Placental restriction of fetal growth decreases IGF1 and leptin mRNA expression in the perirenal adipose tissue of late gestation fetal sheep

Jaime A Duffield¹,³, Tony Vuocolo², Ross Tellam² Bernard S Yuen¹, Beverly Muhlhausler³ and I Caroline McMillen¹,³

¹Discipline of Physiology, School of Molecular and Biomedical Sciences, The University of Adelaide, South Australia 5005, Australia, ²CSIRO Livestock Industries, Queensland Biosciences Precinct, 306 Carmody Rd., St Lucia 4067, QLD, Australia, ³Early Origins of Adult Health Research Group, Sansom Research Institute, School of Pharmacy and Medical Sciences, University of South Australia, SA 5005, Australia

Please address all correspondence to:

Professor I C McMillen

Early Origins of Adult Health Research Group, Sansom Research Institute

School of Pharmacy and Medical Sciences,

University of South Australia

South Australia 5005

Australia

Telephone: 61 - 8 - 8302 0038

FAX: 61 - 8 - 8302 2389

Email: caroline.mcmillen@unisa.edu.au
ABSTRACT

Placental restriction (PR) of fetal growth results in a low birth weight and an increased visceral fat mass in postnatal life. We have investigated whether PR alters expression of genes which regulate adipogenesis (IGF1, IGF1R, IGF2, IGF2R, PPARγ, RXRα), adipocyte metabolism (LPL, G3PDH, GAPDH) and adipokine signalling (leptin, adiponectin) in visceral adipose tissue before birth. PR was induced by removal of the majority of endometrial caruncles in non pregnant ewes prior to mating. Fetal blood samples were collected from 116d gestation and perirenal visceral adipose tissue (PAT) collected from PR and control fetuses at 145d. PAT gene expression was measured by qRT-PCR. PR fetuses had a lower weight (PR 2.90 ± 0.32 kg; Control, 5.12 ± 0.24 kg; P<0.0001), mean gestational arterial PO₂ (P<0.0001), plasma glucose (P<0.01) and insulin concentrations (P<0.02), than Controls. The expression of IGF1 mRNA in PAT was lower in the PR fetuses (PR 0.332 ± 0.063; Control 0.741 ± 0.083; P<0.01). Leptin mRNA expression in PAT was also lower in PR fetuses (PR 0.077 ± 0.009; Control, 0.115 ± 0.013; P<0.05), although there was no difference in the expression of other adipokine or adipogenic genes in PAT between PR and control fetuses. Thus restriction of placental and hence fetal substrate supply results in decreased IGF1 and leptin expression in fetal visceral adipose tissue which may alter the functional development of the perirenal fat depot and contribute to altered leptin signalling in the growth restricted new born and the subsequent emergence of an increased visceral adiposity.

Key Words: Fetus, placenta, leptin, adipose tissue, obesity
INTRODUCTION

A world-wide series of epidemiological and clinical studies has demonstrated that there are associations between the patterns of growth in fetal and early postnatal life and the risk of insulin resistance, type 2 diabetes and obesity in adult life (6, 7, 33, 41, 44, 50-52). Small-for-gestational-age (SGA: birth weight and/or length < 2 standard deviations below the mean for gestational age), (3, 72), infants have low circulating insulin-like growth factor-1 (IGF1) concentrations and a reduced body fat mass at birth (18), and then undergo a period of accelerated postnatal growth during the first few years of life (2, 21), with a relative increase in body fat mass from as early as 2 -12 months of age (27) and trunkal fat mass during childhood (56) and in adult life (40, 58). An accelerated postnatal growth rate is associated with an early increased insulin sensitivity, followed by the emergence of insulin resistance (7, 12, 19, 43, 70, 71), and in particular, insulin resistance of adipose tissue in later life (33, 35, 36, 60).

IGF1 is one of the most important growth factors in the development of adipose tissue. The experimental induction of a global IGF1-receptor (IGF1R) deficiency in mice produces growth restriction, and reduces the growth of adipose tissue relative to that of other tissues (30). At physiologic levels, IGF1 stimulates both proliferation and differentiation of preadipocytes in cell culture, and along with cAMP and glucocorticoids, has been shown to be required for induction of adipose tissue differentiation in both serum-containing and serum-free media (24, 68) It has been shown that after experimental induction of differentiation growth arrested preadipocytes go through clonal expansion prior to a significant increase in the expression of proliferator-activated receptor-γ (PPARγ), a prominent adipogenic transcription factor when heterodimerised with retinoid-X-receptor-α (RXRα). This initiates the transcription of the regulatory genes involved in lipid accumulation and glucose metabolism, particularly lipoprotein lipase (LPL), leptin and adiponectin and marks the entry of the cell into terminal differentiation (24, 61). In species such as the sheep or pig, adipogenesis and lipogenesis occur before birth as in the human, and low
birth weight offspring also grow faster in the postnatal period and have a higher proportion of body fat than their higher birth weight counterparts in later life (23, 45, 62). In a cohort of lambs born after experimental restriction of placental growth, there was an increase in relative visceral fat mass and a relationship between visceral adiposity and the action of insulin to suppress circulating free fatty acids at 6 weeks of age (14). In the fetal sheep, body weight is also related to circulating IGF1 concentrations (10, 38, 57), and to hepatic IGF1 mRNA expression (64). Experimental restriction of placental and hence fetal growth in the sheep reduces both circulating IGF1 protein and IGF mRNA expression in fetal tissues such as the liver, kidney and skeletal muscle (38). Whilst there is evidence for a significant autocrine/paracrine role of IGF1 in adipose tissue proliferation (9, 11, 20, 39, 47, 48, 59) and differentiation, the effect of placental restriction on the expression of IGF1 or insulin-like growth factor-2 (IGF2) or on the ligand and clearance receptors, IGF1R and IGF2R in fetal adipose tissue, is not known. We have therefore used the placentally restricted (PR) fetal sheep as a model to test the hypothesis that poor fetal growth results in a decreased expression of IGF1 mRNA in perirenal adipose tissue, the major fetal adipose depot at 140-145d gestation. We further hypothesise that PR of fetal substrate supply will result in an altered pattern of expression of adipogenic, lipogenic and adipokine genes in the visceral adipose tissue before birth. We have therefore investigated the effect of PR on the expression of IGF1, IGF2, IGF1R, IGF2R, PPARγ and RXRα, LPL, glycerol-3-phosphate dehydrogenase (G3PDH), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), leptin, and adiponectin in fetal perirenal adipose tissue.

MATERIALS AND METHODS

Animals and Surgery

All procedures were approved by The University of Adelaide Animal Ethics Committee. Fifteen Merino ewes were used in the study. Nine non-pregnant ewes underwent surgery to remove the
majority of endometrial caruncles from the uterus, leaving 3 to 8 caruncles in each horn in order to induce experimental restriction of placental and fetal growth (65).

Surgery was performed on control (n=6) and carunclectomised (n=9) pregnant ewes under aseptic conditions between 109 and 124 days (d) of gestation (term = 147 ± 3 d) with general anaesthesia induced by sodium thiopentane (1.25 g i.v.; Pentothal; Rhone Merieux, Pinkenba, Qld, Australia) and maintained with 2.5-4% (v/v) halothane (Fluothane; ICI, Melbourne, Vic, Australia) in oxygen. Vascular catheters were implanted in a maternal jugular vein, a fetal carotid artery and jugular vein, and the amniotic cavity as previously described (16). Catheters were filled with heparinised saline, and the fetal catheters were exteriorized through an incision made in the ewes’ flank. During surgery, ewes and fetuses received a 2 ml i.m. injection of antibiotics (procaine penicillin [250 mg/ml], dihydrostreptomycin [250 mg/ml], and procaine hydrochloride [20 mg/ml]; Penstrep Illium; Troy Laboratories, Smithfield, NSW, Australia). Ewes were housed in individual pens in rooms with a 12 h light / dark cycle with lights on at 7 am and a daily temperature range between 19 and 22 °C. Ewes were fed once daily at 1100 h with 1 kg lucerne chaff (85% dry matter (DM), metabolisable energy (ME) content = 8.3 MJ/kg DM) and 0.3 kg concentrated pellets containing: straw, cereal, hay, clover, barley, oats, lupins, almond shells, oat husks and limestone (90% DM, ME content = 8.0 MJ/kg DM; Johnson & Sons, Kapunda, SA, Australia). This diet provided 100% of the energy requirements for the maintenance of a ewe bearing a singleton pregnancy, as specified by the Ministry of Agriculture, Fisheries and Food, England (1). Water was provided ad libitum. Animals were allowed to recover from surgery for at least 4 d before collection of fetal and maternal blood samples commenced.

**Blood Sampling Protocol**
Fetal arterial (3.5 ml) blood samples were collected between 0800 and 1100 h, before the ewes were fed, three times each week between 116 and 140 d of gestation. Blood samples were centrifuged at 1500g for 10 min and plasma separated into aliquots and stored at -20°C. At times fetal blood samples could not be collected due to technical problems (primarily related to blocked vascular catheters). Fetal arterial blood (0.5 ml) samples were also collected for the measurement of arterial blood gas status (ABL 520 blood gas analyzer; Radiometer, Copenhagen, Denmark).

**Tissue Collection**

Ewes were killed between 140 and 145 days of pregnancy with a lethal overdose of sodium pentobarbitone (Virbac Pty Ltd, Peakhurst, NSW, Australia). Fetuses were delivered by hysterectomy, weighed, and killed by decapitation. Fetal perirenal adipose tissue (PAT) was dissected and weighed, and samples from control (n=6) and PR (n=8 out of 9) fetuses were frozen in liquid nitrogen and stored at -80°C for subsequent gene analysis.

**Plasma Non-Esterified Fatty Acids (NEFAs), Glucose and Hormone Assays**

Plasma NEFAs were measured by an *in vitro* enzymatic colorimetric method (Wako Pure Chemicals Industries Ltd, Osaka, Japan). The method relies upon the acylation of coenzyme A (CoA) by the fatty acids in the presence of added acyl-CoA synthetase (ACS). The acyl-CoA thus produced is oxidised by added acyl-CoA oxidase (ACOD) with generation of hydrogen peroxide. Hydrogen peroxide, in the presence of peroxidase (POD) permits the oxidative condensation of 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA) with 4-aminoantipyrine to form a purple coloured adduct which can be measured colorimetrically at 550nm (Konelab 20, Program Version 6.0 automated analysis system, Thermo Fisher Scientific, Suwanee, USA). The sensitivity of the assay was 0.25 mEq/l and the intra- and inter assay coefficients of variation (CVs) were both < 10 %.
Plasma glucose concentrations were measured by enzymatic analysis using hexokinase and glucose-6-phosphate dehydrogenase to measure the formation of NADH photometrically at 340 nm (Konelab 20, Program Version 6.0 automated analysis system, Thermo Fisher Scientific, Suwanee, USA). The sensitivity of the assay was 0.5 mmol/l and the intra- and inter assay CVs were both < 5%.

Fetal plasma insulin concentrations were measured in control (n=6) and carunclectomised (n=6) fetuses using a commercial kit (Phasadeph radioimmunoassay kit; Pharmacia & Upjohn, Uppsala, Sweden). The detection range of the assay was 1.5-240 µU ml⁻¹. Guinea pig anti-insulin antisera and [¹²⁵I]human insulin (100 µl) were added to plasma samples (100 µl), which were then incubated for 2 h at room temperature before the addition of 2 ml of sheep anti-guinea pig immunoglobulin G. Samples were allowed to stand at room temperature for a further 30 min before being centrifuged at 1500 X g for 10 min as described previously (16). The inter- and intraassay coefficients of variation were < 10%.

Plasma leptin concentrations were measured in control (n=6) and carunclectomised (n=6) fetuses using a competitive ELISA (37). Briefly, an ELISA plate was pre-incubated with recombinant bovine leptin in 50 µL of 0.1 M bicarbonate buffer and blocked with 200 µL of 5 % skim milk in ELISA buffer. Chicken anti-recombinant bovine leptin antiserum (50 µL) was added to the wells, followed by the addition of samples (100 µL) in duplicate. Following an overnight incubation at 37 °C, a biotinylated phosphatase-Streapavidin conjugate (Amrad Biotech, Boronia, Vic, Australia) was added, incubated for 1 h, and the plate developed with p-nitrophenylphosphate disodium salt hexahydrate. The sensitivity of the assay was 0.25 ng/ml and the intra- and inter- assay CVs were both < 15.0 %.
Isolation of RNA, production of cDNA and qRT-PCR analysis

RNA was extracted from 100 mg perirenal adipose tissue (Tri Reagent, Prod T9424, Sigma) from 8 fetuses in the PR group and 6 fetuses in the Control group. RNA was treated for genomic DNA contamination using Ambion Dnase1 and after enzyme deactivation, the RNA was run through a secondary purification process using the RNeasy Mini Kit (QIAGEN, Basel, Switzerland). The quality and concentration of the RNA was determined by measuring the absorbance at 260 and 280 nm, and RNA integrity was confirmed by agarose gel electrophoresis. cDNA was then synthesised using the purified RNA (\(\approx 5 \, \mu g\)) and Superscript 3 reverse transcriptase (Invitrogen Australia Pty Limited, Mount Waverley, Australia) with random hexamers. The relative expression of PPAR\(_{\gamma}\), LPL, G3PDH, leptin, and adiponectin mRNA transcripts (53), and RXR\(\alpha\) (Fwd: cattttcgacaggggtctg; Rev: cttggcgaaccttcctgg) and GAPDH (Fwd: cctggagaaacctgccaagt; Rev: gccaaattcattgtgcgtacca) mRNA transcripts were measured for each fetus by quantitative real time reverse transcription-PCR (qRT-PCR) using the Sybr Green system in an ABI Prism 7900 Sequence Detection System (PE Applied Biosystems, Foster City, CA). Each qRT-PCR reaction well (5 \(\mu l\) total volume) contained: 2.5 \(\mu l\) 2x Sybr Green Master Mix (PE Applied Biosystems, Foster City, CA); 0.25 \(\mu l\) of each primer giving a final concentration of 450 nM, 1.0 \(\mu l\) of molecular grade H\(_2\)O and 1.0 \(\mu l\) of a 1:10 dilution of the stock template.

The relative expression of IGF1, IGF2, IGF1R and IGF2R mRNA transcripts (46) were measured for each fetus by qRT-PCR using the Sybr Green system in an ABI Prism 7300 Sequence Detection System (PE Applied Biosystems, Foster City, CA). Each qRT-PCR reaction well (10 \(\mu l\) total volume) contained: 6 \(\mu l\) 2x Sybr Green Master Mix (PE Applied Biosystems, Foster City, CA); 1ul of each primer giving a final concentration of 450 or 900 nM, 1.0 \(\mu l\) of molecular grade H\(_2\)O and 1.0 \(\mu l\) of a 50 ng/ul dilution of the stock cDNA template.
Primers for each transcript were designed with the aid of Primer Express software (PE Applied Biosystems, Foster City, CA) and where possible one primer of each pair was positioned over a splice site to prevent amplification of any residual genomic DNA. For each transcript RT-PCR was performed using the appropriate primers to ensure the amplification of a single amplicon of the expected size. The product was viewed on an ethidium bromide stained electrophoresis gel. Each amplicon, which was designed to be approximately 200 bp in length, was sequenced to ensure the authenticity of the DNA product and qRT-PCR melt curve analysis was performed to demonstrate amplicon homogeneity. Controls containing no reverse transcriptase were also used and for the qRT-PCR measurements, the cDNA primer concentrations were equivalent for all genes. Amplification efficiencies were determined from the slope of a plot of Ct(defined as the threshold cycle with the lowest significant increase in fluorescence) against the log of the cDNA template material (ranging from 1-100ng/µl) and were 0.997 – 0.999. A constant amount of cDNA equating to 10ng of total RNA was used for each qRT-PCR measurement and four technical replicates were performed for each gene. For all qRT-PCR runs the cycling conditions consisted of 40 cycles of 95 °C for 15 min and 60 °C for 1 min.

The abundance of each mRNA transcript was measured and expression relative to that of Acidic Ribosomal Protein P0 (ARP-P0) was calculated using the comparative threshold cycle (Ct) method (Q-gene qRT-PCR analysis software) (54).

**Statistical Analysis**

The effects of placental restriction on fetal size, plasma hormone and nutrient concentrations, the absolute and relative weights of perirenal fat, and the expression of adipose genes were determined using a Students unpaired t test. Relationships between fetal size, plasma hormone and nutrient concentrations or fat mass and PAT gene expression were determined using linear
regression and partial correlation analyses. There was no effect of gestational age on any of the outcomes reported in this study. All data are presented as the mean ± SEM. A probability of <5% (P<0.05) was taken as the level of significance in all analyses.

RESULTS

The effect of placental restriction on arterial PO₂, plasma glucose and insulin concentrations, fetal growth and fat mass

Mean arterial PO₂ (P < 0.001), plasma glucose (P < 0.01) and plasma insulin concentrations (P < 0.02) were lower in PR compared with control fetuses during late gestation (116-145d) (Table 1). There was no effect, however of placental restriction on either the mean plasma NEFA or leptin concentrations during late gestation (Table 1). PR fetuses had a lower weight (P < 0.0001), crown rump length (CRL) (P < 0.0001), and absolute mass of perirenal fat (P < 0.01) at 140-145d gestation compared with controls, but there was no difference in the relative mass of perirenal fat between the 2 groups (Table 1).

IGF1, IGF2, IGF1R, and IGF2R mRNA Expression in Fetal Perirenal Adipose Tissue

IGF1 mRNA expression was significantly lower (P<0.01) in perirenal adipose tissue (PAT) from PR fetuses compared with Controls at 140-145d gestation (Figure 1). IGF1 mRNA expression was directly related to arterial PO₂ (r²=0.61, P<0.001), mean plasma glucose (r²= 0.47, P < 0.05) and insulin concentrations (r²=0.42, P < 0.05) during late gestation. Partial correlation analysis showed that these relationships were interdependent.

There was no difference in the expression of IGF2, IGF1R, or IGF2R mRNA in PAT from PR and Control fetal sheep (Table 2). When data from both treatment groups were combined, the expression of IGF2 mRNA (r²=0.32, P < 0.05) and IGF2R mRNA (r²=0.34, P < 0.05) were each directly related to the expression of PPARγ mRNA.
**Leptin, Adiponectin, PPARγ, RXRα, LPL, G3PDH and GAPDH mRNA expression in fetal visceral adipose tissue**

Leptin mRNA expression in PAT was lower (P < 0.05) in PR fetuses when compared with Controls (Figure 2) and was directly related to the absolute mass of perirenal fat ($r^2=0.35$, $P < 0.01$). Leptin and PPARγ mRNA expression were also directly related ($r^2=0.55$, $P < 0.01$).

There was no difference in the expression of adiponectin, PPARγ, RXRα, LPL, G3PDH and GAPDH mRNA in PAT between the PR and Control groups at 140-145d gestation (Table 2). Adiponectin mRNA expression was strongly related to G3PDH mRNA expression ($r^2=0.64$, $P < 0.001$) and PPARγ mRNA expression was related to the expression of both RXRα ($r^2=0.31$, $P < 0.05$) and adiponectin mRNA ($r^2=0.35$, $P < 0.05$).

**DISCUSSION**

In this study we have investigated the extent to which the expression of the insulin-like growth factors (IGF1, IGF2), their receptors (IGF1R, IGF2R) and other genes involved in adipogenesis, (PPARγ, RXRα) adipocyte metabolism (G3PDH, GAPDH) and adipokine signalling (leptin, adiponectin) are regulated by placental substrate supply during fetal life. We have demonstrated that the expression of IGF1 and leptin mRNA in adipose tissue were each decreased in response to placental restriction of fetal substrate supply.

Consistent with previous studies of the placentally restricted (PR) sheep model, PR fetuses in the current study were chronically hypoxaemic, hypoglycaemic and hypoinsulinaemic. The changes in fetal blood gas status and nutrient supply in PR sheep fetuses are similar to those measured in cordocentesis studies of SGA human infants (15). In the current study the absolute mass of perirenal fat in PR fetuses at 140d gestation was significantly lower than that of control fetuses, but when fat mass was expressed relative to body weight there was no difference in fat mass.
between PR and control fetuses at 140d gestation. In a previous study it was shown that PR lambs have an increased perirenal and visceral fat mass relative to body weight at 45 days of postnatal life (14). Furthermore, there was an inverse relationship between the insulin sensitivity of glucose, circulating amino acids and free fatty acid metabolism and birth weight in both PR and control lambs, such that a lower birth weight corresponded with a higher whole body insulin sensitivity at 45 days of postnatal life (13).

We have shown that PR results in a decreased expression of IGF1, but not IGF2, IGF1R or IGF2R mRNA expression in fetal perirenal adipose tissue at 140-145d gestation. It has previously been reported that IGF1 mRNA expression is decreased in the fetal liver, kidney and skeletal muscle, and that circulating IGF1 concentrations are decreased in PR, compared to control fetuses at 121d gestation (38, 57). It is currently unknown whether IGF1 mRNA expression in adipocytes is directly related to protein expression, however in hepatocytes IGF1 has been shown to be readily synthesised and secreted, with little preformed or stored (49, 67). It is therefore possible that the decreased expression of IGF1 mRNA in the perirenal adipose tissue of PR fetuses may have consequences for the stimulation of the proliferation and differentiation of perirenal adipose tissue. At physiologic levels IGF1 stimulates both proliferation and differentiation of preadipocytes in cell culture, and along with cAMP and glucocorticoids, is required for induction of differentiation in both serum-containing and serum-free media (24, 68). Furthermore, preadipocyte proliferation can be prevented by stripping IGF1 from adipose tissue conditioned culture medium (47). Given the role of IGF1 in adipocyte proliferation and differentiation, it may initially appear paradoxical that there is a decrease in adipose IGF1 mRNA expression in IUGR fetuses given the association between being born SGA and the emergence of increased adiposity and insulin resistance in later life (56, 58). Recently it has been suggested, however, that low proliferation and differentiation capacity of adipocytes may be a key factor in a propensity to develop larger fat cells which in turn are associated with insulin
It has been proposed that when proliferation of adipocytes is reduced, mesenchymal stem cells are able to differentiate along several mutually exclusive pathways to form other cell types and that the existing adipocytes are susceptible to hypertrophy under conditions of an increased nutrient supply or fat excess (28).

It has been shown previously that the induction of terminal differentiation of preadipocyte cultures by IGF1, or supraphysiological concentrations of insulin acting at the IGF1-receptor (55) results in a marked increase in the expression of PPARγ mRNA (24, 61, 63). PPARγ in a heterodimer with RXRα acts as a transcription factor to initiate transcription of the regulatory genes involved in lipid accumulation and metabolism, particularly LPL, leptin and adiponectin, which all have functional peroxisome proliferator response elements (PPREs) in their promoter regions (29, 32, 66). There is evidence that the effect of IGF1R signalling on PPARγ mRNA expression may be via the p38MAPK downstream signaling pathway (42), which modulates the transcriptional activity of C/EBPβ, an adipocyte differentiation factor that induces PPARγ mRNA expression (5). Whilst IGF1 expression in perirenal adipose tissue was decreased in PR fetal sheep, there was no effect of PR on PPARγ mRNA expression in this tissue. Interestingly, however, the expression of IGF2 related to the expression of PPARγ mRNA in PAT at 140-145d gestation, which may indicate that during fetal life IGF2 binding at the IGF1R significantly contributes to the regulation of PPARγ mRNA expression in adipose tissue. Alternatively PR may act to alter the expression of the IGF binding proteins and hence influence IGF bioavailability within the perirenal adipose tissue. There was no effect of PR on the expression of RXRα, adiponectin LPL, G3PDH, or GAPDH in fetal perirenal adipose tissue although there was a positive relationship between PPARγ and adiponectin mRNA indicating that there may be functional activation of the PPRE in the promoter region of the adiponectin gene in perirenal adipose tissue of PR and normally grown fetal sheep in late gestation.
There was a significant decrease in the expression of leptin mRNA in the perirenal adipose tissue of PR fetal sheep at 140-145d gestation. It has previously been shown that the expression of leptin mRNA and the size of lipid locules in the perirenal adipocytes were increased in the hyperglycaemic sheep fetuses of overnourished ewes (53). Therefore, the reduction in leptin mRNA expression in the PR fetus could reflect a decrease in lipid content of the fetal perirenal adipose tissue.

The leptin gene promoter region contains C/EBP (26, 31, 69), SP1, cAMP, glucocorticoid (22), and hypoxia (4, 25) response elements in addition to the PPRE. In the present study, there was a relationship between the expression of leptin and PPARγ mRNA in perirenal fat, which could reflect either direct activation of the PPRE in the promoter of the leptin gene, or indirect activation of both the leptin and PPARγ promoters by the same transcription factor, such as C/EBP.

In contrast to adult life, there was no relationship between the expression of leptin mRNA in adipose tissue and circulating plasma leptin concentrations in the fetal sheep at 140-145d gestation. It has been suggested that leptin may be derived from other fetal tissues, such as the liver and lung (17), or from the placenta or maternal circulation (8). It is possible that after the transition to postnatal life and the induction of adipose tissue as the main source of circulating leptin that the reduction in leptin mRNA expression in the adipose tissue of lambs which were placentally restricted might result in a functional immaturity of the leptin-fat mass axis with consequences for appetite regulation, lipid metabolism and insulin sensitivity. Interestingly, evidence for a reduction in basal circulating leptin and higher relative increases in BMI have been described in adults who were born IUGR (birth weight < 3rd percentile) (34, 41).

PERSPECTIVES AND SIGNIFICANCE
We have demonstrated that the expression of both IGF1 and leptin mRNA in fetal adipose tissue is decreased by placental restriction of fetal substrate supply. It is possible that a reduced expression of IGF1 mRNA in adipose tissue is associated with a direct reduction in the concentration of IGF1 available for paracrine activation of differentiation in developing adipose tissue. One intriguing possibility is that the low expression of IGF1 in adipose tissue of IUGR fetuses results in a low proliferation and differentiation capacity of adipose tissue. After the transition of the SGA newborn to a nutrient rich environment at birth, however, the adipocytes undergo hypertrophy which in turn has been associated with the emergence of insulin resistance (28). This mechanism could contribute in part to explaining how a mismatch between a nutrient poor prenatal environment and a nutrient rich postnatal environment results in obesity and insulin resistance. The reduction in leptin mRNA expression in perirenal adipose tissue of fetal sheep also raises the possibility that events that occurred in utero which result in a growth restricted fetus may result in a functional immaturity of the leptin – fat mass axis with consequences for the regulation of lipid metabolism and insulin sensitivity during postnatal life.

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FIGURE LEGENDS

**Figure 1:** The expression of IGF1 mRNA (expression relative to ARP-P0 mRNA expression) in fetal perirenal adipose tissue in PR and Control fetuses. There was a significantly lower expression of IGF1 mRNA in the perirenal adipose tissue of PR fetuses (dark bar) compared to Control fetuses (shaded bar) at 140-145d gestation.

**Figure 2:** The expression of leptin mRNA (expression relative to ARP-P0 mRNA expression) in fetal perirenal adipose tissue in PR and Control fetuses. Adipose expression of leptin mRNA was significantly lower in PR (dark bar) than Control fetuses (shaded bar) at 140-145d gestation.
Figure 1
Figure 2

[Bar chart showing leptin mRNA expression with a significant difference indicated by an asterisk (*)]
Table 1: Fetal weight, crown rump length, perirenal fat mass and mean fetal arterial PO2, and plasma NEFA, glucose, insulin and leptin concentrations (116-144d), in PR and Control fetuses.

<table>
<thead>
<tr>
<th></th>
<th>PR (n = 6-9)</th>
<th>CONTROL (n = 6)</th>
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<tbody>
<tr>
<td>Gestational Age (days)</td>
<td>143.6 ± 0.7</td>
<td>144.8 ± 0.2</td>
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<tr>
<td>Fetal Weight (kg)</td>
<td>2.90 ± 0.32*</td>
<td>5.13 ± 0.24</td>
</tr>
<tr>
<td>Fetal Crown Rump Length (cm)</td>
<td>48.6 ± 1.6 *</td>
<td>60.6 ± 1.5</td>
</tr>
<tr>
<td>Absolute mass of perirenal fat (g)</td>
<td>13.4 ± 1.3 *</td>
<td>21.6 ± 1.8</td>
</tr>
<tr>
<td>Relative mass of perirenal fat (g/kg)</td>
<td>4.53 ± 0.57</td>
<td>4.20 ± 0.29</td>
</tr>
<tr>
<td>Mean arterial PO2 116-145d (mmHg)</td>
<td>13.8 ± 0.5 *</td>
<td>21.8 ± 1.3</td>
</tr>
<tr>
<td>Mean plasma glucose 116-145d (mmol/l)</td>
<td>0.68 ± 0.07 *</td>
<td>1.01 ± 0.03</td>
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<tr>
<td>Mean plasma insulin 116-145d (uU/ml)</td>
<td>5.16 ± 0.83 *</td>
<td>8.65 ± 0.76</td>
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<tr>
<td>Mean plasma NEFA 116-145d (uEq/ml)</td>
<td>0.053 ± 0.004</td>
<td>0.054 ± 0.004</td>
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<tr>
<td>Mean plasma leptin 116 – 145d (ng/ml)</td>
<td>1.96 ± 0.23</td>
<td>2.28 ± 0.31</td>
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**Table 2:** IGF2, IGF1R, IGF2R, PPARγ, RXRα, Adiponectin, LPL, Glycerol-3-Phosphate Dehydrogenase (G3PDH) and Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) mRNA expression (expression relative to ARP-P0 mRNA expression) in perirenal adipose tissue in PR and Control fetal sheep.

<table>
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<tr>
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<th>PR (n = 8)</th>
<th>CONTROL (n = 6)</th>
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<tr>
<td>IGF2</td>
<td>0.66 ± 0.05</td>
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<tr>
<td>IGF1R</td>
<td>0.004 ± 0.000</td>
<td>0.005 ± 0.000</td>
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<td>IGF2R</td>
<td>0.003 ± 0.000</td>
<td>0.004 ± 0.000</td>
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<tr>
<td>PPARγ</td>
<td>0.94 ± 0.06</td>
<td>1.16 ± 0.14</td>
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<tr>
<td>RXRα</td>
<td>0.05 ± 0.00</td>
<td>0.07 ± 0.01</td>
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<tr>
<td>LPL</td>
<td>0.31 ± 0.05</td>
<td>0.28 ± 0.03</td>
</tr>
<tr>
<td>G3PDH</td>
<td>0.66 ± 0.08</td>
<td>0.53 ± 0.07</td>
</tr>
<tr>
<td>GAPDH</td>
<td>1.84 ± 0.10</td>
<td>1.91 ± 0.20</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>2.79 ± 0.29</td>
<td>2.49 ± 0.36</td>
</tr>
</tbody>
</table>