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Impact of periconceptional nutrition on maternal and fetal leptin and fetal adiposity in singleton and twin pregnancies

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Edwards, L. J., J. R. McFarlane, K. G. Kauter, and I. C. McMillen. Impact of periconceptional nutrition on maternal and fetal leptin and fetal adiposity in singleton and twin pregnancies. Am J Physiol Regul Integr Comp Physiol 288: R39–R45, 2005. First published June 10, 2004; doi:10.1152/ajpregu.00127.2004.—It has been proposed that maternal nutrient restriction may alter the functional development of the adipocyte and the synthesis and secretion of the adipocyte-derived hormone, leptin, before birth. We have investigated the effects of restricted periconceptional undernutrition and/or restricted gestational nutrition on fetal plasma leptin concentrations and fetal adiposity in late gestation. There was no effect of either restricted periconceptional or gestational nutrition on maternal or fetal plasma leptin concentrations in singleton or twin pregnancies during late gestation. In ewes carrying twins, but not singletons, maternal plasma leptin concentrations in late gestation were directly related to the change in ewe weight that occurred during the 60 days before mating [maternal leptin concentration in late gestation was directly related to the change in ewe weight]. In twin, but not singleton, pregnancies, there was also a significant relationship between maternal and fetal leptin concentrations (maternal leptin = 0.5 fetal leptin + 4.2, r = 0.63, P < 0.005). The relative mass of perirenal fat was also significantly increased in twin fetal sheep in the control-restricted group (6.0 ± 0.5) compared with the other nutritional groups (control-control: 4.1 ± 0.4; restricted-restricted: 4.4 ± 0.4; restricted-control: 4.3 ± 0.3). In conclusion, the impact of maternal undernutrition on maternal plasma leptin concentrations during late gestation is dependent on fetal number. Furthermore, we have found that there is an increased fetal adiposity in the twins of ewes that experienced restricted nutrition throughout gestation, and this may be important in the programming of postnatal adiposity.

fetus; undernutrition; leptin; adipose tissue

LEPTIN IS A 16-kDa protein hormone that is predominantly synthesized and secreted by adipocytes, and, during adult life, plasma leptin concentrations are directly related to body fat content and to the prevailing level of nutrient intake (1, 4, 6). Leptin plays a key role in the regulation of energy homeostasis through its actions at central receptors to suppress appetite and decrease food intake and increase fat mobilization and energy expenditure in the adult (1). It has been proposed that prolonged exposure to high circulating leptin concentrations may result in a resistance to the actions of leptin, leading to a dysregulation of energy balance and obesity (1). Recent evidence has also suggested that leptin resistance in adult life may be programmed by exposure to periods of undernutrition in fetal life (1, 5). Rats exposed to maternal undernutrition during the first 2 wk of pregnancy become obese in adult life (2, 16, 17), and offspring of pregnant rats that were severely undernourished throughout pregnancy became hyperphagic, relatively obese, and hyperleptinemic when fed a hypercaloric diet after weaning (33).

Although people who were small babies tend to have a lower weight for height [body mass index (BMI)] in adult life than people who were larger at birth, these individuals tend to have a greater abdominal distribution of obesity, a significantly reduced muscle mass, and a high body fat content in adolescent and adult life despite their lower BMI (11, 20, 21, 25, 30). Exposure to a reduced nutrient supply in early pregnancy, as occurred in the Dutch Winter Famine in 1944–1945, also results in increased adiposity in later life (27, 28). Although plasma leptin concentrations are low in growth-restricted infants at birth (14), they increase to become higher in these infants at one year of age compared with their normal-birth-weight counterparts, regardless of BMI or gender (15). People with low birth weight also have higher leptin concentrations in adult life compared with individuals with a higher birth weight but same adult BMI (26).

In the sheep, as in the human, it has been demonstrated that leptin is synthesized in fetal adipose tissue and is present in the fetal circulation through late gestation (7, 10, 12, 23, 34, 35). Furthermore, there is evidence that leptin synthesis in fetal adipose tissue is regulated by fetal insulin concentrations and that circulating leptin concentrations are correlated with the relative mass of unilocular fat present in the adipose tissue depots in the sheep fetus during late gestation (term = 150 days gestation; see Refs. 7 and 24). A recent study reported that restriction of maternal nutrient intake between 28 and 80 days gestation in the pregnant ewe was also associated with an increase in fetal adiposity in late gestation (3). Although these studies highlight the importance of the nutritional environment during early pregnancy in determining subsequent adiposity, there have been no studies that have specifically investigated the impact of maternal undernutrition before pregnancy and during the early preimplantation period on the development of fetal adiposity and on the circulating profile of maternal and fetal leptin during late gestation.
The current study aimed to determine if maternal nutrient restriction during the periconceptional period would result in the programming of an increased adiposity before birth, thus leading to an increase in fetal fat mass and circulating leptin concentrations during late gestation. Furthermore, we aimed to determine if this effect of periconceptional undernutrition would occur independently of restriction of maternal nutrition during the remainder of pregnancy. We have therefore investigated the effects of restriction of maternal nutrient intake to 70% of maintenance energy requirements from 60 days before until 7 days after conception (periconceptional undernutrition) compared with gestational undernutrition (from 8 days to 145 days gestation) on fetal fat deposition and maternal and fetal plasma concentrations of leptin throughout late gestation. Pregnant ewes carrying singleton or twin pregnancies were fed either 100% (control) or 70% (restricted) of their maintenance energy requirements from 60 days before conception independently of restriction of maternal nutrition during the remainder of pregnancy. We have therefore investigated the effects of restriction of maternal nutrient intake to 70% of maintenance energy requirements from 60 days before until 7 days after conception (periconceptional undernutrition) compared with gestational undernutrition (from 8 days to 145 days gestation) on fetal fat deposition and maternal and fetal plasma concentrations of leptin throughout late gestation. Pregnant ewes carrying singleton or twin pregnancies were fed either 100% (control) or 70% (restricted) of their maintenance energy requirements from 60 days before until 7 days after conception. Ewes in each group were then maintained on either a maintenance diet (i.e., control-control or restricted-control) or were fed 70% of the maintenance requirements (control-restricted or restricted-restricted) for the remainder of gestation.

MATERIALS AND METHODS

All procedures were approved by The University of Adelaide Standing Committee on Animal Ethics and Experimentation.

Nutritional Protocols

Forty-five Border-Leicester × Merino ewes were used in this study. Before mating (60 days), ewes were randomly assigned to one of two feeding regimes, control (n = 20), which received 100% of nutritional requirements, or restricted (n = 25), which received 70% of the control allowance. The nutritional requirements for the control animals were calculated to provide sufficient energy for the maintenance of a nonpregnant ewe (7.8 MJ/day for a 60-kg ewe; see Ref. 22). All animals were housed in individual pens and had free access to water. The diet consisted of lucerne chaff and pellets containing straw, cereal, hay, clover, barley, oats, lupins, almond shells, oat husks, and limestone. Eighty percent of the total energy requirements were obtained from the lucerne chaff and 20% of the energy requirements from the pellet mixture. The lucerne chaff provided 8.3 MJ/kg metabolizable energy (ME) and 193 g/kg crude protein and contained 85% dry matter, and the pellets provided 8.0 MJ/kg ME and 110 g/kg crude protein and contained 90% dry matter. All of the dietary components were reduced by an equal amount in the restricted diet.

After maintenance on either the control or restricted diet for a minimum period of 60 days, a ram was introduced, and 7 days after mating, ewes from each feeding regime were randomly assigned to the control or restricted plane of nutrition for the remainder of pregnancy (term = 147 ± 3 days gestation). Therefore, animals were maintained either on a control or restricted diet during the periconceptional period (~60 to +7 days after mating) and then either on a control or restricted diet during the gestational period (+8 days until postmortem). Four treatment groups were therefore generated: control-control (C-C, n = 12); control-restricted (C-R, n = 8); restricted-restricted (R-R, n = 14), and restricted-control (R-C, n = 11).

 Pregnancy and fetal number were confirmed by ultrasonography at 60 days gestation. Twenty-three ewes carried singleton fetuses (C-C, n = 6; C-R, n = 3; R-R, n = 9; R-C, n = 5), and 22 ewes carried twin fetuses (C-C, n = 6; C-R, n = 5; R-R, n = 5; R-C, n = 6). The nutritional intake for animals on the restricted diet was maintained at 70% of control energy requirements, and both nutritional regimes were adjusted for gestational age and fetal number, as outlined by the Ministry of Agriculture, Fisheries, and Food. The feed intake was increased every 10 days after 90 days gestation by 7.5% for ewes carrying singleton fetuses and 11% for ewes carrying twin fetuses.

Animals and Surgery

Pregnant ewes were transported to the Animal House between 90 and 100 days gestation. Surgery was performed under aseptic conditions between 105 and 110 days gestation with general anesthesia initially induced by an intravenous injection of sodium thiopentone (1.25 g Pentothal; Rhone Merieux, Pinkenba, Qld, Australia) and maintained with inhalational halothane (2.5-4% Fluothane; ICL, Melbourne, Vic, Australia) in oxygen. In all ewes, vascular catheters were implanted in a fetal carotid artery and jugular vein, a maternal jugular vein, and the amniotic cavity, as previously described (9). Vascular catheters were only inserted in one fetus in twin pregnancies. All catheters were filled with heparinized saline, and the fetal catheters were exteriorized through an incision made in the ewes’ flank. All ewes and fetal sheep received a 2-ml intramuscular injection of antibiotics (250 mg/ml procaine penicillin; 250 mg/ml dihydrostreptomycin; and 20 mg/ml procaine hydrochloride, Penstrep Illium; Troy Laboratories, Smithfield, NSW, Australia) at the time of surgery. The ewes were housed in individual pens in animal holding rooms with a 12:12-h light-dark cycle and were fed one time daily at 1100 with water provided ad libitum. Animals were allowed to recover from surgery for at least 4 days before experimentation.

Blood Sample Collection

Fetal arterial blood (0.5-ml) samples were collected every day for 4 days after surgery and then three times per week thereafter for the measurement of arterial PO2, PCO2, pH, oxygen saturation, and Hb (ABL 520 blood gas analyzer; Radiometer, Copenhagen, Denmark). The fetal arterial blood gas variables measured across late gestation in fetuses in all of the nutritional groups were in the normal range previously reported for healthy fetuses in late gestation (9, 29). Fetal arterial blood samples (3.5 ml) were collected in chilled heparinized tubes (125 IU; Sarstedt, South Australia, Australia) three times per week between 0800 and 1100 for the measurement of plasma glucose, insulin, and leptin concentrations throughout late gestation. Similarly, maternal venous blood samples (5 ml) were collected in chilled tubes three times per week between 0800 and 1100 for the measurement of plasma glucose and leptin concentrations. All blood samples were centrifuged at 1,500 g for 10 min, and plasma was separated into aliquots and stored at −20°C for subsequent hormone and metabolite assays.

Postmortem

Ewes were killed with an overdose of pentobarbitone sodium (Virbac, Peakhurst, NSW, Australia) between 140 and 147 days gestation, and the fetuses were delivered by hysterotomy, weighed, and killed by decapitation. Fetal perirenal adipose tissue was collected and weighed, and, in the case of twins, perirenal adipose tissue was collected and weighed from both fetuses.

Glucose Assay

Plasma concentrations of glucose were measured by enzymatic analysis using hexokinase and glucose-6-phosphate dehydrogenase to measure the formation of NADH photometrically at 340 nm (COBAS MIRA automated analysis system; Roche Diagnostica, Basel, Switzerland; see Ref. 8). The sensitivity of the assay was 0.5 mmol/l and the intra- and interassay coefficients of variation were both <5%.

Leptin Assay

Plasma leptin concentrations were measured using a competitive ELISA previously validated for sheep plasma (18). The ELISA plate was coated with 6 ng recombinant bovine leptin in 50 µl 0.1 M bicarbonate buffer, pH 9.0, overnight at 37°C. The plate was blocked...
with 200 ml 5% skim milk in ELISA buffer for 1 h at 37°C. Samples (100 μl) were assayed in duplicate and added to the wells containing chicken antirecombinant bovine leptin antiserum in 10% Triton X, 0.5% SDS, and 5% sodium deoxycholate (50 μl), and the plate was incubated overnight at 37°C. Streptavidin conjugated to alkaline phosphatase (Amrad Biotech, Boronia, Vic, Australia) was incubated for 1 h, and the plate was developed with p-nitrophenylphosphate disodium salt hexahydrate. The sensitivity of the assay was 0.5 ng/ml, and the interassay and intra-assay coefficients were 11.5 and 6.1%, respectively.

Statistical Analysis

Data are presented as means ± SE. Hormonal data were log transformed where required to normalize data variance for parametric analysis.

The weights of the nonpregnant ewes assigned to the restricted and control periconceptional nutrition groups were compared using an unpaired Student’s t-test. The effects of nutritional group on fetal weight and fetal perirenal fat mass, expressed as absolute values and relative to body weight, were compared separately in singleton and twin fetuses using a one-way ANOVA. The effects of periconceptional and gestational nutrition on maternal and fetal plasma leptin concentrations were compared separately in singleton- and twin- pregnant groups were combined (maternal leptin concentrations throughout late gestation in any of the nutritional protocols, and there was no correlation between maternal and fetal plasma leptin concentrations in ewes carrying singletons. Maternal, but not fetal plasma, leptin concentrations were positively related to circulating glucose concentrations during late gestation when data from all four nutritional treatment groups were combined (maternal leptin = 3.3 maternal glucose + 2.3; r = 0.53, P < 0.05).

Periconceptional and gestational undernutrition and fetal weight and fat mass. There was no effect of restricted maternal nutrition during either the periconceptional or gestational periods on the fetal weights at postmortem or on the absolute or relative mass of perirenal adipose tissue in singleton fetuses (Table 1). Plasma leptin concentrations were not related to

used to determine the relationship between mean fetal leptin concentrations and fetal perirenal fat mass.

RESULTS

Periconceptional Undernutrition and Ewe Weight Loss

There was no difference in the weights of the nonpregnant ewes assigned to the control (55.8 ± 1.0 kg, n = 20) or restricted (56.5 ± 0.9 kg, n = 25) diet before the start of the feeding regime. Ewes in the restricted periconceptional nutrition group lost significantly more weight than those in the control group during the prepregnancy period (Fig. 1).

Singleton Pregnancies

Periconceptional and gestational undernutrition and plasma glucose and leptin concentrations. Maternal plasma glucose concentrations were significantly lower in ewes in which nutrition was restricted during the gestational period, and this occurred independently of the level of nutrition during the periconceptional period (Table 1). Similarly, restricted maternal nutrition during the gestational period, but not the periconceptional period, resulted in significantly lower fetal plasma glucose concentrations (Table 1).

There was no effect of either restricted periconceptional or gestational nutrition on maternal or fetal plasma leptin concentrations in singleton pregnancies during late gestation (Table 1). There was also no change in maternal or fetal plasma leptin concentrations throughout late gestation in any of the nutritional protocols, and there was no correlation between maternal and fetal plasma leptin concentrations in ewes carrying singletons. Maternal, but not fetal plasma, leptin concentrations were positively related to circulating glucose concentrations during late gestation when data from all four nutritional treatment groups were combined (maternal leptin = 3.3 maternal glucose + 2.3; r = 0.53, P < 0.05).

Singleton Pregnancies

Periconceptional and gestational undernutrition and fetal weight and fat mass. There was no effect of restricted maternal nutrition during either the periconceptional or gestational periods on the fetal weights at postmortem or on the absolute or relative mass of perirenal adipose tissue in singleton fetuses (Table 1). Plasma leptin concentrations were not related to

Table 1. Effect of nutritional group on maternal and fetal plasma leptin and glucose concentrations and fetal weight and fat mass in singleton pregnancies

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Maternal glucose, mmol/l</th>
<th>Maternal leptin, ng/ml</th>
<th>Fetal glucose, mmol/l</th>
<th>Fetal leptin, ng/ml</th>
<th>Fetal weight, kg</th>
<th>Absolute fetal perirenal fat mass, g</th>
<th>Relative fetal perirenal fat mass, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-C</td>
<td>10</td>
<td>6.8 ± 0.8</td>
<td>6.8 ± 0.8</td>
<td>1.6 ± 0.1</td>
<td>3.8 ± 0.8</td>
<td>4.9</td>
<td>18.2 ± 1.9</td>
<td>4.1</td>
</tr>
<tr>
<td>C-R</td>
<td>10</td>
<td>6.8 ± 1.0</td>
<td>6.8 ± 1.0</td>
<td>1.7 ± 0.2</td>
<td>4.0 ± 1.2</td>
<td>4.9</td>
<td>21.5 ± 2.7</td>
<td>4.3</td>
</tr>
<tr>
<td>R-R</td>
<td>10</td>
<td>6.8 ± 0.8</td>
<td>6.8 ± 0.8</td>
<td>1.6 ± 0.1</td>
<td>3.8 ± 0.8</td>
<td>4.9</td>
<td>22.0 ± 2.7</td>
<td>4.3</td>
</tr>
<tr>
<td>R-C</td>
<td>10</td>
<td>6.8 ± 0.8</td>
<td>6.8 ± 0.8</td>
<td>1.7 ± 0.2</td>
<td>4.0 ± 1.2</td>
<td>4.9</td>
<td>23.1 ± 4.2</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Data are represented as means ± SE from a minimum of 2 and a maximum of 9 animals/treatment group C, control; R, restricted. Different superscripts (a and b) denote a significant effect of nutritional group on maternal and fetal glucose concentrations, P < 0.05. There was no significant effect of nutritional group on maternal and fetal leptin concentrations or on fetal weight or absolute and relative fetal fat mass.

Fig. 1. Restricted periconceptional nutrition (●, n = 25) resulted in a significant loss of weight in nonpregnant ewes between weeks 5 and 7 after the start of the feeding regime (*significant change in ewe weight, P < 0.05) compared with the change in ewe weight after 1 wk of feeding. There was no significant effect, however, of control periconceptional nutrition (○, n = 20) on the change in nonpregnant ewe weight during the 7 wk after the start of the feeding regime.
either absolute or relative perirenal fat mass in singleton fetal sheep.

**Twin Pregnancies**

Periconceptional and gestational undernutrition and maternal plasma leptin. Maternal glucose concentrations were significantly lower in ewes carrying twins in which nutrition was restricted during the gestational period, and this effect occurred independently of the level of nutrition during the periconceptional period (Table 2). Similarly, restricted maternal nutrition during the gestational period, but not the periconceptional period, resulted in significantly lower fetal plasma glucose concentrations in twin fetal sheep (Table 2). There was no significant effect of restricted nutrition during either the periconceptional or gestational periods on maternal or fetal plasma concentrations of leptin in twin pregnancies during late gestation (Table 2). Maternal plasma leptin concentrations between 135 and 146 days gestation were directly related, however, to the change in ewe weight experienced during the prepregnancy period [maternal leptin = 0.9 (change in ewe weight) + 7.8; \( r = 0.6, P < 0.05 \); Fig. 2]. In contrast to ewes carrying singleton pregnancies, there was no significant relationship between maternal leptin and glucose concentrations during late gestation in ewes carrying twins.

There was, however, a significant positive relationship between maternal and fetal plasma leptin concentrations in ewes carrying twins (maternal leptin = 0.5 fetal leptin + 4.2, \( r = 0.63, P < 0.005 \); Fig. 3).

**Periconceptional and gestational undernutrition and fetal weight and fat mass.** There was a significant effect of nutritional group on fetal weight at postmortem such that fetuses in the C-R group were significantly smaller compared with fetuses in the other nutritional groups (Table 2). There was no significant effect of restricted periconceptional or gestational nutrition on perirenal fat mass expressed as absolute weight (Table 2). The relative mass of fetal perirenal fat was significantly higher, however, in fetuses in the C-R group compared with the other nutritional treatment groups (Fig. 4). There was no significant relationship between plasma leptin concentrations and perirenal fat mass, expressed as absolute or relative amounts, in twin fetal sheep.

**Table 2. Effect of nutritional group on maternal and fetal plasma leptin and glucose concentrations and fetal weight and fat mass in twin pregnancies**

<table>
<thead>
<tr>
<th>Nutritional Group</th>
<th>Maternal Glucose (mmol/l)</th>
<th>Maternal Leptin (ng/ml)</th>
<th>Fetal Glucose (mmol/l)</th>
<th>Fetal Leptin (ng/ml)</th>
<th>Fetal Weight (kg)</th>
<th>Absolute Fetal Perirenal Fat Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-C</td>
<td>2.3 ± 0.2 ( ^{a} )</td>
<td>6.2 ± 0.1</td>
<td>1.3 ± 0.02 ( ^{b} )</td>
<td>4.7 ± 1.2</td>
<td>4.5 ± 0.3</td>
<td>18.8 ± 1.2</td>
</tr>
<tr>
<td>C-R</td>
<td>2.1 ± 0.2 ( ^{b} )</td>
<td>6.5 ± 1.2</td>
<td>1.1 ± 0.03 ( ^{b} )</td>
<td>6.4 ± 1.5</td>
<td>2.9 ± 0.3</td>
<td>18.0 ± 1.2</td>
</tr>
<tr>
<td>R-R</td>
<td>1.9 ± 0.2 ( ^{b} )</td>
<td>6.6 ± 1.3</td>
<td>1.0 ± 0.02 ( ^{b} )</td>
<td>4.3 ± 1.4</td>
<td>3.9 ± 0.2</td>
<td>17.2 ± 1.5</td>
</tr>
<tr>
<td>R-C</td>
<td>2.5 ± 0.1 ( ^{a} )</td>
<td>5.6 ± 1.4</td>
<td>1.3 ± 0.02 ( ^{b} )</td>
<td>4.6 ± 1.0</td>
<td>4.1 ± 0.3</td>
<td>19.2 ± 0.8</td>
</tr>
</tbody>
</table>

Data are represented as means ± SE from a minimum of 4 and a maximum of 12 animals/treatment group. Different superscripts (a and b) denote a significant effect of nutritional group on maternal and fetal glucose concentrations, \( P < 0.05 \). *Significant decrease in fetal weight in the C-R group compared with the other treatment groups (\( P < 0.05 \)). There was no significant effect of nutritional group on maternal or fetal leptin concentrations or on the absolute fetal fat mass.

**Fig. 2.** There was a significant positive relationship between maternal plasma leptin concentrations of leptin between 115 and 146 days gestation [maternal leptin = 0.5 (fetal leptin) + 4.2, \( r = 0.63, P < 0.005 \)].

**Fig. 4.** Perirenal fat mass, expressed relative to body weight, was significantly increased in twin fetal sheep in the C-R group (\( n = 4 \)) compared with twin fetal sheep in the other nutritional groups (C-C, \( n = 8 \); R-R, \( n = 3 \); R-C, \( n = 7 \)). *\( P < 0.05 \).
DISCUSSION

In this study, we have demonstrated that a decrease in maternal nutrition after the 1st wk in pregnancy results in an increase in relative perirenal fat mass in twin, but not singleton, fetuses.

Although there was an increase in fetal adiposity in twin fetuses of ewes that were undernourished from the end of the 1st wk of gestation, there was no increase in circulating leptin concentrations in these fetuses. In twin pregnancies, maternal leptin concentrations were lower in those ewes that lost the most weight before pregnancy, whereas, in singleton pregnancies, maternal leptin concentrations were lower in those ewes with lower prevailing glucose concentrations during late gestation. There was also a correlation between maternal and fetal leptin concentrations in twin pregnancies.

In sheep, the impact of maternal undernutrition on plasma leptin concentrations appears to be dependent on the breed and age of the ewe and on the level of nutrient restriction. In growing adolescent ewes (Suffolk or Dorset Horn × Greyface: body wt 40–45 kg) pregnant as a result of embryo transfer, increases or decreases in maternal nutrition during the first 100 days of gestation were associated with parallel increases or decreases in maternal plasma leptin concentrations (32). When data were pooled across nutritional treatments, circulating leptin concentrations at 104 days gestation were positively correlated with maternal weight and body condition score (32). A 40% restriction of ME requirements between 28 and 80 days gestation also resulted in a decrease in maternal leptin concentrations in Welsh Mountain ewes (body wt 35–40 kg; see Ref. 3), whereas a 50% restriction from 115 days gestation resulted in a fall in plasma glucose, but not leptin, concentrations in Merino ewes (body wt 50–60 kg) during late gestation (35). Similarly, in the present study in Merino ewes carrying singleton fetuses, plasma glucose, but not leptin, concentrations were lower in ewes that had been undernourished from day 8 of pregnancy. Thus, in large adult ewes carrying singleton fetuses, maternal leptin concentrations are not as sensitive a measure of the level of prevailing nutrition as maternal glucose concentrations. There was, however, a significant relationship between maternal plasma leptin and glucose concentrations during late gestation when all nutritional groups were combined. Interestingly, in ewes carrying twin pregnancies, although maternal plasma leptin concentrations were not lower in the restricted nutrition groups during late gestation, they were directly related to the amount of weight loss experienced before pregnancy when all nutrition treatment groups were combined. Because there is a greater energy demand on the mother during a twin pregnancy, it is not surprising that relationships between the level of maternal leptin during late gestation and changes in prepregnancy weight emerge in ewes carrying twins. In contrast to ewes carrying singletons, there was no direct relationship between maternal plasma leptin and glucose concentrations in ewes carrying twins during late gestation, presumably reflecting the greater dependence of maternal leptin concentrations in late gestation on the body fat stores at the start of pregnancy in these animals. These results highlight the interaction between the amount of body fat stores at the start of pregnancy, the number of fetuses, and the level of nutrient intake throughout pregnancy in determining circulating leptin concentrations in the pregnant ewe.

We found that there was no impact of maternal undernutrition during either the periconceptional or gestational periods on fetal plasma leptin concentrations during late gestation. Previous studies have found that a 50% decrease in maternal nutrient intake from ~115 days gestation did not decrease fetal plasma concentrations of leptin or the relative abundance of leptin mRNA in fetal perirenal adipose tissue (10, 35). Thus the synthesis and secretion of leptin in the sheep fetus are relatively resistant to the changes in fetal glucose and insulin concentrations associated with moderate maternal undernutrition. Interestingly, in the present study, there was a direct relationship between circulating maternal and fetal leptin concentrations in twin, but not singleton, pregnancies. It is possible that maternal body composition or fatness either at the beginning or during pregnancy determines the leptin synthetic and secretory capacity of both maternal and fetal adipose tissue. An alternative explanation is that there is maternal-fetal transfer of leptin in the sheep, as has recently been shown in the rat during late gestation (31). Whereas leptin mRNA is expressed at negligible levels in the sheep placenta (10, 32), the leptin receptor is expressed, and this receptor may mediate the uptake of leptin from the maternal to the fetal circulation. Clearly, the placental transport of leptin in sheep requires further investigation to determine if differences exist between singleton and twin pregnancies, which would thus explain the observations of the present study.

We found no effect of maternal nutritional treatment on fetal weight, adiposity, or plasma leptin concentrations in singleton fetal sheep. Twin fetal sheep were significantly smaller than singletons, irrespective of maternal nutritional treatment. Although maternal nutritional treatment did not affect fetal plasma concentrations of leptin in twin fetal sheep, there was a relative increase in adiposity in twin fetuses of ewes that were exposed to maintenance nutrition followed by a decrease in nutrition after the 1st wk of pregnancy. Fetal adiposity was not increased in twin fetal sheep of ewes that had been undernourished from before and throughout pregnancy, indicating that increased fetal adiposity was not a result of the greater degree of growth restriction experienced by twin fetal sheep. This “fat-sparing” effect in the twin fetuses in the control-restricted group, therefore, appears to be a specific effect of the level of nutrition during and immediately after the preimplantation period on growth and adiposity in twin fetal sheep. The altered fetal fat deposition is unlikely to reflect the increased metabolic demand of twin fetuses, since the change in the level of nutrition occurred at a time when the metabolic demands of the embryos were minimal. It has been shown, however, that manipulation of the early nutritional environment of the embryo either in vivo or in vitro can alter the allocation of cells within the innercell mass and trophoblast and subsequent fetal somatic and organ growth (19). Maternal hormonal or metabolic responses to the change in the level of nutrition at the end of the 1st wk of pregnancy may act to alter the expression of key genes within the developing blastocyst to result in enhanced adiposity in late gestation. In a recent study, Bispham and colleagues (3) reported that there was an increase in relative fetal adiposity in fetuses in ewes that had been nutrient restricted between 28 and 80 days gestation and then fed to appetite (i.e., 150% ME) until 140 days gestation compared with fetuses in ewes that had been fed to appetite between 28 and 140 days gestation. In this latter study, fetuses...
of ewes that had been nutrient restricted between 28 and 80 days gestation and then fed to energy requirements (i.e., 100% ME) did not have an increase in relative adiposity compared with their control counterparts. It appears, therefore, that there are different critical windows during which the imposition of maternal undernutrition (~8 days gestation) or maternal over-nutrition (~80 days gestation) can result in an increased fetal adiposity. In the present study, although there was an increase in the relative fat mass in twin fetuses in the control-restricted group, there was not an increase in circulating plasma leptin concentrations in this group during late gestation. In the sheep fetus, the perirenal adipose tissue is composed of multilocular cells, which possess an abundance of mitochondria and express uncoupling protein 1, which are the characteristics typical of brown adipocytes (13, 24). There is a positive relationship between circulating leptin concentrations and the relative mass of fetal adipose tissue, which is composed of large or the dominant lipid locules (23). Although perirenal fat mass is increased in the nutrient-restricted group, it may be that this fat is composed predominantly of smaller lipid locules associated with a lower leptin synthetic capacity.

In summary, this study demonstrates that a decrease in maternal nutrition at the end of the 1st wk in pregnancy results in an increase in fetal adiposity in twin, but not singleton, fetuses. The increase in fetal fat mass was not associated with a concomitant increase in fetal plasma leptin concentrations. Although this study highlights the importance of maternal nutrition at the end of the 1st wk in pregnancy results in an increase in fetal plasma leptin concentrations in twin, but not singleton, fetuses. The increase in fetal fat mass was not associated with a concomitant increase in fetal plasma leptin concentrations. Although this study highlights the importance of maternal nutrition at the end of the 1st wk in pregnancy results in an increase in fetal plasma leptin concentrations.

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MATERNAL UNDERNUTRITION AND FETAL LEPTIN


