Periconceptional Undernutrition of Ewes Impairs Glucose Tolerance in Their Adult Offspring

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ABSTRACT: Maternal undernutrition throughout pregnancy can have long-term effects on the health of adult offspring. Undernutrition around the time of conception alters growth, metabolism, and endocrinology of the sheep fetus, but the impact on offspring after birth is largely unknown. We determined the effect of maternal periconceptional undernutrition in sheep on glucose tolerance in the offspring before and after puberty. Undernourished (UN) ewes were fed individually to maintain weight loss of 10–15% bodyweight from 61 d before until 30 d after mating. Offspring (24 UN, 30 control) underwent an i.v. glucose tolerance test at 4 and 10 mo of age. Glucose tolerance was similar in both groups at 4 mo. Insulin area under the curve increased by 33% between 4 and 10 mo (101 ± 8 *versus* 154 ± 12 ng·min·mL^{-1}, $p < 0.0001$). At 10 mo, UN offspring had a 10% greater glucose area under the curve than controls (809 ± 22 *versus* 712 ± 20 mM·min, $p < 0.01$), a reduced first phase insulin response ($p = 0.003$) which was particularly apparent in females and in singletons, and a decreased insulin:glucose ratio ($p = 0.01$). We conclude that maternal undernutrition around the time of conception results in impaired glucose tolerance in postpubertal offspring. (*Pediatr Res* 65: 409–413, 2009)

It is generally accepted that a mother's diet during pregnancy is important for the health of her offspring. Epidemiologic and animal studies have found associations between reduced size at birth and the development of cardiovascular disease and hypertension in adult life (1,2). Impaired glucose tolerance and diabetes are also linked to small birth size (3–5). However, there is growing evidence that altered disease risk in adult offspring may result from an adverse intrauterine environment independent of fetal size (6) or size at birth (7).

Most studies of the relationship between maternal nutritional status during pregnancy and impaired glucose homeostasis in offspring have looked at the effect of reduced maternal nutrition throughout pregnancy. Feeding rats a globally restricted diet, or one reduced specifically in protein content throughout pregnancy, results in offspring with reduced birthweight and altered pancreatic development, including decreased pancreatic blood flow (8); decreased β cell proliferation, islet size, and islet vascularization (9); reduced pancreatic insulin content and β cell mass (10) and hyperinsulinism (11); and also impaired activities of key hepatic enzymes involved in glycolysis and gluconeogenesis (12). In sheep, impaired glucose homeostasis and insulin action was seen in 1-y-old male sheep that experienced placental restriction *in utero* (13).

There is also evidence that disease risk in adulthood may be affected by maternal nutrition during more limited, but critical, periods of intrauterine growth and development. Studies of the Dutch Hunger Winter showed that the consequences for the offspring depended upon the time of maternal exposure to famine (5,7). Most studies have suggested it is the nutritional status of the mother during late gestation that influences glucose homeostasis in the offspring (5,14,15). However, offspring of rat dams fed a low-protein diet only for the 8 wk before pregnancy, showed increased levels of glucose as adults (16), suggesting that maternal nutritional status before conception may also be important. Fetuses of women with poor nutritional status before or in early pregnancy are also more likely to be born small and preterm (17–19).

In sheep, maternal nutrition during the periconceptional period is an important determinant of fetal growth, metabolism, and endocrine status in late gestation. Mild maternal undernutrition during the first 70 d of gestation resulted in elevated blood pressure and altered development of the hypothalamic-pituitary-adrenal (HPA) axis of late gestation fetuses and young lambs (20). Fetuses of ewes undernourished (UN) before until 7 d after mating also showed altered development of the HPA axis (21) and altered fetoplacental growth (22). Late gestation twin fetuses of ewes UN only during the preattachment period (days 0–7) had altered regulation of blood pressure (23). We have shown that maternal undernutrition from 61 d before until 30 d after mating resulted in extensive changes in the late gestation fetus, including altered regulation of fetal growth (6,24) and the fetal somatotrophic axis (25), changes in placental function, and accelerated maturation of the HPA axis (26) resulting in preterm birth (27). There was also altered regulation of the glucose-insulin axis, with evidence of accelerated pancreatic maturation and reduced glucose area under the curve (AUC) during a glucose tolerance test (GTT) (28,29).

There are limited data in sheep regarding the consequences of maternal undernutrition in early gestation on the offspring after birth. Male offspring of ewes undernourished from day 1–31 showed evidence of insulin resistance at 1.5 y of age (30). Impaired glucose tolerance and insulin resistance were reported

in 1-y-old offspring of ewes undernourished in late but not early gestation (15). Undernutrition of ewes during mid gestation (days 28–79) altered insulin responses to GTT in male offspring pre- and postweaning (31). Offspring of ewes with a low body condition score before and during pregnancy showed mild glucose intolerance and impaired insulin secretion at 18 mo (32).

However, there are few data in animals or humans regarding the possible long-term implications of altered maternal nutrition around the time of conception independent of size at birth and, in particular, whether the observed changes in the late gestation fetus in response to periconceptional undernutrition have any implications for the postnatal health and disease risk of offspring. We therefore studied the effect of moderate maternal undernutrition around the time of conception in sheep, on glucose tolerance of the postnatal offspring before and after puberty.

MATERIALS AND METHODS

Animals. Four- to 5-y-old Romney ewes were acclimatized for 10 d to indoor conditions and a pelleted diet (65% lucerne, 30% barley, with limestone, molasses, and trace element supplements; Camtech Nutrition Ltd., Hamilton, New Zealand). Sixty-one days before mating ewes were weighed, then randomly assigned to maintenance feeding ("N," concentrates at 3–4% of bodyweight/d) or low plane feeding ("UN," fasted for 2 d then fed concentrates at 1–2% of bodyweight/d). Ewes were weighed twice weekly and rations for UN ewes were adjusted individually to reduce bodyweight by 10–15% over the first month (*i.e.* by 1 mo before mating), and then maintain this weight (6,33). A fortnight before mating oestrous was synchronized with a progesterone containing intravaginal device (34). The feed restriction of UN ewes continued until 30 d after mating and thereafter all ewes were fed *ad libitum* for the remainder of the experiment.

Ewes were housed indoors in a photoperiod controlled feedlot from 71 d before mating until 2 wk after lambing. They were kept in individual pens with open mesh sides to ensure visual contact with other ewes during the undernutrition period (UN ewes only), and from 3 wk before expected lambing until 2 wk after lambing. At other times all animals were housed in group pens.

After birth lambs were weighed and remained with their mothers indoors for the first 2 wk of life. All animals were then returned to pasture outdoors and managed as part of a single flock. Lambs were weighed again at weaning (12 wk). While on pasture, animals were kept acquainted with concentrate feeds and handled to allow better acclimatization on reentry to the feedlot for metabolic and endocrine tests.

At 4 and 10 mo of age, offspring were again brought indoors, housed in individual pens and fed concentrates. After a 2-d acclimatization period, animals were weighed, and indwelling catheters (internal diameter 0.04 inch, Critchley Electrical, Auburn, Australia) were inserted percutaneously into both jugular veins under local anesthetic, while the animals were standing in their pens. This procedure takes 5–10 min. Food was removed at 17:00 h and animals underwent an i.v. GTT the following day. After a 3 mL baseline blood sample, a bolus of 0.5 g/kg of glucose was given i.v. within 1 min (29). Blood samples (3 mL) were taken from the second catheter at 2, 5, 10, 15, 20, 30, 40, 50, 60, and 120 min for the measurement of glucose and insulin concentrations. Catheters were later removed and the animals returned to pasture.

Ethical approval was obtained from the University of Auckland Animal Ethics Committee.

Biochemical analysis. Blood samples were collected on ice, centrifuged at 3000 rpm for 10 min at 4°C, and the plasma stored at −20°C. Plasma glucose concentrations were measured by an enzymatic colorimetric assay (Roche Diagnostics, Mannheim, Germany) on a Roche/Hitachi 902 Automatic Analyser (Hitachi High-Technologies Corporation, Tokyo, Japan). Plasma insulin concentrations were analyzed by radioimmunoassay (35) with ovine insulin as the standard (batch No. I9254; Sigma Chemical Co., St. Louis, MO). The limit of detection was 0.03 ng/mL. Inter- and intraassay coefficients of variation were 11.6 and 12.6%, respectively.

Statistical analysis. Insulin data were log transformed to more closely approximate a normal distribution. Total insulin and glucose AUC were calculated from baseline. Where a single data point was missing (because of practical difficulties of sampling or assay, or inadequate plasma volume), the missing value was estimated by extrapolation for the purposes of calculating

AUC. If more than one point was missing, the data were excluded from this calculation. Differences between ages in AUC of glucose and insulin were compared using analysis of variance (ANOVA). Bodyweight, plasma concentrations of glucose and insulin, and AUC parameters at each age were analyzed using factorial ANOVA, with nutritional group, sex and single/twin status as independent variables, and Tukey correction for multiple comparisons. The time courses of the plasma glucose and insulin concentrations during the GTT were analyzed using repeated measures ANOVA with group, sex, and single/twin status as the covariates. The independent effects of nutritional group, sex, single/twin status, birth weight and current weight were analyzed using multiple regression. All analyses were performed using JMP 5.1 (SAS Institute Inc., Cary, NC). Data are presented as mean ± SEM.

RESULTS

A total of 54 offspring of 38 ewes were used in the study; 30 offspring of 20 N ewes (7 singles, 23 twins) and 24 offspring of 18 UN ewes (11 singles, 13 twins, Table 1).

Mean birth weight was 5.5 ± 0.1 kg. Males were heavier than females at all ages (Table 1, $p = 0.05$). There was no significant effect of nutritional group or single/twin status on weight at birth or at 4 mo. However, at 10 mo there was an interaction between nutritional group and single/twin status, with singleton offspring of UN ewes heavier than those of N ewes (kg; UN single: 50.5 ± 2.6, N single: 45.5 ± 3.0, UN twin: 44.5 ± 1.9, N twin: 47.3 ± 1.4; $p = 0.004$).

Baseline plasma glucose and insulin concentrations were similar in all animals at 4 and 10 mo (Table 2). In response to the i.v. glucose bolus, plasma glucose concentrations increased sevenfold in the first 2 min, then declined to just above baseline over the 2-h study period (Fig. 1*A*). Mean glucose AUC did not change between 4 and 10 mo, but mean insulin AUC increased by approximately 33% (Table 2).

At 4 mo, plasma glucose AUC, insulin AUC, and the ratio of insulin:glucose AUC were similar in all animals (Table 2).

At 10 mo, offspring of UN ewes had higher plasma glucose concentrations during the GTT than those of N ewes ($p = 0.006$; Fig. 1*A*), with approximately 10% greater glucose AUC (Table 2). Offspring of UN ewes also had a lesser rise in plasma insulin concentrations (group × time interaction $p = 0.003$), particularly in the first 20 min after the glucose bolus (Fig. 1*B*) and a lower insulin:glucose AUC ratio ($p = 0.01$; Table 2). The smaller rise in plasma insulin concentrations was most apparent in females (ng · min · mL^{-1}; UN female: 109 ± 10, N female: 178 ± 32, UN male: 184 ± 28, N male: 148 ± 10; sex effect $p = 0.007$, group × sex interaction $p = 0.003$) and in singletons (ng · min · mL^{-1}; UN single: 122 ±

Table 1. *Weights of offspring of N and UN ewes at birth, 4, and 10 mo of age*

Age	n	Group	n	Male	n	Female	n
Birth*	54	N	30	5.7 ± 0.2	14	5.2 ± 0.2	16
		UN	24	5.7 ± 0.2	10	5.5 ± 0.3	14
4 mo*	53	N	29	31.1 ± 0.8	14	27.8 ± 1.0	15
		UN	24	31.9 ± 1.7	10	29.1 ± 1.1	14
10 mo†	52	N	30	51.4 ± 1.5	14	42.9 ± 1.2	16
		UN	22	54.0 ± 2.2	9	42.5 ± 1.2	13

Data are mean ± SEM (kg).

* $p < 0.05$, † $p < 0.0001$ for sex effect.

N, well nourished; UN, undernourished.

Table 2. *Baseline plasma concentrations and AUC for glucose and insulin following a GTT in 4 and 10 mo offspring of N and UN ewes*

	Age		Group		Male		Female	
	(mo)	n		n		n		n
Glucose								
Baseline (mM)	4	50	N	29	3.7 ± 0.1	14	3.6 ± 0.1	15
			UN	21	3.3 ± 0.2	9	3.6 ± 0.1	12
	10	52	N	30	3.8 ± 0.1	14	3.6 ± 0.1	16
			UN	22	3.7 ± 0.1	9	3.5 ± 0.1	13
AUC (mM · min)	4	50	N	29	728 ± 34	14	798 ± 26	15
			UN	21	749 ± 52	9	781 ± 29	12
	10*	51	N	29	718 ± 33	14	707 ± 26	15
			UN	22	793 ± 37	9	821 ± 30	13
Insulin								
Baseline (ng · mL^{-1})	4	52	N	29	0.07 ± 0.02	14	0.08 ± 0.03	15
			UN	23	0.07 ± 0.02	10	0.07 ± 0.03	13
	10	52	N	30	0.16 ± 0.02	14	0.11 ± 0.02	16
			UN	22	0.17 ± 0.04	9	0.11 ± 0.02	13
AUC† (ng · min · mL^{-1})	4	52	N	29	107 ± 16	14	96 ± 7	15
			UN	23	125 ± 33	10	83 ± 7	13
	10‡	52	N	30	148 ± 10	14	178 ± 32	16
			UN	22	184 ± 28	9	109 ± 10	13
Insulin:glucose ratio								
AUC	4	52	N	29	0.47 ± 0.03	14	0.42 ± 0.01	15
			UN	23	0.44 ± 0.04	10	0.41 ± 0.01	13
	10§	51	N	29	0.52 ± 0.03	14	0.53 ± 0.03	15
			UN	22	0.48 ± 0.03	9	0.42 ± 0.02	13

Data are mean ± SEM.
* $p < 0.01$ for group effect.
† $p < 0.0001$ for age effect.
‡ $p < 0.01$ for group × sex interaction.
§ $p < 0.05$ for group effect.
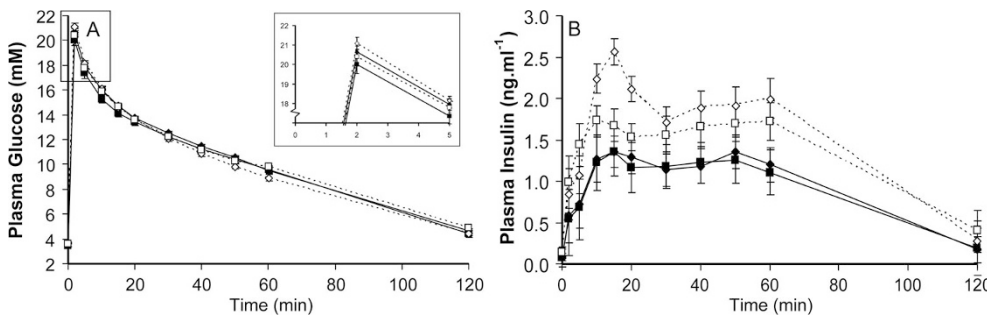AUC, area under curve; N, well nourished; UN, undernourished; GTT, glucose tolerance test.

Figure 1. Plasma glucose (*A*) and insulin (*B*) concentrations during a glucose tolerance test in 4 and 10 mo offspring of well nourished (N) and periconceptionally undernourished (UN) ewes. ◆, 4 mo N; ■, 4 mo UN; ◇, 10 mo N; □, 10 mo UN. Data are mean ± SEM. Insert shows plasma glucose concentrations during the first 5 min after glucose injection.

21, N single 202 ± 70, UN twins: 155 ± 20, N twin: 153 ± 10; group × single/twin interaction $p = 0.02$).

At 4 mo, glucose and insulin AUC both increased with increasing current weight, (glucose: 9.25 ± 4.24 mM · min · kg^{-1}, $p = 0.04$; insulin: 0.03 ± 0.01 ng · min · mL^{-1} · kg^{-1}, $p = 0.001$). At 10 mo, glucose AUC was affected only by nutritional group (UN *versus* N: 46.0 ± 15.7 mM · min; $p = 0.005$) and current weight (5.66 ± 2.86 mM · min · kg^{-1}, $p = 0.05$). None of the other parameters had a significant effect on glucose or insulin AUC at either age.

DISCUSSION

We have demonstrated that moderate maternal undernutrition from 61 d before until 30 d after conception impairs glucose tolerance in the adult offspring. The increased glucose AUC was associated with a decreased insulin response, particularly in females. We have previously demonstrated that periconceptional undernutrition in sheep alters growth, metabolism, and endocrinology in the late gestation fetus, without altering size at birth (6,26) and can lead to preterm birth (27). However, this is the first demonstration that these changes may have important implications for health of the adult offspring.

The period of undernutrition was chosen to incorporate critical periods of development, encompassing development of the oocyte, through early embryonic development and placentation. Most previous studies of the relationship between maternal nutrition during pregnancy and glucose tolerance of the offspring have examined undernutrition throughout pregnancy and/or lactation (30,36) but have not included the period before conception. Low body condition score before and throughout gestation in sheep resulted in mild glucose intolerance in 18-mo-old male offspring (32), but this experiment did not allow any conclusions about the critical time window. Studies in rats (14), sheep (15), and humans (5,7)

have reported impaired glucose tolerance in the offspring after undernutrition late, but not early in pregnancy, suggesting that late gestation is a critical period for the development of fetal pancreatic function.

There are, however, some data suggesting the periconceptional period may also be important. We have previously shown that fetuses of periconceptionally UN ewes had evidence of accelerated pancreatic maturation, and a smaller glucose AUC in response to GTT (28,29). In rats, maternal undernutrition for 8 wk before conception resulted in hyperglycemia in adulthood (16). A low-protein diet confined to the preimplantation period (0–4 d after mating) in rat dams resulted in increased hepatic phosphoenolpyruvate gene expression by late gestation (37). Our study now demonstrates that maternal nutrition around the time of conception has postnatal consequences for glucose homeostasis, and thus potentially for the lifetime of the offspring.

Offspring of UN mothers had a greater plasma glucose AUC at 10 mo, indicating that they were less efficient at clearing glucose. This clearance is determined largely by the capacity to secrete insulin, and insulin sensitivity. The smaller insulin AUC and reduced insulin:glucose AUC ratio strongly suggest impaired insulin secretion. Insulin deficiency may result from inadequate production and/or secretion of insulin, and both may result from maternal undernutrition during pregnancy. Rats born to dams fed a low-protein diet showed a relative deficiency of pancreatic β-cells (38), decreased β-cell proliferation, islet size, and vascularization as newborns (9), and impaired insulin secretion during fetal and young adult life (39). Global feed restriction during late pregnancy resulted in reduced β-cell proliferation and lower pancreatic insulin content in the offspring (40). Similarly, insulin secretion requires glucose metabolism in the β-cell, and activities of the enzymes involved are reduced in offspring of diabetic rats (41), and those fed a low-protein diet (12).

There is also evidence that insulin production and secretion may be influenced by prenatal events in sheep. Lambs subjected to undernutrition during late fetal life showed decreased insulin secretion at 19 wk (42). Similarly, placental restriction resulted in impaired glucose-stimulated insulin secretion in young lambs, although peripheral insulin sensitivity was increased (43).

We have previously shown that periconceptional undernutrition resulted in increased fetal insulin response to i.v. challenge with glucose but not arginine at 119 d gestation (29), suggesting accelerated maturation of the fetal pancreas. Thus, we hypothesized that the adult pancreas would have fewer mature β-cells, potentially leading to impaired insulin secretory capacity in later life. The current study provides the first evidence that this may be the case.

However, we cannot rule out the possibility that insulin resistance may also contribute to the decreased glucose tolerance observed in UN animals. Insulin AUC increased by 33% between 4 (prepuberty) and 10 mo (young adulthood), perhaps reflecting the development of insulin resistance with age as seen in other species such as humans (44) and rats (12,45). In sheep, glucose tolerance, insulin secretion, insulin sensitivity,

and insulin action all decreased with age, predominantly between weaning and adolescence (42).

In UN animals, there was a lower peak of plasma insulin 15 min after the glucose bolus, suggesting the major effect is on the initial phase of insulin secretion. A similar decrease in initial insulin secretory response was also seen in 18-mo offspring of ewes of low body condition score (32) and in lambs exposed to undernutrition during early to mid gestation (31). In most species, the response of insulin to a rapidly changing glucose concentration is biphasic (46–48). The first phase insulin response plays a critical role in maintaining glucose homeostasis, primarily by inhibiting hepatic glucose production (47,48). If early phase insulin release is impaired, increasing glucose concentrations require continued insulin release, placing increased stress on the β-cell, resulting in hyperglycemia and hyperinsulinemia (47). In humans, loss of the early phase insulin response has been associated with progressive impairment of glucose tolerance leading to diabetes (49–51). Therefore, the loss of β-cell function that facilitates the early phase insulin release may play a critical role in the impaired glucose tolerance we observed in the 10 mo UN animals.

We found that periconceptional undernutrition has a more profound effect on female than male offspring at 10 mo, indicated by much lower insulin concentrations despite similar glucose tolerance, perhaps as a result of improved insulin sensitivity. Sex-specific effects of prenatal events on postnatal physiology have been widely recognized but are not always consistent. Aged rats born to dams fed a low-protein diet are glucose intolerant, due more to insulin resistance in males and insulin deficiency in females (12). In offspring born to rats fed a low-protein diet, females were also more susceptible to the insult in mid gestation and males to late gestation (38). In contrast, placental restriction in sheep resulted in greater impairment of insulin sensitivity, insulin secretion, and glucose homeostasis in males than in females (13).

The effect of maternal periconceptional undernutrition was also greater in singles than twins. We have previously shown in sheep that fetal twins show a greater insulin response to i.v. glucose but not arginine challenge than singletons (28), suggesting accelerated pancreatic development similar to that seen in UN singleton fetuses (29). This would be expected to result in a greater reduction in insulin secretion in twins than singletons; the opposite of our findings. However, periconceptional undernutrition abolished the difference in insulin responses between singletons and twins (28), and others have postulated that the effects of intrauterine insults are greater on fetuses that are growing rapidly (1,24). Thus, it is possible that twins, who grow more slowly than singletons, are relatively protected from the effects of periconceptional undernutrition, although the mechanisms by which this occurs remain obscure.

In conclusion, we have shown that maternal periconceptional undernutrition impairs glucose tolerance in postpubertal offspring. Maternal nutrition around the time of conception may have important implications for glycaemic regulation in the next generation.

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