

This rapid communication was first published in the October 1999 issue of *Journal of Endocrinology* **163** R1-R4. Regrettably, the names of the first two authors were printed incorrectly, so the entire article is reprinted here. The journal apologizes for this error and any confusion it has caused.

Abundance of leptin mRNA in fetal adipose tissue is related to fetal body weight

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Abstract

Leptin mRNA was measured in adipose tissue of fetal sheep by reverse transcription polymerase chain reaction (RT-PCR). Abundance of leptin mRNA relative to β -actin mRNA in fetal perirenal adipose tissue increased ($P < 0.02$) with gestation, being higher at 144 d (0.73 ± 0.10 , $n=5$) than at 90-91 d (0.40 ± 0.08 , $n=6$) or 125 d (0.40 ± 0.04 , $n=5$) gestation (term ~147-150 d). There was a positive relationship between relative abundance of leptin mRNA (y) and fetal body weight (x) between 90 and 144 d gestation ($r^2=0.27$, $P < 0.01$). The slope

of the linear dependence of leptin mRNA on fetal weight was 15-fold greater ($P < 0.001$) at 90-91 d ($y = 2.81x - 1.1$, $n=6$, $r^2=0.71$, $P < 0.025$) than between 125-144 d gestation ($y = 0.195x - 0.15$, $n=16$, $r^2=0.39$, $P < 0.01$). Thus the leptin synthetic capacity of fetal adipose tissue appears to increase in late gestation but this is accompanied by constraint of its sensitivity to fetal body weight. We hypothesise that leptin synthesis in fetal adipose tissue is related to fetal nutrient supply and growth rate.

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Introduction

Leptin is a 16 kDa polypeptide hormone synthesised and secreted by adipocytes that acts to suppress appetite and increase energy expenditure in adults (Friedman & Halaas 1998, Houseknecht *et al.* 1998). Abundance of leptin mRNA in adipose tissue and concentrations of leptin protein in blood correlate positively with body weight and adiposity in human adults (Maffei *et al.* 1995, Considine *et al.* 1996). In rodents leptin mRNA is expressed in a number of fetal tissues including cartilage, hair follicles and placenta (Senaris *et al.* 1997, Hoggard *et al.* 1997, Masuzaki *et al.* 1997) and in humans there is a positive association between leptin levels in cord blood and body weight at birth (Schubring *et al.* 1997, Koistinen *et al.* 1997, Ong *et al.* 1999). These data suggest there is a relationship between growth and leptin synthesis before birth, but there have been no reports relating fetal expression of leptin to fetal growth. We have measured leptin mRNA in perirenal fat, the major adipose tissue in the sheep fetus. We also investigated the relationship between leptin mRNA and fetal weight before (90-91 d) and after (125-144 d gestation) the development of sympathetic innervation of the perirenal fat depot (Gemmell & Alexander 1978, Clarke *et al.* 1997).

Materials and Methods

Animals

The study design was approved by the Animal Ethics Committee of the University of Adelaide. Merino ewes ($n=51$)

were mated and provided unrestricted access to feed and water. They were killed between 90 and 145 d of pregnancy (term ~147-150 d) with an overdose of sodium pentobarbitone (6.5 g i.v.) and the fetuses were removed and weighed. In one group ($n=29$, 12 male and 17 female) of singletons ($n=12$) and twins ($n=17$ fetuses from 12 ewes), both left and right fetal perirenal fat depots were collected between 90-99 d ($n=9$) and 137-145 d ($n=20$) and weighed. In another group consisting entirely of twins a biopsy of fetal perirenal adipose tissue was obtained from one twin at 90-91 d ($n=6$), 125 d ($n=5$), 139-141 d ($n=6$) and 144 d ($n=5$), quickly frozen in liquid N₂ and stored at -80 °C for measurement of leptin and β -actin mRNA.

Reverse transcription polymerase chain reactions

RNA was extracted from ~100 mg adipose tissue (Tri Reagent, Prod T9424, Sigma) as recommended. cDNA was obtained by reverse transcription of 2 μ g total RNA with random sequence hexanucleotides (Cat RP-6, GeneWorks, Adelaide, Australia) using Super-Script RNase H⁻ (Cat 18053-017, GIBCOBRL). A fragment of ovine leptin cDNA was amplified through 26 cycles of 60 sec at 94 °C, 15 sec at 53 °C and 60 sec at 72 °C (Hyaid PCR Express, Teddington, UK) from 5 μ l of reverse transcription product using Taq* DNA polymerase (Biotech International, Bently, Australia) as recommended by the manufacturer with 5'-GACATCTCACACACGCAG-3' and 5'-GAGGTTCTCCAGGTCATT-3' (GeneWorks) as primers. This produced 183 bp of ovine leptin ds cDNA (nucleotides 67-249 of the 441 nucleotide cDNA of ovine leptin, Genbank

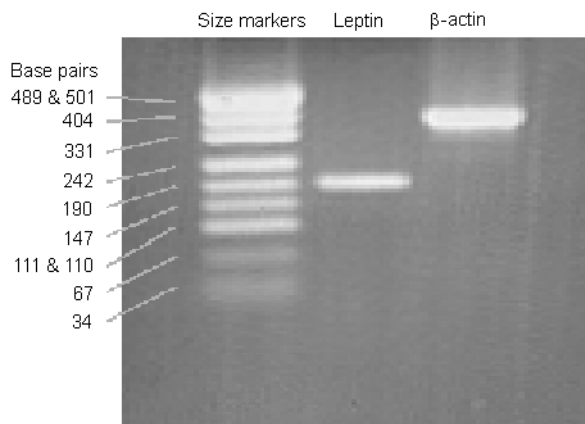


Figure 1 Agarose electrophoresis of RT-PCR products.

Acc. No. U 84247). Sequencing with the ABI PRISM Dye Terminator method (Perkin-Elmer Corp) after QIA-quick purification (QIAGEN Pty. Ltd., Clifton Hill VIC, Australia) confirmed its identity. A 349 bp fragment of ovine β -actin cDNA was similarly amplified with 5'-TGTGATGGTGGGTATATGGGTC-3' and 5'-TAGA-TGGGCACAGTGTGGGT-3' and its identity was confirmed by homology with rat β -actin, GenBank Acc. No. V01217 J00691. Products of RT-PCR (8 μ l) were electrophoresed through a 1.5% (w/v) agarose gel. This was stained with ethidium bromide, transilluminated with UV light and photographed. Intensities of RT-PCR products were measured on the film negative by laser densitometry. Molecular sizes of PCR products were estimated by comparing their electrophoretic migration with those of fragments of pUC19 ds DNA digested with *Hpa* II (GeneWorks). Each leptin RT-PCR product had a mobility similar to that of the 190 bp fragment of pUC19, consistent with the predicted size of 183 bp. The

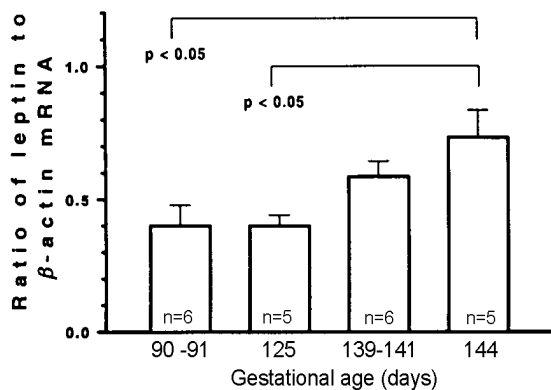


Figure 2 Ontogeny of leptin expression in fetal adipose tissue.

mobility of the β -actin cDNA fragment was intermediate between that of the 331 and 404 bp markers (Fig.1).

Statistics

Results are presented as mean \pm S.E.M. (*n* = number of animals). Total perirenal fat mass is the combined weights of the two perirenal fat depots for each fetus. Relative fat mass is the ratio of total perirenal fat mass to body weight of the fetus. Differences between groups were assessed by one- or two-way analysis of variance with Bonferroni's multiple comparison. Associations were evaluated by linear regression (SigmaStat V1, Jandel Scientific). Differences between the slopes of linear regressions were assessed by Student's *t* test (Zar 1974).

Results

In the first group studied, which included singletons and twins, gender of fetus had no significant effect on fetal weight, perirenal fat weight or relative fat mass. As expected, fetal weight was affected by number of fetuses per pregnancy (*P*<0.01), gestational age (*P*<0.001) and the interaction between these factors (*P*<0.05). Twin fetuses had lower body weights than singletons and this difference was greater after 137 d than before 99 d gestation. Total perirenal fat weight (*P*<0.001), but not relative fat mass, increased with gestation and was unaffected by the number of fetuses per pregnancy.

In the second group, which consisted entirely of twins of which one of each pair was examined, relative abundance of leptin mRNA in fetal perirenal adipose tissue increased (*P*<0.02) with gestation (Fig. 2). Abundance of leptin mRNA relative to β -actin mRNA (*y*) in fetal adipose tissue was positively correlated with the weight (*x*) of the fetus ($y = 0.08x + 0.31, n=22, P<0.01$) such that 27% of the variance in leptin mRNA could be explained by dependence on fetal weight ($r^2=0.27$). The slope of this relationship (Fig. 3) was steeper (*P*<0.001) at 90-91d ($y = 2.81x - 1.1, n=6, r^2=0.71, P<0.025$) than at 125-144 d ($y = 0.195x - 0.15, n=16, r^2=0.39, P<0.01$).

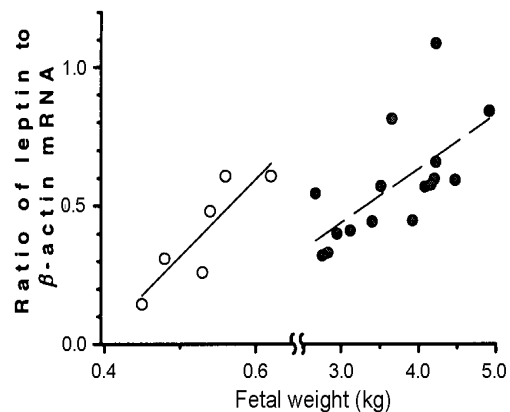


Figure 3 Relationship between leptin mRNA and fetal weight at 90-91 (O) and 125-144 (●) d gestation.

Discussion

We have demonstrated for the first time that leptin mRNA is expressed in fetal adipose tissue. Lipid accumulation in the perirenal area of fetal sheep occurs from ~70 d gestation and there is a marked increase in mitochondria within this tissue between 80 and 90 d (Alexander 1978). After this age, fetal perirenal fat in the sheep consists of brown adipocytes characterised by many mitochondria with numerous cristae (Gemmell & Alexander 1978). In the rat, leptin is expressed in brown adipose tissue during the first 24 h after birth (Dessolin *et al.* 1997). The present study shows that leptin mRNA is expressed in brown adipose tissue before birth.

We found an increase in relative abundance of leptin mRNA in fetal adipose tissue in the last 20 d before delivery in sheep. This may be due to an increase in size of adipocytes in fetal perirenal fat depots, since there is strong evidence that adipocyte cell size is a major determinant of expression and secretion of leptin (Lonnqvist *et al.* 1997) and the mean cell volume of perirenal adipocytes in fetal sheep increases 3- to 4-fold between 90 and 144 d gestation (Vernon *et al.* 1981). Alternatively, increased leptin expression in fetal perirenal fat in sheep during late gestation may be related to the rise in circulating fetal cortisol concentrations in the two weeks before delivery (Bassett & Thorburn 1969). Glucocorticoids stimulate leptin gene expression in adipocytes *in vivo* (De Vos *et al.* 1995) and *in vitro* (Sliker *et al.* 1996). We observed a positive relationship between abundance of leptin mRNA in fetal adipose tissue and fetal body weight as early as 90 d gestation (term is 147-150 d). This relationship may also be a consequence of greater leptin synthesis occurring in larger adipocytes in bigger fetal sheep. Greater leptin expression in larger fetuses might also be a response to stimulation by anabolic hormones such as insulin. Insulin promotes fetal growth in sheep (Fowden 1995) and stimulates leptin synthesis and secretion *in vitro* and *in vivo* postnatally in rats and humans (Gettys *et al.* 1996, Russell *et al.* 1998).

Despite greater abundance of leptin mRNA in fetal adipose tissue after 125 d gestation, the nature of its relationship with body weight changes. The slope of the regression between relative abundance of leptin mRNA and body weight in fetuses of 125 d gestation and older was 15-times lower than at 90-91 d. Thus although leptin synthetic capacity of fetal adipose tissue appears to increase in late gestation, it is less sensitive to variation in fetal body size than at 90-91 d. This implies that an inhibitory influence appears in late gestation that alters the relationship between leptin expression and fetal size. The lower slope for the relationship between leptin mRNA and fetal weight from 125 d gestation may be associated with the onset of sympathetic innervation of fetal perirenal adipose tissue, which occurs at ~120 d gestation in sheep (Gemmell & Alexander 1978, Clarke *et al.* 1997). In bovine fetal adipose tissue the abundance of β_3 -adrenergic receptors and uncoupling protein-1 increase in late gestation (Casteilla *et al.* 1987, 1994). Studies in rats and mice have shown that β_3 -adrenergic

agonists suppress leptin gene expression whilst stimulating expression of uncoupling protein-1 (Fowden 1995, Li *et al.* 1997, Trayhurn *et al.* 1996). Regardless of the mechanism(s) responsible for the change in leptin expression with gestation, the relationship between leptin mRNA abundance and fetal size is consistent with the hypothesis that leptin synthesis in fetal adipose tissue is related to fetal nutrient supply and fetal growth rate.

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