

Leptin expression in offspring is programmed by nutrition in pregnancy

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Abstract

Birth weight is a determinant of blood leptin concentrations in adults. Since nutrition during pregnancy can affect birth weight, the hypothesis that feed intake during pregnancy alters leptin expression in progeny was examined. Leptin mRNA was measured in subcutaneous adipose tissue and leptin protein was measured in blood plasma from 59 day old female pigs whose mothers were fed at the same restricted rate except

that half were permitted to consume 35% more feed during the second quarter of pregnancy. Leptin mRNA abundance in adipose tissue ($P=0.015$) and plasma leptin concentration ($P=0.01$) were higher in progeny from mothers provided with more feed. Body weight at birth was negatively correlated with the abundance of leptin mRNA in subcutaneous fat at 59 days of age ($P=0.01$). This study shows for the first time that maternal nutrition during pregnancy programs postnatal leptin expression in offspring.

Journal of Endocrinology (2000) **165**, R1-R6

Introduction

Leptin is a polypeptide hormone synthesised and secreted into systemic blood principally by adipocytes that acts at the hypothalamus to suppress ingestive behaviour and increase energy expenditure (Friedman & Halaas 1998, Houseknecht *et al.* 1998, Mantzoros & Moschos 1998, Barb 1999, Ramsay 1999). In adults, abundance of leptin mRNA in adipose tissue and concentrations of leptin protein in blood correlate positively with concurrent body weight and adiposity (Maffei *et al.* 1995, Considine *et al.* 1996). Leptin appears to be involved in the relationship between biomass and energy reserve from early in life because there are positive associations between the concentrations of leptin protein in human umbilical cord blood and both body weight and adiposity at birth (Schubring *et al.* 1997, Koistinen *et al.* 1997, Jaquet *et al.* 1999, Ong *et al.* 1999). Furthermore, leptin mRNA abundance in fetal adipose tissue is positively correlated with fetal body weight in sheep (Yuen *et al.* 1999). Blood leptin levels in adults are also influenced by factors acting at or before birth. Independent studies of 502 men and women born in England in the 1920s (Phillips *et al.* 1999) and 1462 women born in Sweden between 1908 and 1930 (Lissner *et al.* 1999) concluded that body weight at birth is related to blood leptin concentrations in adults, independent of adult adiposity. However, unlike the positive correlations seen in fetuses, newborns and adults between concurrent measures of leptin production and body weight or adiposity, the correlation

between body weight at birth and leptin protein concentration in blood from adults is negative. Maternal nutrition during pregnancy affects body weight of the fetus (Lemons *et al.* 1986, Harding & Johnston 1995, Sohlström *et al.* 1998), newborn (Corah *et al.* 1975, Lechtig *et al.* 1975, Godfrey *et al.* 1996, Wallace *et al.* 1997) and adult (Barker 1997). The effect of nutrition during pregnancy on postnatal leptin expression in offspring was therefore examined.

Materials and Methods

Animals

The study was designed in accordance with the *Australian code of practice for the care and use of animals for scientific purposes* (6th edn 1997, National Health & Medical Research Council) and approved by the Animal Experimentation Ethics Committee of Bunge Meat Industries. Large White gilts (primiparous sows, $n=16$) were fed a dry ration containing 13.5 MJ digestible energy and 150 g protein per kg at 2.2 kg.d⁻¹. From the 25th day of pregnancy (140.8 ± 2.4 kg live weight) to the 50th day, half were fed the same diet at 3.0 kg.d⁻¹ (term ~110 days). A female piglet of median birth weight for her litter was killed by barbiturate overdose 59 days after birth. The study was confined to one sex to avoid the confounding effects of gender on growth phenotype (Tanner 1962, Bereskin & Steele 1986) and plasma leptin concentration (Mantzoros & Moschos 1998). Hindquarter

subcutaneous fat was frozen in liquid N₂ and stored at -80°C. Jugular venous EDTA-plasma was stored at -20°C.

Adipose tissue content

DNA content was measured after 1.03 ± 0.02 g of thinly sliced adipose tissue was homogenised and sonicated in 10 ml chloroform:methanol (2:1, vol/vol) and delipidated by repeated inversion at 30 r.p.m. for 15 h at 25°C (Colebrook *et al.* 1988). Cell fragments were recovered from the interphase after centrifugation at 2500 *g* for 10 min at 4°C and their DNA content measured by a modification (Gendimenico *et al.* 1988) of the diphenylamine method (Burton 1956). RNA concentration was determined from absorbance at 260 nm of RNA hydrolysates (Van den Hoff *et al.* 1997) prepared from delipidated homogenates of 207 ± 8 mg of adipose tissue in 3 ml of 10 mmol/l sodium phosphate pH 6. Tissue protein was measured using the BCA (bicinchoninic acid) Protein Assay Reagent according to the manufacturer's instructions (23225X, Pierce Chemical Co, Rockford, IL, USA). Dry matter content was measured after dehydration at 140°C for 24 h. Lipid content was assumed to be the dry residue after subtraction of DNA, RNA and protein content.

Reverse transcription polymerase chain reactions

RNA was extracted with TRIzol (GibcoBRL, Gaithersburg, MD, USA) from one adipose specimen (196 ± 9 mg) from each animal at a yield of 0.63 ± 0.03 mg/g. cDNA was obtained by reverse transcription of 1 µg total RNA with random sequence hexanucleotides (RP-6, GeneWorks, Adelaide, Australia) and SuperScript RNase H⁻ (18053-017, GibcoBRL). A porcine leptin cDNA fragment was amplified with Taq* DNA polymerase (Biotech International, Bently, Australia) through 30 cycles of 15 s at 94°C, 15 s at 53°C and 15 s at 72°C (Hybaid PCR Express, Teddington, UK) with 5'-TTC CTG TGG CTT TGG CCC-3' and 5'-AGC TCT TGG AGG AGG CCA GC-3' (GeneWorks) as primers. This produced 326 bp of ds cDNA. Sequencing by the ABI PRISM Dye Terminator method (Perkin-Elmer, Foster City, California) after QIA-quick purification (Qiagen Pty. Ltd, Clifton Hill, Australia) confirmed its identity as nucleotides 24-349 of the 504 nucleotide porcine leptin cDNA (Genbank Acc. No. U59894). A 349 bp fragment of porcine β-actin cDNA was similarly amplified with 5'-TGT GAT GGT GGG TAT ATG GGT C-3' and 5'-TAG ATG GGC ACA GTG TGG GT-3' (GeneWorks) as primers and its identity confirmed as above (GenBank Acc. No. V01217 J00691). Leptin and β-actin cDNA fragments amplified as above with digoxigenin-dUTP included in the PCR (Cat. 1636120, Roche Diagnostics, Mannheim, Germany) were measured by digoxigenin PCR ELISA (Cat. 1636111, Roche) after immobilisation to streptavidin-coated microtitre plates using 5'-biotinylated oligonucleotides complementary to either porcine leptin cDNA (5'-TCC TCA CCA GTC TGC CC-3') or porcine β-actin cDNA (5'-CAA GAG AGG CAT CCT GAC CC -3'). Assays were calibrated with digoxigenin-

labelled leptin or β-actin cDNA fragments for which the concentration of leptin or β-actin ds cDNA was previously determined by comparing their ethidium bromide staining intensities with those of DNA standards of known mass (DMW-P1, GeneWorks) after agarose gel electrophoresis.

Plasma leptin radioimmunoassay

Leptin protein was measured in plasma by RIA using human [¹²⁵I]-leptin (cat. 9081), guinea pig antiserum (cat. XL-85K) to human leptin (both from Linco Research, St Charles, MO, USA) and human leptin (R&D Systems, Minneapolis, MN, USA) as standard. Dose-responses for pig plasma and human leptin were parallel. Leptin was measured in all plasma

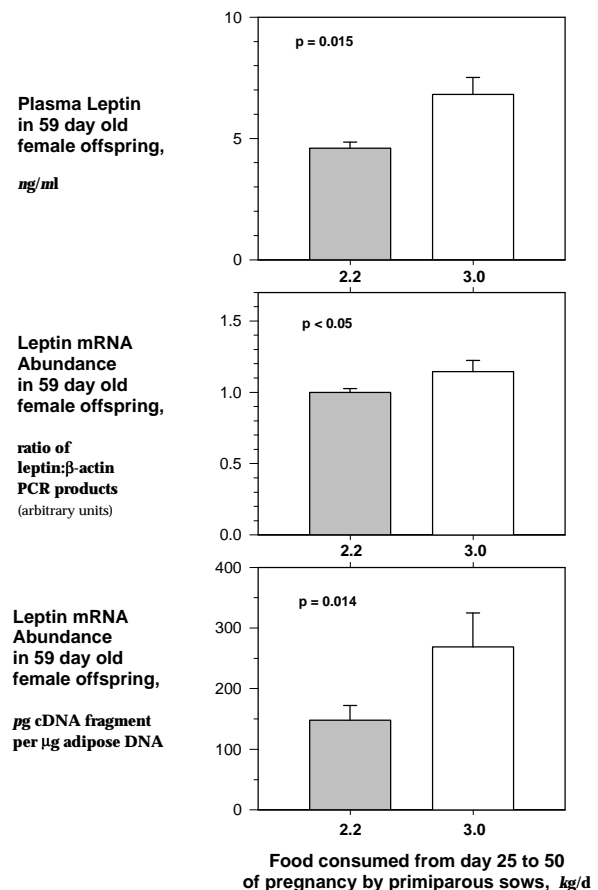


Figure 1 Maternal nutrition in the second quarter of pregnancy and measures of leptin production in 59 day old offspring.

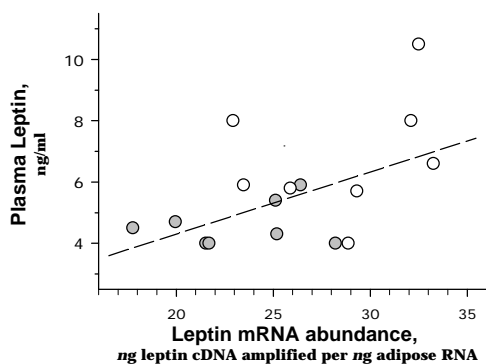


Figure 2 Relationship between leptin mRNA abundance in adipose tissue and leptin protein concentration in plasma 59 days after birth in offspring of mothers fed either 2.2 (shaded circle) or 3.0 (open circle) kg/d during the second quarter of pregnancy ($r=0.54$, $P=0.03$).

samples in a single assay (intra-assay CV=4%) and is expressed in human leptin equivalents.

Statistics

Results are presented as mean \pm S.E.M. Effects of nutrition were assessed by *t*-test. Associations were evaluated by linear regression (SigmaStat V1, Jandel, San Rafael, CA, USA).

Results

Maternal nutrition during the second quarter of pregnancy had no significant effect on the number of animals born alive per pregnancy (10.8 ± 0.4), the number of stillbirths per pregnancy (0.63 ± 0.27) or body weight at birth (1.43 ± 0.05 kg). Feed during pregnancy did not affect body weight at birth or at 59 days of age (20.8 ± 1.2 kg) for median birthweight females selected from each litter.

Neither the content of lipid (500 ± 24 mg/g), water (478 ± 2 mg/g), protein (21.1 ± 2.3 mg/g), RNA (683 ± 49 μ g/g) nor DNA (97.5 ± 8.5 μ g/g) in subcutaneous adipose tissue at 59 days of age in the selected offspring was affected by maternal nutrition during pregnancy. Mass ratios (g/g) of RNA:DNA (8.2 ± 1.4), protein:DNA (280 ± 80) and lipid:DNA (5600 ± 402) were similarly unaffected.

Food availability during the second quarter of pregnancy altered both the concentrations of leptin protein in blood plasma and the abundance of leptin mRNA in subcutaneous adipose tissue of 59 day old progeny. Plasma leptin was ~30% higher, the ratio of leptin/ β -actin mRNA in adipose tissue was ~15% higher, abundance of leptin mRNA per μ g adipose DNA was ~80% higher (Fig. 1) and the amount of leptin cDNA

fragment amplified per ng adipose RNA was ~20% higher (28.4 ± 1.4 versus 23.1 ± 1.3 , $P=0.015$) in offspring from mothers provided more food. Nutrition in pregnancy did not affect abundance of β -actin mRNA in adipose tissue of progeny ($P>0.2$).

Leptin protein concentration in plasma was positively correlated with leptin mRNA abundance per ng adipose RNA (Fig. 2) and tended to correlate with leptin mRNA abundance per μ g adipose DNA ($r=+0.45$, $P=0.09$) but was not related to the ratio of leptin/ β -actin mRNA ($P>0.3$).

Abundance of leptin mRNA per cell (i.e. pg leptin cDNA fragment amplified per μ g adipose DNA) was positively correlated with all measures of adipose cell size (RNA/DNA $r=+0.96$, $P<0.0001$; protein/DNA $r=+0.88$, $P<0.0001$; lipid/DNA $r=+0.64$, $P<0.01$).

Body weight at birth was negatively correlated with leptin mRNA abundance per ng adipose RNA (Fig. 3) and with the ratio of leptin/ β -actin mRNA ($r=-0.51$, $P<0.05$) at 59 days of age.

Discussion

In the present study weight of progeny at birth and at 59 days of age were unaffected by the amount of feed provided to mothers during the second quarter of pregnancy. Adipose cell size and subcutaneous adipose composition of DNA, RNA, protein and lipid were similarly unaffected.

Measures of leptin production in offspring were programmed by maternal nutrition. Greater food availability to pregnant mothers increased the concentration of leptin protein in blood and the abundance of leptin mRNA in adipose tissue. High abundance of leptin mRNA in adipose tissue and high

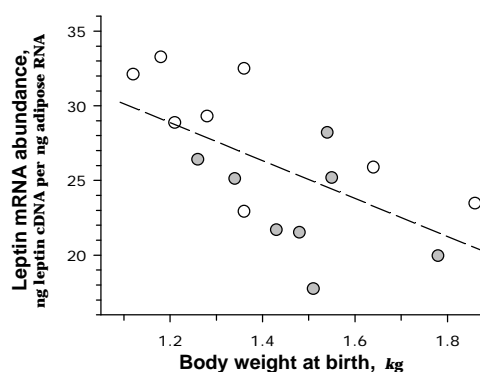


Figure 3 Relationship between weight at birth and leptin mRNA 59 days after birth from mothers fed either 2.2 (shaded circle) or 3.0 (open circle) kg/d during the second quarter of pregnancy ($r=-0.62$, $P=0.01$).

plasma leptin concentrations are associated with greater adiposity in pigs (Robert *et al.* 1998, Spurlock *et al.* 1998, Owens *et al.* 1999) as in other mammals (Maffei *et al.* 1995, Considine *et al.* 1996, Lönnqvist *et al.* 1997). Maternal nutrition during pregnancy in rats can program obesity associated with larger adipocytes (Jones & Friedman 1982, Shepherd *et al.* 1997). However, despite differences in leptin production there is no evidence of programmed obesity in the progeny analysed in the present study. Nutrition in pregnancy had no effect on body weight or adipose cell size. Differences in adiposity of the two groups of progeny might become evident at older ages, because leptin mRNA abundance in adipose tissue, plasma concentrations of leptin protein and body composition of fat increase with age in pigs (Spurlock *et al.* 1998, Barb *et al.* 1999, Owens *et al.* 1999).

The positive relationship observed in this study of adolescent female pigs between leptin mRNA abundance in adipose tissue and leptin protein concentration in blood is similar to that reported for adult women (Lönnqvist *et al.* 1997). In mice, rats and humans, adipocytes appear to be the major sites of leptin expression and secretion into blood (Frederich *et al.* 1995, Maffei *et al.* 1995). The results of this study indicate this is also the case in pigs.

The negative correlation between body weight at birth and subsequent expression of leptin in pigs is similar to recent observations in humans (Lissner *et al.* 1999, Phillips *et al.* 1999). The negative relationships in pigs and humans between body weight at birth and measures of leptin production in adolescents and adults are the opposite of the frequently reported positive correlations between concurrent measures of body weight or adiposity and leptin production seen in fetuses (Yuen *et al.* 1999), infants (Schubring *et al.* 1997, Koistinen *et al.* 1997, Jaquet *et al.* 1999, Ong *et al.* 1999) and adults (Considine *et al.* 1996, Lönnqvist *et al.* 1997). The negative relationship between birth weight and subsequent leptin production appears to be established in the first year of postnatal life in humans. While growth retarded newborn infants have lower than normal concentrations of leptin in their blood at birth, by 12 months of age they have higher than normal plasma leptin levels (Jaquet *et al.* 1999). This may be a consequence of 'catch-up' growth. Infants with lower plasma leptin concentrations at birth and lower body weights at birth tend to gain weight more rapidly in the first months of postnatal life (Ong *et al.* 1999, Jaquet *et al.* 1999). Lower birth weight infants may tend to have higher plasma leptin levels as juveniles and adults because 'catch-up' growth may increase their risk of obesity.

Permanent effects on progeny caused by environmental factors during pregnancy has been termed 'in utero programming' (Barker 1998). Blood pressure (Persson & Jansson 1992, Woodall *et al.* 1996, Barker 1998), cholesterol metabolism (Lucas 1991, Barker 1998, Kind *et al.* 1999), insulin response to glucose (Lucas 1991, Barker 1998), glucose and lipid metabolism (Desai *et al.* 1995) and plasma concentrations of insulin-like growth factors (Muaku *et al.*

1996, Gallaher *et al.* 1998) in adults are all sensitive to influences acting before birth. Greater feed intake in the second quarter of pregnancy could theoretically increase postnatal expression of leptin by increasing the availability of glucose and/or other substrates to the fetus by a number of mechanisms (Lechtig *et al.* 1975, Lemons *et al.* 1986, Harding & Johnson 1995, Gluckman *et al.* 1997) during a critical stage of adipocyte development. Whether substrates such as glucose can directly program leptin expression, possibly acting on preadipocytes in the fetus through UDP-*N*-acetylglucosamine (Wang *et al.* 1998) or other pathways (Fukada & Iritani 1999), or whether this occurs indirectly through actions of fetal hormones such as insulin or insulin-like growth factor-I that are sensitive to nutrition (Gluckman *et al.* 1997) and are capable of regulating fetal adipocyte maturation (Hausman & Hausman 1993, Martin *et al.* 1998) remains to be determined. Fetal tissues express leptin (Hoggard *et al.* 1997), including adipose tissue (Yuen *et al.* 1999), and leptin protein is detectable in fetal plasma from as early as 18 weeks gestation in humans (Jaquet *et al.* 1999). Regardless of the mechanism by which leptin is programmed *in utero*, the present study shows that maternal nutrition during pregnancy is a determinant of postnatal expression of leptin.

Acknowledgements

This work was supported by the Pig Research and Development Corporation of Australia. K L Gatford is a National Health and Medical Research Council (Australia) Peter Doherty Postdoctoral Fellow.

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