

Birth Weight Rather Than Maternal Nutrition Influences Glucose Tolerance, Blood Pressure, and IGF-I Levels in Sheep

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ABSTRACT

Both small size at birth in humans and maternal undernutrition in experimental animals are associated with impaired glucose tolerance and increased blood pressure in the adult offspring. However it is not clear whether maternal undernutrition in late gestation can influence postnatal phenotype, and whether this might occur independent of size at birth. We severely undernourished pregnant ewes for 10 d (UN10) or 20 d (UN20) from 105 d gestation (term = 146 d) and studied the offspring in comparison to those born to *ad libitum* fed ewes (AL20). Glucose tolerance tests and blood pressure recordings were performed at 5 mo of age and repeated at 30 mo together with insulin tolerance tests and GH challenges. UN20 lambs were lighter at birth (UN20, 4.9 ± 0.1 kg, $n = 23$; UN10, 5.3 ± 0.1 kg, $n = 26$; AL20, 5.6 ± 0.2 kg, $n = 26$) but not from weaning onward. Plasma glucose area under the curve at 5 mo and plasma insulin at 30 mo increased with current weight and decreased with increasing birth weight. Blood pressure also increased with current weight and decreased with increasing birth weight at 5 but not 30 mo. Plasma IGF-I concentrations increased with

current weight at all ages and decreased with increasing birth weight at 30 mo. Plasma insulin response to GH challenge increased with current weight. Nutrition group was not related to any of the outcomes measured when birth weight and current weight were taken into account. These data suggest that size at birth is more closely related to processes that determine postnatal phenotype than is maternal nutrition in late gestation. (*Pediatr Res* 52: 516–524, 2002)

Abbreviations

AL20, ewes fed *ad libitum*
AUC, area under the curve
CI, confidence interval
GTT, glucose tolerance test
IGFBP, IGF binding protein
ITT, insulin tolerance test
MAP, mean arterial blood pressure
UN10, ewes undernourished for 10 d (105–115 d of gestation)
UN20, ewes undernourished for 20 d (105–125 d of gestation)

The fetal origins of adult disease hypothesis proposes that small size at birth is associated with a predisposition to metabolic, endocrine, and cardiovascular disease in adult life (1). Epidemiologic studies in human populations have shown that small size at birth is associated with the development of type 2 diabetes and hypertension in adult life (2–5). The reasons for small size at birth are numerous and complex. Studies, particularly in the rodent, demonstrate that both overall maternal undernutrition and protein restriction throughout gestation lead to altered metabolic, endocrine, and cardiovascular function in postnatal life (6–10). Studies of Dutch Hunger Winter survi-

vors suggest that severe maternal undernutrition in pregnant women may also lead to glucose intolerance in their offspring (11).

However a number of questions remain. The favored mechanism to explain the fetal origins of adult disease hypothesis is that an adverse environment, and particularly fetal nutritional demand greater than that supplied by the fetoplacental unit, leads to a series of fetal adaptations. It is proposed that these fetal adaptations to limited nutrient supply result in both reduced fetal growth and permanent changes in structure and function (programming), which in turn lead to predisposition to adult disease (12, 13). However, we have shown in sheep that maternal undernutrition can effect marked changes in fetal growth and metabolism without changing overall weight, length, or head circumference of the lamb (14–16). This raises the question of whether programming of postnatal physiologic function can occur without significant change in size at birth. We hypothesized that reduced size at birth is not on the causal pathway between maternal undernutrition and adult disease,

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and therefore that maternal undernutrition in late gestation would result in altered glucose tolerance and blood pressure of the offspring independent of size at birth.

A further question surrounds the possible underlying endocrine mechanisms of programming. It has been proposed that insulin resistance or GH or IGF-I resistance or excessive glucocorticoid exposure, or some combination of these, could all explain the features of the fetal origins syndrome and thus provide a series of unitary underlying mechanisms (1, 12, 17, 18). However, the effect of changes in maternal nutrition on GH or IGF sensitivity in the adult offspring has received little study.

Finally, the timing and duration of maternal undernutrition required to produce postnatal consequences has received limited study, mostly in early gestation (19–23). We have previously shown that maternal undernutrition in the periconceptual period alters growth trajectory and metabolic, endocrine, and cardiovascular regulation in the late-gestation fetal sheep (14, 15, 24–26). However, the postnatal consequences of varying periods of maternal undernutrition in late gestation have not been determined.

We therefore undertook this long-term study of the effects of maternal undernutrition in sheep. We aimed to distinguish the effects of maternal undernutrition in late gestation from those of birth weight in determining glucose tolerance, blood pressure, and responsiveness to GH in prepubertal and adult offspring.

METHODS

Romney ewes were mated with Dorset rams after synchronization of estrous with the use of intravaginal progesterone-release devices (27). Ewes were scanned by ultrasound 60 d after mating, and only those found to be carrying singleton lambs were used for these experiments. Throughout early and mid gestation ewes remained on pasture but received 100 to 200 g of feed concentrates per day (Multifeed Sheep nuts, NRM Ltd., Auckland, New Zealand) as a supplement and also to aid familiarization to this form of feed. The concentrate diet had 10 MJ metabolizable energy per kilogram (ruminant) and 12.9% crude protein. At 95 d of gestation ewes were placed into individual indoor pens, fed the concentrate diet (28), and allowed free access to water and barley straw of low nutritional value.

Ewes were acclimatized indoors for 10 d before allocation to a treatment group using random number tables. From 105 d control ewes were fed *ad libitum* (AL20, approximately 13 and up to 15 MJ/d at 125 d) while restricted ewes were undernourished for either 10 d (UN10, 0.3 MJ/d until refeeding at 115 d) or 20 d (UN20, 0.3 MJ/d until 115 d then 0.5 MJ/d until refeeding at 125 d). Barley straw rations were increased during periods of restriction to provide bulk and maintain rumen function. At 125 d ewes were returned to supplemented pasture feeding in preparation for lambing.

Soon after birth lambs had their ears tagged, their blood was sampled, they were weighed, and their body proportions were measured. Weights and other measurements were repeated at weaning (3 mo) and at 5 and 30 mo of age. Details of these

measurements are available elsewhere (28). At 5 mo lambs (ewes: AL20, $n = 11$; UN10, $n = 11$; UN20, $n = 12$; rams: AL20, $n = 7$; UN10, $n = 10$; UN20, $n = 10$) were acclimatized to concentrate feeding in the farm feedlot for approximately 10 d before being transported into the laboratory for GTTs and blood pressure monitoring.

On arrival at the laboratory lambs were housed in individual metabolic cages with access to water and food *ad libitum*. Our sheep laboratory has temperature control ($16 \pm 1^\circ\text{C}$, humidity $50 \pm 10\%$) and a 12-h light–dark cycle. After at least 1 wk of laboratory acclimatization lambs were weighed and fasted the night before surgical implantation of catheters. Antibiotics were administered intramuscularly (5 mL of Streptopen, Pitman Moore, Wellington, New Zealand) before the start of surgery. Surgery was performed under general anesthesia (2% halothane in O_2). Catheters were placed in a femoral artery and vein, and a subcutaneous catheter was placed under the skin at an equivalent vertical position to the tip of the arterial catheters. Five to 7 d of recovery was allowed before experiments commenced. Arterial blood pressure and subcutaneous pressure measurements were collected for 12 h via pressure transducer recording devices (Novotrans II, MX860; Medex, Dublin, OH, U.S.A.). Subcutaneous pressure was used as a zero reference to correct for postural changes and was subtracted electronically. Pressure signals were low-pass filtered at 20 Hz. Pressure data were collected on-line using real-time software according to published methods (29). Lambs were fasted overnight and then injected i.v. with 0.5 g/kg body weight glucose. Heparinized blood samples were taken before fasting and –10, 0, 1, 3, 5, 7, and 10 min from i.v. glucose injection. Lambs were refed after the completion of the sampling. All blood samples were collected on ice, then centrifuged at 3000 rpm. Plasma aliquots were stored at -20°C until analysis. Once experiments were completed, the catheters were removed and the lambs were returned to the research farm.

At 30 mo of age ewes ($n = 11$ per group) were bought back into the laboratory for GTT, ITT, blood pressure monitoring, and GH sensitivity testing. Acclimatization, surgical and recovery procedures, and blood pressure monitoring procedures were essentially the same as those described above. From our experience with GTTs at 5 mo we decided to reduce the size of the glucose dose and extend the period of sampling. Ewes were fasted overnight and then injected i.v. with 0.2 g/kg body weight glucose. Heparinized blood samples were taken in a fed state and –10, 0, 2.5, 5, 7, 10, 20, 30, 40, 50, 60, 90, 120, 150, and 180 min from i.v. glucose injection. Ewes were refed after the completion of the sampling. ITTs were performed approximately 1 wk later using essentially the same sampling protocol with the addition of extra samples taken at 70 and 80 min after i.v. injection of regular insulin (0.15 U/kg body weight, Humulin R; Eli Lilly, Indianapolis, IN, U.S.A.). Blood samples were handled in the manner described above. One week after the ITTs ewes were again fasted overnight before receiving an intramuscular injection of bovine GH (bGH, 0.15 mg/kg). Immediately after the injection ewes were refed and further injections of bGH at the same dose were given 12, 24, and 36 h later. Blood samples were collected before fasting and –10, 0, 6, 12, 24, 36, and 48 h after the first bGH injection. Forty-eight

hours after the start of GH treatment ewes were euthanized with an overdose of pentobarbitone. Experiments were approved by the Animal Ethics Committee of the University of Auckland.

Assays. Plasma glucose concentration was analyzed using standard enzymatic micromethods modified for 96-well microplates (30, 31). Plasma insulin concentrations were measured by an RIA established and validated for ovine plasma in this laboratory. The insulin RIA was identical to our previously published method (32) except that ovine insulin (batch No. I9254; Sigma Chemical Co., St. Louis, MO, U.S.A.) was used as the standard. The standard curve displaced in parallel with ovine plasma samples and cross-reactivity with IGF-I or IGF-II was lower than 0.01%. The minimal detectable concentration was 60 pg/mL plasma, and the inter- and intraassay coefficients of variation were 11.1 and 6.7%, respectively.

Plasma IGF-I concentrations were measured by IGFBP-blocked RIA (33) using a polyclonal antibody (No. 878/4) that has very high affinity and specificity for IGF-I and low cross-reactivity with IGF-II (<0.01%). Dilution and acidification of plasma to pH 2.8 is followed by addition of excess IGF-II (batch No. 099EM9, 25 ng/tube; Eli Lilly) to block IGFBP interference. The RIA exhibits complete parallelism between serial dilutions of ovine plasma and the rhIGF-I standard (No. GO80AB; Genentech, South San Francisco, CA, U.S.A.). The recovery of unlabeled rhIGF-I added before assay was $95 \pm 6.6\%$ ($n = 16$). The ED_{50} was 0.1 ng/tube, the detection limit was 0.7 ng/mL, and the inter- and intraassay coefficients of variation were 10.1 and 5.0% respectively.

Plasma IGF-II concentrations were measured using a double-antibody RIA developed for ovine plasma (33). Plasma samples were extracted after an acid-ethanol (AE) cryoprecipitation procedure described previously (34). The standard ovine IGF-II (oIGF-II) was diluted in PBS-Tris-AE buffer pH 7.6. An hIGF-II antibody (No. P53) raised in this laboratory was used at a final concentration of 1:3600 diluted in assay buffer (0.05 M sodium phosphate, 0.1 M NaCl, 0.2% BSA, 0.1% Triton X-100, 0.05% NaN_2 , pH 7.8) containing 37.5 ng of rhIGF-I per 100 μ L (batch No. 56820A51; Pharmacia, Uppsala, Sweden). Parallel displacement curves between purified ovine IGF-II, rhIGF-II, and serial dilutions of sheep plasma were achieved. The recoveries of oIGF-II in different plasma samples were $88 \pm 7\%$ ($n = 9$). The minimal detectable concentration of IGF-II was 10 ng/mL plasma. Inter- and intraassay coefficients of variation were 9.7 and 5.1%, respectively, and cross-reactivity with IGF-I and insulin were <0.005%.

Plasma oIGFBP-2 concentrations were determined by specific RIA (35). Briefly, biIGFBP-2 (batch No. BP2AU500-CJIBP2A2; Gropep, Theburton, Australia) standards and plasma samples were diluted with assay buffer [0.05 M phosphate buffer, pH 7.4, 0.1 M NaCl, 0.2% BSA (Sigma Chemical Co.), 0.1% Triton X-100, 0.05% NaN_3]. The ED_{50} for the assay was 0.2 ng of purified oIGFBP-2 per tube, and the minimal detectable dose was 0.05 ng/tube. The inter- and intraassay coefficients of variation were 12 and 6%, respectively.

Statistical analysis. All data are presented as mean \pm SEM. Morphometric data were analyzed using factorial ANOVA with Fisher's correction for *post hoc* comparisons. MAP data were analyzed as 1-h averages and also after averaging the 12-h collection period. Total AUC was calculated for plasma glucose and insulin responses to GTT. Statistical analysis of treatment group responses during tests was performed using multiple regression with treatment group, birth weight, and current weight as independent variables. Lamb sex was also included as an independent variable up to 5 mo of age. Results of multiple regression analysis are presented in the text as regression coefficients and 95% CI. StatView 5.0.1 (SAS Institute, Cary, NC, U.S.A.) was used for all statistical analyses.

RESULTS

UN20 lambs weighed less than AL20 lambs at birth (Table 1; all $p < 0.05$), but body weight was not different among treatment groups at 3, 5, or 30 mo of age. A detailed description of maternal feed intake during nutritional manipulations, maternal weight, and fetal and placental morphology is presented in a separate publication (28).

Glucose tolerance. At 5 mo, fed and fasted plasma glucose concentrations were not influenced by treatment group, birth weight, current weight, or sex (Table 2). After injection of glucose, plasma glucose AUC was not influenced by treatment group (Fig. 1) but increased with current weight (+1.58 mM/kg body weight; 95% CI, 0.7–2.4; $p < 0.001$) and decreased with increasing birth weight (–6.0 M/kg birth weight; 95% CI, –11.0 to –0.8 mM; $p < 0.05$; Table 3). Fed and fasted plasma insulin concentrations were not influenced by treatment group, birth weight, or sex, but both increased with current weight. For every kilogram of weight at 5 mo, fed plasma insulin concentrations were 12.3 pM higher (95% CI, 4.7–20.0; $p < 0.01$), whereas fasted plasma insulin concentrations were 2.9 pM higher (95% CI, 0.5–5.6; $p < 0.05$; Table 3). Plasma insulin AUC during GTT was not influenced by treatment group, birth weight, current weight, or sex.

At 30 mo, fed and fasted plasma glucose concentrations and plasma glucose AUC after glucose bolus were not influenced by treatment group, birth weight, or current weight (Tables 2 and 3 and Fig. 2). Fed plasma insulin concentrations were not influenced by treatment group (Tables 2 and 3), but increased with current weight (+4.4 pM/kg body weight; 95% CI,

Table 1. Effect of nutrition group on growth of offspring

	AL20	UN10	UN20
Ewes (kg)			
Birth	5.6 \pm 0.2 (13)	5.1 \pm 0.2 (13)	4.8 \pm 0.2 (13)*
3 mo†	28 \pm 1 (11)	28 \pm 1 (12)	27 \pm 1 (12)
5 mo	28 \pm 1 (11)	29 \pm 2 (11)	27 \pm 1 (12)
30 mo	74 \pm 2 (11)	72 \pm 2 (11)	69 \pm 2 (11)
Rams			
Birth	5.3 \pm 0.4 (10)	5.5 \pm 0.2 (13)	5.0 \pm 0.2 (13)
3 mo	30 \pm 1 (8)	29 \pm 1 (12)	29 \pm 1 (13)
5 mo	33 \pm 1 (8)	31 \pm 1 (10)	29 \pm 2 (10)

Values are mean \pm SEM. Brackets contain number of animals at each time.

* Significantly different from AL20 ($p \leq 0.05$).

† Sex difference (rams > ewes, $p < 0.05$).

Table 2. Effect of treatment on fed and fasted plasma glucose and insulin concentrations in ewes

Group	Glucose (mM)		Insulin (pM)	
	Fed	Fasted	Fed	Fasted
5 mo pre-GTT				
AL20 (11)	4.1 ± 0.1	3.7 ± 0.1	120 ± 20	20 ± 10
UN10 (11)	4.3 ± 0.2	3.9 ± 0.2	160 ± 30	30 ± 10
UN20 (12)	4.3 ± 0.2	3.7 ± 0.1	150 ± 40	30 ± 10
30 mo pre-GTT				
AL20 (11)	3.5 ± 0.1	3.3 ± 0.1	70 ± 10	40 ± 10
UN10 (11)	3.5 ± 0.1	3.1 ± 0.0	90 ± 10	40 ± 10
UN20 (11)	3.6 ± 0.1	3.4 ± 0.1	100 ± 20	50 ± 10
30 mo pre-ITT				
AL20 (11)	3.5 ± 0.1	3.3 ± 0.1	80 ± 10	40 ± 10
UN10 (11)	3.3 ± 0.1	3.2 ± 0.1	60 ± 10	40 ± 10
UN20 (11)	3.5 ± 0.1	3.2 ± 0.1	80 ± 10	40 ± 10

Values are mean ± SEM. Numbers in brackets represent number of animals studied. There are no significant differences among treatment groups.

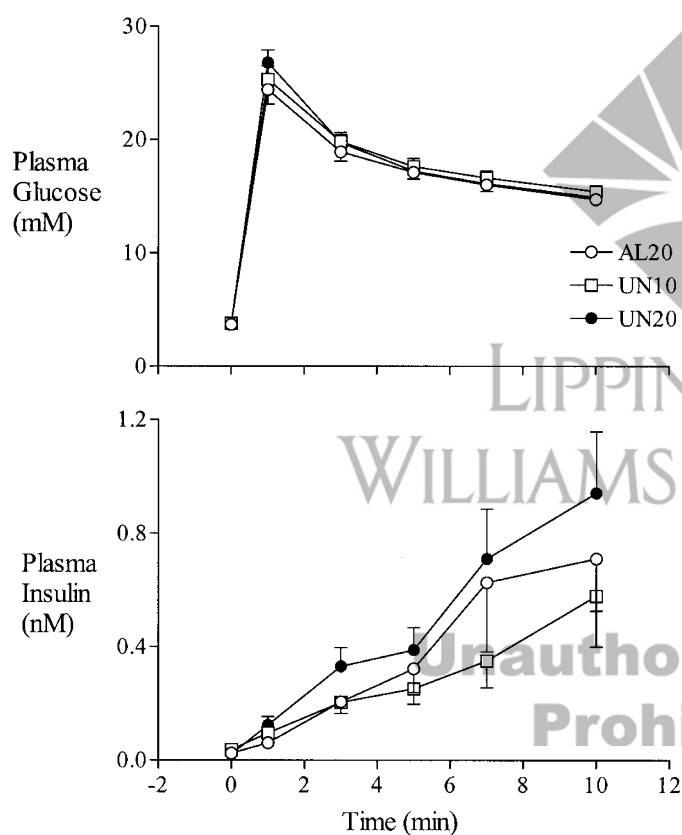


Figure 1. Plasma glucose (top) and insulin (bottom) responses to i.v. GGT (0.5 g glucose/kg body weight) in 5-mo-old lambs. Values are mean ± SEM.

2.0–6.9; $p < 0.01$) and decreased with increasing birth weight (–35 pM/kg birth weight; 95% CI, –59 to –10; $p < 0.01$). Treatment group, birth weight, or current weight did not influence fasted plasma insulin concentrations (Tables 2 and 3 and Fig. 2) or plasma insulin AUC at 30 mo. However when treatment group, birth weight, and current weight are all included in the analysis, plasma insulin AUC increased with current weight (+3.5 pM/kg body weight; 95% CI, –0.09 to 7.1; $p < 0.06$).

Insulin tolerance. At 30 mo, fed and fasted plasma glucose concentrations and fasted plasma insulin concentrations were

not influenced by treatment group, birth weight, or current weight (Table 2 and Fig. 3). Fed plasma insulin concentrations were not influenced by treatment group or birth weight but increased with current weight (+1.9 pM/kg body weight; 95% CI, 0.3–3.6; $p < 0.05$; Table 2). Plasma glucose and insulin responses to insulin injection were not influenced by current weight. Plasma insulin concentrations were higher in UN20 ewes 2.5 min after insulin injection (+959 pM; 95% CI, 357–1561; $p < 0.01$) but also increased with increasing birth weight (+524 pM/kg birth weight; 95% CI, 71–977; $p < 0.05$).

Blood pressure. At 5 mo, MAP recorded during a 12-h period was not influenced by treatment group (Table 4) or sex, but MAP did increase with current weight (+0.9 mm Hg/kg body weight; 95% CI, 0.26–1.5; $p < 0.01$; Table 3) and decreased with increasing weight at birth (–4.0 mm/kg birth weight; 95% CI, –7.8 to –0.15; $p < 0.05$). At 30 mo, MAP was not influenced by treatment group, birth weight, or current weight.

GH-IGF axis. Plasma IGF-I concentrations at birth were not influenced by treatment group (Table 4) or sex but increased with birth weight (+5.2 nM/kg birth weight; 95% CI, 3.3–7.1; $p < 0.0001$; Table 3). At 3 mo of age, plasma IGF-I concentrations were not influenced by treatment group but increased with current weight (+1.0 nM/kg body weight; 95% CI, 0.53–1.5; $p < 0.0001$) and decreased with increasing birth weight (–2.8 nM/kg birth weight; 95% CI, –5.3 to –0.39; $p < 0.05$). Plasma IGF-I concentrations were higher in male than female lambs at 3 mo (+8.1 nM; 95% CI, 4.8–11.3; $p < 0.0001$). Plasma IGF-I concentrations at 5 mo of age were not influenced by treatment group or birth weight but increased with current weight (+0.69 nM/kg body weight; 95% CI, 0.42–0.96; $p < 0.0001$). At 30 mo of age, fed plasma IGF-I concentrations increased with current weight (+0.29 nM/kg body weight; 95% CI, 0.09–0.49; $p < 0.01$) and decreased with increasing birth weight (–2.1 nM/kg birth weight; 95% CI, –4.0 to –0.16; $p < 0.05$; Table 3).

At 30 mo plasma IGF-I, IGF-II, and IGFBP-2 responses to the GH stimulation test were not influenced by treatment group (Table 5), birth weight, or current weight. Plasma insulin responses to GH stimulation increased with current weight (+6.0 pM/kg body weight at 6 h; 95% CI, 0.3–11.7; $p < 0.05$; +62 pM/kg body weight at 48 h; 95% CI, 8.4–116; $p < 0.05$) but were not influenced by treatment group or birth weight. Plasma glucose responses were not influenced by treatment group, birth weight, or current weight.

To clarify the relative magnitude of the interacting effects of birth weight and current weight on outcomes, animals were divided above and below the median for birth weight and current weight. The mean outcome values for each group (higher and lower birth weight, higher or lower current weight) are given in Table 3.

DISCUSSION

The purpose of this study was to distinguish the effects of maternal undernutrition in late pregnancy, size at birth, and postnatal size on metabolic, endocrine, and cardiovascular regulation during postnatal life. Our main finding was that

Table 3. Birth weight and current interactions on plasma glucose, insulin, IGF-I, and MAP

	5 mo (ewes and rams) Birth weight		30 mo (ewes) Birth weight	
	Lower	Higher	Lower	Higher
Fed glucose (mM)				
Current weight lower	4.2 ± 0.2 (17)	4.3 ± 0.2 (14)	3.5 ± 0.1 (11)	3.6 ± 0.1 (5)
higher	4.1 ± 0.1 (14)	4.2 ± 0.1 (17)	3.8 ± 0.1 (5)	3.4 ± 0.1 (12)
Fasted glucose (mM)				
Current weight lower	3.6 ± 0.1	3.6 ± 0.1	3.3 ± 0.1	3.3 ± 0.1
higher	3.7 ± 0.1	4.2 ± 0.3	3.3 ± 0.1	3.3 ± 0.1
GTT glucose AUC (mM)				
Current weight lower	145 ± 3*†	141 ± 4*†	951 ± 30	925 ± 46
higher	157 ± 3*†	152 ± 4*†	961 ± 25	952 ± 25
Fed insulin (pM)				
Current weight lower	120 ± 27†	135 ± 26†	75 ± 11*†	78 ± 4*†
higher	194 ± 62†	154 ± 22†	147 ± 36*†	73 ± 7*†
Fasted insulin (pM)				
Current weight lower	20 ± 5†	19 ± 6†	39 ± 6	42 ± 11
higher	50 ± 12†	29 ± 7†	56 ± 10	40 ± 4
GTT insulin AUC (nM)				
Current weight lower	4.6 ± 0.8	5.0 ± 1.1	20 ± 2	23 ± 5
higher	4.7 ± 1.2	4.1 ± 0.8	30 ± 7	24 ± 3
IGF-I (nM)				
Current weight lower	17 ± 1†	14 ± 1†	17 ± 1*†	16 ± 1*†
higher	21 ± 2†	19 ± 1†	21 ± 1*†	17 ± 1*†
MAP (mm Hg)				
Current weight lower	53 ± 1*†	52 ± 3*†	82 ± 3	83 ± 3
higher	62 ± 3*†	59 ± 3*†	83 ± 3	82 ± 4

Animals are divided into groups above and below the median birth weight and current weight at each age (see text for details). Values are mean ± SEM (number per group for all observations shown in brackets).

* Effect of birth weight ($p < 0.05$).

† Effect of current weight ($p < 0.05$).

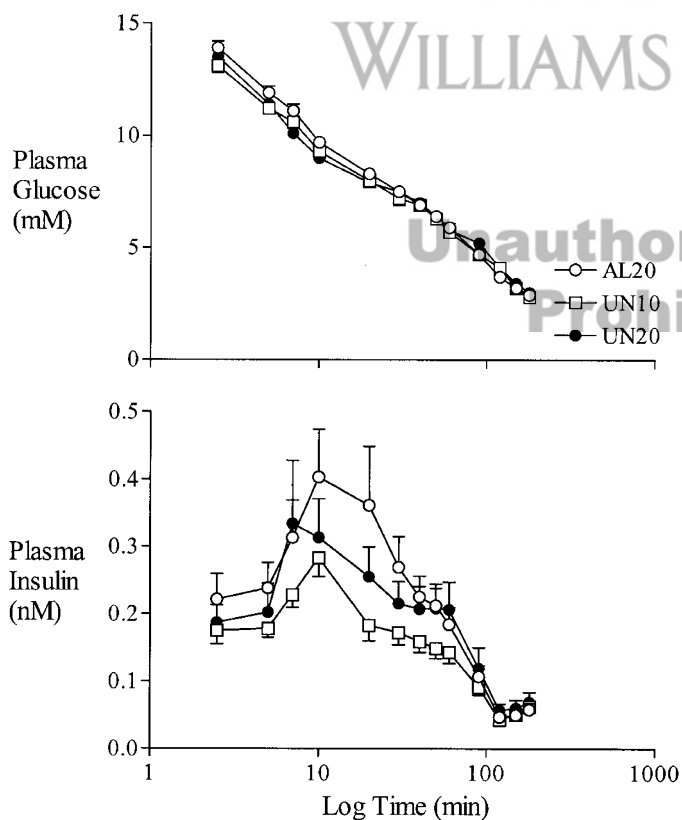


Figure 2. Plasma glucose (*top*) and insulin (*bottom*) responses to i.v. GGT (0.2 g glucose/kg body weight) in 30-mo-old ewes. Values are mean ± SEM.

severe undernutrition of pregnant ewes for 10 or 20 d in late gestation had little effect on glucose tolerance, insulin sensitivity, blood pressure, or IGF-I response to GH in the offspring once birth weight and current weight were taken into account. Furthermore, the negative relationship between birth weight and both glucose tolerance and blood pressure that we observed in 5-mo-old lambs was no longer present in 30-mo-old adult sheep.

Most human studies examining the fetal origins of adult disease hypothesis have studied the relationship between size at birth, usually birth weight or body proportions, and postnatal disease risk factors (1). In contrast, most animal studies have investigated the relationship between maternal nutrition during pregnancy and the physiologic markers of disease risk in their offspring (6–10). However there are limited data from human studies on the direct effects of maternal nutrition on postnatal physiology. The proportions of protein and carbohydrate in the maternal diet during pregnancy have been shown to influence both size at birth (36) and placental size and also blood pressure (37) and insulin resistance (38) in the adult offspring. Studies of survivors of the Dutch famine have also suggested that some of the effects on the offspring of severe maternal undernutrition during pregnancy are independent of size at birth, particularly if the undernutrition occurred in early pregnancy (39). However, in this cohort blood pressure of the offspring was related to size at birth and independent of the presence or timing of maternal undernutrition during gestation (5).

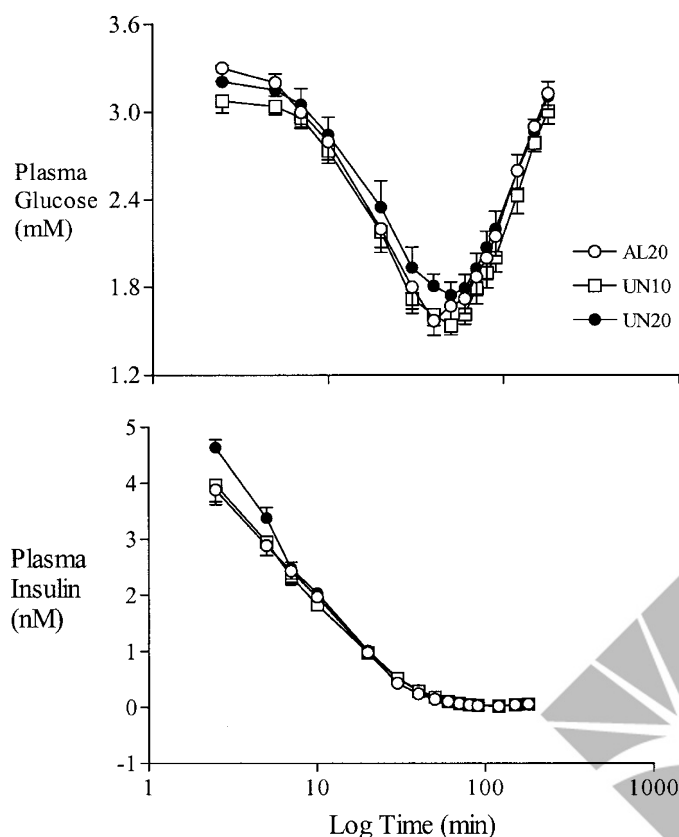


Figure 3. Plasma glucose (top) and insulin (bottom) responses to i.v. ITT (0.15 U insulin/kg body weight) in 30-mo-old ewes. Values are mean \pm SEM.

Table 4. Effect of nutritional treatment group on plasma IGF concentrations and MAP

	AL20	UN10	UN20
Ewes			
IGF-I (nM)			
Birth	20 \pm 2 (13)	22 \pm 2 (12)	22 \pm 2 (12)
3 mo*	23 \pm 1 (11)	24 \pm 1 (12)	24 \pm 1 (12)
5 mo	16 \pm 1 (10)	17 \pm 1 (9)	17 \pm 1 (9)
30 mo	17 \pm 1 (11)	18 \pm 1 (11)	18 \pm 1 (11)
MAP (mm Hg)			
5 m	53 \pm 3 (9)	61 \pm 5 (10)	60 \pm 5 (10)
30 m	82 \pm 3 (11)	84 \pm 3 (11)	81 \pm 3 (11)
Rams			
IGF-I (nM)			
Birth	26 \pm 2 (8)	24 \pm 2 (12)	22 \pm 2 (12)
3 mo	34 \pm 3 (8)	29 \pm 2 (12)	34 \pm 3 (10)
5 mo	20 \pm 4 (6)	17 \pm 2 (9)	18 \pm 2 (9)
MAP (mm Hg)			
5 mo	59 \pm 6 (7)	56 \pm 4 (10)	53 \pm 3 (10)

Values are mean \pm SEM. Numbers in brackets represent animals with data obtained at each time point.

* Sex difference ($p < 0.0001$).

It has been proposed that the fetal origins of adult disease reflect fetal adaptive responses *in utero* to limitation in nutrient supply. These adaptations lead both to altered fetal growth and also to altered physiology, which predisposes to adult disease (12, 13). If this proposal is correct, and fetal nutrient limitation does not necessarily lead to altered birth weight, then birth weight is not necessarily on the causal pathway between fetal

Table 5. Effect of nutritional treatment group on plasma endocrine and glucose responses to 48 h GH challenge at 30 months

	AL20	UN10	UN20
IGF-I (nM)			
0 h	15 \pm 1	17 \pm 1	18 \pm 1
6 h	19 \pm 1	20 \pm 1	20 \pm 1
12 h	25 \pm 1	27 \pm 1	28 \pm 1
24 h	42 \pm 2	43 \pm 2	43 \pm 2
36 h	52 \pm 4	55 \pm 3	53 \pm 2
48 h	59 \pm 4	60 \pm 3	60 \pm 2
IGF-II (nM)			
0 h	21 \pm 2	18 \pm 1	20 \pm 2
6 h	16 \pm 1	15 \pm 1	16 \pm 1
12 h	12 \pm 1	11 \pm 1	9.9 \pm 0.6
24 h	6.6 \pm 0.8	6.1 \pm 0.5	7.2 \pm 0.5
36 h	5.7 \pm 0.7	4.8 \pm 0.5	5.5 \pm 0.5
48 h	6.0 \pm 0.8	4.5 \pm 0.6	5.1 \pm 0.5
IGFBP-2 (nM)			
0 h	17 \pm 1	15.1 \pm 2	19 \pm 1
6 h	19 \pm 2	17 \pm 2	22 \pm 2
12 h	18 \pm 2	16 \pm 2	20 \pm 1
24 h	15 \pm 2	13 \pm 1	17 \pm 1
36 h	11 \pm 1	10 \pm 1	13 \pm 1
48 h	9.0 \pm 0.9	8.4 \pm 0.8	10 \pm 1
Insulin (pM)			
0 h	59 \pm 11	57 \pm 8	76 \pm 12
6 h	206 \pm 33	185 \pm 19	226 \pm 33
12 h	435 \pm 68	344 \pm 52	425 \pm 52
24 h	736 \pm 153	1018 \pm 360	964 \pm 194
36 h	1475 \pm 531	1308 \pm 380	1672 \pm 333
48 h	1257 \pm 268	1138 \pm 198	1467 \pm 351
Glucose (mM)			
0 h	2.3 \pm 0.1	2.4 \pm 0.1	2.4 \pm 0.1
6 h	3.0 \pm 0.1	3.0 \pm 0.1	3.1 \pm 0.2
12 h	3.7 \pm 0.2	4.0 \pm 0.3	3.9 \pm 0.2
24 h	3.2 \pm 0.2	3.6 \pm 0.2	3.5 \pm 0.4
36 h	4.4 \pm 0.4	4.5 \pm 0.5	4.6 \pm 0.5
48 h	5.1 \pm 0.5	4.8 \pm 0.5	5.4 \pm 0.6

Values are mean \pm SEM ($n = 11$ per treatment group). There are no differences between treatment groups.

malnutrition and adult disease (40). This study was specifically designed to address this hypothesis directly. We believe our data are the first to address the effects of maternal nutrition and birth weight independently in determining the effects on the physiology of the offspring.

The findings of this study suggest that birth weight is more important than maternal nutrition in late gestation in determining postnatal glucose tolerance and blood pressure. This was contrary to our expectations, as we have previously argued that important changes in fetal growth, metabolism, and endocrine status may be induced experimentally by maternal undernutrition independently of fetal size (15, 24–26, 41). We had therefore hypothesized that we would observe an effect of nutrition group on postnatal physiology independent of birth weight. Our data did not support this, and indeed may suggest that birth weight may be on the causal pathway between fetal nutrition and adult disease.

However, these data may also have other explanations. First, we used severe maternal undernutrition for 10 or 20 d in late gestation to induce fetal undernutrition. Late-gestation nutritional insults are more likely to alter size at birth than are similar insults in early gestation. However, almost all of the

evidence relating maternal nutrition to postnatal physiology is concerned with altered maternal nutrition in early pregnancy or around the time of conception (42). Indeed, the only other studies of which we are aware demonstrated an association between early gestation undernutrition, postnatal blood pressure (20), and alterations in the hypothalamic-pituitary-adrenal axis in sheep (21, 23). Dexamethasone administration to pregnant sheep also results in increased blood pressure of the offspring if given at 27 but not 64 d gestation, again suggesting the importance of early gestation for the programming phenomenon (43, 44). Similarly, the Dutch famine data suggest that it is exposure to famine early in gestation that influences adult health, lipid profile, and coronary heart disease risk (45). Thus further studies may be required to determine the interaction between maternal undernutrition in early pregnancy and birth weight in determining postnatal physiologic function.

Another reason why our findings were not as expected may be that the period of undernutrition was too short. We chose intervals of 10 and 20 d of undernutrition in late gestation because we had previously shown that a similar 10-d period of maternal undernutrition results in marked changes in fetal growth and body proportions without altering overall fetal weight (14). When the undernutrition was prolonged to 20 d we expected that the growth restriction would not be reversible *in utero* and that these animals would be small at birth (46). This proved to be the case (28). However, the duration of fetal undernutrition required to induce permanent changes in postnatal physiology is not known. It may be that relatively brief periods of undernutrition in late gestation are not sufficient to permanently reset physiologic and homeostatic mechanisms and that earlier and/or more prolonged nutritional restriction is required. Furthermore, although our experimental design involved three nutrition groups, there was substantial overlap in birth weight among the groups. Much larger groups may therefore be required to demonstrate relatively subtle effects of brief periods of maternal undernutrition over and above the relatively large effect demonstrated across the range of birth weights in this study.

Finally, it is possible that size at birth does not lie on the causal pathway between maternal undernutrition and postnatal physiology, but it may nevertheless reflect the underlying programming process more closely than does maternal nutritional group assignment. The assumption that because maternal nutrition may be an important factor determining fetal growth it is also a key factor in the development of adult disease has been challenged (47). We have argued that fetal growth is largely regulated by fetal nutrition in late gestation, but that fetal nutrition may bear little relationship to maternal nutrition (48). This is because a number of steps along the fetal supply line, such as maternal metabolic and endocrine milieu, uterine blood flow, and placental transport capacity, intervene between maternal nutrient intake and fetal nutrient supply. Thus fetal size could be regarded as an indirect integrated measure of fetal nutrient supply, which may bear little relationship to maternal nutrition. If this is so, and if the underlying mechanisms of programming do indeed reflect fetal adaptations to limited nutrient supply, then fetal size may be related more closely than maternal nutrition to the programming mechanism and to

postnatal physiologic consequences without lying on the direct causal pathway between the two.

Most epidemiologic studies of the relationship between birth weight and postnatal outcome have taken current weight into account. Although the validity of this has been debated (47), it is widely accepted that current weight is an important influence on both blood pressure and glucose tolerance, particularly in young animals, and that such adjustment is appropriate (49–51). We therefore adjusted for current weight in all of our analyses. We found, as expected, that glucose tolerance, blood pressure, and circulating IGF-I concentrations are all strongly positively related to current weight at both postnatal ages studied. Although previous studies have suggested that development of postnatal obesity is an important amplifier of the fetal programming process (10, 11), there was no evidence of obesity in our animals. Indeed, we found that the animals whose mothers were undernourished for 20 d in late gestation tended to have reduced rather than increased back fat depth at 30 mo of age (28). It is therefore unlikely that the development of postnatal obesity, whether related to maternal undernutrition or to birth weight, was an important confounder in the results we observed.

We found the relationships between birth weight and both glucose tolerance and blood pressure that we observed at 5 mo of age were no longer evident when animals were restudied at 30 mo of age. This is in contrast to reports in human studies that these relationships can be demonstrated at all ages except possibly during adolescence (52). Indeed, it has been suggested that the relationships may grow stronger rather than weaker with increasing postnatal age (53). The reason for the discrepancy between our findings and those is not clear. Five-month-old lambs are prepubescent, whereas 30-mo-old ewes are in early adulthood rather than elderly. It would have been interesting to restudy these animals as they aged to determine the possible return of these relationships, but such prolonged study was not possible for logistic reasons. However, future studies in large animals should plan on prolonged follow-up to determine the permanence and possible amplification of any relationships with increasing postnatal age.

For logistic reasons we were unable to keep uncastrated rams on our farm long enough to study at 30 mo of age. It is possible that any effects of birth weight on our outcome measures may have been present in rams but not ewes at 30 mo. The relationship between small size at birth, insulin resistance, and hyperinsulinemia is reported to be present in young adult men but not women (54). However, other studies have demonstrated that small size at birth leads to an exaggerated insulin response to a glucose load in both men and women at 20 y of age (55).

It has been proposed that reduced sensitivity to GH or IGF-I may be a core mechanism by which small size at birth is related to increase blood pressure and impaired glucose intolerance in the offspring (17, 18). We found no evidence of altered IGF-I response to GH administration in adult sheep in relation to either maternal nutrition or birth weight. However, plasma IGF-I concentrations were positively related to current weight and negatively related to birth weight in both 5- and 30-mo-old offspring.

The relationship between birth weight and plasma IGF-I is well established in the human (56) and a number of other species (57, 58). Gene knockout studies have demonstrated that IGF-I has a critical role in the regulation of fetal growth (59). The dominant influence of birth weight rather than treatment group on IGF-I concentrations at birth is consistent with this role. In childhood plasma IGF-I concentrations have been reported to be inversely related to birth weight (60). It has been proposed that higher plasma IGF-I levels in children with lower birth weight once current weight is taken into account reflect relative IGF-I resistance in these children. Such an interpretation is also tempting in our studies. It is also possible, although less likely, that the higher IGF-I concentrations in these children simply reflect their more rapid growth, and it is difficult to disentangle these effects in young children or growing lambs. However, the persistence of the negative relationship between birth weight and plasma IGF-I concentrations in adult sheep in our study makes this growth interpretation less likely.

Our GH challenge studies suggest that plasma IGF-I, IGF-II, IGFBP-2, and glucose responses at 30 mo were not influenced by treatment group, birth weight, or current weight. This is consistent with reports that intrauterine growth restricted children have appropriate IGF responses to GH therapy (61). Offspring of rats fed 30% of *ad libitum* diet throughout pregnancy also appear to have no impairment in IGF-I response to postnatal GH treatment despite being smaller at birth (M. Vickers, unpublished observations). Thus, if perturbation of the somatotrophic axis is one possible mechanism for programming of postnatal physiology, it seems likely that GH-independent elements are more involved.

CONCLUSIONS

In summary, we have shown that severe undernutrition of pregnant ewes for 10 or 20 d in late gestation has little effect on glucose tolerance or blood pressure of the offspring once birth weight and current weight were taken into account. These findings may suggest that achieved size at birth is more closely related to the programming process than is maternal undernutrition. Our studies provide some evidence of long-term IGF-I insensitivity in lambs of lower birth weight. However, further studies are required to determine the timing and duration of any nutritional insult in pregnancy that results in permanently altered physiology in the offspring.

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