis JR, Harding JE 2003 A periconceptional nutritional origin for noninfectious preterm birth. Science 300:606
17. Hawkins P, Steyn C, McGarrigle HH, Saito T, Ozaki T, Stratford LL, Noakes DE, Hanson MA 1999 Effect of maternal nutrient restriction in early gestation on development of the hypothalamic-pituitary-adrenal axis in fetal sheep at 0.8-0.9 of gestation. J Endocrinol 163:553–561
18. Pedersen JF 1980 Ultrasound evidence of sexual difference in fetal size in first trimester. BMJ 281:1253
19. Blickstein I 2004 Is it normal for multiples to be smaller than singletons? Best Pract Res Clin Obstet Gynaecol 18:613–623
20. Eriksson JG, Forsen T, Tuomilehto J, Osmond C, Barker DJ 2001 Early growth and coronary heart disease in later life: longitudinal study. BMJ 322:949–953
21. Singhal A, Cole TJ, Fewtrell M, Deanfield J, Lucas A 2004 Is slower early growth beneficial for long-term cardiovascular health? Circulation 109:1108–1113
22. Poore KR, Cleal JK, Newman JP, Boullin JP, Noakes DE, Hanson MA, Green LR 2007 Nutritional challenges during development induce sex-specific changes in glucose homeostasis in the adult sheep. Am J Physiol Endocrinol Metab 292:E32–E39
23. Loos RJ, Derom C, Eeckels R, Derom R, Vlietinck R 2001 Length of gestation and birthweight in dizygotic twins. Lancet 358:560–561
24. Holm P, Walker SK, Seamark RF 1996 Embryo viability, duration of gestation and birth weight in sheep after transfer of in vitro matured and in vitro fertilized zygotes cultured in vitro or in vivo. J Reprod Fertil 107:175–181
25. Oken E, Kleinman KP, Olsen SF, Rich-Edwards JW, Gillman MW 2004 Associations of seafood and elongated n-3 fatty acid intake with fetal growth and length of gestation: results from a US pregnancy cohort. Am J Epidemiol 160:774–783
26. Corva PM, Muccia NC, Evans K, Medrano JF 2004 Diet effects on female reproduction in high growth (hg/hg) mice that are deficient in the Socs-2 gene. Reprod Nutr Dev 44:303–312
27. Challis JR, Matthews SG, Gibb W, Lye SJ 2000 Endocrine and paracrine regulation of birth at term and preterm. Endocr Rev 21:514–550
28. Hawkins P, Steyn C, McGarrigle HH, Saito T, Ozaki T, Stratford LL, Noakes DE, Hanson MA 1999 Effect of maternal nutrient restriction in early gestation on development of the hypothalamic-pituitary-adrenal axis in fetal sheep at 0.8-0.9 of gestation. J Endocrinol 163:553–561