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Periconceptional Events Perturb Postnatal Growth Regulation in Sheep

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ABSTRACT: Periconceptional undernutrition and twin conception alter intrauterine growth and metabolism and are associated with later adverse metabolic outcomes. The contribution of postnatal growth to these outcomes is less well defined. We investigated whether maternal periconceptional undernutrition or twin conception altered postnatal growth regulation in ways that could lead to metabolic disease. Single and twin offspring of ewes undernourished (UN) from 61 d before until 30 d after mating, fed to achieve and maintain 10–15% weight loss (UN), were compared with offspring of maintenance-fed controls (N). At 2 h and 1, 6, and 12 wk after birth, lambs were weighed and plasma hormone and metabolite concentrations analyzed. Milk intake, measured by deuterium oxide dilution, was inversely related to birth weight only in N singles, although twins had the greatest postnatal growth velocity. Positive associations were seen between milk intake, growth velocity, and leptin concentrations in N, but not UN, offspring. We conclude that periconceptional undernutrition alters the relationships between regulators of postnatal growth, including nutrient intake and key hormonal axes, in both singles and twins without affecting size at birth or postnatal growth velocity. Dissociation of growth from its key regulators is one possible mechanism underlying adverse metabolic outcomes after periconceptional undernutrition. **(*Pediatr Res* 70: 261–266, 2011)**

Nutritional insults that result in fetal growth restriction and LBW are associated with altered growth and metabolism after birth (1). Maternal undernutrition around the time of conception does not necessarily result in LBW in the offspring but has been shown to alter fetal growth trajectory (2) and endocrine and metabolic factors that regulate fetal growth, such as the fetal somatotropic (3), hypothalamic-pituitary-adrenal (HPA), and glucose-insulin axes (4). Some of these endocrine changes persist after birth (5), although their relationship to the regulation of postnatal growth has not been described. Twinning, another periconceptional event, alters intrauterine growth and glucose-insulin axis function in late gestation fetal sheep (6) and is also associated with altered postnatal growth (7). The combined effect of periconceptional undernutrition and twin conception on postnatal growth regulation is not known.

Postnatal growth is the result of complex interactions between genetic factors, nutrient intake, and growth-regulating endocrine systems. Hormones that regulate appetite and metabolism, in particular leptin, insulin, and IGF1 (8), are in turn influenced by nutrient intake. Because periconceptional undernutrition alters fetal endocrine development, it is possible that the relationships between growth-regulating hormones and nutrient intake and uptake might also be disrupted after birth in ways that lead to increased metabolic risk in later life, such as impaired glucose-insulin axis function. Similarly, twin fetal sheep have altered HPA and glucose-insulin axis function compared with singletons, but the changes are different from those seen after periconceptional undernutrition in singletons (9). In studies of human twins, the relationship between fetal growth and later disease risk is inconsistent, and the effects of altered postnatal growth on later health are even less clear (10).

It has been suggested that rapid postnatal growth is the "common pathway" for later metabolic risk after nutritional insults *in utero* (11,12), and that postnatal interventions (for example, slowing postnatal growth rate by limiting nutrient supply) could therefore potentially ameliorate adverse outcomes. However, if the regulation of postnatal growth is perturbed by events very early in fetal life, then rapid postnatal growth rate and later metabolic disease both may be manifestations of intrauterine developmental disruption; in which case altering postnatal growth rate may not be achievable simply by regulating nutrient intake and may be only partially effective in decreasing long-term risk. We therefore investigated whether the periconceptional influences of maternal undernutrition or twin conception altered the regulation of postnatal growth in ways that could lead to later metabolic disease.

Aims. The aims of this experiment were to assess the effects of periconceptional undernutrition and twin conception in sheep on lamb growth in the first 12 wk after birth and on the relationships between growth, nutrient intake, and plasma concentrations of metabolites and growth-related hormones.

METHODS

Ethical approval for the study was obtained from the University of Auckland Animal Ethics Committee. Five-yr-old Romney ewes were randomly allocated to one of two treatment groups. Controls (N) were fed a maintenance ration of complete feed (Camtech Nutrition, Ltd., Hamilton, New Zealand) at 3–4% body weight per day. Undernourished (UN) ewes had feeds adjusted on an individual basis to achieve and maintain a weight loss of

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Abbreviations: D_2O, deuterium oxide; **HPA,** hypothalamic-pituitary-adrenal; **N,** nourished (control group); **NMR,** nuclear magnetic resonance; **UN,** undernourished (treatment group)

10–15% body weight, from 61 d before to 30 d after mating, and were fed thereafter in the same way as the control group (6). Ewes were housed indoors in a photoperiod controlled feedlot from 71 d before mating until 2 wk after lambing. Ewes were synchronized for mating using controlled internal drug release devices; four rams were used across all mating groups. UN ewes were kept in individual pens during the undernutrition period and then returned to group pens with the control animals until 3 wk before expected lambing, when all ewes were moved to individual pens. Labor occurred spontaneously. While in individual pens, ewes had visual and limited olfactory contact with other ewes at all times. Lambs returned to the paddock with their ewes at 2 wk of age.

Lambs were weighed as close as possible to 2 h after birth, at 7 d of age, and weekly thereafter until 12 wk. Blood samples for measurement of plasma concentrations of hormones and metabolites were taken by jugular venipuncture as close as possible to 2 h after birth, and at 1, 6, and 12 wk of age. Samples were collected into lithium heparin tubes, placed immediately on ice, centrifuged at 4°C at 3000 rpm, and the plasma separated and frozen at −80°C until analysis. Plasma glucose concentrations were measured by autoanalyzer (Hitachi 902Automatic Analyzer; enzymatic colorimetric assay): interassay variation 2.48% and intraassay variation 1.37%. Amino acid concentrations (valine, leucine, isolecuine, methionine, phenylalanine, lysine, histidine, threonine, taurine, serine, glycine, arginine, tyrosine, ornithine, hydroxyproline, proline, aspartate, citrulline, asparagines, glutamine, and glutamate) were measured by HPLC (13) and summed for analysis. Plasma ovine insulin concentration was measured by RIA, using ovine insulin as the standard (Sigma Chemical Co. Chemical, St. Louis, MO): interassay variation 9.4% and intraassay variation 7.9%. Plasma IGF1 concentrations were measured using an IGFBP-blocked RIA: interassay variation 9.1% and intraassay variation 11.0% (14) Plasma cortisol concentrations were measured using mass spectrometry: interassay variation 5.7% and intraassay variation 3.5% (15). Plasma leptin concentrations were measured in a subset of lambs by RIA (Perth, Western Australia) (16).

Milk intake was estimated between 1 and 2 wk of age in a subset of lambs using deuterium oxide (D_2O) dilution, which also assessed body water (17,18). During this time, lambs were in a pen with their mother with no access to any alternative milk source and were removed from their mother only briefly during blood collection and weighing. Lambs were weighed at the beginning and end of the study. Plasma samples were analyzed for D_2O concentration using nuclear magnetic resonance spectroscopy (NMR). The NMR spectrometer (Bruker DRX-400; Bruker BioSpin, Karlsruhe, Germany) was fitted with a 10-mm broadband probe. Acquisition and analysis was carried out using XwinNMR 3.5. The spectrometer was locked using DMSO and then the lock power was turned down and the lock cable removed. Either 2 mL of plasma or standard D_2O solution was added to a 10-mm NMR tube, and a 5-mm NMR tube containing the internal standard (1% C_6D_6 in C_6H_6) was suspended in it. Samples were run in nonspinning mode at room temperature with the sweep off. Initial shimming was carried out on the proton-free induction decay to optimize the field for each sample and then the deuterium spectrum was recorded (32 scans). The integral of the D_2O signal was measured relative to that of C_6D_6 for both standards (0–1.0 mg/mL) and unknown samples. The concentration of D_2O in the unknowns was determined from the calibration curve. Body water provided a proxy measure of lean body mass, with a greater percentage of body water reflecting greater proportional lean mass (19).

Statistics. Statistical analysis was performed using JMP v8 (SAS Institute Inc., Cary, NC). Nutritional groups, singles/twins, and males and females were compared using three-way ANOVA. Where no difference was found between sexes, data are presented for both sexes together. If there were significant differences between sexes, data were analyzed independently. To account for nonindependence of twins within a pair, ewe tag number was included as a random variable in the analysis. Relationships between lamb growth parameters, milk intake, and metabolite and hormone concentrations were analyzed using simple and multiple linear regression. The level of significance was taken at $p < 0.05$. Results are expressed as mean ± SEM.

RESULTS

One hundred twenty-four lambs were studied: 63 N [single (S) = 23, Twin (Tw) = 40] and 61 UN (S = 26, Tw = 35; Table 1); making a total of 75 twin lambs out of 41 ewes, so that 7 twins did not have a co-twin alive throughout the whole period of analysis, hence the odd number of twin lambs.

Lamb growth. Birth weight was similar in N and UN groups, but singles were heavier than twins, particularly in the UN group (singles *versus* twins $p = 0.0002$, nutrition group × twinning, $p = 0.006$), and males were heavier than females ($p = 0.008$, Table 1). At 12 wk of age, weights remained similar in N and UN groups, but singles were still heavier than twins ($p = 0.005$ for males and $p < 0.0001$ for females). Male single lambs of UN ewes were heavier than those in the N group at birth ($p = 0.02$) and also tended to have higher % body water in the second week of life, suggesting they were the leanest animals (nutrition group × sex interaction, $p = 0.06$; Table 1). Growth velocity was not different overall between groups in the first 12 wk of life but UN twins grew faster than UN singles (19.6 ± 0.5 *versus* 18.2 ± 0.4 $g \cdot kg^{-1} \cdot day^{-1}$; nutrition × twinning interaction, $p = 0.02$).

Milk intake. Mean milk intake was 166 ± 3 $mL \cdot kg^{-1} \cdot day^{-1}$ and was similar in all groups (Table 1).

Postnatal metabolites. Plasma glucose concentrations 2 h after birth were higher in singles than twins ($p < 0.0001$) and in females than males ($p = 0.01$), but were similar thereafter in all

Table 1. *Lamb weight, growth velocity, and nutrient intake from birth to 12 wk of age in single and twin offspring of N and UN ewes*

	Single lambs $n = 49$				Twin lambs $n = 75$			
	N ($n = 23$)		UN ($n = 26$)		N ($n = 40$)		UN ($n = 35$)	
	Male ($n = 8$)	Female ($n = 15$)	Male ($n = 13$)	Female ($n = 13$)	Male ($n = 16$)	Female ($n = 24$)	Male ($n = 14$)	Female ($n = 21$)
Weight (kg)								
Birth†‡	5.6 ± 0.4	5.3 ± 0.2	6.5 ± 0.2§	5.8 ± 0.2	5.5 ± 0.1	5.1 ± 0.1	5.2 ± 0.2	5.0 ± 0.2
1 wk	8.0 ± 0.5	7.9 ± 0.3	8.8 ± 0.3	8.1 ± 0.3	7.6 ± 0.3	7.0 ± 0.2	7.4 ± 0.2	7.1 ± 0.2
6 wk	17.9 ± 1.2	17.6 ± 0.8	17.7 ± 0.7	17.8 ± 0.6	16.8 ± 0.4	14.5 ± 0.5	16.5 ± 0.6	15.3 ± 0.6
12 wk†	27.0 ± 2.6	27.7 ± 1.4	27.8 ± 1.4	28.1 ± 1.1	26.1 ± 0.8	23.5 ± 0.6	27.6 ± 1.3	25.4 ± 1.0
Growth velocity ($g \cdot kg^{-1} \cdot day^{-1}$)								
0–2 wk	45 ± 6	49 ± 2	45 ± 1	44 ± 3	44 ± 2	41 ± 3	48 ± 3	44 ± 2
0–12 wk‡	19 ± 2	20 ± 0.5	18 ± 0.6	19 ± 0.6	19 ± 0.4	18 ± 0.5	20 ± 0.7	19 ± 0.7
Milk intake $mL \cdot kg^{-1} \cdot day^{-1}$	165 ± 18	167 ± 9	176 ± 9	165 ± 8	167 ± 8	165 ± 5	152 ± 12	170 ± 12
Total body water (%)	76 ± 4	76 ± 3	83 ± 3	72 ± 3	73 ± 3	74 ± 2	72 ± 2	73 ± 2

Values are mean ± SEM.

† $p < 0.05$ for difference between singles and twins.

‡ $p < 0.05$ for twin × nutrition group interaction.

§ $p < 0.05$ for difference between sexes.

Table 2. *Plasma concentrations of metabolites and growth related hormones in single and twin offspring of N and UN ewes in the first 12 wk of life*

	Single lambs (n = 49)				Twin lambs (n = 75)			
	N (n = 23)		UN (n = 26)		N (n = 40)		UN (n = 35)	
	Male (n = 8)	Female (n = 15)	Male (n = 13)	Female (n = 13)	Male (n = 16)	Female (n = 24)	Male (n = 14)	Female (n = 21)
Glucose (mmol · L^{-1})								
Birth*	4.8 ± 0.7	8.5 ± 2.0†	6.2 ± 0.8	8.0 ± 1.3	4.0 ± 0.5	4.7 ± 0.4	3.8 ± 0.4	4.3 ± 0.3
7 d	6.6 ± 0.	6.8 ± 0.2	7.0 ± 0.2	6.9 ± 0.2	6.8 ± 0.1	6.6 ± 0.2	6.8 ± 0.3	6.7 ± 0.1
6 wk	5.4 ± 0.2	5.7 ± 0.2	4.8 ± 0.6	5.5 ± 0.2	5.6 ± 0.2	5.1 ± 0.2	5.5 ± 0.2	5.5 ± 0.1
12 wk	4.6 ± 0.2	5.0 ± 0.2	4.8 ± 0.4	5.0 ± 0.3	4.6 ± 0.1	4.7 ± 0.2	4.9 ± 0.2	4.8 ± 0.1
Summed amino acids (mmol · L^{-1})								
Birth	6464 ± 1255	7696 ± 1391	5421 ± 1554	6592 ± 1054	5613 ± 637	5635 ± 623	6595 ± 2070	6370 ± 362
6 wk	3961 ± 488	3723 ± 311	3783 ± 151	4064 ± 206	4257 ± 126	3847 ± 202	4029 ± 267	4136 ± 268
12 wk	3275 ± 577	3211 ± 228	3290 ± 107	3257 ± 190	3669 ± 177	3490 ± 101	3270 ± 224	3632 ± 99
Insulin (ng · mL^{-1})								
Birth*	1.3 ± 0.7	2.5 ± 1.0	0.8 ± 0.4	0.6 ± 0.2	0.2 ± 0.03	0.3 ± 0.07	0.6 ± 0.2	0.8 ± 0.2
1 wk	0.9 ± 0.3	0.5 ± 0.05	1.2 ± 0.3	0.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.07	1.3 ± 0.4	0.8 ± 0.3
6 wk*	0.2 ± 0.05	0.3 ± 0.2	0.2 ± 0.06	0.3 ± 0.06	0.2 ± 0.05	0.1 ± 0.02	0.1 ± 0.03	0.2 ± 0.03
12 wk	0.1 ± 0.02	0.1 ± 0.02	0.2 ± 0.04	0.2 ± 0.05	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.01
IGF 1 (ng · mL^{-1})								
Birth*‡	91 ± 26	97 ± 20	104 ± 9	112 ± 14	84 ± 9	62 ± 5	80 ± 7	87 ± 9
1 wk*‡	149 ± 30	210 ± 22	202 ± 12	188 ± 15	171 ± 12	148 ± 8	191 ± 28	148 ± 12
6 wk*†	172 ± 30	149 ± 24	176 ± 29	126 ± 12	149 ± 18	94 ± 7	154 ± 31	120 ± 12
12 wk†	148 ± 44	129 ± 28	125 ± 26	111 ± 17	118 ± 14	98 ± 9	124 ± 20	93 ± 8
Cortisol (ng · mL^{-1})								
Birth§	53 ± 11	20 ± 7	50 ± 17	50 ± 8	47 ± 10	43 ± 8	24 ± 5	26 ± 6
1 wk§	14 ± 4	12 ± 6	12 ± 4	15 ± 3	10 ± 2	11 ± 2	6 ± 2	8 ± 2
6 wk	9 ± 3	7 ± 2	10 ± 3	11 ± 2	8 ± 1	10 ± 1	4 ± 1	8 ± 2
12 wk*‡§	10 ± 4	8 ± 2	13 ± 2	11 ± 3	11 ± 2	13 ± 2	4 ± 1	6 ± 1
Leptin (ng · mL^{-1})								
Birth	0.4 ± 0.01	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.1	0.6 ± 0.0	0.6 ± 0.1
1 wk	0.8 ± 0.3	1.7 ± 0.0	1.6 ± 0.1	1.5 ± 0.2	1.2 ± 0.2	1.6 ± 0.2	1.6 ± 0.5	1.4 ± 0.2
6 wk	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.7 ± 0.0	0.6 ± 0.1	0.7 ± 0.1
12 wk†	0.6 ± 0.0	0.8 ± 0.1	0.7 ± 0.0	0.9 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1

Values are mean ± SEM.
* $p < 0.05$ for difference between singles and twins.
† $p < 0.05$ for difference between sexes.
‡ $p < 0.05$ for difference between nutritional groups.
§ $p < 0.05$ for twin × nutrition group interaction.

groups (Table 2). Summed plasma amino acid concentrations were similar in all groups in the first 12 wk after birth (Table 2).

Growth-related hormones. Plasma insulin concentrations were similar in N and UN groups at all measured time points. Plasma insulin concentrations were higher in single than twin lambs at birth ($p = 0.01$) and at 6 wk of age ($p = 0.005$) but not at later time points (Table 2).

Plasma IGF1 concentrations were not different between nutritional groups in the first 12 wk of life. Plasma IGF1 concentrations were higher in singles than in twins at birth ($p = 0.01$) and still tended to be higher at 6 wk but were not different thereafter. At 6 wk, males had higher IGF1 concentrations than females ($p = 0.003$), but these differences were no longer significant by 12 wk.

Plasma cortisol concentrations were lowest in UN twins at birth (nutrition group × twin interaction, $p = 0.02$) and this pattern persisted throughout the first 12 wk of life (Table 2). There was no difference in cortisol concentrations between sexes. Plasma leptin concentrations were similar in all groups until 12 wk of age, when males had lower concentrations than females ($p = 0.006$).

Relationships between regulators of postnatal growth. Milk intake in the second week after birth was inversely related to birth weight in N single lambs but not significantly in N twins or in UN singles or twins (N singles: $R^2 = 0.56$, $p = 0.005$; N twins: $R^2 = 0.1$, $p = 0.06$ and UN singles: $R^2 = 0.003$, $p = 0.8$; UN twins: $R^2 = 0.12$, $p = 0.15$). Milk intake was positively related to growth velocity in the first 2 wk after birth in N single and twin lambs but not UN singles or twins (singles and twins combined, N: $R^2 = 0.17$, $p = 0.009$ and UN: $R^2 = 0.04$, $p = 0.2$).

Milk intake was not related to plasma glucose or amino acid concentrations, or to plasma insulin or cortisol concentrations in the first 12 wk after birth. Milk intake was positively related to IGF1 concentrations at birth and 1 wk of age only in N twins (birth: $R^2 = 0.14$, $p = 0.03$ and 1 wk: $R^2 = 0.14$, $p = 0.03$). Milk intake was positively related to plasma leptin concentrations at 1 wk of age only in the N group (singles and twins combined, N: $R^2 = 0.20$, $p = 0.03$ and UN: $R^2 = 0.002$, $p = 0.9$).

Plasma IGF1 concentrations were positively related to current weight in N but not UN single and twin lambs at 1 wk (N:

$R^2 = 0.25$, $p = 0.004$ and UN: $R^2 = 0.08$, $p = 0.1$; singles and twins combined) and in all lambs at 6 and 12 wk of age (6 wk N: $R^2 = 0.25$, $p < 0.0001$; UN: $R^2 = 0.21$, $p = 0.002$ and 12 wk N: $R^2 = 0.50$, $p < 0.0001$; UN: $R^2 = 0.27$, $p = 0.002$; singles and twins combined).

Plasma IGF1 concentrations were positively related to insulin concentrations only in UN singles and twins of both sexes at 1 wk (N: $R^2 = 0.03$ $p = 0.3$ and UN: $R^2 = 0.25$, $p = 0.003$) and 6 wk of age (N: $R^2 = 0.07$, $p = 0.08$ and UN: $R^2 = 0.14$, $p = 0.04$; singles and twins combined). There was no relationship between plasma IGF-1 and plasma cortisol concentrations in any group at 1, 6, or 12 wk.

Plasma IGF1 concentrations at 1 wk of age were strongly positively related to plasma leptin concentrations in N lambs ($R^2 = 0.63$, $p < 0.0001$), but not in UN lambs ($R^2 = 0.13$, $p = 0.1$), with similar findings in males and females. These relationships were not present at 6 wk. At 12 wk, plasma IGF1 concentrations were positively related to plasma leptin concentrations only in N twins (N single: $R^2 = 0.17$, $p = 0.40$; N twins: $R^2 = 0.26$, $p = 0.02$ and UN single: $R^2 = 0.05$, $p = 0.5$; UN twin: $R^2 = 0.06$, $p = 0.40$).

In summary, early postnatal plasma concentrations of growth-related hormones IGF-1 and insulin were lower in twins than singles, and cortisol concentrations were lowest in UN twins, who also had the fastest early growth. Maternal nutritional group had a stronger effect than twin conception on growth regulation, with the significant relationships between nutrient intake, growth-regulating hormones, and growth velocity seen in the N group in both singles and twins being abolished by maternal periconceptional undernutrition.

DISCUSSION

The postnatal growth trajectory of an individual reflects the complex interactions between genetic potential (including sex effects), nutrient intake and uptake, and the action of growth-related hormones. We found that periconceptional undernutrition altered these interactions from as early as the first postnatal week of life, suggesting that the regulation of postnatal growth is strongly influenced by events that occur very early in gestation.

Nutrient intake is a major regulator of postnatal growth. In our study, both milk intake and growth velocity were similar among groups, but the expected positive relationship between the two was abolished in both single and twin offspring of periconceptionally UN ewes. The major regulator of growth in these lambs is therefore not clear. This is important because modification of postnatal growth rate may not therefore be achievable solely by altering nutrient intake except at the extremes. Dissociation of growth rate from milk intake may be due to altered lamb metabolism but other possibilities include differences in milk composition and/or hormone content.

Nutrient intake also regulates hormones that influence growth and appetite, including IGF1 and leptin. Previous studies have shown derangement of the fetal somatotropic axis after periconceptional undernutrition. Gallaher *et al.* (3) showed that growth trajectory in fetuses of periconceptionally UN ewes did not change in response to an acute decrease in maternal nutrition later in pregnancy, despite a fall in plasma IGF1 concentrations, whereas fetuses of normally nourished ewes slowed and accelerated growth in parallel with the changes in maternal nutrition (2). Fetuses of periconceptionally UN ewes also had decreased plasma IGF1 and IGF-binding protein concentrations in mid-late gestation (4). These findings suggest that not only is the regulation of the fetal somatotropic axis altered by periconceptional undernutrition but also that changes in plasma IGF1 concentrations are dissociated from both nutrient intake and growth responses in these lambs. The mechanisms underlying this are not clear, but possibilities include altered tissue production of, or sensitivity to, IGF-1 that is not reflected in plasma hormone concentrations. Studies in mice suggest that postnatal nutrition also has a long-term influence on the function of the somatotropic axis. In newborn mice, postnatal under- and overnutrition resulted in short-term changes in blood glucose and leptin concentrations (high in overfed and low in underfed) that normalized when fed *ad libitum* after weaning; but longer term effects on growth trajectory, insulin concentrations, and somatotropic axis function. Glucose tolerance was poorer in both underfed and overfed groups at 3 and 12 mo of age, but for different reasons. There was inadequate initial insulin response in the underfed and high plasma insulin concentrations and insulin resistance in the overfed. Pituitary GH and plasma IGF-1 concentrations were low in previously nutrient-restricted mice and high in the overfed group at 3 mo of age. The authors suggested that IGF-I, together with other hormones like leptin, may influence adult somatotropic axis activity in mice by direct effects on the development of GH releasing hormone (GHRH) neurons in the hypothalamus during early postnatal life (20).

Somatotropic axis dysfunction is associated with manifestations of the metabolic syndrome (21). A study of normal children found that lower birth weight and IGF1 concentrations were associated with poor compensatory insulin secretion, suggesting a link between early somatotropic axis regulation and later metabolic derangement; these effects were independent of postnatal growth (22). Growth-restricted infants are also reported to have perturbed somatotropic axis regulation in the postnatal period independent of current nutritional status and have a higher rate of later metabolic disease (23). LBW followed by rapid growth in childhood is associated with the worst health outcomes in later life (24), including glucose intolerance, cardiovascular disease, and obesity (25,26). However, it is not clear whether rapid postnatal and childhood growth result from intrauterine endocrine adaptations, altered body composition, or from factors such as perturbed appetite regulation resulting in inappropriate nutrient intake (27,28).

The adipokine leptin also has roles related to the control of metabolism and energy homeostasis (29), with anorectic, antiobesity, and insulin-sensitizing properties (30). In sheep and humans, plasma leptin concentrations are positively associated with body fatness and nutritional status (31). Serum leptin concentration has been found in humans to be a biomarker for components of the metabolic syndrome in both lean and obese subjects (32). In long-standing type 2 diabetics, decreased

leptin response to glucocorticoids was associated with inability to augment insulin secretion during glucocorticoid treatment, suggesting that deranged regulation of leptin may contribute to the obesity and insulin resistance seen in type 2 diabetes (30).

Given that the normal relationships between nutrient intake, IGF1, and leptin concentrations were altered in offspring after periconceptional undernutrition, and that deranged function of somatotropic and leptin-insulin axes has been implicated in later development of metabolic syndrome (20,21), these findings suggest a possible mechanism underlying, for example, the poorer glucose tolerance in adult offspring of periconceptionally UN ewes (5).

In both humans and sheep, there is increasing evidence to suggest that there are physiological differences between singles and twins that are independent of nutrition or birth weight; *i.e.* an effect of twinning itself, which must therefore originate *in utero* (33–36). The physiology of twin pregnancy is the result of factors operating in both early and late gestation. The relationship between reduced size at birth and later metabolic disease risk, well described in singletons, is less clear in twins (37), and the possible compounding effect of postnatal growth rate in twins is not well described.

The effects of periconceptional undernutrition and twinning on fetal growth and metabolism, and the interaction between the two, have been studied in sheep (6,9,38). The lighter twin of a pair in a normally nourished ewe slowed growth rate during a maternal fast in late gestation significantly more than the light fetus of a periconceptionally UN ewe (6). The fetal glucose-insulin axis was affected differently by maternal nutrition in twins and singletons, with twins of normally nourished ewes having a greater insulin response to glucose than singles; this difference was abolished in fetuses of UN ewes, with insulin response the same in both twins and singles (6).

Periconceptional undernutrition resulted in decreased ACTH and cortisol response in both twins and singletons (9). There was also an effect of being a twin on fetal HPA axis function independent of maternal nutrition with similar ACTH, but lower baseline cortisol concentrations in twins compared with singles, but a greater ACTH response to corticotrophin-releasing hormone/arginine vasopressin (CRH/AVP) challenge in twins. In our experiment, postnatal cortisol concentrations were lowest in UN twins, who also had the lowest birth weight and fastest early growth. Twins from both nutritional groups had lower postnatal plasma concentrations of IGF-1 and insulin than singletons.

Given that HPA and somatotropic axes are likely to be important mediators of the relationship between fetal and early postnatal adaptations and later metabolic disease, these early differences may provide an explanation for the variation in outcomes between singles and twins. The periconceptional events of twinning and nutritional insult, in combination with the lower birth weight in twins of periconceptionally UN ewes, may compound the adverse effects on later metabolic health in this group.

Summary. Periconceptional undernutrition alters the relationships between key regulators of postnatal growth, including nutrient intake and somatotropic, leptin-insulin, and HPA axis function in both singles and twins without affecting size at birth or postnatal growth velocity. These changes are evident very early in postnatal life and persist for at least the first 12 wk, suggesting that they are likely to have been determined predominantly before, rather than after, birth. The dissociation of growth from its key endocrine regulators suggests one possible mechanism underlying later adverse metabolic outcomes in offspring of periconceptionally UN mothers.

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