Impact of Maternal Periconceptional Overnutrition on Fat Mass and Expression of Adipogenic and Lipogenic Genes in Visceral and Subcutaneous Fat Depots in the Postnatal Lamb

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Women entering pregnancy with a high body weight and fat mass have babies who are at increased risk of becoming overweight or obese in later life. We investigated whether maternal overnutrition in the periconceptional period results in an increased fat mass and expression of adipogenic and lipogenic genes in offspring and whether dietary restriction can reverse these changes. Nonpregnant donor ewes (n = 23) were assigned to one of four groups: control-control fed at 100% maintenance energy requirements (MER) for at least 5 months, control-restricted fed 100% MER for 4 months and 70% MER for 1 month, high-high (HH) fed *ad libitum* (170–190% MER) for 5 months, or high-restricted (HR) fed *ad libitum* for 4 months and 70% MER for 1 month. Single embryos were transferred to nonobese recipient ewes, and lamb fat depots were weighed at 4 months. Peroxisome proliferator-activated receptor-γ, glyceraldehyde-3-phosphate dehydrogenase, lipoprotein lipase, leptin, and adiponectin mRNA expression was measured in the lamb fat depots. Total fat mass was higher in female lambs in the HH but not HR group than controls. There was a relationship between donor ewe weight and total fat mass and *G3PDH* mRNA expression in perirenal fat in female lambs. There was no effect of periconceptional nutritional treatment on peroxisome proliferator-activated receptor-γ, glyceraldehyde-3-phosphate dehydrogenase, lipoprotein lipase, leptin, and adiponectin mRNA expression in any fat depot. Thus, exposure to maternal overnutrition in the periconceptional period alone results in an increased body fat mass in the offspring and that a short period of dietary restriction can reverse this effect. (**Endocrinology** **151: 5195–5205, 2010**)

Currently more than half of all adults in the United States, United Kingdom, and Australia are either overweight or obese, and there are increasing rates of overweight and obesity in all age groups including women of reproductive age (1–6). In one large study in the United States, it was reported that the incidence of women being overweight or obese at the start of pregnancy increased from 25 to 35% between 1991 and 2001 and that the incidence of maternal obesity at delivery rose from 29 to 39% across the same period (5). A high maternal body mass index (BMI) increases the risk of developing preeclampsia and gestational diabetes and giving birth to a macrosomic infant (birth weight > 4000 g) (7). Although it is not unexpected that the maternal nutritional environment would influence the fetal nutrient supply and infant body composition, the effects of the *in utero* nutritional environment persist beyond fetal life. There is a U-shaped relationship between birth weight and adult fat mass, with

Abbreviations: BCS, Body condition score; BMI, body mass index; CC, control-control; CR, control restricted; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; HH, high-high restricted; HR, high restricted; LPL, lipoprotein lipase; MER, metabolizable energy requirements; PCON, periconceptional overnutrition; PPAR, peroxisome proliferator-activated receptor; qRT-PCR, quantitative real-time RT-PCR.

a higher prevalence of adult obesity occurring in individuals with birth weights at either the low or high end of the birth weight distribution (8–11). A recent retrospective study of 8400 children in the United States reported that children born to obese mothers were twice as likely to be obese by as early as 2 yr of age (12).

In pregnancies complicated by gestational diabetes or even mildly impaired glucose tolerance, the offspring are also at risk of developing obesity and glucose intolerance in later life (9, 13–15). It has been reported that prepregnancy maternal weight and the associated level of maternal insulin resistance are correlated with an infant's fat mass at birth, whereas the level of maternal insulin resistance in later pregnancy is correlated with birth weight and an infant's fat-free mass (7). Similarly, other studies have shown that there appears to be independent contributions of maternal prepregnancy weight and maternal glucose intolerance during pregnancy to birth weight and the risk of adolescent obesity (16). It is not known, however, whether there are specific mechanisms that underlie the impact of a high BMI at the time of conception on postnatal fat development.

We have previously shown that exposure of the sheep fetus to maternal overnutrition in late pregnancy results in an increase in the expression of key adipogenic, lipogenic, and adipokine genes [peroxisome proliferator-activated receptor (*PPARγ*), lipoprotein lipase (*LPL*), and leptin] mRNA in fetal visceral fat and an increased mass of sc fat in postnatal life (17–19). *PPARγ* coordinately induces the expression of a suite of adipocyte specific genes, resulting in growth arrest of the preadipocyte and the terminal differentiation of adipocyte cells (20). *PPARγ* in combination with retinoid-X-receptor-α functions as a heterodimeric transcription factor, which binds to peroxisome proliferator response elements on DNA (21) to initiate transcription of the genes involved in lipid accumulation and metabolism, *e.g. LPL* and glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*), and to regulate expression of the adipokines including adiponectin and leptin.

In human studies, it is difficult to isolate the impact of a high maternal body weight during the periconceptional period from the impact of a high maternal body weight during later gestation on the development of body fat in the offspring. We have developed a model in which nonpregnant ewes were either overnourished on a high plane of nutrition or normally nourished on a control plane of nutrition for a period of at least 4 months before artificial insemination. In two subgroups, a 4-wk period of dietary restriction was imposed before and after artificial insemination, which resulted in four nutritional treatment groups: CC, CR, HH, or HR. Around a week after conception, single embryos were transferred to adult nono-

bese recipient ewes, which were maintained on a control diet for the remainder of pregnancy. We used this model to test the hypothesis that an increase in maternal nutrition and fat mass before and immediately after conception will result in an increase in body fat mass in the offspring and in the expression of *PPARγ*, lipogenic, and adipokine genes in the visceral and sc fat depots in postnatal life. We also determined whether a short period of dietary restriction decreases the impact of periconceptional overnutrition on the development of adiposity in the offspring.

Materials and Methods

Animals and nutritional feeding regimen

All procedures were approved by the University of Adelaide Animal Ethics Committee. Eighty-nine South Australian Merino ewes were used in this study. Donor ewes (n = 23) were moved into an enclosed shed and housed in pens 20 d before the start of the feeding regimen. All ewes were weighed and a body condition score (BCS) was assessed by an experienced assessor using a scale of 1 (emaciated) to 5 (obese), with intervals of 0.25 (22). During this 20-d period, ewes were acclimatized to a pelleted diet containing cereal hay, lucerne hay, barley, oats, almond shells, lupins, oat bran, lime, and molasses (Johnsons & Sons Pty. Ltd., Kapunda, South Australia, Australia). The pellets provided 9.5 MJ/kg^{-1} of metabolizable energy and 120 g/kg^{-1} of crude protein and contained 90.6% dry matter. All ewes received 100% of metabolizable energy requirements (MER) for the maintenance of a nonpregnant ewe according to weight, as defined by the Ministry of Agriculture, Fisheries, and Food (23).

At the end of this acclimatization period, ewes were randomly assigned to one of four treatment groups: control-control (CC), control-restricted (CR), high-high (HH) or high-restricted (HR) nutritional treatment groups. CC ewes (n = 6) were a control group maintained at 100% MER for 4 months before conception with a BCS of 3.0–3.5. CR ewes (n = 6) were maintained at 100% MER for the first 3 months and then 1 month before conception were placed on a dietary restriction of 70% MER to achieve a BCS of 2.0–2.5. HH ewes (n = 6) were maintained at a high BCS of 4.0–4.5 and were fed an *ad libitum* diet (170–190% MER) for 4 months before conception. HR (n = 5) ewes were maintained at a high body condition and were fed between 170 and 190% MER for 3 months and then 1 month before conception were placed on an energy-restricted diet of 70% MER to reduce the BCS to 3.0–3.5.

Donor and recipient ewe synchronization and pregnancy

Superovulation protocol

The reproductive cycles of all experimental ewes were synchronized, and superovulation was induced by the administration of an intravaginal progestagen pessary (45 mg flugestone acetate; Intervet, Paris, France) for 12 d, followed by the administration of FSH (Folltropin, equivalent to National Institutes of Health-FSH-P1 standard) to the donor ewes, administered in six equal injections given over 3 d, twice daily, commencing 48 h before pessary removal. Each donor ewe (Bioniche Animal

Health, Belleville, Ontario, Canada) also received equine chorionic gonadotropin (Pregnecol Bioniche Animal Health) and GnRH (Fertagyl, Invervet/Schering-Plough Animal Health, Millsboro, DE) after pessary removal.

Artificial insemination and embryo collection

Fresh semen was collected from a single nonobese ram of proven fertility (24). The donor ewes were inseminated by laparoscopy 36 h after pessary withdrawal. During the insemination procedure, ewes were lightly sedated with iv administration of Rompum (xylazine, 0.1 ml, Bayer, Leverkusen, Germany) and lignocane (Sigma Chemical, St. Louis, MO).

Donor ewes were anesthetized 6–7 d after insemination, and embryos collected by laparoscopy by flushing the oviducts with saline. There was no effect of periconceptional overnutrition and/or dietary restriction on the number of embryos collected or on the developmental stage of the collected embryos (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org).

Embryo transfer protocol

Donor embryos (eight cells and acceptable morphology) were transferred to adult recipient ewes maintained on a control diet at 6–7 d by laparoscopy. A single embryo was transferred to the uterine horn in 63 ewes. These recipient ewes were maintained on a diet calculated to provide 100% energy requirements as specified by the Ministry of Agriculture, Fisheries, and Food (23). Pregnancy was confirmed at 49 d of gestation and 47 singleton pregnancies were confirmed (CC, n = 9; CR, n = 12; HH, n = 13; HR, n = 13). Pregnant ewes were allowed to give birth naturally.

Blood sampling of the donor and recipient ewes

Venous blood samples (10 ml) were collected from donor ewes into heparinized tubes on ice. Baseline samples were collected at 1 month and 2 wk before the start of the dietary intervention. Blood samples were then collected at 2 and 4 wk into the dietary intervention period. Blood samples were also taken from recipient ewes before embryo transfer and at d 49 of gestation. Samples were centrifuged at $1500 \times g$ for 10 min at 4 C and plasma stored at -20 C for subsequent determination of glucose and insulin concentrations.

Blood sampling of the postnatal lamb at birth and at 4 months of age

Venous blood samples (5 ml) were collected on the day of birth and on the morning of postmortem into heparinized tubes on ice. Blood samples were centrifuged at $1500 \times g$ for 10 min at 4 C and plasma was stored at $\times 20$ C.

Postmortem and adipose tissue collection

At 4 months of age, the lambs were weighed and then humanely killed with an overdose of sodium pentobarbitone (Virbac Pty. Ltd., Peakhurst, New South Wales, Australia). All fat from internal and sc fats depot was dissected and weighed to determine total body fat mass in all lambs. Weights of the omental, perirenal, and sc depots were also recorded, and samples from each depot were immediately collected, weighed, snap frozen, and stored at $\times 80$ C.

RNA extraction

RNA from omental, perirenal, and sc adipose tissue (~100 mg) was extracted using Trizol reagent (Invitrogen Australia Pty. Ltd., Mount Waverley, Australia) and chloroform. RNA was treated with 70% ethanol and purified using the RNeasy minikit (QIAGEN Pty. Ltd., Doncaster, Australia). Quality and concentration of the RNA were determined by measuring absorbance at 260 and 280 nm, and RNA integrity was confirmed by agarose gel electrophoresis. cDNA was then synthesized using the purified RNA (~5 μg), Superscript 3 reverse transcriptase (Invitrogen Australia) and random hexamers.

Quantitative real-time RT-PCR (qRT-PCR)

The relative expression of *PPARγ*, *G3PDH*, *LPL*, leptin, and adiponectin mRNA transcripts was measured by qRT-PCR using the Sybr Green system in an ABI prism 7900 sequence detection system (PE Applied Biosystems, Foster City, CA) (25, 26). All primers were designed with the aid of Primer Express software (PE Applied Biosystems), and, where possible, one primer of each pair was positioned over a splice site. For each transcript, RT-PCR was performed using specific primers (17, 27). Each amplicon was designed to be approximately 200 bp in length, sequenced to ensure the authenticity of the DNA product, and qRT-PCR melt curve analysis performed to demonstrate amplicon homogeneity. Controls containing no reverse transcriptase were also used. Primer concentrations were equivalent for all genes, and the amplification efficiencies were 0.981–0.999. A constant amount of cDNA equating to 10 ng of total RNA was used and three technical replicates were performed.

Each qRT-PCR reaction (5 μl total volume) contained 2.5 μl $2 \times$ Sybr Green master mix (Applied Biosystems); 0.25 μl of each primer giving a final concentration of 450 μl, 1.0 μl of molecular grade H$_2$O; and 1.0 μl of a 1:10 dilution of the stock template. The cycling conditions consisted of 40 cycles of 95 C for 15 sec and 60 C for 1 min. At the end of each run, a dissociation melt curve was obtained. The abundance of each mRNA transcript was measured and its expression relative to that of ribosomal protein large subunit P0 was calculated using Q-gene qRT-PCR analysis software (Applied Biosystems, Mulgrave, Victoria, Australia).

Plasma glucose and nonesterified free fatty acid assays

Plasma glucose concentrations (Thermo Scientific, Waltham, MA) were determined spectrophotometrically using a Konelab 20XTi automated sample analyzer (Thermo Electron, Melbourne, Victoria, Australia) using hexokinase and glucose-6-phosphate dehydrogenase to measure the formation of nicotinamide adenine dinucleotide photometrically at 340 nM.

Plasma nonesterified fatty acid concentrations were measured by an *in vitro* enzymatic colorimetric method (Wako Pure Chemical Industries Ltd., Osaka, Japan). This method relies on the acylation of coenzyme A by the fatty acids in the presence of added acyl-coenzyme A synthetase. The acyl-coenzyme A thus produced is oxidized by added acyl-coenzyme A oxidase with the generation of hydrogen peroxide, resulting in the formation of a purple-colored adduct, which can be measured colorimetrically at 550 nM using a Konelab 20XTi automated sample analyzer (Thermo Electron). Both assays have been previously validated for use in sheep plasma (18, 28,
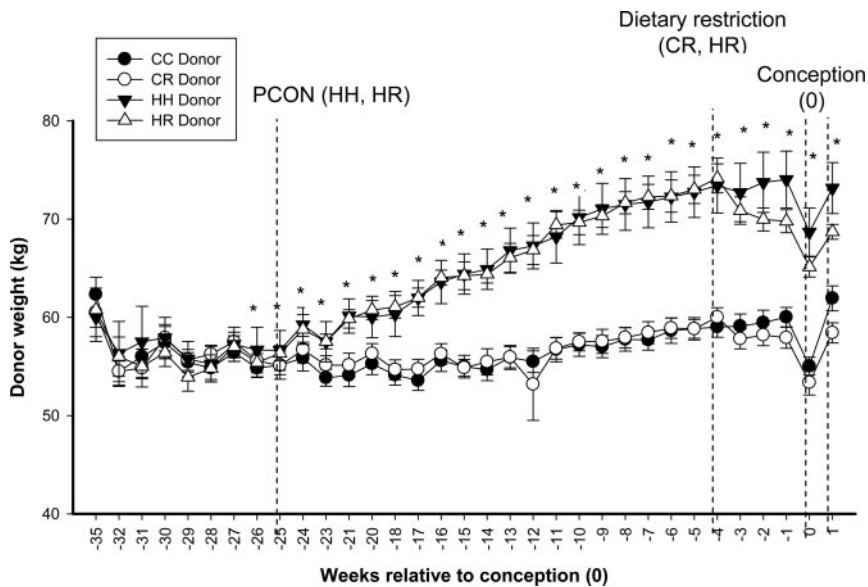
FIG. 1. Effect of PCON and/or dietary restriction on the weight of donor ewes during the nutritional feeding protocol from 35 wk before conception to 1 wk after conception. CC, *Closed circle*; CR, *open circle*; HH, *closed triangle*; HR, *open triangle*. *, Significant difference between the weight of the donor ewes in the HH and HR groups compared with the CC and CR groups ($P < 0.05$).

29), and intra- and interassay coefficients were both less than 10%.

Plasma insulin RIA

Plasma insulin concentrations were measured using a RIA (rat insulin kit; Linco Research, Inc., St. Charles, MO), validated for use with sheep plasma (17, 27). The sensitivity of the assay was 0.01 ng/ml, and the intra- and interassay coefficients of variance were both less than 10%.

Statistical analyses

All data are presented as mean ± SEM. Data were analyzed using the Statistical Package for the Social Sciences version 17.0 (SPSS Inc., Chicago, IL) and STATA10 (Stata Corp., College Station, TX) data analysis and statistical software for repeated measures. The weights of the donor ewes assigned to the four treatment groups during the periconceptional period were compared using two-way ANOVA with repeated measures. The effects of nutritional treatment on maternal or lamb plasma concentrations of glucose, nonesterified fatty acid, and insulin were compared using multifactorial ANOVA. Specified factors for the ANOVA included group (CC, CR, HH, or HR), time, and lamb gender. The effect of periconceptional nutrition on birth weight was analyzed using either a multifactorial ANOVA in which the specified factors included group (CC, CR, HH, or HR) or gender. The effects of periconceptional overnutrition and/or dietary restriction on the expression of adipogenic and lipogenic genes were compared using multifactorial ANOVA. Specified factors for the ANOVA included treatment group (CC, CR, HH, or HR) and gender. The depot-specific expressions of adipogenic and lipogenic genes were compared using multifactorial ANOVA with repeated measures. When a significant interaction between major factors was identified, the data were split on the basis of the interacting factors and reanalyzed. The Duncan's multiple range test was used *post hoc* and a probability level of 5% ($P < 0.05$) was taken as significant.

Results

Donor ewe weights

The weights of the nonpregnant donor ewes assigned to CC (54.5 ± 1.53 kg, n = 7), CR (54.5 ± 1.04 kg, n = 10), HH (56.3 ± 3.24 kg, n = 12), or HR (56.0 ± 2.00 kg, n = 12) groups were not different before the start of the feeding regimen (32 wk before conception).

At 4 wk before conception, after a minimum of 4 months on the feeding regimen, there was a significant difference in the weights of the donor ewes in the different treatment groups. The ewes that were overnourished were heavier (HH, 73.4 ± 2.8 kg, n = 12; and HR, 74.1 ± 1.5 kg, n = 12) than the ewes on the control level of nutrition (CC, 59.0 ± 1.1 kg, n = 7; and CR, 60.0 ± 0.9 kg, n = 10) ($P < 0.001$) (Fig. 1). At 4 wk before conception, the BCS of the donor ewes in the HH and HR groups (HH: 4.17 ± 0.08 and HR: 4.35 ± 0.11) was higher than in the CC and CR groups (CC: 3.08 ± 0.05 and CR: 3.04 ± 0.08) ($P < 0.0001$).

The CR and CC groups gained significantly less weight during the period extending from 25 wk from before conception to the day of embryo transfer when compared with both the HH and HR groups ($P < 0.05$, Fig. 1). A period of moderate dietary restriction in the CR and HR groups resulted in a weight loss in these groups (CR: −0.40 ± 0.40 kg, HR: −4.32 ± 1.93 kg) in contrast to the increase in weight ewes in the CC group (3.10 ± 0.41 kg) and maintained weight in the HH group (0.42 ± 0.56 kg) during this period (Fig. 1).

Plasma glucose and insulin concentrations in donor ewes

There was no effect of periconceptional overnutrition on plasma glucose concentrations in ewes at 5, 4, or 1 wk before conception (Table 1). Plasma insulin concentrations were significantly higher in the HR group when compared with the CC and CR groups, but not HH group, between 5 wk before conception and conception ($P < 0.05$) (Table 1).

Body condition and weight of recipient ewes

Recipient ewes were maintained at a normal BCS (3.00 ± 0.03, n = 47) from the start of the donor ewe feeding regimen to the time of conception (3.30 ± 0.03, n = 47). There was no difference between the weights of the recipient ewes allocated to carry the CC, CR, HH, or

TABLE 1. Plasma glucose and insulin concentrations in donor ewes at 5, 4, and 1 wk prior to conception and at conception

	−5 wk	−4 wk	−1 wk	Conception
Plasma glucose concentration (mmol/liter)				
CC (n = 7)	3.28 ± 0.20	3.80 ± 0.57	4.24 ± 0.25	3.76 ± 0.15
CR (n = 10)	3.32 ± 0.26	3.13 ± 0.60	3.82 ± 0.27	3.73 ± 0.26
HH (n = 12)	4.21 ± 0.61	4.07 ± 0.37	4.00 ± 0.22	3.66 ± 0.13
HR (n = 12)	3.36 ± 0.23	3.53 ± 0.09	3.68 ± 0.18	3.53 ± 0.14
Plasma insulin concentration (ng/ml)				
CC (n = 7)	0.26 ± 0.03[a]	0.56 ± 0.06[a]	0.83 ± 0.15[a]	0.50 ± 0.09[a]
CR (n = 10)	0.24 ± 0.06[a]	0.48 ± 0.09[a]	0.55 ± 0.12[a]	0.40 ± 0.09[a]
HH (n = 12)	0.48 ± 0.06[a,b]	0.72 ± 0.11[a,b]	0.85 ± 0.15[ab]	0.59 ± 0.08[a,b]
HR (n = 12)	0.49 ± 0.13[b]	0.86 ± 0.15[b]	1.25 ± 0.28[b]	0.71 ± 0.15[b]

Different superscripts (*e.g.* a, b) denote significant differences between the mean plasma insulin concentrations in different nutritional treatment groups from −5 wk until conception.

HR embryos 1 wk before embryo transfer (CC, 60.04 ± 2.46 kg, n = 9; CR, 64.28 ± 2.23 kg, n = 12; HH, 58.92 ± 2.03 kg, n = 14; HR, 61.09 ± 2.42 kg, n = 12).

Lamb birth weight

There was no significant effect of either periconceptional overnutrition and/or dietary restriction on lamb weight at birth and at 4 months of age (Supplemental Table 2). Male lambs were significantly heavier at birth and at 4 months of age, however, than females ($P < 0.05$) (Supplemental Table 2).

Plasma glucose, insulin, and nonesterified free fatty acid concentrations in the postnatal lamb

There was no effect of periconceptional overnutrition and/or lamb gender on the plasma glucose, insulin, or free fatty acid concentrations in the postnatal lamb at 4 months of age (Supplemental Table 3).

Adipose tissue mass in lambs

There was an interaction between the effects of maternal nutritional treatment and lamb gender on total adipose tissue mass ($P < 0.05$). Periconceptional overnutrition alone (HH group) resulted in a higher total fat mass in female lambs compared with lambs in the CC and CR groups ($P < 0.05$, Fig. 2). There was no significant effect of periconceptional overnutrition and/or dietary restriction on the total fat mass in male lambs at 4 months of age (Fig. 2). There was a significant relationship between the weight of the donor ewe at conception and the total fat mass of female [total fat mass = 63 (donor weight) − 1466.7, $r^2 = 0.31$, $P < 0.05$] but not male lambs at 4 months (Fig. 3). There was no effect of maternal nutritional treatment on the total adipose tissue mass relative to body weight in the offspring. Female lambs, however, had more total fat mass relative to body weight than male lambs at 4 months of age ($P < 0.001$).
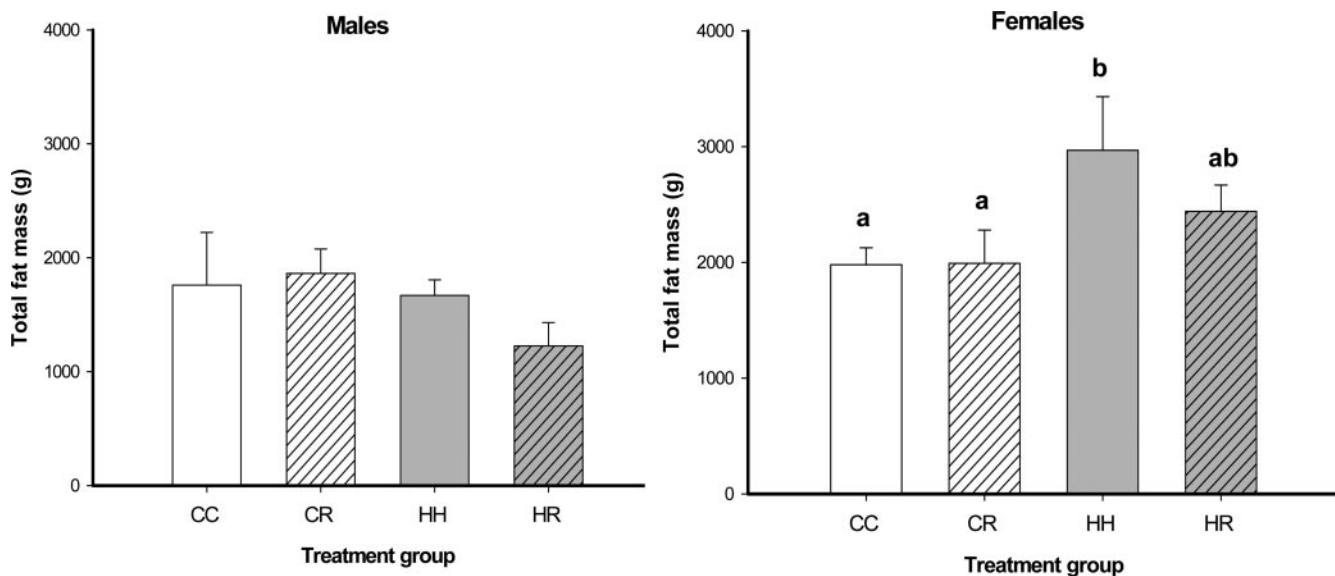

FIG. 2. Effect of PCON and/or dietary restriction on the total fat mass of male and female lambs at 4 months of age. CC, *Open bar*; CR, *striped bar*; HH, *gray bar*; HR, *gray striped bar*. Different superscripts (*e.g.* a, b) denotes a significant difference of total fat mass between the treatment groups ($P < 0.05$).
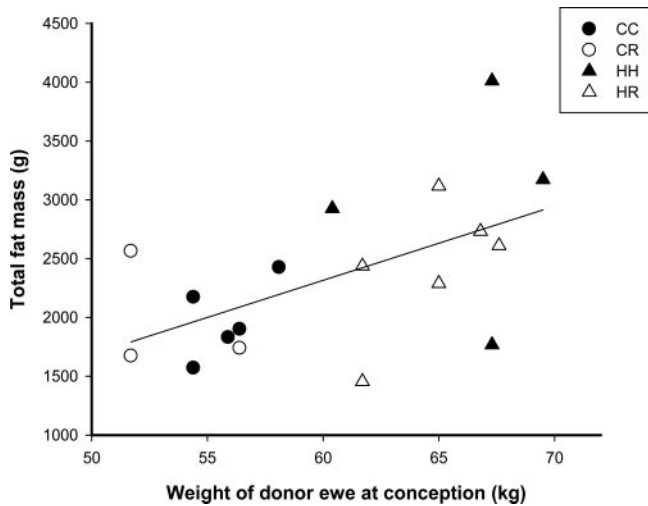
FIG. 3. Relationship between donor ewe weight at conception and total fat mass in female lambs at 4 months of age. CC, *Closed circle*; CR, *open circle*; HH, *closed triangle*; HR, *open triangle*. There was a significant positive relationship between the total fat mass in female offspring at 4 months of age and the weight of the donor ewe at conception [total fat mass = 63 (donor ewe weight) − 1466.7, r^2 = 0.31, $P < 0.05$].

Weight of adipose tissue depots

There was no effect of periconceptional overnutrition and/or dietary restriction on the absolute mass and absolute mass relative to body weight of each of the separate omental, perirenal, and sc fat depots in either male or female lambs (Fig. 4). Although there was a significant effect of maternal nutritional treatment on the absolute and relative omental fat mass ($P < 0.05$), *post hoc* analyses found no difference between treatment groups at the $P < 0.05$ level. When data from all lambs were combined, female lambs had a higher absolute [absolute omental fat mass: 551.1 ± 61.3 g, n = 19 (females) *vs.* 324.6 ± 35.7 g, n = 21 (males), $P < 0.001$] and relative [relative omental fat mass; 18.3 ± 8.9 g/kg, n = 19 (females) *vs.* 9.7 ± 1.1 g/kg, n = 21 (males), $P < 0.001$] omental fat mass when compared with male lambs at 4 months. Female lambs also had a higher absolute [absolute perirenal fat mass, 557.9 ± 74.1 g, n = 20 (females) *vs.* 343.7 ± 53.4 g, n = 21(males), $P < 0.05$] and relative [relative perirenal fat mass: 15.1 ± 1.0 g/kg, n = 20 (females) *vs.* 8.7 ± 0.7 g/kg, n = 21 (males), $P < 0.05$] perirenal fat mass when compared with male lambs at 4 months. There was no difference, however, between male and female lambs in absolute and relative sc fat mass at 4 months of age.

PPARγ, G3PDH, LPL, leptin, and adiponectin mRNA expression in adipose tissue depots

Omental adipose tissue

There was no effect of periconceptional overnutrition and/or dietary restriction on the relative expression of PPARγ, LPL, leptin, adiponectin, and G3PDH (Table 2) in the omental fat depot. The relative expression of G3PDH mRNA was higher ($P < 0.01$), however, in the omental fat of female lambs compared with male lambs at 4 months of age (Table 2). There were also significant relationships between PPARγ mRNA expression and LPL ($P < 0.05$) and leptin ($P < 0.05$) mRNA expression in the omental adipose tissue in male and female lambs (Supplemental Table 4).

Perirenal adipose tissue

There was no effect of periconceptional overnutrition and/or dietary restriction on the relative expression of PPARγ, LPL, leptin, and G3PDH (Table 2) in the perirenal fat depot. There was a significant positive relationship between donor ewe weight at conception and G3PDH expression in the perirenal adipose tissue in the lamb at 4 months of age ($P < 0.05$, Fig. 5). The relative expression of G3PDH ($P < 0.04$) and LPL ($P < 0.01$) mRNA in perirenal fat was higher in female compared with male lambs at 4 months of age independent of nutritional treatment (Table 2).

There was an interaction between the effects of maternal periconceptional nutritional treatment and gender ($P < 0.05$) on the relative expression of adiponectin in the perirenal fat depot. In male lambs, but not in female lambs, there was a lower expression of adiponectin in the perirenal fat in the HR group compared with the CC group ($P < 0.05$).

There were significant relationships between PPARγ mRNA expression and adiponectin ($P < 0.05$), G3PDH ($P < 0.05$), and LPL ($P < 0.05$) mRNA expression in the perirenal adipose tissue in male and female lambs (Supplemental Table 4).

Subcutaneous adipose tissue

There was no effect of periconceptional overnutrition and/or dietary restriction on the relative expression of PPARγ, LPL, leptin, adiponectin, and G3PDH (Table 2) in the sc fat depot. The relative expression of G3PDH ($P < 0.05$) and LPL ($P < 0.05$) were higher and leptin mRNA expression tended to be higher ($P = 0.07$) in the sc fat of female compared with male lambs at 4 months of age (Table 2). There were also significant relationships between PPARγ mRNA expression and adiponectin ($P < 0.05$) and LPL ($P < 0.05$) mRNA expression in the sc adipose tissue of male and female lambs (Supplemental Table 4).

Discussion

The objective of this study was to investigate whether the plane of nutrition before conception and during early em-
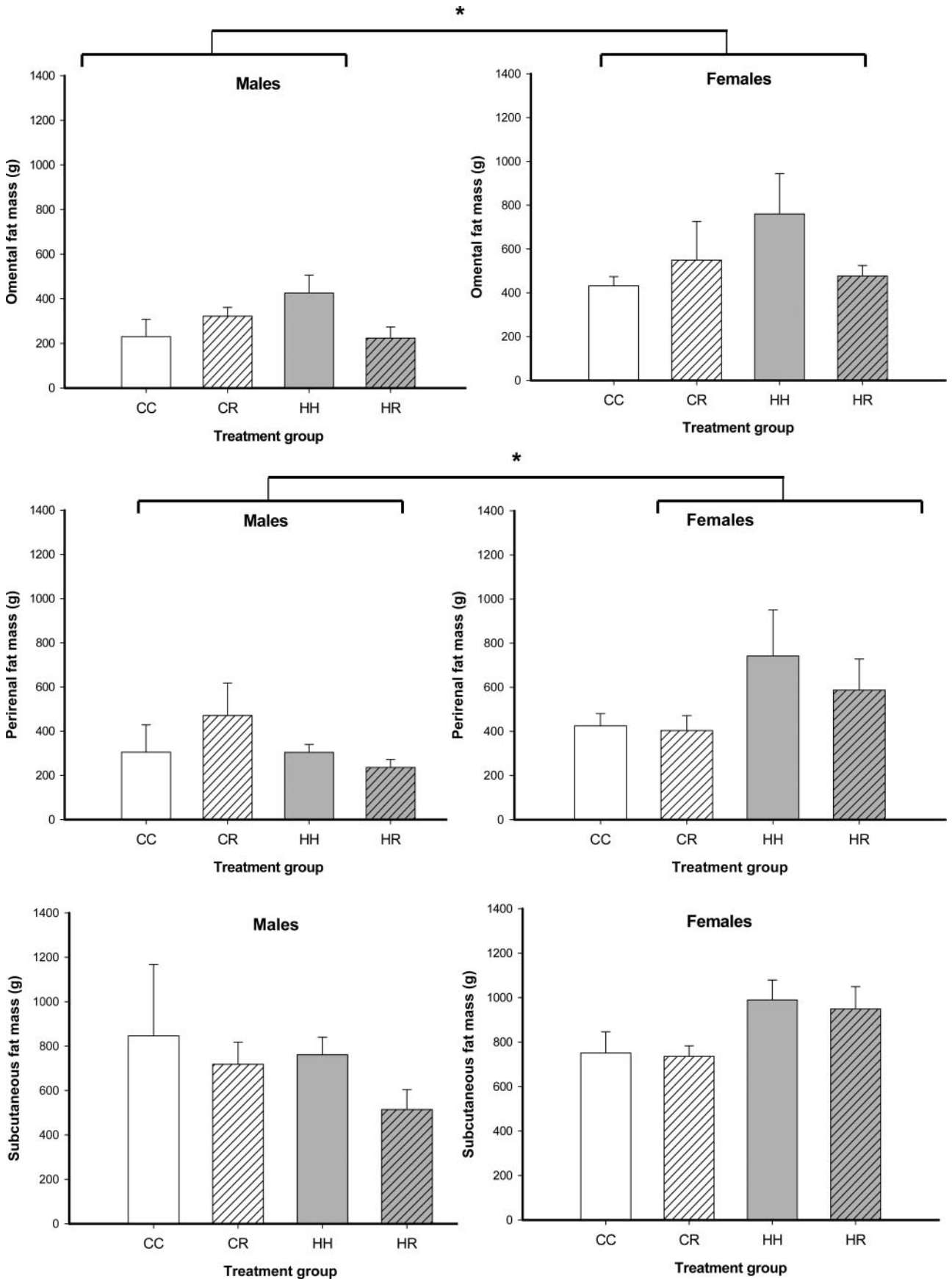
FIG. 4. The effect of PCON and/or dietary restriction on the mass of the omental, perirenal, and sc fat. CC, *Open bar*; CR, *striped bar*; HH, *gray bar*; HR, *gray striped bar*. *, Significant difference of fat mass between male and female lambs at 4 months of age (*P* < 0.05).

TABLE 2. The effect of PCON and/or dietary restriction on the expression of adipogenic and lipogenic genes in omental, perirenal and sc adipose tissue depots of lambs at 4 months of age

	PPARγ mRNA expression		LPL mRNA expression		Leptin mRNA expression		Adiponectin mRNA expression		G3PDH mRNA expression	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Omental adipose tissue										
CC	22.1 ± 8.5	15.2 ± 3.2	129.9 ± 5.5	162.3 ± 20.0	74.5 ± 5.4	93.7 ± 14.0	41.4 ± 8.8	54.4 ± 5.9	7.4 ± 0.5	11.9 ± 2.1*
CR	12.9 ± 3.1	12.9 ± 3.9	89.8 ± 10.2	172.6 ± 30.0	33.7 ± 4.2	75.8 ± 2.8	44.3 ± 6.7	46.6 ± 6.4	7.1 ± 1.2	9.6 ± 1.7*
HH	15.8 ± 6.8	12.5 ± 6.2	113.7 ± 25.7	169.9 ± 30.2	39.9 ± 9.2	59.1 ± 12.8	44.7 ± 6.8	35.5 ± 2.1	5.4 ± 1.3	14.5 ± 3.2*
HR	16.0 ± 5.2	13.4 ± 3.6	196.0 ± 11.5	121.5 ± 29.4	64.5 ± 20.4	44.1 ± 16.8	46.4 ± 7.4	50.3 ± 8.5	7.3 ± 1.7	7.5 ± 1.3*
Perirenal adipose tissue										
CC	1.69 ± 0.01	1.83 ± 0.13	12.77 ± 0.49	18.61 ± 4.84*	0.17 ± 0.08	0.39 ± 0.10	7.29 ± 1.90[a]	6.90 ± 1.11	1.03 ± 0.18	0.84 ± 0.19*
CR	1.92 ± 0.14	1.82 ± 0.22	10.48 ± 1.36	12.92 ± 2.65*	0.20 ± 0.06	0.27 ± 0.12	6.52 ± 0.72[ab]	4.53 ± 0.53	0.75 ± 0.14	0.93 ± 0.25*
HH	1.86 ± 0.27	1.85 ± 0.29	10.70 ± 1.77	22.50 ± 5.20*	0.24 ± 0.14	0.65 ± 0.33	5.17 ± 0.68[ab]	5.91 ± 1.21	0.61 ± 0.13	1.56 ± 0.37*
HR	1.70 ± 0.44	1.87 ± 0.25	10.16 ± 0.79	15.38 ± 2.24*	0.16 ± 0.05	0.27 ± 0.07	3.81 ± 0.46[b]	7.29 ± 0.83	0.69 ± 0.22	1.11 ± 0.16*
Subcutaneous adipose tissue										
CC	0.33 ± 0.06	0.29 ± 0.04	3.36 ± 0.23	3.69 ± 1.09*	0.09 ± 0.03	0.11 ± 0.02	5.28 ± 0.11	5.51 ± 0.37	1.84 ± 1.0	1.51 ± 0.46*
CR	0.44 ± 0.06	0.43 ± 0.09	3.56 ± 0.82	7.25 ± 1.84*	0.11 ± 0.08	0.16 ± 0.07	5.30 ± 0.71	6.65 ± 1.04	1.24 ± 0.18	2.05 ± 0.66*
HH	0.38 ± 0.01	0.37 ± 0.03	3.42 ± 0.46	5.27 ± 0.77*	0.09 ± 0.08	0.17 ± 0.04	5.66 ± 0.61	6.95 ± 1.39	1.19 ± 0.24	2.69 ± 0.63*
HR	0.49 ± 0.12	0.42 ± 0.08	3.99 ± 0.21	5.07 ± 1.19*	0.08 ± 0.05	0.14 ± 0.05	4.63 ± 0.42	6.13 ± 1.07	1.28 ± 0.42	2.20 ± 0.47*

* Significant difference in gene expression within a fat depot between male and female lambs at 4 months of age ($P < 0.05$). Different superscripts (*e.g.* a, b) denotes a significant difference of gene expression between treatment groups ($P < 0.05$).

bryo development is an independent determinant of body fat mass in the offspring, using the sheep as an animal model.

Periconceptional overnutrition (PCON) resulted in a significant increase in maternal body weight and body condition in the ewe entering pregnancy, and this was associated with a decrease in maternal insulin sensitivity as evidenced by higher circulating fasting insulin concentrations (7). PCON also resulted in a significant increase in total fat mass in female but not male lambs, and there was a positive relationship between donor ewe weight at con-

ception and the total fat mass in female offspring at 4 months of age. The greatest impact of PCON appeared to be on the visceral fat depots, *i.e.* the perirenal and omental fat depots in the female lamb, and the weights of these depots were also higher in female than male lambs. In contrast, there was no difference in the weight of the sc fat depot between female and male lambs and no effect of PCON on the weight of this adipose depot. Thus, the effects of PCON may be dependent on mechanisms that result in a greater deposition of triglyceride in the visceral fat depots in the female lamb.
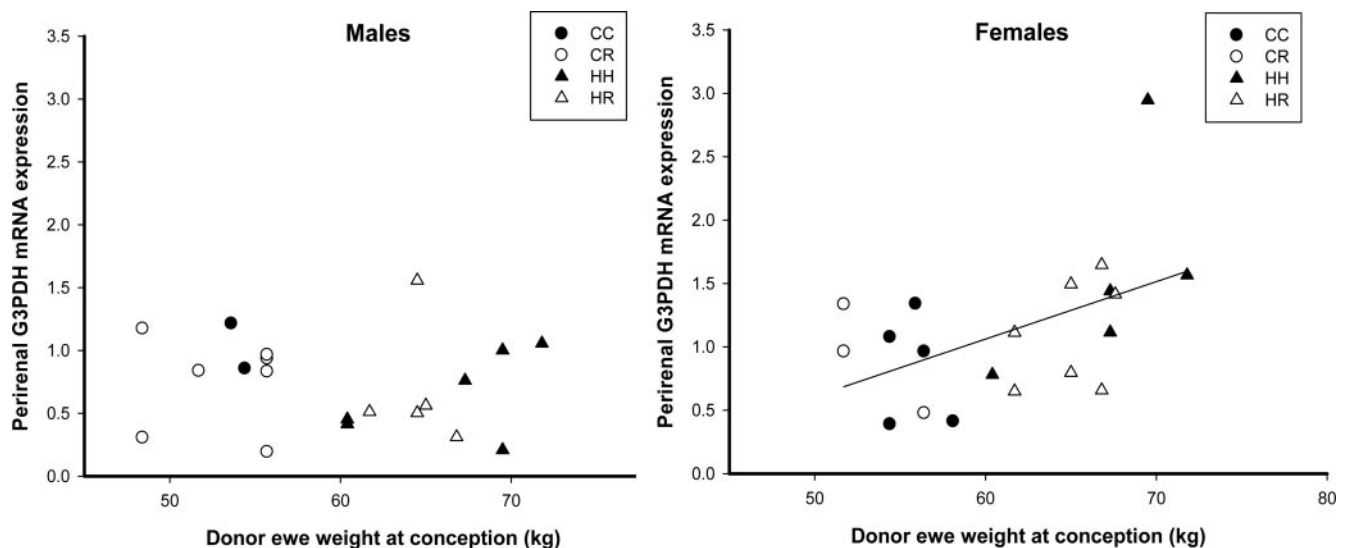

FIG. 5. The relationship between perirenal G3PDH mRNA expression and donor ewe weight at conception in the lambs at 4 months of age. CC, *Closed circle*; CR, *open circle*; HH, *closed triangle*; HR, *open triangle*. There was a significant positive relationship between the perirenal G3PDH mRNA expression of 4-month-old female lambs and donor ewe weight at conception [perirenal G3PDH mRNA expression = 63 (donor weight at conception) − 1466.7, $r^2 = 0.24$, $P < 0.05$].

Alterations in the nutrient environment of the developing embryo *in vivo* and *in vitro* can alter the allocation of cells within the inner cell mass and trophectoderm (30, 31). In the present study, we found there was no effect of PCON or dietary restriction in the periconceptional period on lamb birth weight, suggesting that these treatments did not directly influence either the growth or nutrient transfer capacity of the placenta. A recent study has investigated the impact of the prepregnancy BMI of the adolescent ewe on placental and lamb weights at birth (32). In this model placental and lamb birth weights were influenced by initial BMI with heavier ewes having heavier lambs. There may be some key differences in the impact of periconceptional overnutrition on the developing embryo in the young, growing adolescent ewe when compared with the mature, postpubertal animal.

It has been shown that reduced insulin sensitivity in women before pregnancy is correlated with the fat mass of the offspring at birth, whereas reduced maternal insulin sensitivity in late gestation is more strongly correlated with birth weight and fat-free mass (7, 33, 34). In the present study, we found that the effects of PCON on fat deposition in the female lamb were partially ablated when overnourished ewes were exposed to a 1-month period of dietary restriction before conception. There was no difference, however, in fasting insulin concentrations between ewes in the HH and HR groups, although it is possible that the period of dietary restriction may have increased peripheral insulin sensitivity in the HR group, which was not reflected in the circulating insulin levels.

In rats, maternal consumption of high fat diets containing lard for 10 d before conception through to weaning led to increased adiposity in the offspring at 6 months of age (35, 36). A recent study (37) determined the impact of feeding nonpregnant rats with 15% excess calories/d for 3 wk before mating. Offspring from the obese dams gained greater body weight and higher percentage body fat when fed a high-fat diet in postnatal life. The key difference between the present and prior studies is that we have used a model in which the embryo is transferred from an overnourished to a normally nourished ewe, which limits the effects of maternal overnutrition to the periconceptional period alone.

We have previously shown that maternal overnutrition imposed in the sheep during late gestation results in an increase in the expression of the transcriptional coactivator *PPARγ* and in *LPL*, adiponectin, and leptin mRNA in perirenal adipose tissue in the fetus (17). We also reported that there was an increase in sc fat mass at 1 month of age in both male and female lambs exposed to maternal overnutrition during late gestation (17). We therefore proposed that an increase in maternal and hence fetal nutri-

tion in late gestation increases the expression and activation of the adipogenic gene, *PPARγ*, in perirenal adipose tissue and that there was subsequent signaling between the perirenal and sc adipose tissue to result in an increase in sc fat mass (17).

Maternal overnutrition in late gestation results in an increase in sc fat mass in male and female lambs in postnatal life, whereas the effects of periconceptional overnutrition appear to be more dominant on the visceral fat mass depots and only occur in the female lamb. Furthermore, we found no effect of PCON with or without dietary restriction on the expression of *PPARγ*, *G3PDH*, *LPL*, or leptin in the perirenal, omental, or sc fat depots in either the female or male lamb at 4 months after birth. There were the expected relationships present between *PPARγ* mRNA and *LPL* mRNA expression in each of the perirenal, omental and sc depots, and these relationships were not altered by the different periconceptional nutritional environments. Although the expression of the lipogenic gene, *G3PDH*, was higher in all fat depots in the female compared with the male lamb, we also found that maternal donor weight at conception was directly related to the level of expression of *G3PDH* mRNA in the perirenal fat of female but not male lambs at 4 months of age. This suggests that the perirenal fat depot, which is the first major fat depot to appear during development in the sheep, is more susceptible to the effects of maternal overnutrition during the periconceptional period. Interestingly, there also appeared to be an impact of periconceptional undernutrition on adiponectin expression in the perirenal fat of the male lamb as perirenal adiponectin expression was lowest in lambs in the HR group. The persistence of a decrease in adiponectin expression and secretion from this depot would potentially result in a decrease in peripheral insulin sensitivity (38). It has previously been shown that a more severe nutritional restriction imposed across the periconceptional and early gestation period (from 60 d before until 30 d after mating) resulted in a decreased glucose tolerance in the 10-month-old offspring (39). Thus, not all of the effects of dietary restriction in the periconceptional period on adipose tissue may be beneficial in the longer term.

In summary, we have demonstrated that exposure to overnutrition in the periconceptional period results in an increase in maternal weight and body condition and in an increase in the total body fat mass of her female offspring. Exposure of the oocyte and early embryo to a high plane of maternal nutrition therefore results in a greater postnatal capacity to synthesize and store triglycerides, an effect that is more prominent in females compared with males. One speculation is that this increased capacity represents the effects of nutritional programming of the early

embryo to predict that the postnatal nutritional environment will match that experienced during the periconceptional period. We have also shown that, unlike the effects of maternal overnutrition in late gestation, this increased adipogenic and/or lipogenic capacity does not appear to be the result of increased expression of *PPARγ*, *G3PDH*, *LPL*, or leptin in any of the major fat depots. Although we have found that a period of moderate dietary restriction ablates the effect of periconceptional overnutrition on the development of an increased body fat mass, it will be important to ensure that any periconceptional dietary restriction regimen does not incur a further metabolic cost in postnatal life.

Acknowledgments

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