Ontogeny and nutritional programming of adiposity in sheep: potential role of glucocorticoid action and uncoupling protein-2

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Gnanalingham, Muhuntha G., Alison Mostyn, Michael E. Symonds, and Terence Stephenson. Ontogeny and nutritional programming of adiposity in sheep: potential role of glucocorticoid action and uncoupling protein-2. Am J Physiol Regul Integr Comp Physiol 289: R1407–R1415, 2005. First published July 7, 2005; doi:10.1152/ajpregu.00375.2005.—Increased glucocorticoid action and adipose tissue inflammation contribute to excess adiposity. These adaptations may be enhanced in offspring exposed to nutrient restriction (NR) in utero, thereby increasing their susceptibility to later obesity. We therefore determined the developmental ontogeny of glucocorticoid receptor (GR), 11β-hydroxysteroid dehydrogenase (11βHSD) types 1 and 2, and uncoupling protein (UCP)-2 mRNA in perirenal adipose tissue between late gestation and 6 mo after birth in the sheep, as well as the effect of maternal NR targeted between early to mid (28–80 days, term ∼147 days)- or late (110–147 days) gestation. GR and 11βHSD1 mRNA increased with fat mass and were all maximal within the 6-mo observation period. 11βHSD2 mRNA abundance demonstrated a converse decline, whereas UCP2 peaked at 30 days. GR and 11βHSD1 mRNA abundance were strongly correlated with total and relative perirenal adipose tissue weight, and UCP2 was strongly correlated with GR and 11βHSD1 mRNA. Early- to midgestational NR increased GR, 11βHSD1, and UCP2 mRNA, but decreased 11βHSD2 mRNA abundance, an adaptation reversed with late-gestational NR. We conclude that the continual rise in glucocorticoid action and fat mass after birth may underlie the development of later obesity. The magnitude of this adaptation is partly dependent on maternal food intake through pregnancy.

Adipose tissue; glucocorticoids; mitochondria

Obese individuals exhibit a perturbed feedback control of the hypothalamic-pituitary-adrenal (HPA) axis that is associated with insulin resistance, hypertension, and the metabolic syndrome (1a). One mechanism by which adipose tissue may contribute to insulin resistance is related to the effects of glucocorticoids on preadipocytes and adipocytes (27). Thus glucocorticoids control adipogenic differentiation, influence adipocyte metabolism, and regulate adipocyte gene expression (22). Consequently, changes in glucocorticoid metabolism, as seen, for example, in Cushing’s syndrome, markedly influence adipocyte morphology and physiology (38). Glucocorticoid action is determined not only by the HPA axis and systemic glucocorticoid levels, but also by local enzymatic conversion, determined by the expression of glucocorticoid receptor (GR, type 2) and isoforms of 11β-hydroxysteroid dehydrogenase (11βHSD) (40). 11βHSD type 1 (11βHSD1) behaves predominantly as an 11-oxoreductase, catalyzing the conversion of inactive cortisone to active cortisol, amplifying activation of intracellular GR, while 11βHSD type 2 (11βHSD2) behaves as an 11-dehydrogenase, catalyzing the inactivation of cortisol to inert cortisone, and thereby maintaining the specificity of the mineralocorticoid receptor for aldosterone (3, 40). In addition, increased expression of GR and 11βHSD1 in visceral adipose tissue has been associated with the development of obesity in humans (43) and in rats who were overfed neonatally (10). Surprisingly, however, the developmental ontogeny of GR and 11βHSD isoforms in adipose tissue has not been established in any species to date.

Fetal adiposity and glucocorticoid action are strongly influenced by maternal nutrient restriction (NR) (8, 47), although in the sheep, there does not appear to be a substantial effect of maternal NR on fetal plasma cortisol concentration and there is a transient increase in maternal plasma cortisol concentration in late gestation (18). When maternal NR occurs in early to midgestation (i.e., 28 to 80 days gestation, term ∼147 days), coincident with the period of maximal placental growth, there is a resultant enhancement of fat deposition in the fetus (8). This occurs in conjunction with increased GR and 11βHSD1 mRNA abundance in adipose tissue sampled from NR neonatal offspring compared with control animals (47). These findings suggest that the increased incidence of obesity in adults born to mothers exposed to the Dutch famine during early pregnancy (39) may be a direct consequence of adaptations in the endocrine sensitivity of fetal adipose tissue. Alternatively, maternal NR in late gestation (i.e., 110 to 147 days gestation), coincident with the period of maximal fetal growth and a parallel rise in fetal fat depots, results in reduced adiposity if this energy restriction is accompanied by a reduction in fetal glucose supply (42). This has led to the hypothesis that suboptimal nutrition during discrete periods of pregnancy modifies programs (i.e., results in persistent changes beyond the immediate period of nutrient restriction) fetal morphology, metabolism, and blood pressure regulatory pathways (4). As a consequence, and in association with maladaptation to the postnatal environment, periods of NR during fetal life confer greater risk of metabolic and cardiovascular disease in adult life (5, 20).

The inner mitochondrial protein, uncoupling protein (UCP)-2, has been genetically linked to obesity and insulin resistance (21) and has postulated roles in reactive oxygen species production and apoptosis (29, 34), as well as in energy regulation (9). In the sheep lung, the peak abundance in GR and 11βHSD1 mRNA occurs close to term, whereas UCP2 mRNA peaks just after birth and is barely detectable after 1 mo of age (25). In addition, irrespective of the period of gestational NR, UCP2, GR, and 11βHSD1 mRNA were upregulated in the fetal and postnatal lung (25). It remains to be established if the developmental ontogeny and impact of maternal NR extend to UCP2 mRNA abundance in

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adipose tissue. UCP2 may have additional roles in the development of white adipose tissue characteristics in postnatal life (15) and in macrophage accumulation within adipose tissue, which has been implicated in the development of obesity (46).

In this study, we investigated whether glucocorticoid action and UCP2 mRNA abundance in perirenal adipose tissue would increase with developmental age, coincident with the marked increase in fat mass (15, 23). At the same time, we examined whether maternal NR during defined periods of gestation has an impact on glucocorticoid action and UCP2 mRNA expression in perirenal adipose tissue, the largest fat depot in sheep, which constitutes ~80% of fetal fat mass at term. The aims of the study were thus to determine: 1) the ontogeny of GR, 11βHSD1, 11βHSD2, and UCP2 mRNA abundance in fetal and postnatal perirenal adipose tissue up to 6 mo of age; 2) whether maternal NR in early to mid- or late gestation resulted in altered abundance of GR, 11βHSD1, 11βHSD2, and UCP2 mRNA in fetal and postnatal perirenal adipose tissue up to 6 mo of age; and 3) significant associations between glucocorticoid action and UCP2 mRNA abundance and perirenal fat mass with developmental age.

**MATERIALS AND METHODS**

### Ontogeny of Perirenal Adipose Tissue Development

For the ontogeny study, a mixture of Welsh Mountain and Border Leicester Swaledale sheep were used. We have previously established that with respect to the molecular measurements made in the present study, there are no distinguishable differences between breeds at the same developmental age (Gnanalingham MG, Dandrea J, Symonds ME, and Stephenson T, unpublished data). Perirenal adipose tissue was sampled from fetuses at 140- and 146-days gestation (term ~147 days) and sheep at 1, 7, 30, and 180 days (i.e., 6 mo) after birth (n = 6–8 at each sampling age, 42 sheep in total) after euthanasia with an overdose of barbiturate (200 mg/kg iv pentobarbital sodium; Euthatal, RMB, Animal Health, Stoke, UK). In sheep, perirenal adipose tissue forms 80% of total fat stores up to the time of birth (14) and is then maintained at 40–50% of total fat stores postnatally (23). Over this period, perirenal fat mass increases exponentially from <1g in the midgestation fetus to ~400 g in prepubertal adolescent (Fig. 1). All sheep were born normally at term to mothers that were fed 100% of their total metabolizable energy (ME) requirements (taking into account requirements for both ewe maintenance and growth of the conceptus to produce a 4.5-kg lamb at term; Ref. 1). Perirenal adipose tissue was rapidly dissected, weighed, and then placed in liquid nitrogen and stored at ~80°C until analyzed.

**Maternal Nutritional Manipulation of Perirenal Adipose Tissue Development**

**Study 1. Early- to midgestational NR.** This study was designed to examine the effects of early- to midgestational NR, coinciding with the period of maximal placental growth, on perirenal adipose tissue sampled from fetal and adolescent offspring. Twenty-four singleton bearing Welsh Mountain sheep of similar age (median 3 years) and weight (36.1 ± 0.9 kg; means ± SE) were entered into the study and individually housed at 28 days gestation, as described by Bishpham et al. (8). Animals were allocated to one of two nutritional groups using stratified randomization by body weight. They were offered either 60% (i.e., nutrient restricted) or 225% (i.e., allowed to feed to appetite) of their calculated ME requirements for both ewe maintenance and growth of the conceptus on the basis of producing a 4.5-kg lamb at term (1). Food intakes were measured daily, and NR ewes consumed all of the feed offered, whereas ewes fed to appetite consumed 150% of ME requirements because not all of the hay provided was eaten. Food consumption between 28 and 80 days gestation was 3.2–3.8 MJ/day of ME in the NR group (~60% of ME requirements) or 8.7–9.9 MJ/day of ME in the group fed to appetite (~150% of ME requirements) (Fig. 1). The amount of feed given to each ewe was increased at 43 and 61 days gestation to meet the higher energy requirements associated with growth of the conceptus (1). The diet comprised chopped hay [estimated ME content of 7.91 MJ/kg dry matter; crude protein content (nitrogen × 6.25) of 69 g/kg dry matter] and barley-based concentrate (estimated ME content of 11.6 MJ/kg dry matter; crude protein content of 162 g/kg dry matter). The proportion of hay to concentrate fed was ~3:1, with respect to dry weight. All diets contained adequate minerals and vitamins. After 80 days gestation, all ewes were offered sufficient feed to meet 100% of the ME requirements as calculated to produce a 4.5-kg lamb. These animals consumed between 6.5 and 7.5 MJ/day of ME. For these animals, the amount of feed provided was increased at 100 and 120
days gestation to meet the increased ME requirements that accompany the increase in fetal weight with gestation. In those sheep allowed to go to term, all gave birth normally, and the offspring were weaned at 3 mo of age. Throughout lactation, ewes were fed hay ad libitum and 1 kg concentrate daily (500 g AM and 500 g PM), and they had ad libitum access to water.

Six sheep within each nutrition group were randomized to tissue sampling at 140 days gestation. Each animal was humanely euthanized after 200 mg/kg iv pentobarbital sodium administration. Fetal perirenal adipose tissue was rapidly dissected and weighed, and a representative portion was placed in liquid nitrogen and stored at −80°C until further analysis. The remaining offspring (n = 6 per nutritional group) were sampled at 180 days (6 mo) after birth.

Study 2. Late gestational NR. This study was designed to examine the effects of late gestational NR, coinciding with the period of maximal fetal growth, on postnatal perirenal adipose tissue development, that is, immediately after birth and at 1 mo, when UCP abundance is changing most rapidly in the sheep (33). To reduce the number of pregnant animals recruited into the study, twin-bearing ewes were used. This also meant that any potential confounding maternal influence on perirenal adipose tissue development was minimized. Fourteen twin-bearing Border Leicester cross Swaledale sheep of similar weight and body condition score were randomly assigned to receive either 60% (NR, n = 8) or 100% of ME requirements (controls, n = 6) for the final (110 to 147 days) mo of gestation, as described by Mostyn et al. (33) (Fig. 1). All mothers gave birth normally at term, with one randomly selected twin being humanely euthanized (200 mg/kg iv pentobarbital sodium) within 24 h of birth, while the other was reared with the ewe until it was euthanized at 30 days of age. Perirenal adipose tissue was rapidly dissected and weighed, and a representative portion was placed in liquid nitrogen and stored at −80°C until further analysis. The maternal diet composition was identical to that described for the early-to mid-gestational NR study. All operative procedures and experimental protocols had the required Home Office approval as designated by the Animals (Scientific Procedures) Act (1986).

**Laboratory Analyses**

**Messenger RNA detection.** Total RNA was isolated from a known amount of perirenal adipose tissue (1–2 g) using Tri-Reagent (Sigma, Poole, UK). From the RNA concentration of this preparation (μg/μl), taking into account the volume (μl) of DEPC H2O needed to dissolve the initial RNA pellet and the total perirenal fat mass, the total RNA concentration was then calculated for each animal. To maximize sensitivity, a two-tube approach to reverse transcription (RT) was adopted. The conditions used to generate first-strand cDNA RT were 70°C (5 min), 4°C (5 min), 25°C (5 min), 25°C (10 min), 42°C (1 h), 72°C (10 min), and 4°C (5 min). The RT reaction (final volume 20 μl) contained: 5× cDNA (first-strand), buffer (250 mM Tris·HCl, 40 mM MgCl₂, 150 mM KCl, 5 mM diithioerythritol pH 8.5), 2 mM dNTPs, 1× hexanucleotide mix, 10 units RNase inhibitor, 10 units M-MLV reverse transcriptase, and 1 μg total RNA. All of these commercially available products were purchased from Roche Diagnostics (Lewes, UK).

The expression of UCP2, GR (type 2), and 11βHSD type 1 and 2 mRNA was determined by semiquantitative RT-PCR, as previously described (25). The analysis used oligonucleotide cDNA primers to UCP2, GR (type 2), and 11βHSD type 1 and 2 genes generating specific intron-spanning products (Table 1). Briefly, the PCR program consisted of an initial denaturation (95°C, 15 min), amplification [stage I, 94°C (30 s); stage II, annealing temperature (30 s); stage III, 72°C (60 s)], and final extension [72°C (7 min); 8°C hold]. The PCR mixture (final volume 20 μl) contained 7 μl DEPC H₂O, 10 μl Thermo-Start PCR Master Mix (50 μl contains 1.25 units Thermo-Start DNA Polymerase, 1× Thermo-Start reaction buffer, 1.5 mM MgCl₂, and 0.2 mM each of dATP, dCTP, dGTP, and dTTP, catalog no. AB-0938-DC-15, Abgene, Epsom, UK), 1 μl forward primer, 1 μl reverse primer, and 1 μl RT (cDNA) product. The PCR reaction for the individual mRNA product was performed separately from the 18S rRNA reaction. The annealing temperature and cycle number of all primers were optimized and used in their linear range (see Table 1). Agarose gel electrophoresis (2.0–2.5%) and ethidium bromide staining confirmed the presence of both the product and 18S at the expected sizes. Densitometric analysis was performed on each gel by image detection using a Fujifilm LAS-1000 cooled charge-coupled device camera, and UCP2, GR, 11βHSD type 1 and 2 mRNA, and 18S rRNA abundance were determined. Consistency of lane loading for each sample was verified, and all results are expressed as a ratio of a reference sample to r18S abundance. All analyses and gels were conducted in duplicate, with appropriate positive and negative controls, as well as a range of molecular weight markers. The resultant PCR product was extracted (QIAquick gel extraction kit, catalog no. 28704, Qiagen, West Sussex, UK) and sequenced, and results were cross-referenced against the Genbank Web site to determine specificity of the target gene.

**Statistical Analyses**

All data are presented as means ± SE. Significant differences (P < 0.05) between values obtained from different ages were determined by one-way ANOVA with a post hoc Bonferroni test and between control and nutrient-restricted groups by Mann-Whitney U-test. Significant correlations between the physiological and molecular parameters measured in this study were determined by the Spearman rank order test (SPSS v11.0, SPSS).

### Table 1. Primer sequences and optimal PCR conditions used in perirenal adipose tissue in the sheep

<table>
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<tr>
<th>Primer Set</th>
<th>Product Size, BP</th>
<th>Primer Sequence</th>
<th>Annealing Temperature, °C</th>
<th>Cycle Number</th>
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<tr>
<td>UCP2</td>
<td>513</td>
<td>F 5’-GGG ACT CTG GAA AGG GAC AT-3'</td>
<td>59.0</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R 5’-AAG AGA GGG ATG GGA AGA GA-3’</td>
<td>58.7</td>
<td>32</td>
</tr>
<tr>
<td>GR (type 2)</td>
<td>150</td>
<td>F 5’-ACT GGC CCA AGT GAA AAG AGA-3’</td>
<td>58.7</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R 5’-ATG AAC AGA AAT GGC AGA CAT T-3’</td>
<td>58.5</td>
<td>32</td>
</tr>
<tr>
<td>11βHSD1</td>
<td>160</td>
<td>F 5’-GGT CGG GAT GCC TTG GAT GAT-3’</td>
<td>58.5</td>
<td>32</td>
</tr>
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<td></td>
<td>R 5’-GGG ATG ATC CCA TCT TT-3’</td>
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<td>38</td>
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<tr>
<td>11βHSD2</td>
<td>260</td>
<td>F 5’-GGC ATT GTG ACC GTA AGC-3’</td>
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<tr>
<td>18S</td>
<td>324</td>
<td>Ambion Classic II 18S Internal Standards</td>
<td>Catalogue no. 1717</td>
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UCP2, uncoupling protein-2; GR, glucocorticoid receptor; 11βHSD1, 11β-hydroxysteroid dehydrogenase type 1; and 11βHSD2, 11β-hydroxysteroid dehydrogenase type 2.
RESULTS

Ontogeny of GR, 11βHSD1, 11βHSD2, and UCP2 mRNA abundance with increased perirenal adipose tissue mass. GR, 11βHSD1, 11βHSD2, and UCP2 mRNA were detected in perirenal adipose tissue at all sampling ages, with their abundance being developmentally regulated (Fig. 2). GR and 11βHSD1 mRNA abundance increased with postnatal age and were maximal at 6 mo of age. UCP2 mRNA abundance similarly increased with age, peaking at 30 days, before declining up to 6 mo postnatal age. In contrast, the abundance of 11βHSD2 mRNA declined with postnatal age, having been maximal at 140 days gestation.

Overall, there were positive associations between GR and 11βHSD1 mRNA with total (GR: $R^2 = 0.85, P < 0.0001$; 11βHSD1: $R^2 = 0.81, P < 0.0001$) and relative (total perirenal adipose tissue weight per kilogram body weight) adipose tissue weight (Fig. 3, A and B), and with each other (Fig. 3C), in all control samples, irrespective of age. UCP2 mRNA was also positively associated with total ($R^2 = 0.38, P < 0.0001$) and relative adipose tissue weight ($R^2 = 0.42, P < 0.0001$), as well as with GR ($R^2 = 0.58, P < 0.0001$) and 11βHSD1 ($R^2 = 0.66, P < 0.0001$) mRNA. In contrast, 11βHSD2 mRNA was negatively correlated to total ($R^2 = 0.42, P < 0.001$) and relative ($R^2 = 0.36, P < 0.001$) adipose tissue weight and with UCP2 ($R^2 = 0.73, P < 0.001$), GR ($R^2 = 0.62, P < 0.001$) and 11βHSD1 mRNA ($R^2 = 0.67, P < 0.001$) abundance.

Total and relative (total RNA concentration per gram of total perirenal adipose tissue weight) RNA concentration decreased with postnatal age, having peaked at 146 days gestation, and were negatively correlated with UCP2 (total RNA: $R^2 = 0.51, P < 0.0001$; relative RNA concentration: $R^2 = 0.61, P < 0.0001$), GR (total RNA: $R^2 = 0.54, P < 0.0001$; relative RNA concentration: $R^2 = 0.63, P < 0.0001$), and 11βHSD1 (total RNA: $R^2 = 0.55, P < 0.0001$; relative RNA concentration: $R^2 = 0.68, P < 0.0001$) mRNA and total and relative adipose tissue weight, which increased with postnatal age (Fig. 1).
Ontogeny of glucocorticoid action and UCP2 mRNA abundance with increased fat mass. We have shown for the first time the developmental ontogeny of glucocorticoid sensitivity in fetal and postnatal adipose tissue as determined by measuring the abundance of GR and 11βHSD type 1 and 2 mRNA, which has also demonstrated their close relationship with fat growth. Surprisingly, although numerous studies have examined local glucocorticoid action in visceral obesity in adulthood, this is the first to examine the developmental ontogeny of these genes in adipose tissue between midgestation to early juvenile life and to link this directly to changes in fat mass over the same period. To this extent, both GR and 11βHSD1 mRNA abundance increased with postnatal age and were maximal at 6 mo, whereas 11βHSD2 mRNA abundance showed a converse relationship with adipose tissue weight. These changes in potential glucocorticoid sensitivity were directly related to the increase in adipose tissue mass over the first 6 mo of life, with both GR and 11βHSD1 mRNA abundance positively correlated to total and relative adipose tissue weight. Furthermore, both GR and 11βHSD1 mRNA were negatively correlated to total and relative RNA abundance, indicating that the observed ontogeny was not simply a reflection of increased RNA in adipose tissue per se. Our observations were made in perirenal adipose tissue, which forms up to 50% of total fat stores after the peripartum period in sheep (23), and in contrast to all other tissues is capable of unlimited growth in postnatal life (15, 23). In particular, the developmental ontogeny of local glucocorticoid action contrasts with other tissues such as the lung, where GR and 11βHSD1 mRNA abundance both peak during late gestation before declining with postnatal age (25). This differential regulation of glucocorticoid action in adipose tissue compared with other tissues demonstrates the potential importance of glucocorticoids in regulating fat growth. In the case of obesity, glucocorticoids may act as pathophysiological mediators (12).

In adipose tissue, especially at a visceral level, increased local conversion of cortisone to cortisol, driven by 11βHSD1, has been described in patients with obesity (12, 35, 37), with cortisol known to increase 11βHSD1 gene expression in human adipocytes in vitro (19). Interestingly, 11βHSD2 gene expression is reduced in obese women, in which increased 11βHSD1 gene expression is positively correlated with both waist circumference and insulin resistance (19). Furthermore, transgenic mice selectively overexpressing 11βHSD1 in adipose tissue develop visceral obesity and are glucose intolerant (32). Conversely, homozygous 11βHSD1 knockout mice are protected from features of the metabolic syndrome and obesity (30). Changes in GR response could also be important, either at the level of the central nervous system, by modulating the negative glucocorticoid feedback (17), or, peripherally, by regulating preadipocyte differentiation and adipocyte metabolism in a site-specific fashion (28). These alterations in glucocorticoid action have been observed when cortisol secretion and clearance are known to be dysregulated in obesity, leading to affect total or relative adipose tissue weight, and/or total or relative RNA concentration, in any of the sampling ages examined (data not shown), and there was no direct effect of sex on any of the parameters measured in this study.

DISCUSSION

Effect of early to mid- and late-gestational NR on UCP2, GR, and 11βHSD type 1 and 2 mRNA abundance in perirenal adipose tissue. Early- to midgestational NR increased GR, 11βHSD1, and UCP2 mRNA in fetal adipose tissue at 140 days gestation but decreased 11βHSD2 mRNA abundance. These effects were still evident in the adipose tissue of NR offspring at 6 mo postnatal age, compared with age-matched controls (Fig. 4). In contrast, late-gestational NR decreased GR, 11βHSD1, and UCP2 mRNA and increased 11βHSD2 mRNA abundance at both 1 and 30 days postnatal age (Fig. 5). Gestational NR in either early to mid- or late gestation did not
to normal, increased, or decreased plasma cortisol levels (26, 37). Indeed, our observed postnatal increase in glucocorticoid action in adipose tissue occurred during the decline in plasma cortisol and leptin (7) and are likely to be accompanied by parallel changes in 11βHSD activity, which are closely related to mRNA abundance (47).

Our study has also shown for the first time the developmental ontogeny of UCP2 mRNA and its relationship with adipose tissue development, extending previous genetic linkage studies with obesity and insulin resistance (21). In adipose tissue, UCP2 mRNA abundance peaked at 30 days, before declining up to 6 mo postnatal age, in contrast to the peak at 1 day of age in the lung (25). In addition, like GR and 11βHSD1, UCP2 mRNA abundance was positively correlated to total and relative adipose tissue weight, reflecting its potential importance in postnatal adipose tissue development. Possible roles may include the acquisition of white adipose tissue characteristics postnatally and the accumulation of macrophages, which has been implicated in the development of visceral obesity (36, 46). Specific proinflammatory adipokines such as TNF-α and IL-6 also increase the expression of UCP2 (31). Our findings suggest a role for UCP2 in adipose tissue development, although further studies are clearly warranted.

Maternal NR and programming of glucocorticoid action and UCP2 mRNA abundance in perirenal adipose tissue. We have shown for the first time the long-term programming and differential regulation of glucocorticoid action and UCP2 mRNA abundance in adipose tissue through reduced maternal nutrition through pregnancy. Whereas early- to mid-gestational NR increased glucocorticoid action and UCP2 mRNA abundance in adipose tissue through reduced maternal nutrition through pregnancy. Whereas early- to mid-gestational NR increased glucocorticoid action and UCP2 mRNA abundance both near term and at 6 mo of age, the reverse pattern was found with late-gestational NR during pregnancy can result in a reduction in plasma concentrations of a range of anabolic hormones including insulin, insulin-like growth factors, and thyroid hormones (6, 16), which all regulate adipose tissue development (41), although there is only a transient rise in maternal, but not fetal, plasma cortisol (18). Indeed, maternal cortisol actually decreases during long-term NR (8). Hence, the observed effects on glucocorticoid action and UCP2 mRNA abundance in adipose tissue after gestational NR are likely to reflect changes in the mitochondria, independent of changes in plasma cortisol. Compared with adipose tissue, both periods of gestational NR

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Fig. 4. Effect of early- to midgestational NR on the abundance of GR mRNA (A), 11βHSD1 mRNA (B), 11βHSD2 mRNA (C) and UCP2 mRNA (D) in perirenal adipose tissue sampled in the fetus at 140 days (140D) gestation (term ~147 days) and at 6 mo (6M) postnatal age in the sheep, from ewes that consumed 60% (NR) or 100% (control) of their metabolizable energy requirements for maternal metabolism and fetal growth between 28 and 80 days gestation. Examples of mRNA expression are given in each nutritional group. Values are means ± SE (n = 6–8 per group). *P < 0.05, **P < 0.01, mean value significantly different from control group.
increased glucocorticoid action and UCP2 mRNA abundance in the lung of the fetus and postnatal sheep (25).

The increase in glucocorticoid action at 6 mo after early- to mid-gestational NR extends previous findings by Whorwood et al. (47), who showed increased GR and 11βHSD1 expression in adipose tissue sampled from nutrient-restricted newborn sheep compared with controls. These findings also suggest that the increased incidence of obesity in adults born to mothers exposed to the Dutch famine during early pregnancy (39) may be as a direct consequence of sustained adaptations in the endocrine sensitivity of adipose tissue. In contrast, the decrease in glucocorticoid action and UCP2 mRNA abundance after late-gestational NR, which was coincident with the period of maximal fetal growth and a parallel rise in fetal fat depots, may be protective against the development of visceral obesity. Indeed, reduced maternal NR in late gestation results in smaller fat depots (42), in conjunction with lower fetal plasma glucose and insulin, but has no effect on fetal leptin or prolactin receptor mRNA abundance in adipose tissue (42, 48). Then after birth, there are compensatory increases in mitochondrial proteins, including UCP2, in adipose tissue of NR offspring that are maintained up to at least 1 mo of age (33).

The exact significance of these modifications in glucocorticoid action and UCP2 mRNA abundance in adipose tissue have yet to be determined, although increased UCP2 (13), GR (11), and 11βHSD1 (12, 19, 35, 37) and decreased 11βHSD2 (19) expression have all been observed in patients with visceral obesity. Furthermore, to increase fat mass in these offspring after gestational NR examination beyond 6 mo of age may be necessary. Indeed, late-gestational NR has been found to increase adipose tissue weight and glucose intolerance in nutrient-restricted offspring at 1 yr of age compared with controls (24). In addition, the physical environment in which animals are reared may also have a bearing on the development of obesity. Indeed, we have recently found that when the offspring are maintained within an environment of reduced physical activity, the nutrient-restricted offspring show increased subcutaneous fat depth compared with controls (Gardner DS and Symonds ME, unpublished observations). These latter factors may explain why we did not observe any differences in adipose tissue weights with gestational NR, although at a cellular and mitochondrial level, there were discrete and consistent effects on glucocorticoid action and UCP2 mRNA abundance. Although there are no other comparable models that have examined specific periods of gestational NR on glucocorticoid action or UCP2 mRNA abundance on adipose tissue development, there is evidence that the postnatal diet contributes to obesity. Overfeeding in the immediate postnatal period in rats induces early-onset obesity and accelerated maturation of the HPA axis, increased corticosterone secretion together with an upregulation of GR and 11βHSD1 mRNA abundance in adipose tissue (10). These findings, in conjunction with our own previous findings, demonstrate that glucocorticoid sensitivity and UCP2 can be affected by the nutritional status in utero and postnatally, thereby having an impact on adipose tissue development and potentially visceral obesity.

Fig. 5. Effect of late-gestational NR on the abundance of GR mRNA (A), 11βHSD1 mRNA (B), 11βHSD2 mRNA (C), and UCP2 mRNA (D) in perirenal adipose tissue sampled at 1 and 30 days postnatal age, from ewes that consumed 60% (D, NR) or 100% (control) of their metabolizable energy requirements for maternal metabolism and fetal growth between 110 and 147 days gestation (term ∼147 days). Examples of mRNA expression are given in each nutritional group. Values are means ± SE (n = 6–8 per group). * P < 0.05, ** P < 0.01, mean value significantly different from control group.
Although our studies are predominantly observational, they provide markers for identifying those most at risk for visceral obesity at whom therapeutic strategies could subsequently be targeted. In this respect, inhibition of 11βHSD1 activity shows considerable promise, especially since compared with other strategies for manipulating glucocorticoid action, there is no impairment of circulating cortisol (44). Initial studies with the nonselective inhibitor, carbenoxolone, have shown enhanced hepatic insulin sensitivity, both in healthy controls and in patients with type II diabetes (2, 45), although specific inhibition of adipose tissue 11βHSD1 may be required in obese patients to obtain distinct therapeutic benefits (44). Complimentary studies examining 11βHSD1 inhibition in conjunction with selective periods of maternal NR may therefore be productive in the development of new strategies aimed at preventing later obesity.

In conclusion, we have shown for the first time that the continual rise in glucocorticoid action is associated with the increase in fat mass postnatally. This adaptation in conjunction with greater abundance of UCP2 mRNA is affected by maternal NR in utero, which may subsequently lead to the pathophysiological development of visceral obesity in later life.

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