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Nutritional challenges during development induce sex-specific changes in glucose homeostasis in the adult sheep

Kirsten R. Poore, Jane K. Cleal, James P. Newman, Julian P. Boullin, David E. Noakes,[1] Mark A. Hanson, and Lucy R. Green

Centre for the Developmental Origins of Health and Disease, University of Southampton, Southampton;
[1]Department of Veterinary Reproduction, Royal Veterinary College, North Mymms, United Kingdom

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Poore KR, Cleal JK, Newman JP, Boullin JP, Noakes DE, Hanson MA, Green LR. Nutritional challenges during development induce sex-specific changes in glucose homeostasis in the adult sheep. *Am J Physiol Endocrinol Metab* 292: E32–E39, 2007. First published July 25, 2006; doi:10.1152/ajpendo.00253.2006.—The early-life environment has implications for risk of adult-onset diseases, such as glucose intolerance, insulin insensitivity, and obesity, effects that may occur with or without reduced birth weight. We determined the consequences of nutrient restriction in early gestation and early postnatal life and their interactions on postnatal growth, body composition, and glucose handling. Ewes received 100% (C, $n = 39$) or 50% nutritional requirements (U, $n = 41$) from 1 to 31 days gestation and 100% thereafter. Male and female offspring (singleton/twin) from C and U ewes were then fed either ad libitum (CC $n = 22$, UC $n = 19$) or to reduce body weight to 85% of target from 12 to 25 wk of age (CU $n = 17$, UU $n = 22$) and ad libitum thereafter. At 1.5 and 2.5 yr, glucose handling was determined by area under the curve (AUC) for glucose and insulin concentrations following intravenous glucose (0.5 g/kg body wt). Insulin sensitivity was determined at 2.5 yr following intravenous insulin (0.5 IU/kg). In females, postnatal undernutrition reduced ($P < 0.05$) glucose AUC at both ages, regardless of prenatal nutrition. Postnatal undernutrition did not affect insulin secretion in females but enhanced insulin-induced glucose disappearance in singletons. Poor early postnatal growth was associated with increased fat in females. In males, glucose tolerance was unaffected by undernutrition despite changes in insulin AUC dependent on age, treatment, and single/twin birth. Nutrition in early postnatal life has long-lasting, sex-specific effects on glucose handling in sheep, likely due, in females, to enhanced insulin sensitivity. Improved glucose utilization may aid weight recovery but have negative implications for glucose homeostasis and body composition over the longer term.

nutrition; glucose tolerance; developmental origins of health and disease

EPIDEMIOLOGICAL STUDIES show that the environment in early life may have long-term effects on the risk of adult-onset metabolic diseases. Low birth weight or thinness at birth confer an increased risk of glucose intolerance (15), insulin resistance (29), and the metabolic syndrome (3) in adult life. These effects are independent of adult lifestyle factors but may be compounded by increased adiposity, which itself is associated with poor early growth (18). Studies of adults who were in utero at the time of the Dutch Famine (November 1944 to May 1945) provide direct evidence that maternal undernutrition, acting at critical windows of development, can affect adult health. Male and female offspring of women exposed to famine during mid- and late gestation had lower birth weights and reduced glucose tolerance in adulthood, which worsened in the presence of obesity (31). Birth weight was not reduced in those affected by famine in early gestation, but nonetheless, these individuals had an increased risk of later obesity. This effect was first recorded in young adult men (33) but in older age was evident only in women (32). It is therefore clear that birth weight alone is an inadequate marker of altered fetal development. Animal studies demonstrate that maternal undernutrition postconception influences fetal insulin secretion in late gestation (21) and brief fetal exposure to glucocorticoids alters glucose metabolism (19) and blood pressure (11) in adult life, all of which occur without changes in birth weight.

Postnatal growth patterns also influence later health, with reduced size at birth, persistent thinness in infancy but accelerated childhood growth, and later obesity conferring an increased risk of insulin resistance (2, 6, 8). In addition, boys malnourished during their first year have reduced glucose tolerance and insulin sensitivity as young adults, independent of birth weight, which worsened as present body mass index increased (13). By contrast, the relative growth-limiting effects of breast feeding, compared with formula feeding, reduces adult diabetes, obesity, and hypertension risks (28, 36, 39). Nutrient restriction in many mammalian species prevents obesity-related metabolic disorders and has beneficial effects for enhanced lifespan, which itself is associated with improved insulin sensitivity (17).

Recently, it has been suggested that, although responding to changes in maternal nutrition is immediately beneficial to the fetus, the long-term effects of these adaptations may prove detrimental if postnatal nutrition does not match that predicted by the fetus on the basis of its prenatal environment (12). This "predictive adaptive response" theory suggests that the degree of mismatch, e.g., in nutrition, between the pre- and postnatal environments determines disease risk (12). If correct, the theory suggests that the consequences of a nutritional restriction in postnatal life will differ in individuals exposed to a prenatal nutritional restriction from those that were not. Testing this concept directly in relation to metabolic disease necessitates using a large animal model such as sheep, which are of similar maturity at birth to humans for key organs and systems, have a long gestation period that allows manipulation of maternal nutrition for specific periods, and have a smaller number of fetuses than rodents. Hence, we aimed to determine the long-term consequences of moderate nutrient restriction in

Address for reprint requests and other correspondence: K. R. Poore, Centre for DOHaD, University of Southampton, Princess Anne Hospital, Level F (MP 887), Coxford Road, Southampton SO16 5YA, UK (e-mail: kpoore@soton.ac.uk).

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early gestation and in early postnatal life, and their possible interactions, on postnatal growth, body composition, and glucose handling in adulthood. The study was specifically designed to examine any potential sex differences in these effects.

MATERIALS AND METHODS

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

Welsh Mountain ewes ($n = 80$) in their second or third parity and of uniform good body condition [~3 on a scale of 1–5, (35)] were housed on straw in open barns at the Royal Veterinary College, Hertfordshire, UK. They were allowed to acclimatize, for ≥1 wk prior to conception, to a complete pelleted diet (GFW Titmus, Hertfordshire, UK) that provided 100% of their nutritional requirements as per standard guidelines (1). 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% crude protein.

The estrous cycle of groups of 3–4 ewes was synchronized by withdrawing vaginal medroxyprogesterone acetate-impregnated sponges (Veramix; Upjohn, Crawley, UK) 12 days after insertion. One of three raddled Welsh Mountain rams was introduced for 3 days, and *day 0* of gestation was taken as the first day that an obvious raddle mark was observed. The choice of ram for each group of ewes was completely random. Pregnancy was confirmed by measuring plasma progesterone concentrations on *day 16* of gestation using an enzyme immunoassay kit (Ridgeway Science, Avington, UK).

Before conception, ewes were randomly assigned to a control or a dietary-restricted group. Nutritional requirements were calculated individually for each ewe on the basis of initial body weight and fed in a pelleted form, without access to hay. Control ewes [prenatal control (C) $n = 39$] received 100% of their nutritional requirements before and then throughout gestation. From 1 to 31 days of gestation (term = 147 days), ewes in the dietary-restricted group [prenatal undernutrition (U), $n = 41$] received 50% of their nutritional requirements (by restricting the pelleted diet ration) and 100% of requirements for the remainder of gestation. Feed rations were adjusted according to gestational age based on guidelines for pregnant sheep (1). Water was provided ad libitum. Ewes were individually penned from 7 days before conception to 37 days of gestation and group housed thereafter with animals at a similar gestational age. Ewes were allowed to deliver and suckle their lambs naturally and during this time continued to receive 100% of their nutritional requirements, with further adjustments of the feed ration according to needs during lactation and standard guidelines (1). Lambs were weaned at 12 wk of age after a gradual introduction of the postweaning diet (see below).

After being weaned, lambs were group housed on straw in open barns. The postweaning lamb diet consisted of free access to water and hay. Creep pellets (Prestige Lamb Pellets + Decox; BOCM Pauls, Loughborough, UK) were provided to the group each morning and afternoon, such that each lamb had access to a standard ration according to body weight as per standard guidelines (1). As fed, creep pellets provided 10.51 MJ/kg (metabolizable energy) and 18% crude protein.

Between 12 and 25 wk of age, lambs from C and U ewes were grouped with ~10 others of similar body weight and treatment group and were fed either 100% of nutritional requirements [prenatal control/postnatal control (CC) $n = 22$, prenatal undernutrition/postnatal control (UC) $n = 19$] or at an intake level that reduced body weight to 85% of their target weight [prenatal control/postnatal undernutrition (CU) $n = 17$, prenatal undernutrition/postnatal undernutrition (UU) $n = 22$]. To keep body weight on the desired trajectory, lambs were monitored individually by weekly weighing and feed ration adjustment. Their individual target weight at each week was calculated by a linear trajectory projected from body weights taken at birth, 4, 8, and 12 wk of age. Weight reduction was achieved by restricting the pelleted diet but maintaining free access to hay. If necessary, lambs were removed from group housing to maintain weight at the desired level.

From 25 wk of age onwards, lambs were returned to larger group housing and received 100% of nutritional requirements. At ~32 wk of age, lambs were transferred onto a standard ration of an adult complete pelleted diet (Ewbol 18; BOCM Pauls) according to body weight, as per standard guidelines (1), plus ad libitum hay. As fed, adult pellets provided 10.38 MJ/kg (metabolizable energy) and 18% crude protein.

Each group contained approximately equal numbers of males and females, and the ratio of singleton to twin lambs was ~4:6. Group numbers according to singleton and twin birth for males were CC: singleton $n = 5$, twin $n = 8$; CU: singleton $n = 3$, twin $n = 5$; UC: singleton $n = 4$, twin $n = 5$; UU: singleton $n = 5$, twin $n = 6$; and for females they were CC: singleton $n = 2$, twin $n = 7$; CU: singleton $n = 4$, twin $n = 5$; UC: singleton $n = 2$, twin $n = 8$; UU: singleton $n = 4$, twin $n = 7$. The number of observations for each experimental data set is indicated in the legend of each table or figure or in RESULTS. There was a small reduction in the number of experimental observations at 2.5 compared with 1.5 yr due to technical difficulties (e.g., catheter loss shortly after surgery).

All lambs were weighed again at 35 wk of age and just prior to both experimental ages [16.5 ± 0.1 mo (1.5 yr) and 29.6 ± 0.2 mo (2.5 yr) of age]. At each experimental age, fat and muscle depths in the third lumbar region were measured by ultrasound (Aloka SSD 210 DX11; BCF Technology, Livingstone, UK) with a 7.5 MHz linear array transducer.

Surgical techniques. At 9.9 ± 0.1 mo of age, lambs were fasted overnight. Anesthesia was induced by thiopentone sodium (10 mg/kg iv; Link Pharmaceuticals, Horsham, UK) and maintained by halothane (2–4% in O_2, Vetothane Halothane; Virbac, Cambridge, UK), and carotid artery loops (externalization of artery within a flap of skin) were created. Male lambs were also vasectomized during the surgery. Immediately after surgery, analgesic (1.7 mg/kg sc, Finadyne Meglumine; Schering-Plough Animal Health, Welwyn Garden City, UK) and antibiotic (15 mg/kg im, LA Betamox, amoxicillin; Norbrook Laboratories, Newry, UK) treatments were administered and the normal feeding regime was restored. At least 4 mo recovery was allowed prior to experimentation.

At 1.5 yr of age, sheep were moved into individual metabolism cages and acclimatized for 4 days, with no change in their feeding regime. After an overnight fast, temporary indwelling carotid artery (via the loop) and jugular vein catheters were inserted under general anesthesia (2–4% halothane in O_2 by face mask). Antibiotic treatment was administered (15 mg/kg im, Betamox), and catheters were maintained for 3 days. Temporary indwelling catheters were removed at the completion of the experiments from all sheep, and they were returned to group housing for later study.

At 2.5 yr of age, sheep were moved from the Royal Veterinary College to the University of Southampton, where they were housed in individual metabolism cages. Feeding regimes remained unaltered, and sheep were allowed 6 days to recover from the transport. After an overnight fast, general anesthesia was induced by thiopentone sodium (10 mg/kg iv) and maintained by halothane (2–4% in O_2), and indwelling carotid artery (via the loop) and jugular vein catheters were inserted. Antibiotic treatment was administered (15 mg/kg im, Betamox), and catheters were maintained for up to 17 days.

Experimental protocol. At 1.5 and 2.5 yr of age, a glucose tolerance test (GTT) was performed following an overnight fast (1600–0900). Glucose (0.5 g/kg body wt) was administered as an intravenous bolus of 3 min duration, and arterial blood samples (3 ml into chilled EDTA tubes) were collected for analysis of plasma glucose and insulin concentrations at 30 min, 15 min, and immediately (0 min) before and

5, 10, 15, 20, 30, 45, 60, 90, and 120 min after the start of the glucose administration (*time 0*). At 2.5 yr only, an insulin tolerance test (ITT) was performed (1 day after the GTT) following an overnight fast (1600–0900). Insulin (0.5 IU/kg body wt) was administered as a rapid intravenous bolus at *time 0*, and arterial blood samples (4–10 ml into chilled EDTA tubes) were collected for analysis of plasma glucose concentrations at 30 min, 15 min, and immediately (0 min) before and 5, 10, 15, 20, 30, 45, 60, 90, and 120 min after insulin administration. All blood samples were centrifuged immediately (10 min at 4°C), and after determination of plasma glucose levels plasma was stored at −20°C.

Postmortem. At the completion of all experimental protocols, sheep were killed by a barbiturate (pentobarbitone sodium) overdose. Approximately 1 g of pancreatic tissue was collected from a consistent region and placed in 10 ml HCl (180 mmol/l) in 75% (vol/vol) ethanol on ice (27). The tissue was minced finely with scissors, sonicated for 30 s, and then extracted overnight at 4°C. After centrifugation (3,000 rpm for 20 min), the supernatant was removed and stored at −20°C for later analysis.

Biochemical analyses. Plasma glucose concentrations were measured using a blood gas analyzer (ABL 735; Radiometer, Crawley, UK). Insulin concentrations in plasma and extracted pancreatic tissue were measured by ELISA (DRG Sheep Insulin ELISA; Immuno-Diagnostic Systems, Tyne and Wear, UK). The inter- and intra-assay coefficients of variation for the insulin assay were 8 and 5%, respectively.

Data analysis. For each GTT experiment, the area under the glucose and insulin response curve (AUC) was calculated [integrated plasma concentrations following glucose administration (5–120 min) above the mean pre-GTT (−30 to 0 min) concentrations]. For each ITT experiment, the minimum glucose level achieved relative to baseline [Δminimum glucose = minimum glucose following insulin administration − mean pre-ITT (−30 to 0 min)] and the slope of the fall in glucose following insulin administration from 5 to 20 min {glucose slope = ([glucose] at 20 min − [glucose] at 5 min)/15 min} were calculated. All measurements of fat and muscle depth were corrected for present body weight.

Data are expressed as means ± SE. Data were analyzed in two ways. First, the effects of prenatal diet, postnatal diet, sex, and number of offspring per pregnancy were tested on the data set as a whole using multifactorial analyses of variance (4-way ANOVA). Where significant effects of any factor or interactions were found, further analyses were performed, having split the data by that factor(s). Second, the relationship between two factors was analyzed separately within each sex using linear regression analysis. Statistical analyses were performed using GraphPad Prism version 3.0 and SPSS version 8. Significance was accepted at $P < 0.05$.

RESULTS

Postnatal growth patterns and body composition. Early gestation undernutrition had no effect on birth weight or size (Table 1). From 12 wk female sheep were lighter than male sheep, but at 1.5 and 2.5 yr females had more fat and muscle relative to their body weight (Table 1). In males and females, the reduction in body weight induced by postnatal undernutrition (12–25 wk) occurred independently from prenatal nutritional group (Table 1). This reduction persisted until 35 wk in all males and in prenatally undernourished females but was no longer evident by 1.5 (young adult) or 2.5 yr (mature adult) in any group (Table 1). However, body weight at 2.5 yr tracked with age, being related to weight at 25 wk, 35 wk, and 1.5 yr in males ($r^2 = 0.11$, 0.25, and 0.58, respectively; $n = 41$, $P < 0.05$) and to weight at birth, 12 wk, 25 wk, 35 wk, and 1.5 yr in females ($r^2 = 0.11$, 0.26, 0.40, 0.39, and 0.82, respectively; $n = 39$, $P < 0.05$).

In the female population as a whole, fat depth tracked with age from 1.5 to 2.5 yr ($r^2 = 0.19$, $P < 0.05$). Fat depth was increased in 1.5-yr-old female, but not male, sheep that received both pre- and postnatal undernutrition (UU) compared with prenatal undernutrition alone (UC; Table 1), although this was no longer significant at 2.5 yr (Table 1). Poor growth between 12 and 25 wk ($r^2 = 0.11$, $P < 0.05$; Fig. 1*C*), low body weight at 25 and 35 wk, and accelerated growth rate from 35 wk to 1.5 yr were each associated with increased body fat in young adult female sheep ($r^2 = 0.13$, 0.11, and 0.24, respectively; $P < 0.05$).

Table 1. *Body weights, fat, and muscle depths in male and female sheep undernourished in early gestation and/or early postnatal life*

	Males				Females			
	CC	UC	CU	UU	CC	UC	CU	UU
Weight at birth, kg	3.5±0.2	3.9±0.2			3.8±0.1	3.6±0.2		
Body mass index at birth, kg/m^2	22.8±1.0	24.4±1.6			23.6±1.4	22.5±1.3		
Crown rump length at birth, cm	39.1±0.9	40.3±0.9			40.8±0.9	40.4±1.0		
Weight at 12 wk, kg[ac]	22.5±1.3	25.3±1.1	24.2±1.6	26.3±1.2	21.2±0.8	20.9±1.0	24.9±1.4	21.2±0.9
Weight at 25 wk, kg[ace]	43.7±1.3	44.8±1.4	34.9±1.6	35.5±0.9	36.8±1.0	36.4±1.4	34.3±1.8	29.2±0.5
Weight at 35 wk, kg[ac]	48.2±1.4	49.8±1.3	41.5±1.7[e]	44.7±1.0[e]	41.1±1.9	42.4±1.4	42.7±2.0	34.9±1.3[f]
At 1.5 yr of age								
Weight, kg[ac]	61.5±2.0	59.9±2.1	59.5±2.3	62.2±1.7	52.2±2.2	51.4±1.9	54.1±3.2	47.6±1.1
Corrected fat depth, mm/kg[bh]	0.082±0.004	0.072±0.006	0.066±0.007	0.073±0.004	0.129±0.014	0.117±0.010	0.124±0.010	0.160±0.011[g]
Corrected muscle depth, mm/kg[bd]	0.484±0.017	0.461±0.015	0.448±0.017	0.469±0.015	0.468±0.023	0.524±0.022	0.512±0.016	0.550±0.015
At 2.5 yr of age								
Weight, kg[a]	75.2±2.0	74.3±2.2	75.4±2.5	75.8±3.1	62.7±2.7	62.4±2.3	63.0±4.2	56.9±1.2
Corrected fat depth, mm/kg[b]	0.052±0.007	0.057±0.013	0.060±0.006	0.056±0.008	0.089±0.006	0.090±0.008	0.084±0.009	0.113±0.009
Corrected muscle depth, mm/kg[bd]	0.420±0.011	0.416±0.016	0.428±0.018	0.450±0.025	0.431±0.017	0.520±0.023	0.496±0.021	0.549±0.019

Values are means ± SE. CC, prenatal control/postnatal control; UC, prenatal undernutrition/postnatal control; CU, prenatal control/postnatal undernutrition; UU, prenatal undernutrition/postnatal undernutrition. Significant findings (ANOVA; $P < 0.05$): [a]male > female; [b]female > male; [c]twin < single (see text for details); [d]twin > single (see text for details); [e]postnatal undernutrition < postnatal control; [f]UU < UC; [g]UU > UC; [h]interaction (ANOVA) between prenatal and postnatal treatment group (see text for details). Statistical findings in the left column apply to male and female sheep; otherwise see markings within each sex. Males: CC $n = 13$, UC $n = 9$, CU $n = 8$, UU $n = 11$. Females: CC $n = 9$, UC $n = 10$, CU $n = 9$, UU $n = 11$.

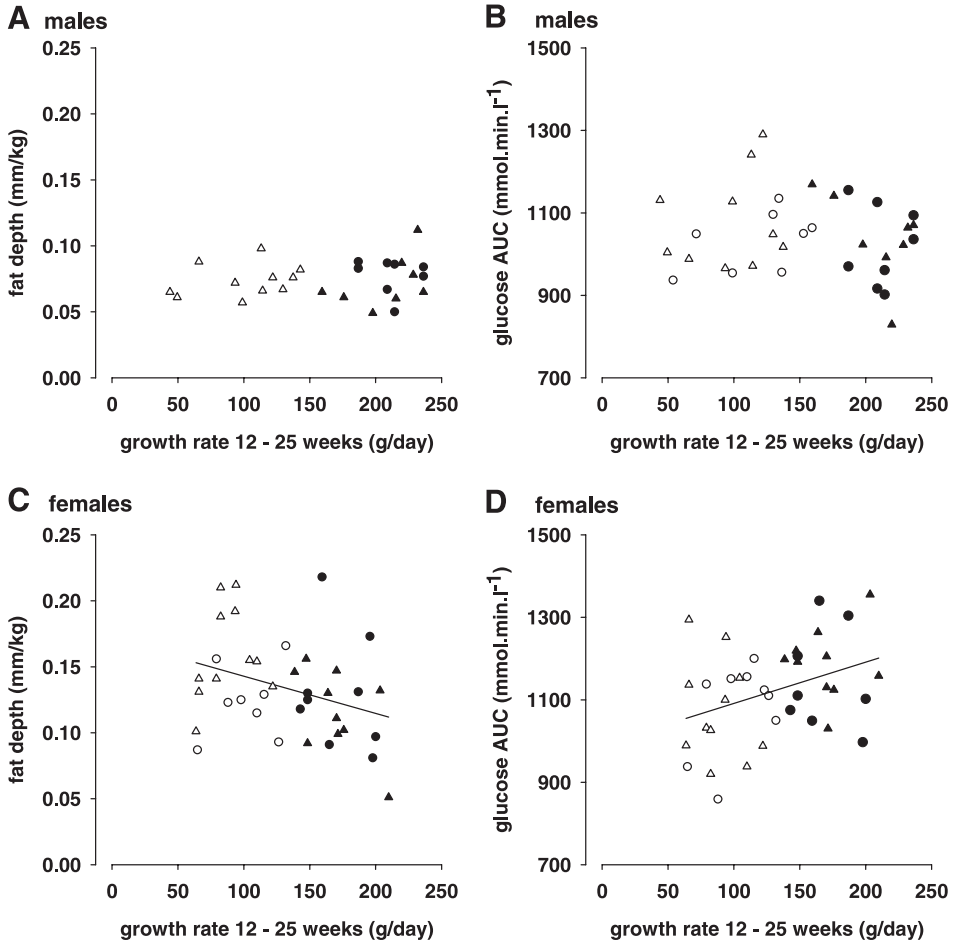

Fig. 1. Relationships between fat depth corrected for body weight (*A* and *C*) and area under the glucose curve (AUC) during a glucose tolerance test (*B* and *D*) and growth rate from 12 to 25 wk of age in male and female sheep at 1.5 yr of age. ●, prenatal control/postnatal control (CC); ▲, prenatal undernutrition/postnatal control (UC); ○, prenatal control/postnatal undernutrition (CU); △, prenatal undernutrition/postnatal undernutririon (UU). *C*: $r^2 = 0.11$, $P < 0.05$; *D*: $r^2 = 0.14$, $P < 0.05$.

Muscle depth was unaffected by pre- or postnatal diet (Table 1). However, muscle depth at 1.5 yr was negatively influenced by weight at 35 wk in males ($r^2 = 0.18$, $P < 0.05$) and by weight at 12, 25, and 35 wk in females ($r^2 = 0.14$, 0.33, and 0.24, respectively; $P < 0.05$). Muscle depth tracked with age from 1.5 to 2.5 yr in males ($r^2 = 0.13$, $P < 0.05$) and females ($r^2 = 0.33$, $P < 0.05$).

Glucose tolerance and insulin sensitivity in female sheep. At both 1.5 and 2.5 yr of age, glucose tolerance was greater (lower glucose AUC during GTT) in females exposed to postnatal undernutrition (Fig. 2, *C* and *D*). This result was not affected by prenatal nutritional status or singleton/twin birth. There was no change in the insulin response to glucose at either study age in the postnatally undernourished groups (Table 2). Moreover, there were no overall effects of pre- or postnatal diet on insulin sensitivity (glucose slope or Δminimum glucose during ITT; Table 2). However, insulin sensitivity at 2.5 yr was enhanced in singleton females (increased glucose slope) by postnatal undernutrition, regardless of prenatal diet (CU and UU: 0.092 ± 0.004 mmol·l^{-1}·min^{-1}, $n = 6$; CC and UC: 0.077 ± 0.005 mmol·l^{-1}·min^{-1}, $n = 4$, $P < 0.05$). In addition, a reduction in basal insulin concentrations in the postnatal undernourished groups indicated improved insulin sensitivity in 1.5-yr-old singleton females (CC and UC: 0.52 ± 0.07 μg/l, $n = 4$; CU and UU: 0.32 ± 0.05 μg/l, $n = 8$, $P < 0.05$).

In females as a whole, improved glucose tolerance at 1.5 yr was associated with both slower growth rate between 12 and 25 wk (Fig. 1*D*) and accelerated fractional growth rate from 25 to 35 wk ($r^2 = 0.11$, $P < 0.05$). However, higher growth rates from 1.5 to 2.5 yr and higher body weight in the 2.5-yr-old female population were associated with poorer glucose tolerance (increased glucose AUC during GTT; $r^2 = 0.21$ and 0.18, respectively, $P < 0.05$). Increasing fat depth was associated with elevated insulin AUC and the insulin AUC:glucose AUC ratio at 1.5 yr ($r^2 = 0.10$ and 0.13, respectively, $P < 0.05$) and elevated basal insulin concentrations and the insulin:glucose ratio at 2.5 yr ($r^2 = 0.39$ and 0.39, respectively, $P < 0.05$), indicative of reduced insulin sensitivity. Low weight and body mass index at birth were associated with low pancreatic insulin content at 2.5 yr ($r^2 = 0.15$ and 0.14, respectively, $P < 0.05$). Overall, glucose tolerance (as indicated by glucose AUC and basal glucose concentrations) was worse in females than in males at 1.5 yr (Fig. 2*C* and Table 2).

Glucose tolerance and insulin sensitivity in male sheep. In males at 1.5 yr, prenatal undernutrition did not alter glucose handling but did increase the insulin AUC and the ratio of insulin AUC to glucose AUC during GTT (Table 2). However, postnatal undernutrition reduced insulin AUC (CC and UC: $1{,}065 \pm 155$ μg·min^{-1}·l^{-1}, $n = 5$; CU and UU: 466 ± 142 μg·min^{-1}·l^{-1}, $n = 7$, $P < 0.05$) and the ratio of insulin AUC to glucose AUC (CC and UC: 1.01 ± 0.15, $n = 5$; CU and UU: 0.45 ± 0.14, $n = 7$, $P < 0.05$) at 2.5 yr (singletons only), regardless of the prenatal diet.

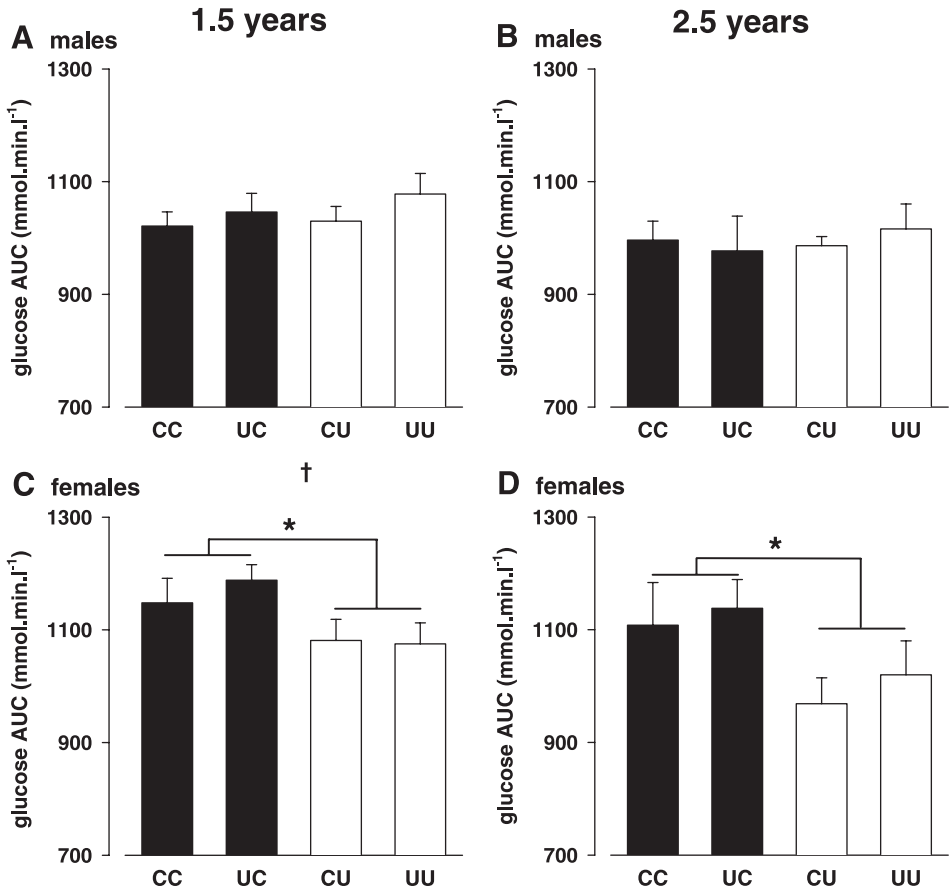

Fig. 2. AUC during a glucose tolerance test in male (*A* and *B*) and female (*C* and *D*) sheep at 1.5 and 2.5 yr of age. Sheep were born to ewes that received either 100 [group C (control): male $n = 21$, female $n = 18$] or 50% of nutritional requirements [group U (undernutrition): male $n = 18$, female $n = 21$] from *day 1* to *day 31* of gestation and 100% thereafter. Offspring were fed ad libitum (closed bars, CC: male $n = 13$, female $n = 9$; UC: male $n = 9$, female $n = 10$) or to reduce body weight to 85% of target from 12 to 25 wk postnatal age and ad libitum thereafter (open bars, CU: male $n = 8$, female $n = 9$; UU: male $n = 11$, female $n = 11$). $*P < 0.05$, ANOVA; $\dagger P < 0.05$, ANOVA, females vs. males.

In males as a whole, faster growth rate in the first 12 postnatal weeks and higher body weight at 12 wk were associated with increased glucose AUC at 2.5 yr ($r^2 = 0.20$ and 0.16, respectively, $P < 0.05$), indicative of poorer glucose tolerance. In addition, insulin sensitivity in males was reduced (lower Δminimum glucose during ITT) as body condition increased (as assessed by weight and relative fat depth; $r^2 = 0.13$ and 0.20, respectively, $P < 0.05$). Fat depth as well as

Table 2. *Glucose and insulin concentrations in the fasted state and during glucose and insulin tolerance tests at 1.5 and 2.5 yr of age in male and female sheep undernourished in early gestation and/or early postnatal life*

	Males				Females			
	CC	UC	CU	UU	CC	UC	CU	UU
GTT at 1.5 yr of age								
Basal glucose, mmol/l[a]	3.5±0.1	3.7±0.1	3.5±0.1	3.5±0.1	3.7±0.1	3.8±0.1	3.9±0.2	3.9±0.1
Basal insulin, μg/l[f]	0.32±0.05	0.41±0.10	0.28±0.04	0.43±0.05	0.42±0.07	0.34±0.03	0.40±0.07	0.38±0.05
Basal insulin:glucose	0.09±0.01	0.11±0.03	0.08±0.01	0.13±0.01	0.11±0.02	0.09±0.01	0.10±0.01	0.10±0.01
Insulin AUC, $\mu g \cdot min^{-1} \cdot l^{-1}$[df]	280±36	372±48[b]	272±34	390±44[b]	391±68	278±36	279±54	387±38
Insulin AUC:glucose AUC[df]	0.27±0.03	0.36±0.05[b]	0.27±0.04	0.37±0.05[b]	0.34±0.06	0.23±0.03	0.26±0.04	0.36±0.04
GTT at 2.5 yr of age								
Basal glucose, mmol/l	3.4±0.1	3.2±0.1[c]	3.2±0.1	3.4±0.1	3.2±0.1	3.3±0.1	3.4±0.1	3.4±0.1
Basal insulin, μg/l	0.49±0.07	0.78±0.27	0.47±0.06	0.52±0.07	0.53±0.02	0.48±0.03	0.43±0.06	0.63±0.06
Basal insulin:glucose	0.14±0.02	0.24±0.08	0.15±0.02	0.15±0.02	0.17±0.01	0.15±0.01	0.13±0.02	0.19±0.02
Insulin AUC, $\mu g \cdot min^{-1} \cdot l^{-1}$[f]	555±98	739±260	515±93	517±119	669±69	554±65	697±147	1184±406
Insulin AUC:glucose AUC[f]	0.58±0.11	0.72±0.22	0.53±0.10	0.49±0.11	0.67±0.07	0.50±0.06	0.72±0.15	1.27±0.48
ITT at 2.5 yr of age								
Glucose slope, $mmol \cdot l^{-1} \cdot min^{-1}$[f]	0.081±0.004	0.081±0.007	0.080±0.005	0.079±0.005	0.079±0.004	0.082±0.003	0.084±0.004	0.084±0.004
ΔMinimum glucose, mmol/l[e]	1.86±0.058	1.89±0.122	1.829±0.060	1.904±0.097	1.863±0.148	1.811±0.089	1.833±0.068	1.894±0.047
Pancreatic insulin at 2.5 yr, μg/g tissue	221±8	215±13	225±17	219±14	205±9	202±25	224±6	192±21

Values are means ± SE. GTT, glucose tolerance test; AUC, area under curve; ITT, insulin tolerance test. Significant findings (ANOVA; $P < 0.05$): [a]male < female; [b]prenatal undernutrition > prenatal control; [c]UC < CC; [d]twin < single; [e]twin > single (see text for details); [f]interaction (ANOVA) with singleton/twin birth (see text for details). Statistical findings in the left column apply to all sheep; otherwise see markings within each sex. GTT at 1.5 yr: males: CC $n = 13$, UC $n = 9$, CU $n = 8$, UU $n = 10$; females: CC $n = 8$, UC $n = 10$, CU $n = 9$, UU $n = 11$. GTT at 2.5 yr: males: CC $n = 9$, UC $n = 6$, CU $n = 6$, UU $n = 9$; females: CC $n = 6$, UC $n = 9$, CU $n = 6$, UU $n = 9$. ITT at 2.5 yr: males: CC $n = 11$, UC $n = 7$, CU $n = 7$, UU $n = 9$; females: CC $n = 9$, UC $n = 9$, CU $n = 7$, UU $n = 11$. Pancreatic insulin at 2.5 yr: numbers same as for Table 1.

high growth rate from 1.5 to 2.5 yr of age were also negatively related to the glucose slope during ITT ($r^2 = 0.12$ and 0.14, respectively, $P < 0.05$) and to pancreatic insulin content ($r^2 = 0.45$ and 0.35, respectively, $P < 0.001$).

The effect of singleton or twin birth. Birth weight was not different between twins (3.6 ± 0.1 kg, $n = 29$) and singletons (3.9 ± 0.2 kg $n = 51$). However, twin sheep at birth were shorter than singletons (single: 43.2 ± 0.7 cm; twin: 38.4 ± 0.5 cm, $P < 0.005$), and thus body mass index was greater in twins than in singletons (single: 21.3 ± 1.1 kg/m^2; twin: 24.6 ± 0.8 kg/m^2, $P < 0.05$). Twin sheep were lighter than singletons at 12 wk (single: 26.7 ± 0.9 kg; twin: 23.0 ± 0.8 kg, $P < 0.005$), 25 wk (single: 39.2 ± 0.8 kg; twin: 35.8 ± 0.6 kg, $P < 0.005$), 35 wk (single: 45.5 ± 0.9 kg; twin: 42.0 ± 0.7 kg, $P < 0.005$), and 1.5 yr (single: 59.0 ± 1.2 kg; twin: 54.4 ± 0.9 kg, $P < 0.005$), and this was not influenced by sex. By 2.5 yr there was no longer any effect of singleton or twin birth on body weight (Table 1). Fat depth was unaffected by singleton or twin birth (Table 1), but muscle depth was greater in twins than in singletons at 1.5 (single: 0.47 ± 0.01 mm/kg; twin: 0.50 ± 0.01 mm/kg, $P < 0.05$) and 2.5 yr (single: 0.44 ± 0.01 mm/kg; twin: 0.49 ± 0.01 mm/kg, $P < 0.05$).

Overall, twin sheep were more insulin sensitive than singletons at 2.5 yr, as shown by a larger fall in glucose concentrations relative to baseline during ITT (single: 1.75 ± 0.06 mmol/l, $n = 24$; twin: 1.91 ± 0.04 mmol/l, $n = 46$, $P < 0.05$) and a reduction in the peak glucose achieved during the GTT (single: 24.3 ± 0.6 mmol/l; twin: 22.6 ± 0.4 mmol/l, $P < 0.05$).

In addition, insulin AUC and insulin AUC to glucose AUC ratio were reduced ($P < 0.05$) at 1.5 yr in twin (307 ± 18 and 0.29 ± 0.12 $\mu g \cdot min^{-1} \cdot l^{-1}$, respectively, $n = 49$) compared with singleton sheep (377 ± 24 and 0.35 ± 0.02 $\mu g \cdot min^{-1} \cdot l^{-1}$, respectively, $n = 29$).

Pancreatic insulin content. Overall, pancreatic insulin content at 2.5 yr was unaffected by exposure to pre- and/or postnatal undernutrition (Table 2).

DISCUSSION

This study has shown for the first time that a short nutritional challenge in early postnatal life, when the young animal is establishing regulation of nutritional intake independent of its mother, has a long-lasting effect on glucose handling in a sex-specific manner. In adult female, but not male, sheep glucose tolerance was improved by moderate postweaning undernutrition. This may reflect the different strategies adopted by males and females following impaired nutrient supply at a critical developmental stage. The ability to recover body weight may have a higher priority in females aimed at protecting later reproductive function, which is dependent on a critical body weight and nutrient stores in the form of fat (4). An improvement in glucose handling may therefore be an adaptive strategy that has an immediate survival advantage, increasing the ability to utilize available glucose in a poor nutrient environment as well as providing a longer-term mechanism to facilitate a return of body weight when adequate nutrition is restored. However, the long-term implications of improved glucose handling following early life nutrient restriction in the present study remain unknown, since in other species poor early life nutrition enhances (22–24) but then subsequently reduces glucose tolerance (26). Increased glucose tolerance may also predispose to increased fat accumulation, which may become detrimental to glucose handling with time, particularly if nutrition is restored to adequate or abundant levels. Indeed, across the adult population as a whole, heavier sheep were less glucose tolerant. We hypothesize that, if the discrepancy between the predicted nutritional environment and that actually experienced in adult life was increased, the adaptive response of improved glucose tolerance following undernutrition in early postnatal life may have negative implications for later glucose homeostasis.

The improvement in glucose tolerance following postnatal undernutrition in females, with no change in the insulin response to glucose, indicates that glucose is cleared more rapidly from the circulation or that there is greater insulin-induced suppression of glucose production in these animals. Slower growth rates during, and accelerated rates after, the postnatal nutrient challenge directly predicted improved glucose tolerance in females. In addition, the amount of body fat and muscle was related to growth patterns in early postnatal life. Growth trajectories that increase the amount of these key tissues may have implications for whole body insulin sensitivity and may, in part, explain the improved glucose handling in postnatally undernourished females. In singleton-born females, the rate of insulin-induced glucose disappearance was increased by postnatal nutrient restriction. Taken together, these data suggest that heightened peripheral insulin sensitivity, rather than increased insulin secretion, may underlie the improvement in glucose clearance following poor postnatal nutrition in females. In other species, the mechanisms whereby the environment in early life may affect glucose tolerance and insulin sensitivity include changes in insulin receptor number, components of the insulin-signaling pathway, glucose transport and hepatic synthetic capacity, and pancreatic structure and function (7, 22–24, 30, 37). In this study, we found no effect of the early life nutritional regimes on pancreatic insulin content in 2.5-yr-old females. However, low body weight and thinness at birth in females were associated with a reduction in pancreatic insulin content. Given that body weight in females tracked with age from birth, it remains possible that the ability of the adult pancreas to secrete insulin may be influenced by early life growth trajectories and, in combination with altered insulin sensitivity, determine adult glucose tolerance. It is also possible that early life nutrient restriction provides a stressful stimulus that may influence later insulin sensitivity via changes in the hypothalamo-pituitary-adrenal axis (20).

In male sheep, glucose tolerance was unaffected by the imposed early life nutritional regimes despite changes in glucose-stimulated insulin secretion that were dependent on postnatal age, pre- or postnatal nutrition and single/twin birth. In rats, low-protein diets during postnatal life, for discrete (38) or sustained periods (16), persistently impair insulin secretion without reducing glucose tolerance, an effect exacerbated by subsequent high-fat feeding (16). An impaired pancreatic ability to respond to the nutrient environment may predispose to diabetes in later life (14, 38). In the present study, pancreatic insulin content in 2.5-yr-old males was negatively influenced by present adult body condition. An increase in adipose tissue may increase the demand on pancreatic β-cell function, and a critical weight/fatness threshold may exist over which glucose tolerance deteriorates. In addition, we found that accelerated

growth patterns during suckling (first 12 wk) predicted poorer glucose tolerance in the adult male population, suggesting that, as in females, there are critical developmental periods in males when postnatal growth patterns can have long-lasting effects on later metabolic homeostasis. Although body weight is likely to be important for males competing for dominance, i.e., access to females, an improvement in glucose handling does not appear to be a strategy employed by males to regain body weight after a nutritional challenge, as it is in females.

We found no direct evidence in either male or female sheep for a role of the maternal nutritional environment in very early gestation, or any interaction between the early gestation and early postnatal environments, on offspring weight at mid-gestation (unpublished observations taken from an additional set of animals) or birth, or on the adult offspring's glucose handling. However, insulin resistance, in terms of an increased insulin response to GTT, was indicated in young, but not mature, male sheep that had experienced prenatal undernutrition. Poor nutrition in early gestation may have a greater impact on male fetuses, since there is evidence in humans that males grow more quickly than females at this time (25). However, the timing of a gestational insult is likely to be important, since, in human (exposure to the Dutch famine) and animal studies, glucose tolerance is most affected by undernutrition in mid- to late gestation (10, 31), whereas blood pressure is influenced by early gestation undernutrition (9, 34). In the present study, exposure to undernutrition in both early gestation and early postnatal life increased fat depth in young adult females in the absence of any changes in current body weight. Pre- or postnatal undernutrition alone had no such effect. This suggests that repeated nutrient challenges in early life may set in place mechanisms, such as altered regulation of appetite or activity levels, that predispose to increased fat stores during times of abundant nutrition. In the sheep, it appears that the period of developmental plasticity for such an effect extends until adolescence.

The sheep used in this study came from both singleton and twin pregnancies. In twin lambs with discordant birth weight, the lighter twins had enhanced glucose tolerance in early life, but this effect disappeared by 12 mo of age (5). Although twin lambs were lighter and smaller than singletons in the present study, the improved glucose tolerance observed in females following postnatal nutrient restriction was not affected by twin/singleton birth. However, there was evidence that twins were generally more insulin sensitive than singletons in adulthood. A poorer nutrient environment both in utero and during suckling, brought about by a more competitive early life environment for twin lambs relative to singletons, may have induced changes in the glucose homeostatic set point.

In conclusion, this study has found that glucose tolerance in adult life is improved by a discrete period of undernutrition in early postnatal life. This effect is accompanied by increased sensitivity to insulin and is long-lasting but is restricted to females, suggesting that females employ different life course strategies from males in metabolic responses to nutrient restriction. The long-term implications of this increased glucose tolerance and increased propensity to accumulate body fat remain to be determined but could result in a deterioration in glucose handling with advancing age and could be particularly detrimental in an abundant nutrient environment. These results have implications for food choices made by mothers of young infants and for populations in transition (e.g., economic or migrant) and may inform future strategies for optimizing growth trajectories in early life.

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GRANTS

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