Maternal Nutritional Programming of Fetal Adipose Tissue Development: Differential Effects on Messenger Ribonucleic Acid Abundance for Uncoupling Proteins and Peroxisome Proliferator-Activated and Prolactin Receptors

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Maternal nutrient restriction at specific stages of gestation has differential effects on fetal development such that the offspring are programmed to be at increased risk of a range of adult diseases, including obesity. We investigated the effect of maternal nutritional manipulation through gestation on fetal adipose tissue deposition in conjunction with mRNA abundance for uncoupling protein (UCP)1 and 2, peroxisome proliferator-activated receptors (PPAR)α and γ, together with long and short forms of the prolactin receptor (PRLR). Singleton-bearing ewes were either nutrient-restricted (3.2–3.8 MJ day−1 metabolizable energy) or fed to appetite (8.7–9.9 MJ day−1) over the period of maximal placental growth, i.e., between 28 and 80 d gestation. After 80 d gestation, ewes were either fed to calculated requirements (6.7–7.5 MJ day−1), or to appetite (8.0–10.9 MJ day−1). At term, offspring of nutrient-restricted ewes possessed more adipose tissue, an adaptation that was greatest in those born to mothers that fed to requirements in late gestation. This was accompanied by an increased mRNA abundance for UCP2 and PPARα, an adaptation not seen in mothers re-fed to appetite. Maternal nutrition had no effect on mRNA abundance for UCP1, PPARγ, or PRLR. Irrespective of maternal nutrition, mRNA abundance for UCP1 was positively correlated with PPARγ and the long and short forms of PRLR, indicating that these factors may act together to ensure that UCP1 abundance is maximized in the newborn. In conclusion, we have shown, for the first time, differential effects of maternal nutrition on key regulatory components of fetal fat metabolism. (Endocrinology 146: 3943–3949, 2005)

A N INCREASING AMOUNT of animal and epidemiological evidence suggests that the amount of feed consumed by the mother through pregnancy can have a significant impact on fetal and later adipose tissue development (1–3). As a consequence, the resulting offspring can be at increased risk of obesity and obesity-related diseases (4, 5). It is therefore important to enhance our understanding of how fetal adipose tissue growth is regulated and what impact changes in maternal nutrition at defined stages of pregnancy have on its endocrine sensitivity. In both the human and sheep fetus, adipose tissue is comprised of brown and white adipocytes (6–8), which have a common mesenchymal stem cell precursor lineage (9). Because the energetic requirement for lipid synthesis (39 MJ/kg) is greater than for carbohydrate or protein (15–25 MJ/kg), growth of fat in the fetus is usually constrained in an environment in which oxygen and the majority of metabolic substrates are limited (10, 11).

Despite the small amount of fat present at birth in most species, including sheep, its abundance and endocrine sensitivity are highly sensitive to the maternal and fetal nutritional regime throughout gestation (2). In this regard, nutrient restriction coincident with the period of maximal placental growth (i.e., 28–80 d gestation), followed by refeeding up to term, results in fatter fetuses. The magnitude of this adaptation is, however, partly dependent on the amount of food consumed by the mother in late gestation. When she is allowed to feed ad libitum, the amount of fetal adipose tissue is less than in fetuses whose mothers were fed to metabolic requirements only (2). With ad libitum feeding in late gestation, however, the abundance of the brown adipocyte-specific uncoupling protein (UCP)1 (11) is enhanced, whereas leptin mRNA abundance in adipose tissue is reduced (2). Taken together, these findings suggest that there is an inverse relationship between brown adipocyte distribution and total fat mass. Brown fat is also characterized as having a high abundance of both the long and short forms of the prolactin receptor (PRLR) (12, 13). The abundance of the long, but not the short, form of the PRLR is also nutritionally regulated (11), and activation of the PRLR after birth can act to maximize heat production in the newborn (13). The extent to which the association between mRNA expression for UCP1, PRLR, and other lipogenic factors is established in utero is not known. One aim of the present study, therefore, was to determine the relative contribution of changes in maternal

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Abbreviations: ME, Metabolizable energy; NR, nutrient-restricted from 28–80 d gestation; NR-A, fed to appetite from 81–140 d gestation; NR-R, fed to requirements from 81–140 d gestation; PPAR, peroxisome proliferator-activated receptor; PRLR, prolactin receptor; UCP, uncoupling protein.

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feed intake through pregnancy, in conjunction with adaptations in fetal fat mass, on UCP1 and PRLR mRNA abundance.

The rapid rise in UCP1 abundance at birth is accompanied by a peak in UCP2 mRNA. The role of UCP2 in fetal (or adult) adipose tissue is not known, but it has been genetically linked to obesity (14). UCP2 is highly conserved among all species examined to date (15) and has been linked to a range of physiological functions, including the regulation of reactive oxygen species production and apoptosis (16–18). An increase in UCP2 mRNA expression is observed in both rodents and humans after diabetes, obesity, and fasting (19, 20).

The gestational increase in both UCP1 and 2 mRNA within fetal adipose tissue is mediated, in part, by cortisol acting through its receptor (21, 22). It has been demonstrated that adipose tissue sampled from offspring born to previously nutrient-restricted (from 28–80 d gestation) (NR) fetuses exhibits a higher mRNA abundance for the glucocorticoid receptor (23), but it is not known what impact this has on UCP mRNA abundance. A further aim of the present study was therefore to determine whether manipulation of the maternal diet through gestation had similar effects on UCP1 and 2 mRNA and whether these responses are separate from adaptations in fat mass in the fetus.

Finally, the present study investigated whether maternal nutrient intake can act to regulate mRNA abundance of the peroxisome proliferator-activated receptors (PPARs), transcription factors that have a primary role in regulating fat deposition in adults. PPAR-γ is involved in the cascade of events that leads to adipogenesis, promotes differentiation of preadipocytes, and regulates the expression of fat cell-specific genes. PPAR-γ is most abundant in adipose tissue, where it is considered to be a major regulator of fat cell development and is necessary for the maintenance of normal adipocyte function (24). In contrast, PPAR-α is highly abundant in the liver, where it regulates fatty acid oxidation (25). Both PPAR-α and γ, however, use fatty acids as endogenous ligands (26), which suggests that they are both nutritionally regulated. Furthermore, PPAR-α and γ differentially modulate the expression of UCP1 and 2 (27), whereas PPAR-γ regulates the expression of PRLR in bone marrow stroma (28). Neither the degree to which PPAR-α and γ are differentially regulated in fetal adipose tissue, nor whether their abundance is related to that of UCP and PRLR, has previously been established. In summary, the aim of the present study was to use our established model of nutritional programming of fetal adipose tissue development to determine the magnitude by which the abundance of UCP, PRLR, and PPAR can be nutritionally regulated, together with the extent that such adaptations are related to differences in fetal fat mass.

Materials and Methods

Animals and diets

Twenty Welsh Mountain ewes of similar age (median, 3 yr) and weight [36.1 ± 0.9 kg (mean ± SEM)] were entered into the study and mated with one of two Texel rams. Breeding dates were established from the last date of observed mating. Throughout the study, the body condition score was assessed fortnightly by the same individual who was blinded to the nutritional group to which each ewe belonged. Each animal’s physical characteristics were assessed in the lumbar region, on and around the backbone, in the loin, and immediately behind the last rib. This was undertaken using a scale of 0–5, with 0 being very thin and 5 being grossly fat (29), and the score was 2.7 ± 0.2 arbitrary units at the start of the study. After mating, sheep were fed 100 g concentrate/d and allowed access to hay ad libitum. At 28 d gestation, they were individually housed and fed daily at approximately 0900 h. Thereafter, the metabolizable energy (ME) requirements for each animal were calculated according to body weight, taking into account requirements for both maternal maintenance and growth of the conceptus on the basis of producing a 4.5-kg lamb at term (30). The diet comprised chopped hay that had an estimated ME content of 7.91 MJ/kg dry matter and a crude protein content (nitrogen 6.25) of 69 g/kg dry matter and a barley-based concentrate that had an estimated ME content of 11.6 MJ/kg dry matter and a crude protein content of 162 g/kg dry matter. The proportion of hay to concentrate was approximately 3:1 with respect to dry weight. All diets contained adequate minerals and vitamins. Diets were adjusted fortnightly in order that the feed provided met the increased ME requirements that accompany the increase in fetal weight with gestation. However, feed was not reduced if maternal body weight decreased. All studies were conducted on sheep that were pregnant between December to April, during which time they were all housed indoors within an open barn under natural lighting.

Mothers were allocated to one of two nutritional groups using a stratified randomization by body weight. They were offered either 60% (i.e. NR) or 122% (i.e. allowed to feed to appetite, A) of their calculated ME requirements, with feed intake measured daily. NR ewes consumed all feed or consumed 135 g/d less than the ME requirements, because not all hay was eaten. Food consumption between 28 and 80 d gestation was, therefore, either 3.2–3.8 MJ/d in the NR group (60% of ME requirements) or 8.7–9.9 MJ/d in the group fed to appetite (~150% of ME requirements).

Between 80 and 140 d gestation, equal numbers of ewes from each group were either fed to appetite [A: consumed 8–10.9 MJ/d (ME (150% requirements, as calculated to produce a 4.5 kg lamb)] or were fed to requirement [R: consumed 6.5–7.5 MJ/d of ME (100% requirements as calculated to produce a 4.5 kg lamb)]. At 140 d gestation, five ewes from each nutritional group [NR-R (fed to requirements from 81–140 d gestation), NR-A (fed to appetite from 81–140 d gestation), A-R, A-A] were killed by iv administration of 100 mg/kg pentobarbitial sodium (Euthatal). The entire uterus was removed, and the fetus was killed with barbiturate. Perirenal adipose tissue, which constitutes at least 80% of fetal fat, was completely dissected, weighed, placed in liquid nitrogen, and stored at −80 C until analyzed. All operative procedures and experimental protocols had the required Home Office approval designated by the Animals (Scientific Procedures) Act (1986).

Laboratory analyses

Total RNA was isolated from adipose tissue using Tri-Reagent (Sigma, Poole, UK). All PCR primer sets were designed so that amplicons spanned at least one exon/intron boundary to identify any potential DNA contamination (described in Table 1). The abundance of mRNA was determined by RT-PCR (31). Cycles ranged from 24–35 cycles, dependent on the levels of expression of the genes in question. The range of temperatures used varied from 55–60 C and was specific to each gene primer set. Amplicons were separated by agarose gel electrophoresis. Ethidium bromide staining confirmed the presence of both test amplicon and 185 rRNA, an internal standard used to normalize RNA loading. The identity of all PCR products was confirmed through sequencing.

In the case of PPAR-γ, quantitative real-time PCR was performed on a Rotorgene 3000 (Corbett Research Australia, Sydney, Australia), using a 2 × SYBR Green I master mix (Abgene AB-1159; Abgene House, Epson, UK) in a 20-µL reaction vol, containing 1 µL reverse transcriptase reaction. A sequenced and isolated PCR amplicon was used to produce a standard curve, to ensure equal PCR amplification efficiency. Each assay was performed in duplicate on all samples from each group of fetuses.

Statistical analyses

Statistical analysis with respect to significant differences (P < 0.05) between mean values obtained from offspring of control and nutritionally manipulated mothers was carried out using Kruskal-Wallis H and
Mann-Whitney U tests (SPSS 11.0.1) (SPSS, Inc., Chicago, IL) that investigated the effect of maternal nutrition in both early to mid-, as well as late gestation. Correlations associating mRNA species were performed using Spearman’s rho test (SPSS 11.0.1).

**Results**

**Fetal adipose tissue weight and PPAR mRNA abundance**

Fetal weight was significantly lower in mothers fed to appetite up to 80 d gestation and then fed to 100% of ME requirements to term, when compared with all other nutritional groups (A-A, 4.9 ± 0.26; NR-A, 4.9 ± 0.30; A-R, 3.9 ± 0.28; NR-R, 4.8 ± 0.35 kg; *P* < 0.05). Fetuses sampled from sheep nutrient restricted between 28 and 80 d had significantly more adipose tissue than those fed to appetite (A-A, 18.3 ± 2.7; NR-A, 21.8 ± 2.2; A-R, 19.7 ± 1.7; NR-R, 23.5 ± 1.7 g; *P* < 0.05). This difference was irrespective of maternal nutrition over the second half of gestation, although the fetuses of ewes fed to appetite in late gestation had less adipose tissue than those sampled from mothers fed to requirements. These results are as we have previously demonstrated (2), and the weights of all other major organs were not significantly affected by the nutritional regimes (data not shown).

Maternal nutrition had differential effects on the mRNA abundance for PPARα, which was highly abundant in all adipose tissues sampled. Nutrient restriction between 28 and 80 d gestation, followed by refeeding to 100% of ME requirements, resulted in a significantly increased PPARα mRNA abundance, an adaptation that was reversed when mothers were re-fed to appetite (Fig. 1). In contrast, mRNA abundance for PPARγ tended to be higher in adipose tissue sampled from fetuses whose mothers had been previously nutrient restricted, although this difference was not statistically significant (Table 2). Maternal feed intake after 80 d gestation had no effect on PPARγ mRNA abundance.

**Maternal nutrition and UCP and PRLR mRNA abundance in fetal adipose tissue**

Maternal nutrient restriction between 28 and 80 d gestation resulted in up-regulation of UCP2 mRNA abundance in fetal adipose tissue but only when the mothers were fed to requirements after 80 d gestation (Fig. 2). In contrast, although mRNA abundance for both UCP1 and the short form of the PRLR tended to be higher in adipose tissue sampled from NR mothers, this was not statistically significantly (Table 2). Maternal food intake after 80 d gestation had no effect on UCP1 mRNA abundance. There was also no nutritional effect on mRNA abundance for either form of the PRLR.

### TABLE 1. Primer sequences and reaction conditions used for detection of mRNA abundance of PPARs, UCPs, and long (l) and short (s) forms of the PRLR using PCR

<table>
<thead>
<tr>
<th>Gene (accession no.)</th>
<th>Product (bp)</th>
<th>Primer sequences</th>
<th>Annealing temperature (C)</th>
<th>Cycle number</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARα (AY369138)</td>
<td>151</td>
<td>F 5'–CGT GTG AAC ATG ACC TAG AAG–3'</td>
<td>58.2</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R 5'–ACG AAG GGC GGA TTG TTG–3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPARγ (AY315429)</td>
<td>250</td>
<td>F 5'–CCG TTG ACT TCT CCA GCA TT–3'</td>
<td>59.4</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R 5'–TGG AAC CCT GAC GCT TTA TC–3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCP1 (X14064)</td>
<td>301</td>
<td>F 5'–AAA GTC CCG CTA CAG ATC CA–3’</td>
<td>60.0</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R 5'–TGA CCT TGA CCA CCT CTT TG–3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCP2 (AF127029)</td>
<td>513</td>
<td>F 5'–GGG ACT CTT GAG AGG GAC AT–3’</td>
<td>59.2</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R 5'–AAG AGA GGG ATG GGG AGA GA–3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPR1 (AF041257)</td>
<td>200</td>
<td>F 5'–CCA GAT ACC TAA TGA CTT CCC–3’</td>
<td>55.0</td>
<td>32</td>
</tr>
<tr>
<td>sPRLR (AF041977)</td>
<td>229</td>
<td>F 5'–CCA GAT ACC TAA TGA CTT CCC–3’</td>
<td>55.0</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R 5'–GCC CTT CTA TTA AAA CAC AGA C–3’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F, Forward; R, reverse.

**Fig. 1. Influence of maternal nutrition during gestation on PPARα mRNA abundance in adipose tissue sampled from near-term fetuses.** Each mother was either nutrient restricted (NR) or fed to appetite (A) between 28 and 80 d gestation and then to fully meet maintenance requirements or to appetite up to term. **Bar graphs.** Means, with their SE values (n = 5 per group) with significant differences between groups (*, *P* < 0.05).
### TABLE 2. Effects of maternal nutritional manipulation during gestation on mRNA abundance for PPARγ, UCP1, long (l) and short (s) forms of the PRLR mRNA in perirenal adipose tissue sampled from near-term fetuses

<table>
<thead>
<tr>
<th>Nutritional manipulation</th>
<th>Nutrient restriction 28–80 d</th>
<th>Appetite 28–80 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>To requirements</td>
<td>To requirements</td>
</tr>
<tr>
<td></td>
<td>(NR-R)</td>
<td>(NR-A)</td>
</tr>
<tr>
<td>PPARγ</td>
<td>1.1 ± 0.27</td>
<td>1.2 ± 0.16</td>
</tr>
<tr>
<td>UCP1</td>
<td>51.0 ± 13.8</td>
<td>56.4 ± 7.6</td>
</tr>
<tr>
<td>1 PRLR</td>
<td>166.9 ± 25.8</td>
<td>164.0 ± 14.3</td>
</tr>
<tr>
<td>s PRLR</td>
<td>45.7 ± 10.1</td>
<td>43.8 ± 5.9</td>
</tr>
</tbody>
</table>

Mean values with their SE values are given in arbitrary densitometric units with the exception of PPARγ, for which the units are fg/μl and (n = 5) per nutritional group.

Discussion

The major finding of the present study is that we have shown differential effects of maternal food intake through gestation on the abundance of key genes involved in adipose tissue growth and metabolism in the resulting fetus near to term. Importantly, these adaptations are not all linked with changes in fat mass per se but relate, in part, to metabolic control within the adipocyte itself. Furthermore, some of the observed responses appear to be within brown, rather than white, adipocytes, given the very close association we have established in the present study between the mRNA abundance of both PPARγ and PRLR with the brown adipocyte-specific UCP1. It is also notable that the up-regulation of mRNA abundance, for both PPARα and UCP2, with nutrient restriction, was only observed in those fetuses in which fat mass was greatest. PPARα and UCP2 are both highly abundant in white adipose tissue, and it is possible that increased fat deposition in these fetuses is primarily related to enhanced lipid deposition, a characteristic of white, rather than brown, fat. Our study, therefore, emphasizes the potential significance of PPARα in regulating early adipose tissue development (32, 33), possibly in those adipocytes displaying white adipose tissue characteristics.

PPARα is known to regulate fatty acid oxidation through the citric acid cycle, thereby generating a proton electrochemical gradient, although the significance of this process in regulating fat mass is not established. It is possible that a parallel increase in UCP2 could promote lipid deposition through an increased rate of uptake of glucose (34, 35). Indeed, we have previously shown that glucose transporter 1 abundance is raised in the placenta of fetuses whose mothers were fed to requirements rather than to appetite, following an earlier period of maternal nutrient restriction (36), which would support an increase in fetal glucose supply. Consequently, a combination of raised PPARα and UCP2 in conjunction with increased sensitivity to IGFs due to up-regulation of mRNA of both IGF-I and II receptor, but not their ligands, within the adipocyte (2) could explain why fat mass is greater in these fetuses compared with all other nutritional groups in the present study. The specific nutritional or endocrine signal by which maternal nutrient status affects expression of PPARs and UCP2, however, appears to be complex. A period of nutrient restriction alone is sufficient to alter the expression of PPARα mRNA, whereas the level of refeeding after the period of nutrient restriction appears to be a primary factor regulating the expression of UCP2 mRNA. Surprisingly, when mothers are allowed to feed to appetite after a period of nutrient restriction, mRNA abundance for PPARα is reduced, whereas UCP2 is unchanged.

We have also been able, for the first time, to describe, by way of association, a regulatory cascade for the modulation of UCP1 mRNA expression involving both PPARγ and
PRLR. Increasing maternal nutrition from midgestation has been shown to have substantial effects on brown adipose tissue development in the ovine fetus, by increasing the abundance and thermogenic activity of UCP1 (11). It is of interest to note that, in the present study, there was no direct effect of maternal nutrition on mRNA abundance for either UCP1 or PRLR, indicating that the previously described stimulatory effect of maternal nutrition on both UCP1 and the long form of PRLR protein (11) are the result of post-translational modifications. Our results do suggest that, over the range of maternal nutrient intakes adopted in the present study, there is a very close association among mRNA abundance for PPARγ, the long and short forms of PRLR, and UCP1. These adaptations may be mediated by changes in sympathetic innervation of fetal adipose tissue, which can influence the abundance of UCP1 and PPARγ (37–39), although this has yet to be confirmed for the PRLR. Taken together, our findings extend previous data relating UCP1 and PRLR, in which the postnatal loss of UCP1 is paralleled by a reduction in abundance of the PRLR (13). For example, PPARγ is capable of up-regulating PRLR expression (28). The parallel increase in both PPARγ and UCP1 mRNA is in accord with the finding of a promoter region within the UCP1 gene that directly responds to PPARγ after its binding (40, 41). Furthermore, PPARγ and its agonists can also increase the expression of UCP1 mRNA and protein both in vitro and in vivo (40, 42–44).

The potential unifying mechanism by which PPAR, PRLR, and UCP1 mRNA abundance are nutritionally regulated in adipose tissue of the fetus remains to be determined. PPARα and γ are both under nutritional regulation because they bind to fatty acids (45); although, in the fetus, this mechanism may be limited, because plasma free fatty acid concentrations are normally very low. One potential candidate that could regulate these genes is cortisol, acting through its receptor (21, 22), because its abundance is increased in adipose tissue sampled from previously NR fetuses (23). We have shown, for the first time, the differential effects of maternal nutrient restriction on mRNA abundance for both PPARα and UCP2. Potentially they could have pronounced effects on fat mass as this increases during postnatal (43) and later life, although these are yet to be investigated. Indeed, they could contribute to the increased risk of obesity that has been associated with maternal exposure to nutrient restriction in early pregnancy in human populations (4). At the same time, we have uncovered a potentially important mechanism by which UCP1 mRNA is maximized at the molecular level that involves PPARγ and PRLR. This may be critical in ensuring that UCP1 abundance is optimized in the newborn, ensuring ade-
quate thermoregulatory responses after exposure to the relatively cold extrauterine environment.

Indeed, there may be many initial advantages to being able to rapidly lay down fat immediately around the time of birth, including increased insulation and access to an energy store that can be rapidly mobilized during periods of dietary insufficiency. However, the ability to rapidly lay down fat as an adult could become deleterious when whole-body energy requirements are greatly reduced. Therefore, discrepancies in the internal monitoring of energy intake can lead to an increased incidence of obesity in the adult, the outcome of which can manifest as type II diabetes and cardiovascular heart disease. Finally, these data suggest that maternal feeding levels throughout pregnancy are important and that it is not just the periods of nutritional insufficiency that may shape the physiological outcome of the resulting offspring.

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References


23. Erickson CB, Niesman IR, Harmon BD, Massague J 1987 Thiazolidinediones on plasma triglyceride metabolism are mediated by distinct peroxisome proliferator-activated receptor-α and -β and a mediate in vivo regulation of uncoupling protein (UCP1, UCP2, UCP3) gene expression. Endocrinology 139:4092–4097


27. Forman BM, Chen J, Evans RM 1997 Hypolipidemic drugs, polyunsaturated fatty acids, eicosanoids are ligands for peroxisome proliferator-activated receptors α and β. Proc Natl Acad Sci USA 94:4312–4317


controls the concentration of the uncoupling protein in brown adipose tissue. FEBS Lett 166:393–396


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