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Intrauterine Growth Restriction and the Sex Specific Programming of Leptin and Peroxisome Proliferator-Activated Receptor γ (PPARγ) mRNA Expression in Visceral Fat in the Lamb

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ABSTRACT: Being born small is associated with an increased risk of visceral obesity and insulin resistance in adult life. We have investigated the effect of IUGR on adipogenic and lipogenic gene expression in visceral fat in the lamb at 3 wk of age. Perirenal fat mass, but not adipocyte size was greater in females than males, independent of birth weight. Plasma insulin concentrations during the first 24 h after birth predicted the size of the adipocytes and expression of adiponectin in visceral adipose tissue in both males and females. In females, plasma nonesterified fatty acids (NEFA) concentrations during the first 24 h after birth were directly related to peroxisome proliferator-activated receptor γ (PPARγ) mRNA expression in the perirenal fat depot at 3 wk of age. In the males, in contrast to the females, PPARγ and leptin expression in perirenal visceral fat were significantly lower in IUGR compared with control lambs. Thus, the early nutritional environment programs adipocyte growth and gene expression in visceral adipose tissue. The differential effect of sex and IUGR on PPARγ and leptin expression in visceral fat may be important in the subsequent development of visceral obesity and the insulin resistant phenotype in later life. **(*Pediatr Res* 66: 59–65, 2009)**

A worldwide series of epidemiologic and clinical studies has demonstrated that there are associations between patterns of growth in fetal and early postnatal life and the risk of obesity, insulin resistance, type 2 diabetes mellitus in adult life (1–4). In particular, individuals born small for gestational age (<10th centile for gestational age) who grow rapidly in the early postnatal period have a higher body fat mass from as early as 2 to 12 mo of age (5) and increased body fatness and abdominal fat accumulation during childhood (6) and adulthood (7,8). Although rapid postnatal "catch-up" growth is associated with increased insulin sensitivity, people who are thin at birth and later develop obesity have the highest risk of insulin resistance in adulthood (2,3,9). There is also evidence that obesity after fetal growth restriction is associated with insulin resistance of adipose tissue (2,10,11).

Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear hormone receptor that plays a central role in the regulation of adipogenesis and lipogenesis. PPARγ, as a heterodimer with retinoid-X-receptor (RXR)α, modulates the synthesis and secretion of adipokines, such as adiponectin and leptin, and the induction of lipoprotein lipase (LPL), which in turn regulate peripheral insulin sensitivity, nonesterified fatty acid (NEFA) metabolism, and uptake, respectively (12). Interestingly, the impact of Pro12Ala polymorphisms of the PPARγ2 gene on insulin sensitivity are greater in low-birthweight individuals, suggesting that PPARγ plays a pivotal role in the pathway from fetal growth restriction to later insulin resistance (13,14). It is not known, however, whether a suboptimal nutritional environment before birth programs altered expression of PPARγ or other genes within the adipocyte, which regulate adipogenesis and lipogenesis in postnatal life.

In sheep and pigs, adipogenesis and lipogenesis occur before birth as in the human, and low-birthweight offspring also undergo rapid postnatal growth and have a higher proportion of body fat in later life (15–18). We have previously shown that fetal growth restriction in the sheep is associated with decreased expression of leptin mRNA in the visceral adipose tissue before birth (19), which may have consequences for the regulation of lipid metabolism and insulin sensitivity during postnatal life. It is not known, however, whether IUGR is associated with changes in gene expression within the visceral adipocyte after birth, which may contribute to increased accumulation of visceral adipose tissue. We have therefore, tested the hypothesis that IUGR in association with rapid postnatal growth results in an altered expression of genes within the visceral adipocyte, which regulate adipogenesis and lipogenesis, including PPARγ, RXRα, leptin, adiponectin, LPL, glycerol-3-phosphate dehydrogenase

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Abbreviations: G3PDH, glycerol phosphate dehydrogenase; **LPL,** lipoprotein lipase; **NEFA,** nonesterified fatty acid; **PPAR,** peroxisome proliferator-activated receptor; **qRTPCR,** quantitative real-time PCR

(G3PDH), which promotes *de novo* fatty acid synthesis in adipose tissue, and the hypoxia sensitive glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

METHODS

Animals and surgery. All procedures were approved by The University of Adelaide Animal Ethics Committee. Twenty-two Merino ewes were used in the study. Six nonpregnant ewes underwent surgery to remove the majority of endometrial caruncles (>70) from the uterus, leaving 3–8 caruncles in each horn to induce experimental restriction of placental and fetal growth.

From 110-d gestation, all ewes fed to energy requirements once daily at 1000 h with lucerne chaff and concentrated pellets (Johnson & Sons, Kapunda, South Australia, Australia) (20). Ewes with a twin pregnancy were provided with additional feed to provide 100% of the energy requirements for the maintenance of a singleton or twin bearing ewe, as specified by the UK Ministry of Agriculture (20). After parturition, additional 1 kg lucerne chaff was provided daily.

Blood sampling, growth measurements, and postmortem. Lambs were weighed daily between 1000 and 1400 h. Venous blood samples were collected after ~60 min of nonsuckling on alternate days between 0900 and 1300 h, centrifuged at 1500× g for 10 min, and stored at −20°C. Plasma NEFA, glucose, insulin, and leptin concentrations were determined using methods that have been previously described by this group.

On d 21, lambs were killed with an overdose of sodium pentobarbitone (Virbac Pty Ltd, Peakhurst, New South Wales, Australia) and all fat depots dissected and weighed. Fat samples from each depot were frozen in liquid nitrogen and fixed in 0.4% paraformaldehyde before embedding in paraffin wax.

Adipose tissue histology. Tissue sections were cut (4–5 μm) and stained with hematoxylin and eosin. Standard point counting techniques were used with Video Image Analysis using Video Pro software (Leading Edge, Adelaide, South Australia, Australia) to measure the area of 100 adipocytes within each fat depot (21).

Isolation of RNA, production of cDNA, and quantitative real-time reverse transcription-PCR (qRT-PCR). RNA from perirenal adipose tissue (~600 mg) was isolated using Trizol reagent (Invitrogen Australia Pty Limited, Australia) and chloroform, treated for genomic DNA contamination using Ambion Dnase1, and run through a secondary purification using the RNeasy Mini Kit (QIAGEN Pty Ltd-Australia, Doncaster, Australia). RNA integrity was confirmed by agarose gel electrophoresis and cDNA synthesized using 5 μg purified RNA, Superscript-3 Reverse Transcriptase (Invitrogen Australia Pty Limited, Australia), and random hexamers.

The relative expression of mRNA transcripts were measured by qRTPCR using the Sybr Green system in an ABI Prism 7900 Sequence Detection System (PE Applied Biosystems, Foster City, CA) (22). For each transcript, qRTPCR was performed using the appropriate primers (Table 1). Melt-curve analysis was performed to demonstrate amplicon homogeneity and controls containing no reverse transcriptase were also used. For the qRTPCR measurements, primer concentrations were equivalent for all genes and amplification efficiencies were 0.997–0.999. A constant amount of cDNA, equating to 10 ng of total RNA was used for each qRTPCR measurement, and four technical replicates were performed for each gene.

Each qRTPCR reaction (5 μL total volume) contained 2.5 μL 2 × Sybr Green Master Mix (Applied Biosystems), 0.25 μL of each primer giving a final concentration of 450 nM, 1.0 μL of molecular grade H_2O, 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 s and 60°C for 1 min. The abundance of each mRNA transcript was expressed relative to that of acidic ribosomal-protein large subunit-P0 (RPLP0) and calculated using Q-gene qRTPCR analysis software (23).

Statistical analysis. A frequency distribution curve of birth weights of control Merino singleton lambs from a separate cohort of 45 animals born during the past 5 y was used to determine the birthweight range for IUGR in this population. Newborn lambs in this study were classified as IUGR when their birth weight was less than 2 SD below the cohort mean (IUGR, <4.3 kg; $n = 9$) or normally grown if their birth weight was within 2 SD on either side of the mean (control: 4.5–6.7 kg, $n = 14$). Using these criteria, three male and three female placentally restricted singleton lambs, a male and female pair of twin siblings and one female control singleton were allocated into the IUGR group. Therefore, there were four males and five females in the IUGR group and eight males and six females in the control group.

BMI for each lamb was calculated (weight/crown-rump length^2). Daily growth rate (%) was calculated as body weight gained per day as a percentage increase from the previous days' body weight.

The effects of IUGR and sex on the absolute and relative weights of fat, size of adipocytes, levels of plasma insulin, glucose, NEFA and leptin, and on expression of adipocyte genes were determined using two-way ANOVA. The Duncan's multiple range test was used posthoc to identify the differences between mean values. Relationships between the variables were determined using linear regression analyses. Where there were potential associations between independent variables, multiple linear regression analyses were used and partial correlation coefficients were derived. Where relationships between variables were found to be independent, simple linear regression analysis was then used. All data are presented as the mean ± SEM and a probability of <5% ($p < 0.05$) was taken as significant.

RESULTS

The effect of IUGR on birth weight, plasma NEFA and insulin during the first 24 h, and postnatal growth. IUGR lambs were lighter and shorter at birth ($p < 0.001$) (Table 2) and had a higher daily growth rate during wk 1 ($p < 0.001$) and wk 2 ($p < 0.05$) (Fig. 1*A*). IUGR lambs also had lower plasma NEFA concentrations during the first 24 h compared with controls ($p < 0.05$). Partial correlation analysis of the relationships between birth weight, NEFA during the first 24 h after birth, and growth rate during wk 1 showed that in males NEFA was an independent predictor of wk 1 growth rate ($r^2 = 0.36$, $p < 0.05$). In female lambs, however, NEFA was not related to wk 1 growth rate, and birth weight was an independent predictor of daily growth rate during wk 1 ($r^2 = 0.38$, $p < 0.05$). In female lambs, but not in males, there was a positive relationship between birth weight and plasma insulin concentrations during the first 24 h after birth (Table 3) and insulin and growth during wk 1; however, these relationships did not persist when the effects of glucose and NEFAs were controlled for. The daily growth rate decreased ($p < 0.05$) between wk 1 and 3 in both IUGR and control groups (Fig. 1*A*). At 21 d, IUGR lambs were lighter ($p < 0.001$) and shorter ($p < 0.05$) than control lambs and there was no effect of sex on weight or length (Table 2).

Plasma NEFA, glucose, insulin, and leptin concentrations during wk 1–3. During wk 1–3, plasma NEFA concentrations were lower in IUGR than control lambs. Plasma insulin, but

Table 1. *Oligonucleotide primer sequences for Acidic RPLP0, 18s rRNA, RXRα, PPARγ, Leptin, Adiponectin, LPL, G3PDH, and GAPDH*

Gene name (Genbank accession)	Forward	Reverse	Amplicon size (bp)
RPLP0 (AF013214)	5′caaccctgaagtgcttgacat3′	5′aggcagatggatcagcca3′	220
18S rRNA (AY779625)	5′gtaacccgttgaaccccatt3′	5′ccatccaatcggtagtagcg3′	151
PPARγ (AY179866)	5′atgtctcataatgccatcaggtt3′	5′gataacaaacggtgatttgtctgtc3′	225
RXRα (DQ100361)	5′cattttcgacagggtgctg3′	5′cttggcgaaccttcctgg3′	220
Leptin (NM173928)	5′atctcacacacgcagtccgt3′	5′ccagcaggtggagaaggtc3′	202
Adiponectin (NM174742)	5′atcaaactctggaacctcctatctac3′	5′ttgcattgcaggctcaag3′	232
LPL (M16966)	5′ taccctgcctgaagtttccac 3′	5′ cccagtttcagccagactttc 3′	302
G3PDH (BT020681)	5′gctttggcgacaacacca3′	5′agctgctcaatggactttcc3′	208
GAPDH (U85042)	5′cctggagaaacctgccaagt3′	5′gccaaattcattgtcgtacca3′	226

Table 2. *Size at birth and plasma glucose and insulin concentrations during the first 24 h after birth and during the first 3 wk of life*

	IUGR		Control	
	Male ($n = 4$)	Female ($n = 5$)	Male ($n = 8$)	Female ($n = 6$)
Birth weight (kg)	3.85 ± 0.16*	3.78 ± 0.21*	6.02 ± 0.18	5.65 ± 0.10
Crown rump length at birth (cm)	43.0 ± 0.4*	42.3 ± 1.3*	47.4 ± 1.1	47.3 ± 0.8
Body Mass Index at birth (kg/m^2)	0.21 ± 0.01*	0.21 ± 0.01*	0.27 ± 0.01	0.25 ± 0.00
Plasma NEFA during 24 h after birth (mEq/L)	0.65 ± 0.13*	0.70 ± 0.10*	0.87 ± 0.09	0.98 ± 0.11
Plasma insulin during 24 h after birth (ng/mL)	2.65 ± 0.88	1.05 ± 0.35†	2.57 ± 0.28	2.00 ± 0.31†
Plasma glucose during 24 h after birth (ng/mL)	5.32 ± 0.61	4.34 ± 0.58	6.32 ± 1.18	5.52 ± 0.38
Weight at 21 d (kg)	9.69 ± 0.51*	9.82 ± 0.53*	13.43 ± 0.23	12.94 ± 0.19
Crown rump length at 21 d (cm)	59.3 ± 1.0*	63.0 ± 1.7*	66.1 ± 2.2	66.9 ± 1.3
Body Mass Index at 21 d (kg/m^2)	0.27 ± 0.01*	0.24 ± 0.01*	0.31 ± 0.02	0.29 ± 0.01
Mean plasma NEFA (wk 1–3) (mEq/L)	0.47 ± 0.06*	0.50 ± 0.08*	0.61 ± 0.06	0.71 ± 0.09
Mean plasma insulin (wk 1–3) (ng/mL)	2.69 ± 0.69	1.43 ± 0.18†	2.31 ± 0.19	1.79 ± 0.25†
Mean plasma glucose (wk 1–3) (mMol/L)	6.00 ± 0.41	5.66 ± 0.17	5.90 ± 0.28	5.73 ± 0.26
Mean plasma leptin (wk 1–3) (ng/mL)	5.67 ± 1.37	5.39 ± 0.60	5.18 ± 0.50	6.74 ± 0.33

* Significant differences between mean values in the IUGR and control groups.
† Significant differences between mean values in the male and female groups.

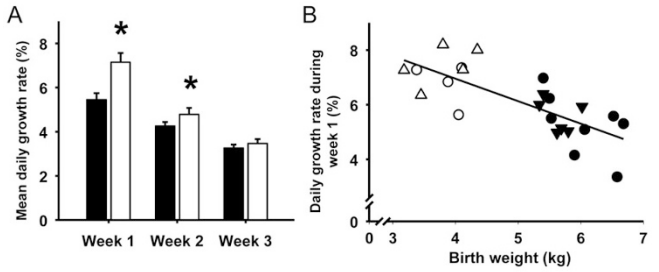

Figure 1. Mean daily growth rate (%) during wk 1–3 of IUGR (□) and control (■) lambs. IUGR lambs grew faster during wk 1 (*, $p < 0.001$) and wk 2 (*, $p < 0.05$) (*A*). The significant relationship ($r^2 = 0.53$, $p < 0.0001$) between birth weight (kg) and the average daily growth rate during wk 1 (%) in IUGR male (●), IUGR female (▼), control male (○), and control female (△) lambs (*B*).

not glucose, concentrations were higher in males than females, in both the IUGR and control groups (Table 2). There were no differences in circulating leptin concentrations between IUGR and control lambs or between males and females.

Visceral fat mass and adipocyte cell size. At 21 d, there was no difference in the relative perirenal or omental fat mass, or the mean size of perirenal or omental adipocytes between IUGR and control lambs (Fig. 2). At 21 d, both absolute and relative perirenal fat mass were lower ($p < 0.05$) in males than females (Fig. 2). In males, relative perirenal fat mass at 21 d was directly related to plasma insulin concentrations during the first 24 h after birth (Table 3 and Fig. 4) and this relationship persisted when the effects of birth weight were controlled for ($r^2 = 0.42$, $p < 0.05$). In females, however, relative perirenal fat mass at 21 d was directly related to plasma NEFA concentrations during the first 24 h after birth ($r^2 = 0.79$, $p < 0.01$; Table 3 and Fig. 4), independent of the effects of birth weight or plasma insulin concentrations during the first 24-h postpartum. The relative perirenal fat mass in female lambs was also directly related to mean plasma glucose ($r^2 = 0.51$, $p < 0.05$) and insulin concentrations ($r^2 = 0.52$, $p < 0.05$) during wk 1–3; however, these relationships did not persist when the effects of plasma NEFA concentrations during the first 24 h after birth were controlled for. Plasma insulin concentrations during the first 24 h after birth, predicted the mean size of the perirenal adipocytes at 21 d in both male and female lambs (Table 3) and this relationship persisted when the effects of glucose and insulin concentrations during the first 3 wk of postnatal life were controlled for.

Adipocyte gene expression. In males and females, adiponectin mRNA and LPL mRNA levels in perirenal fat were each related to plasma insulin concentrations during the first 24 h after birth (adiponectin, $r^2 = 0.28$, $p < 0.05$; LPL, $r^2 = 0.28$, $p < 0.05$) and to the daily growth rate during wk 1 (adiponectin, $r^2 = 0.27$, $p < 0.05$; LPL, $r^2 = 0.18$, $p < 0.05$); however, these relationships did not persist when the effects of birth weight were controlled for. There was no effect of either IUGR or sex on the levels of RXRα, adiponectin, LPL, G3PDH, and GAPDH mRNA expression in perirenal fat (Table 4). There was a significant interaction ($p < 0.05$) between the effects of IUGR and sex on PPARγ and leptin mRNA expression in perirenal fat (Fig. 3). In female lambs, there was no difference in PPARγ and leptin mRNA expression in perirenal fat between the IUGR and control groups (Fig. 3). PPARγ mRNA expression was directly related to plasma NEFA during the first 24 h after birth in both IUGR and control female lambs ($r^2 = 0.69$, $p < 0.01$; Table 3 and Fig. 4) and this relationship persisted when the effects of birth weight were controlled for. PPARγ mRNA expression was also directly related to mean insulin concentrations during wk 1–3 ($r^2 = 0.61$, $p < 0.05$), and this relationship persisted when the effects of mean plasma glucose and NEFA concentrations during wk 1–3 were controlled for. In females, the relative perirenal fat mass was directly related to PPARγ mRNA expression in both the IUGR ($r^2 = 0.93$, $p < 0.01$) and control lambs ($r^2 = 0.85$, $p < 0.01$) (Fig. 4). This relationship persisted when the effects of mean plasma insulin concentrations were controlled for when data from control and IUGR lambs were combined ($r^2 = 0.45$, $p < 0.05$). There was also a relationship between PPARγ and leptin mRNA expression in perirenal fat ($r^2 = 0.54$, $p < 0.01$) and this relationship persisted when the effect of relative perirenal fat mass was controlled for ($r^2 = 0.79$, $p < 0.01$).

Table 3. *Relationships between measures of growth and perirenal fat mass and plasma insulin or NEFA concentrations during the first 24 h of life in male and female lambs*

	Male		Female	
	Insulin (n = 10)	NEFA (n = 12)	Insulin (n = 9)	NEFA (n = 11)
Birth weight (kg) (x)	NS	$y = 0.14x + 0.06$ $r^2 = 0.35$, $p < 0.05$	$y = 0.56x - 1.05$ $r^2 = 0.46$, $p < 0.05$	$y = 0.18x - 0.02$ $r^2 = 0.45$, $p < 0.05$
Daily growth rate wk 1 (%) (y)	NS	$y = -3.7x + 8.7$ $r^2 = 0.57$, $p < 0.01$	$y = -0.84x + 7.7$ $r^2 = 0.50$, $p < 0.05$	NS
Relative perirenal fat mass at 21 d (g/kg) (y)	$y = 1.73x + 6.16$ $r^2 = 0.43$, $p < 0.05$	NS	NS	IUGR: $y = 9.0x + 7.2$, $r^2 = 0.94$, $p < 0.001$; Control: $y = 11.5x + 3.3$, $r^2 = 0.66$, $p < 0.05$
Size of perirenal adipocytes at 21 d (mm^2) (y)	$y = 177x + 1023$ $r^2 = 0.40$, $p < 0.05$	NS	$y = 245x + 957$ $r^2 = 0.85$, $p < 0.001$	NS
PPARγ mRNA expression at 21 d (y)	NS	NS	NS	IUGR: $y = 2.4x + 0.24$, $r^2 = 0.98$, $p < 0.002$ Control: $y = 1.6x + 0.12$, $r^2 = 0.73$, $p < 0.03$
Adiponectin mRNA expression at 21 d (y)	NS	$y = 0.697x + 9.99$ $r^2 = 0.61$, $p < 0.01$	NS	NS
LPL mRNA expression at 21 d (y)	NS	$y = -0.548x + 3.01$ $r^2 = 0.57$, $p < 0.01$	NS	NS
G3PDH mRNA expression at 21 d (y)	NS	$y = 1.16x + 1.88$ $r^2 = 0.33$, $p < 0.05$	NS	NS

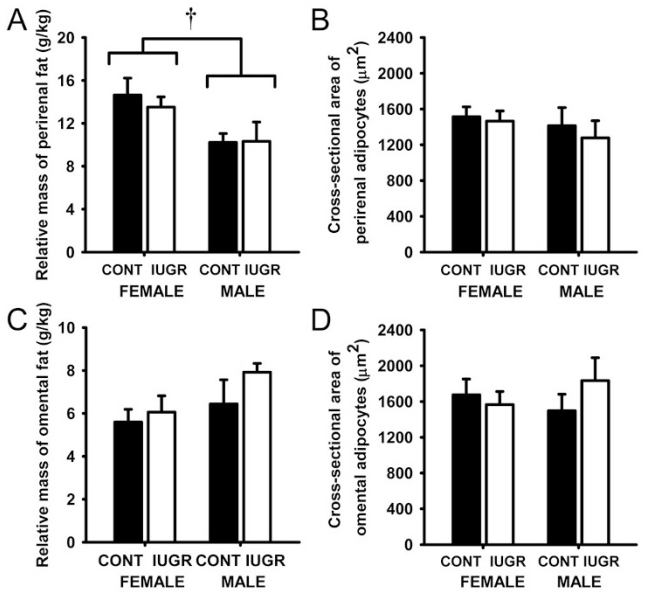

Figure 2. The relative mass and mean cross sectional areas of adipocytes for perirenal fat (*A* and *B*) and omental fat (*C* and *D*) in control (■) and IUGR (□) female and male lambs at 21 d. Relative fat mass was higher in female lambs (†, $p < 0.05$) compared with males.

In females, adiponectin and LPL mRNA expression in perirenal fat were strongly related to each other ($r^2 = 0.78$, $p < 0.001$), and this relationship was independent of the effect of PPARγ mRNA expression ($r^2 = 0.74$, $p < 0.01$). There was also an independent relationship between LPL mRNA expression and G3PDH mRNA expression in perirenal fat ($r^2 = 0.46$, $p < 0.05$).

In male lambs, PPARγ and leptin mRNA levels were each lower in the perirenal fat of IUGR when compared with control lambs (Fig. 3), and PPARγ mRNA expression was directly related to birth weight ($r^2 = 0.37$, $p < 0.05$). In addition, partial correlation analysis revealed that leptin mRNA expression was also directly related to birth weight independent of the effects of PPARγ mRNA expression ($r^2 = 0.42$, $p < 0.05$). In contrast to females, PPARγ mRNA expression was not related to plasma NEFA concentrations during the first 24 h after birth (Table 3) and there was no relationship between the relative mass of perirenal fat and PPARγ mRNA expression.

In males, adiponectin and LPL mRNA expression in perirenal fat were strongly related to each other ($r^2 = 0.79$, $p < 0.0001$) and this relationship persisted when the effects of PPARγ mRNA expression ($r^2 = 0.74$, $p < 0.0001$) or G3PDH mRNA expression ($r^2 = 0.88$, $p < 0.0001$) were controlled for. There was also a relationship between plasma NEFA during the first 24 h after birth and the expression of adiponectin mRNA, LPL mRNA, and G3PDH mRNA in the male lambs (Table 3).

DISCUSSION

IUGR and postnatal growth. The IUGR lambs in this study were smaller and thinner at birth and had a higher daily fractional growth rate in the first 2 wk of life compared with control lambs. Consistent with previous studies, IUGR lambs grew faster and had not caught up to their control counterparts in terms of weight or length by 21 d (15,17,18). IUGR lambs had lower plasma NEFA concentrations during the first 24 h after birth and throughout the first 3 wk after birth. There was also a direct relationship between birth weight and plasma NEFA concentrations, which suggests that a reduced fetal

Table 4. *RXRα, adiponectin, LPL, G3PDH, and GAPDH mRNA expression in perirenal adipose tissue*

	IUGR		Control	
Target: reference gene	Male (n = 4)	Female (n = 5)	Male (n = 8)	Female (n = 6)
RXRa: RPLP0	0.17 ± 0.01	0.18 ± 0.02	0.20 ± 0.02	0.18 ± 0.01
Adiponectin: RPLP0	8.50 ± 0.65	7.10 ± 1.68	8.76 ± 01.53	7.64 ± 1.04
LPL: RPLP0	1.77 ± 0.25	1.39 ± 0.43	1.89 ± 0.47	1.37 ± 0.22
G3PDH: RPLP0	2.07 ± 0.36	2.82 ± 0.51	2.95 ± 0.30	3.00 ± 0.41
GAPDH: RPLP0	1.29 ± 0.28	1.43 ± 0.16	1.32 ± 0.10	1.36 ± 0.07

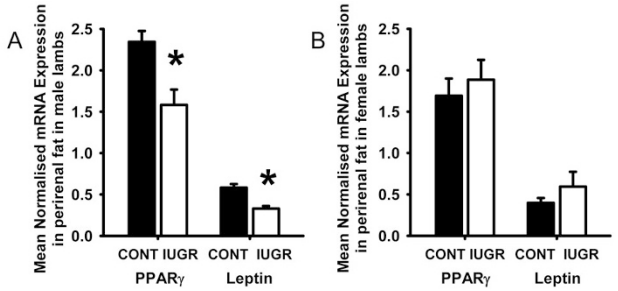

Figure 3. PPARγ and leptin mRNA expression in perirenal fat in IUGR male (□) and control male lambs (■) (*A*) and in IUGR female (□) and control female lambs (■) (*B*) at 21 d. PPARγ mRNA (*, $p < 0.05$) and leptin mRNA levels (* $p < 0.001$) were significantly lower in the perirenal fat of IUGR compared with control male lambs.

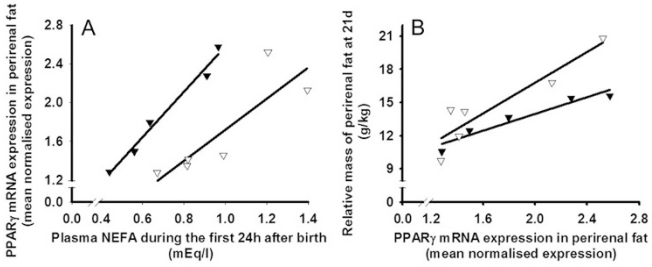

Figure 4. The relationship between plasma NEFA concentrations during the first 24 h after birth and PPARγ mRNA expression in perirenal fat at 21 d in female IUGR (▼; $r^2 = 0.98$, $p < 0.001$) and female control lambs (▽; $r^2 = 0.73$, $p < 0.05$) (*A*), and the relationship between the expression of PPARγ mRNA in perirenal fat and the relative mass of perirenal fat at 21 d in female IUGR (▼: $r^2 = 0.93$, $p < 0.01$) and control lambs (▽: $r^2 = 0.85$, $p < 0.01$) (*B*).

substrate supply, may lead to altered NEFA handling during postnatal life.

IUGR and the development of perirenal adipose tissue. There was no difference between IUGR and control lambs in the relative mass of perirenal or omental adipose tissue present at 21 d of life, or between the mean size of adipocytes. In previous studies, lambs that were IUGR were found to have a higher relative mass of perirenal and total visceral fat at 6 wk of age when compared with controls, a higher total body fat content than their higher birthweight counterparts when measured at the same body weight (20 kg) 40–50 d after birth (15) and a higher abdominal fat mass at 2 y of age (17).

IUGR and the expression of adipogenic and lipogenic genes in the female lamb. Female lambs had a significantly greater relative mass of perirenal fat, but do not have larger perirenal adipocytes when compared with male lambs at 3 wk of age. This suggests that the increased mass of the perirenal fat depot in females is predominantly a result of hyperplasia rather than hypertrophy. It has been previously shown that clonal expansion of growth arrested preadipocytes is predominantly modulated by stimulation of IGF1-receptor by both plasma insulin and IGF1 (24). In this study, the mean size of the perirenal adipocytes was directly related to plasma insulin concentrations during the first 24 h after birth in both males and females, whereas perirenal fat mass in females, but not males, was related to plasma glucose and insulin concentrations during the first 3 wk after birth. Although modulators of adipocyte hyperplasia other than insulin, in particular IGF1, may also contribute to fat deposition during this period, the results of this study provide evidence that, in females, there is an impact of insulin both immediately after birth and during the first 3 wk of postnatal life on adipogenesis and therefore suggest that there is an increased responsiveness of adipose tissue to insulin in the female lamb, compared with the male lamb. Fatty acids are one of the principal inducers of adipocyte differentiation (25,26), and in both IUGR and control female lambs, there was a relationship between plasma NEFA concentrations during the first 24 h after birth and relative perirenal fat mass at 3 wk of life. There was also a direct relationship between plasma NEFA concentrations during the first 24 h and perirenal PPARγ mRNA expression. Although the slope of this relationship was similar in IUGR and control females, perirenal PPARγ mRNA expression was higher at any given plasma NEFA concentration on d 1 in the IUGR, compared with the control group. This may suggest, therefore, that female lambs, in which plasma insulin concentrations are low relative to the male, fatty acids may act as transcriptional activators of PPARγ expression resulting in enhanced insulin signaling and hyperplasia of the perirenal adipocytes and resulting in a greater relative mass of perirenal fat in females than in males at 3 wk of age (25) (Fig. 5*A*).

IUGR and the expression of adipogenic and lipogenic genes in the male lamb. In male lambs, the size of the perirenal adipocytes and relative perirenal fat mass at 3 wk of age were each related to plasma insulin concentrations during the first 24 h after birth. Therefore, in males, insulin in the early perinatal period seems to be an important determinant of the subsequent triglyceride storage capacity of perirenal adipocytes and perirenal fat mass in early life. In male lambs, in contrast to females, PPARγ and leptin mRNA expression in perirenal fat were each lower in the IUGR compared with control group. Interestingly, in males there was also no relationship between plasma NEFA concentrations on d 1of life and either perirenal fat mass or PPARγ mRNA expression. One possibility is that the presence of the relatively higher insulin concentrations in the male lamb compared with the

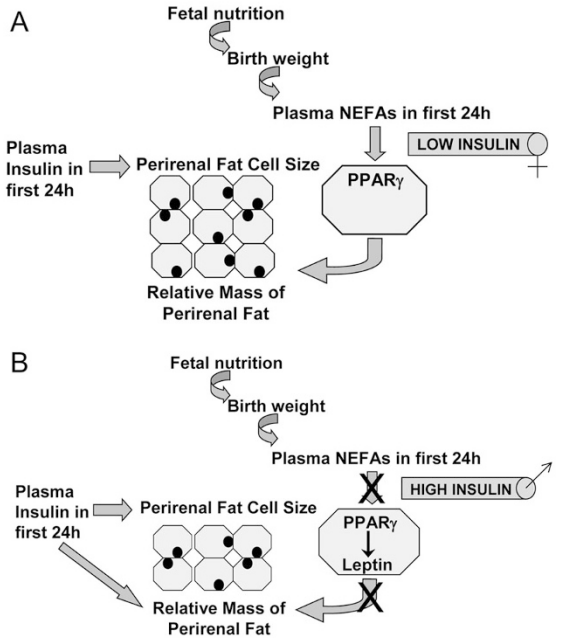

Figure 5. Summary diagrams highlighting the effects of early insulin exposure on the development of perirenal fat and on PPARγ and leptin mRNA expression in the female and male lamb. Decreased intrauterine substrate supply results in IUGR and lower circulating plasma NEFA concentrations during the first 24 h after birth. In female lambs (*A*), in the presence of lower plasma insulin concentrations, plasma NEFA concentrations during the first 24 h after birth drive the expression of PPARγ mRNA and the relative mass of perirenal fat at 21 d. This may occur through NEFA activation of PPARγ expression, resulting in enhanced insulin signaling and hyperplasia of the perirenal adipocytes and resulting in a greater relative mass of perirenal fat in females than in males at 3 wk of age. In the male lamb (*B*), in the presence of higher plasma insulin concentrations, there is reduced NEFA activation of PPARγ and leptin gene expression and plasma insulin concentrations immediately after birth represent the predominant drive to both perirenal adipocyte size and relative perirenal fat mass at 3 wk of age.

female is associated with a reduced activation and/or expression of PPARγ when fatty acid concentrations are low (Fig. 5*B*). An alternate explanation is that the levels of other modulators of PPARγ expression, such as IGF1, are affected differently by growth restriction in males and females. A decrease in PPARγ mRNA expression in adipose tissue is associated with a decrease in adipose tissue and hepatic insulin sensitivity, particularly during periods of high caloric intake (27). Therefore, the reduced expression of PPARγ mRNA in the perirenal visceral fat depot of male IUGR lambs may be associated with the emergence of insulin resistance of adipose tissue during the early postnatal period and predispose male IUGR lambs to the subsequent development of an insulin resistant phenotype.

We have previously reported that there was a decrease in leptin expression in perirenal adipose tissue in male and female IUGR compared with control fetal sheep at 140–145-d gestation (19). Thus, this study suggests that this decrease persists into postnatal life in males. In this study, we found that the lower leptin expression in the IUGR male lambs was not associated with a reduction in circulating plasma leptin levels. This may be a consequence of the relatively small contribution of the perirenal depot to systemic leptin concentrations, but relatively lower leptin secretion from this or other visceral fat depots may have an important impact on the paracrine or hepatic regulation of lipid metabolism and insulin sensitivity.

In this study, we have found that plasma insulin concentrations during the first 24 h after birth predicted the level of adiponectin expression in perirenal fat in both male and female lambs at 3 wk of age, and that plasma NEFA concentrations during the first 24 h after birth predicted the expression of adiponectin mRNA in the perirenal adipose tissue in male lambs. Whether there is a longer term impact of the early low circulating plasma NEFA concentrations in IUGR male lambs on subsequent adiponectin expression in the visceral fat after the initial postnatal growth period will be important to determine. A novel finding of this study was that here was a strong relationship between the expression of adiponectin and LPL in the perirenal fat in both male and female lambs.

Thus, the early nutritional environment, as represented by birth weight and plasma insulin and NEFA concentrations during the first 24 h of life, determines the subsequent growth and functional development of visceral adipose tissue. There are sex specific differences in the impact of IUGR on the regulation of PPARγ and leptin expression in perirenal adipose tissue and separately, plasma insulin and NEFA concentrations in early postnatal life predict the subsequent level of expression of adiponectin in visceral fat. We have demonstrated that there are early changes in the expression of PPARγ and leptin mRNA in the visceral adipose tissue of IUGR male lambs, which precede the emergence of an obese phenotype. Therefore, this study clearly highlights that the early postnatal period is a potentially critical time for nutritional intervention to limit the adverse metabolic consequences of being small for gestational age.

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