Impact of maternal undernutrition before and during pregnancy on maternal and fetal leptin and fetal adiposity in singleton and twin pregnancies

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ABSTRACT

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 which occurred during the 60 days before mating (maternal leptin = 0.9 (change in ewe weight) + 7.8; r = 0.6, P < 0.05). 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) when compared to 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 which experienced restricted nutrition throughout gestation and this may be important in the programming of postnatal adiposity.
INTRODUCTION

Leptin is a 16 kDa protein hormone which is predominantly synthesised 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 mobilisation 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 weeks of pregnancy become obese in adult life (2, 16, 17) and offspring of pregnant rats which were severely undernourished throughout pregnancy, became hyperphagic, relatively obese and hyperleptinaemic when fed a hypercaloric diet after weaning (33).

Whilst 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). Whilst 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 when compared to their normal birth weight counterparts, regardless of bodymass index or gender (15). People with low birth weight also have higher leptin
concentrations in adult life when compared to 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 synthesised 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=150d gestation) (7, 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). Whilst these studies highlight the importance of the nutritional environment during early pregnancy in determining subsequent adiposity, there have been no studies which 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) when 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 cross Merino ewes were used in this study. Sixty days prior to mating, ewes were randomly assigned to one of two feeding regimes, Control (C, n = 20) which received 100% of nutritional requirements or Restricted (R, 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 non-pregnant ewe (7.8 MJ/day for a 60 kg ewe) (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 twenty percent of the energy requirements from the pellet mixture. The lucerne chaff provided 8.3 MJ/kg metabolisable energy (ME), 193 g/kg of crude protein and contained 85% dry matter and the pellets provided 8.0 MJ/kg ME, 110 g/kg of 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 (d), a ram was introduced and 7 d 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 d gestation). Therefore animals were maintained either on a Control or Restricted diet during the periconceptional period (-60 to +7 d after mating) and then either on a Control or Restricted diet during the gestational period (+8 days until post mortem). 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 ultrasound at 60 d 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 into the Animal House between 90 and 100 d gestation. Surgery was performed under aseptic conditions between 105 and 110 d gestation with general anaesthesia initially induced by an intravenous (iv) injection of sodium thiopentone (1.25 g; Pentothal, Rhone Merieux, Pinkenba, Qld, Australia) and maintained with inhalational halothane.
(2.5 - 4%; Fluothane, ICI, 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 into one fetus in twin pregnancies. All catheters were filled with heparinised saline and the fetal catheters exteriorised through an incision made in the ewes’ flank. All ewes and fetal sheep received a 2 ml intramuscular injection of antibiotics (procaine penicillin 250 mg/ml; dihydrostreptomycin 250 mg/ml; procaine hydrochloride 20 mg/ml, 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 hour light/dark cycle and fed once daily at 1100 h with water provided ad libitum. Animals were allowed to recover from surgery for at least 4 days prior to experimentation.

**Blood sample collection**

Fetal arterial blood (0.5 ml) samples were collected every day for 4 days after surgery and then 3 times per week thereafter, for the measurement of arterial PO$_2$, PCO$_2$, pH, oxygen saturation and haemoglobin (ABL 520 blood gas analyser, 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 heparinised tubes (125IU, Sarstedt, South Australia, Australia) 3 times per week between 0800 h - 1100 h, 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 3 times per week between 0800 h - 1100 h, for the measurement of plasma glucose and leptin concentrations. All blood samples...
were centrifuged at 1500 x g for 10 minutes (min) and plasma was separated into aliquots and stored at -20°C for subsequent hormone and metabolite assays.

*Post Mortem*

Ewes were killed with an overdose of sodium pentobarbitone (Virbac, Peakhurst, NSW, Australia) between 140 and 147 d gestation and the fetuses 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) (8). The sensitivity of the assay was 0.5 mmol/l and the intra- and interassay coefficients of variation were both less than 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 antisera 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 developed with p-nitrophenylphosphate disodium salt hexahydrate. The sensitivity of the assay was 0.5 ng/ml and the inter assay and intra assay coefficients were 11.5% and 6.1% respectively.

**Statistical Analysis**

Data are presented as the mean ± standard error of the mean (SEM). Hormonal data were log transformed where required, in order to normalise data variance for parametric analysis.

The weights of the non-pregnant 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 bearing ewes and their fetuses using a multifactorial ANOVA with repeated measures. Specified factors for the ANOVA included periconceptional nutrition (C or R), gestational nutrition (C or R) and gestational age. When a significant interaction between major factors was identified by ANOVA, the data were split on the basis of the interacting factor and reanalysed using ANOVA. The Duncan’s New Multiple Range Test was used post-ANOVA to identify significant differences between mean values and a probability level of 5% ($P < 0.05$) was taken as significant.

Mean plasma leptin and glucose concentrations were determined between 115 and 145 d gestation for each ewe and fetus. Linear regression analysis was then used to determine the
relationship between maternal leptin concentrations and the change in ewe weight during the prepregnancy period, maternal glucose concentrations and fetal leptin concentrations. Similarly, linear regression analysis was 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 non-pregnant ewes assigned to the Control (55.8 ± 1.0 kg, n = 20) or Restricted (56.5 ± 0.9 kg, n = 25) prior to 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 (Figure 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 4 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 post mortem or on the absolute or relative mass of perirenal adipose tissue in singleton fetuses (Table 1). Plasma leptin concentrations were not related to 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 pre-pregnancy period (maternal leptin = 0.9 (change in ewe weight) + 7.8; r = 0.6, P < 0.05) (Figure 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, \( r = 0.63, P < 0.005 \)) (Figure 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 to 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 when compared to the other nutritional treatment groups (Figure 4). There was no significant relationship between plasma leptin concentrations and perirenal fat mass, expressed as absolute or relative amounts, in twin fetal sheep.

DISCUSSION
In this study we have demonstrated that a decrease in maternal nutrition after the first week in pregnancy results in an increase in relative perirenal fat mass, in twin, but not singleton fetuses. Whilst there was an increase in fetal adiposity in twin fetuses of ewes which were undernourished from the end of the first week of gestation, there was no increase in circulating leptin concentrations in these fetuses. In twin pregnancies maternal leptin concentrations were lower in those ewes which lost 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 x Greyface: body weight 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 weight 35-40 kg) (3) whereas a 50% restriction from 115 days gestation resulted in a fall in plasma glucose, but not leptin concentrations in Merino ewes (body weight 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 which 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, whilst 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. As 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 pre-pregnancy 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 is 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). Whilst 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
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. Whilst 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 which were exposed to maintenance nutrition followed by a decrease in nutrition after the first week of pregnancy. Fetal adiposity was not increased in twin fetal sheep of ewes which 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, as 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 inner cell mass and trophectoderm 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 first week 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 (2003) reported that there was an increase in relative fetal adiposity in fetuses in ewes which had been nutrient restricted between 28 and 80 days gestation and then fed to appetite (i.e. 150% ME) until 140 days gestation when compared with fetuses in ewes which
had been fed to appetite between 28 and 140 days gestation. In this latter study, fetuses of ewes which 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 when compared to their control counterparts. It appears therefore that there are different critical windows during which the imposition of maternal undernutrition (~8 days gestation) or maternal overnutrition (~80 days gestation) can result in an increased fetal adiposity. In the present study, whilst there was an increase in the relative fat mass in twin fetuses in the C-R 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 comprised of multilocular cells, which possess an abundance of mitochondria and express UCP1 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 comprised of large or the dominant lipid locules (23). Whilst perirenal fat mass is increased in the nutrient restricted group, it may be that this fat is comprised 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 first week 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. Whilst this study highlights the importance of maternal nutrition in early pregnancy for adipose tissue development, future work is required to determine if these changes persist into postnatal life and represent the initial steps in the early programming of adult obesity.
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Figure Legends

Figure 1. Restricted periconceptional nutrition (close symbols, n = 25) resulted in a significant loss of weight in non-pregnant ewes between weeks 5 and 7 after the start of the feed regime (* denotes significant change in ewe weight, $P < 0.05$) compared with the change in ewe weight after 1 week of feeding. There was no significant effect, however, of control periconceptional nutrition (open symbols, n = 20) on the change in non-pregnant ewe weight during the 7 weeks after the start of the feeding regime.

Figure 2. There was a significant positive relationship between maternal plasma concentrations of leptin in ewes carrying twin pregnancies between 135 and 146 d gestation and the change in ewe weight during the pre-pregnancy period (maternal leptin = 0.9 (change in ewe weight) + 7.8, $r = 0.6, P < 0.05$).

Figure 3. There was a significant positive relationship between maternal and fetal plasma concentrations of leptin between 115 and 146 d gestation (maternal leptin = 0.5 (fetal leptin) + 4.2, $r = 0.63, P < 0.005$).

Figure 4. The perirenal fat mass, expressed relative to body weight, was significantly increased in twin fetal sheep in the C-R group (n = 4) compared to twin fetal sheep in the other nutritional groups (C-C n = 8; R-R n = 3; R-C n = 7) $P < 0.05$. 
Table 1. Effect of nutritional group on maternal and fetal plasma leptin and glucose concentrations and fetal weight and fat mass in singleton pregnancies

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<tr>
<td>Maternal glucose (mmol/l)</td>
<td>2.8 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Maternal leptin (ng/ml)</td>
<td>6.8 ± 0.8</td>
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<td>6.9 ± 1.0</td>
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<td>Fetal glucose (mmol/l)</td>
<td>1.6 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.47 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Fetal leptin (ng/ml)</td>
<td>3.8 ± 0.8</td>
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<td>Fetal weight (kg)</td>
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<td>Absolute fetal perirenal fat mass (g)</td>
<td>18.2 ± 1.9</td>
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<td>23.1 ± 4.2</td>
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<td>Relative fetal perirenal fat mass (g/kg)</td>
<td>4.1</td>
<td>4.3</td>
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<td>5.5 ± 0.9</td>
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Data are represented as mean ± SEM, from a minimum of 2 and a maximum of 9 animals per treatment group. Different superscripts 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.
Table 2. Effect of nutritional group on maternal and fetal plasma leptin and glucose concentrations and fetal weight and fat mass in twin pregnancies

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<td>Maternal glucose (mmol/l)</td>
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<td>Fetal leptin (ng/ml)</td>
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<td>Fetal weight (kg)</td>
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<td>Absolute fetal perirenal fat mass (g)</td>
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Data are represented as mean ± SEM, from a minimum of 4 and a maximum of 12 animals per treatment group. Different superscripts denote a significant effect of nutritional group on maternal and fetal glucose concentrations, *P < 0.05. * denotes a significant decrease in fetal weight in the C-R group compared to 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.
Figure 1.

![Graph showing the weight change in non-pregnant ewes over the number of weeks after the start of feeding regimes. The graph compares control periconceptional nutrition with restricted periconceptional nutrition.](image-url)
Figure 2.

Change in ewe weight from -60d until mating

Maternal plasma leptin (ng/ml)

Change in ewe weight from -60d until mating

-8 -6 -4 -2 0 2 4
Figure 3.

Mean fetal plasma leptin (ng/ml)

Mean maternal plasma leptin (ng/ml)

C-C
C-R
R-R
R-C
Figure 4.