Developmental Control of Plasma Leptin and Adipose Leptin Messenger Ribonucleic Acid in the Ovine Fetus during Late Gestation: Role of Glucocorticoids and Thyroid Hormones

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In developed countries, the increasing incidence of obesity is a serious health problem. Leptin exposure in the perinatal period affects long-term regulation of appetite and energy expenditure, but control of leptin production in utero is unclear. This study investigated perirenal adipose tissue (PAT) and placental leptin expression in ovine fetuses during late gestation and after manipulation of plasma glucocorticoid and thyroid hormone concentrations. Between 130 and 144 d of gestation (term at 145 ± 2 d), plasma leptin and PAT leptin mRNA levels increased in association with increments in plasma cortisol and T₃. Fetal adrenalectomy prevented these developmental changes, and exposure of intact 130 d fetuses to glucocorticoids, by cortisol infusion or maternal dexamethasone treatment, caused premature elevations in plasma leptin and PAT leptin gene expression. Fetal thyroidectomy increased plasma leptin and PAT leptin mRNA abundance, whereas intravenous T₃ infusion to intact 130 d fetuses had no effect on circulating or PAT leptin. Leptin mRNA expression was low in the ovine placenta. Therefore, in the sheep fetus, PAT appears to be a primary source of leptin in the circulation, and leptin gene expression is regulated by both glucocorticoids and thyroid hormones. Developmental changes in circulating and PAT leptin may mediate the maturational effects of cortisol in utero and have long-term consequences for appetite regulation and the development of obesity. (Endocrinology 148: 3750–3757, 2007)
plasma leptin are mediated by the thyroid hormones is unknown.

Therefore, the aims of this study were 1) to determine whether developmental and glucocorticoid-induced changes in circulating leptin in utero are attributable to changes in adipose leptin mRNA abundance and 2) to establish the role of thyroid hormones in the control of circulating leptin and leptin mRNA levels in fetal adipose tissue. The study tested the hypothesis that developmental and glucocorticoid-induced changes in plasma leptin before birth are caused by changes in adipose leptin gene expression that, in turn, may be mediated by thyroid hormones. Glucocorticoid and thyroid hormone concentrations in the sheep fetus were manipulated by endocrine gland removal and exogenous hormone treatment.

Materials and Methods

Animals

Forty-three Welsh Mountain sheep fetuses of known gestational age were used in this study; 30 were singletons, and 13 were twin fetuses. In total, there were 26 male and 17 female fetuses. The ewes were housed in individual pens and had free access to hay, water, and a salt-lick block. Food, but not water, was withheld for 18–24 h before surgery. All surgical and experimental procedures were in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986 and were approved by the local animal ethics committee.

Surgical procedures

All surgical operations were performed under halothane anesthesia (1.5% in O₂-N₂O with positive pressure ventilation. Five fetuses were thyroidectomized (TX) between 105 and 110 d of gestation, and an additional five fetuses were adrenalectomized (AX) between 116 and 120 d of gestation (term at 145 ± 2 d) using surgical techniques described previously (17, 18). For two of the AX and one of the TX fetuses, the unoperated twin acted as a control intact fetus.

In 15 of the remaining intact fetuses, intravascular catheters were inserted into the femoral artery and vein between 115 and 118 d of gestation (19). All catheters were exteriorized through the flank of the ewe and secured in a plastic bag sutured to the skin. The catheters were flushed daily with heparinized saline solution (100 IU/ml heparin, 0.9% saline wt/vol) from the day after surgery. At surgery, all plasma aliquots were stored at −20°C until analysis. Plasma leptin concentration was measured by RIA using recombinant bovine-ovine leptin standards as described previously (20); the lower limit of detection was 0.09 ng/ml, and the interassay coefficient of variation was 12%. Plasma cortisol concentration was measured by RIA (22) in which the lower limit of detection was 1.0–1.5 ng/ml and the interassay coefficient of variation was 12%. Plasma T₃ and T₄ concentrations were also measured by RIA using a commercial kit (23). The lower limits of detection were 0.07 ng/ml for T₃ and 7.6 ng/ml for T₄, and the interassay coefficients of variation were 10% for both assays. All RIAs were validated for use with ovine plasma.

Biochemical and molecular analyses

Plasma hormone concentrations. All blood samples were immediately placed into EDTA-containing tubes and centrifuged for 5 min at 1000 × g at 4°C. The plasma aliquots were stored at −20°C until analysis. Plasma cortisol concentration was measured by RIA (22) in which the lower limit of detection was 1.0–1.5 ng/ml and the interassay coefficient of variation was 12%. Plasma T₃ and T₄ concentrations were also measured by RIA using a commercial kit (23). The lower limits of detection were 0.07 ng/ml for T₃ and 7.6 ng/ml for T₄, and the interassay coefficients of variation were 10% for both assays. All RIAs were validated for use with ovine plasma.

Immunohistochemistry. The fixed tissue samples were dehydrated in ethanol, transferred to a 50:50 dilution of ethanolic ethanol and xylene (Histoclear; National Diagnostics, Atlanta, GA) for 1–2 h and then incubated in xylene overnight. Samples were incubated in three changes of paraffin wax at 60°C for 2 h and then blocked out in fresh wax. Sections of 7 μm thickness were cut and mounted on microscope slides. Sections were dewaxed in xylene and transferred to 100% ethanol for 10 min. After washing in distilled water for 20 min, the sections were incubated with PBS/1% BSA/0.05% thiomersal alone or PBS/1% BSA/0.05% thiomersal alone (negative control) overnight at 4°C. Unbound primary antibody was removed by washing with PBS. The sections were incubated at room temperature for 40 min with the secondary antibody (4 nm gold-labeled goat antirabbit; Jackson ImmunoResearch, West Grove, PA) at a dilution of 1:140. All sections were washed in PBS and distilled water and then incubated with silver intensifying solution (IntenSE M silver enhancement kit; GE Healthcare, Little Chalfont, UK) for up to 20 min. The sections were air dried and mounted.

In situ hybridization. Oligonucleotide probes to ovine leptin were commercially synthesized (Sigma-Genosys, Cambridge, UK). Antisense and sense probes were 5'-ACT GCG TGG GTG AGA TGT CAT TGC TCC TGG TGA CAA TCG TCT TGA GGA 3' and 5' CCT CAT CAA GAC GAT TGT CAC CAG CAT GAT CAC CAA CAC CCA GCA GT-3', respectively, and were labeled with digoxigenin (DIG) using DIG oligonucleotide tailing kit (Roche Diagnostics, Welwyn Garden City, UK) according to the instructions of the manufacturer.

Sections of 10 μm thickness were cut from frozen tissue and mounted on poly-lysine-coated microscope slides. Sections were fixed in 4% paraformaldehyde/PBS on ice for 5 min and washed twice with 2.5 min in PBS. The slides were then transferred to 50%, 70%, and 95% ethanol for 5 min each. The slides were stored in 95% ethanol at 4°C until needed.

Sections were incubated in prehybridization solution [47% deionized formamide, 2× sodium chloride and sodium citrate (SSC), 10% dextran...
sulfate, 1 X Denhardt's solution, 50 mm sodium phosphate (pH 7.5), 250 μg/ml salmon sperm DNA, and 500 μg/ml RNA) for 2 h at 37 C. The sections were then washed in PBS and 70, 95, and 100% ethanol for 3 min each and allowed to air dry. The sections were incubated overnight with hybridization solution (prehybridization solution and labeled probe) at 37 C. Sections were washed at 37 C in 2 X SSC, 1 X SSC, and 0.5 X SSC twice for 10 min each. The slides were washed at room temperature in 100 mm Tris-HCl (pH 7.5), 150 mm NaCl [Tris-buffered saline (TBS)] twice for 10 min each wash. The sections were incubated with blocking solution (TBS, 2% sheep serum, and 0.1% Triton X-100) for 30 min at room temperature and then with the detecting antibody (anti-DIG alkaline phosphatase conjugate; Roche Diagnostics) at 1:100 overnight at 4 C. The sections were washed in TBS twice for 10 min each wash and then transferred to 100 mm Tris-HCl (pH 9.5), 100 mm NaCl, 50 mm MgCl2 for an additional 10 min. Nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate color developing solution [stock solution diluted 1:40 in 100 mm Tris-HCl (pH 9.5), 100 mm NaCl, 50 mm MgCl2] (Roche Diagnostics) was applied to the sections, which were incubated in the dark at room temperature for up to 4 h. The reaction was stopped with the application of 10 mm Tris-HCl (pH 7.5), 1 mm EDTA. Sections were allowed to air dry and were mounted.

Quantitative real-time PCR. Tissue samples of approximately 100 mg were homogenized on ice in 1 ml Trig reagent (Sigma) using a Polytron homogenizer, and RNA extraction was performed following the method of Chomczynski and Sacchi (24). The RNA samples were resuspended in diethylpyrocarbonate-H2O and were treated with deoxyribonuclease 1 (Roche Diagnostics). The RNA samples were resuspended in 1 ml Tri reagent (Sigma) using a Polytron homogenizer, and RNA extraction was performed following the method of Chomczynski and Sacchi (24). The RNA samples were resuspended in diethylpyrocarbonate-H2O and were treated with deoxyribonuclease 1 (Roche Diagnostics) was applied to the sections, which were incubated in the dark at room temperature for up to 4 h. The reaction was stopped with the application of 10 mm Tris-HCl (pH 7.5), 1 mm EDTA. Sections were allowed to air dry and were mounted.

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Leptin protein and mRNA were localized in maternal PAT, fetal PAT, and placenta in control animals at 130 d of gestation. In maternal and fetal PAT, leptin protein was detected evenly across the sections and was localized to the periphery of the adipocytes (Fig. 1, B and C). Expression of leptin mRNA was identified in maternal and fetal PAT in a similar pattern to the leptin protein (Fig. 1, E and F).

In the ovine placenta at 130 d of gestation, leptin protein and mRNA were present on the membranes of the fetomaternal interdigitating villi (Fig. 1, D and G). However, it was not possible to distinguish whether staining was localized on the fetal trophoblast layer or the fetomaternal syncytiotrophoblast layer. The sense probes showed negligible staining for leptin mRNA in all tissues (Fig. 1, H–J). Similar localization of leptin protein and mRNA were observed in all three tissues taken from animals at 144 d of gestation.

Effect of gestational age

In the intact control fetuses, plasma concentrations of cortisol, T3, and leptin, but not T4, increased significantly between 130 and 140 d of gestation (P < 0.05) (Table 1 and Fig. 2A). Over this period of gestation, the developmental change in plasma leptin concentration was associated with a significant rise in leptin mRNA abundance in fetal PAT. Leptin mRNA expression in fetal PAT at 144 d of gestation was significantly greater than at 130 d of gestation (P < 0.05) (Fig. 2B). Between 130 and 144 d of gestation, there were no significant differences in either absolute or relative PAT weights (Table 2). In the placenta, although leptin protein and mRNA expression were identified by immunohistochemistry and in situ hybridization at 130 and 144 d of gestation, leptin mRNA abundance was very low and did not change with gestational age (data not shown).

Effect of manipulation of plasma glucocorticoid concentration

Fetal adrenalectomy, which prevented the prepartum surge in both plasma cortisol and T3, abolished the gestational rise in circulating leptin concentration (Table 1 and Fig. 2A). At 144 d of gestation, AX fetuses had significantly lower plasma concentrations of cortisol, T3, and leptin compared with intact fetuses (P < 0.05) (Table 1 and Fig. 2A). However, there was no significant difference in plasma T4 concentration between the two groups of fetuses (Table 1). The gestational rise in leptin mRNA abundance in PAT was also prevented in the AX fetuses; at 144 d of gestation, mean leptin mRNA abundance in the AX fetuses was significantly lower than in the intact fetuses (P < 0.05) (Fig. 2B). There were no significant differences in absolute or relative PAT weights between the intact and AX fetuses at 144 d of gestation (Table 2).

Exogenous infusion of cortisol into the fetus for 5 d caused with repeated measures, followed by Tukey’s test as appropriate. Data from the dexamethasone-treated animals and from the AX and TX fetuses were compared with values from their respective control groups by unpaired t test. Differences at P < 0.05 were regarded as significant.

Results

Localization of leptin protein and mRNA

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Exogenous infusion of cortisol into the fetus for 5 d caused
significant increases in plasma concentrations of cortisol, T₃, and leptin but not T₄ (Table 1 and Fig. 3A). Compared with the pretreatment baseline, a significant rise in plasma leptin concentration was observed on all days of the infusion in the cortisol-treated fetuses (P < 0.05) (Fig. 3A). Furthermore, within 24 h of the start of the infusion and on each day thereafter, plasma leptin in the cortisol-infused fetuses was significantly higher than in those infused with saline (P < 0.05) (Fig. 3A). The cortisol-induced rise in plasma leptin was associated with a significant increase in leptin mRNA expression in fetal PAT; on the fifth day of infusion, leptin mRNA abundance was significantly higher in the cortisol-infused fetuses compared with the saline-infused fetuses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of fetuses</th>
<th>Gestational age at delivery (d)</th>
<th>Plasma cortisol (ng/ml)</th>
<th>Plasma T₃ (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fetus</td>
<td>Fetus</td>
</tr>
<tr>
<td>Intact/saline infusion</td>
<td>7</td>
<td>130</td>
<td>13.8 ± 1.4*</td>
<td>104.3 ± 13.7</td>
</tr>
<tr>
<td>Intact</td>
<td>6</td>
<td>144</td>
<td>89.5 ± 20.1</td>
<td>107.1 ± 21.7</td>
</tr>
<tr>
<td>AX</td>
<td>5</td>
<td>144</td>
<td>7.1 ± 0.6*</td>
<td>84.0 ± 6.9</td>
</tr>
<tr>
<td>TX</td>
<td>5</td>
<td>144</td>
<td>110.0 ± 25.9</td>
<td>ND*</td>
</tr>
<tr>
<td>Maternal saline</td>
<td>5</td>
<td>127</td>
<td>57.8 ± 12.2</td>
<td>132.5 ± 11.7</td>
</tr>
<tr>
<td>Maternal dexamethasone</td>
<td>5</td>
<td>127</td>
<td>3.2 ± 0.1***</td>
<td>126.3 ± 22.0</td>
</tr>
</tbody>
</table>

* P < 0.05, significantly different from intact fetuses at 144 d (t test); **, P < 0.05, significantly different from d 0 within treatment group (two-way ANOVA with repeated measures and Tukey’s test); ***, P < 0.05, significantly different from saline-treated animals (t test); ND, not detectable. Values within columns with different letters are significantly different from each other (P < 0.05, two-way ANOVA with repeated measures and Tukey’s test). Plasma concentrations in the fetuses from the ewes treated with saline or dexamethasone have been reported previously (31).
Effect of manipulation of plasma thyroid hormone concentration

At 144 d of gestation, plasma leptin and PAT leptin mRNA levels were significantly higher in TX fetuses than in intact fetuses ($P < 0.05$) (Fig. 2, A and B). In the TX fetuses, plasma concentrations of T3 and T4 were significantly lower than in the intact fetuses, but plasma cortisol remained similar ($P < 0.05$) (Table 1). Fetal body weight was significantly lower and relative PAT weight was significantly higher in the TX fetuses compared with the intact fetuses at 144 d ($P < 0.05$) (Table 2).

An exogenous infusion of T3 in utero for 5 d had no effect on plasma leptin or leptin mRNA abundance in fetal PAT (Fig. 3). There was no significant difference in plasma leptin concentration between the T3 and saline-infused fetuses on any day of the infusion (Fig. 3A). Furthermore, on the fifth day of the infusion, PAT leptin mRNA abundance and absolute and relative PAT weights in the T3-infused fetuses were similar to those seen in fetuses treated with saline (Fig. 3B and Table 2).

Discussion

The present study demonstrates that circulating leptin and PAT leptin mRNA levels are regulated in utero by glucocorticoids and thyroid hormones. The normal increments in plasma leptin and PAT leptin mRNA seen in the sheep fetus near term were abolished by fetal adrenalec-tomy, and both endogenous and synthetic glucocorticoids induced up-regulation of PAT leptin gene expression and, in turn, plasma leptin concentration. Therefore, the developmental rise in leptin production may be caused, at least in part, by the prepartum cortisol surge and can be induced prematurely by antenatal glucocorticoid treatment. In contrast, thyroid hormones had an inhibitory effect on PAT leptin synthesis and secretion in fetal sheep close to term. Although exogenous T3 infusion earlier in gestation had no effect on circulating leptin or PAT leptin mRNA, hypothyroidism in utero was associated with elevated circulating leptin and PAT leptin mRNA abundance. Therefore, in the ovine fetus during late gestation, ontogenic changes in PAT leptin production may result from the balance of stimulatory and inhibitory influences of the glucocorticoids and thyroid hormones, respectively.

Developmental and endocrine-induced changes in plasma leptin concentration observed in the fetuses of the present study were associated with changes in PAT leptin gene expression. This is in agreement with previous findings in normally fed and undernourished sheep in which circulating leptin in utero correlated with leptin mRNA abundance in fetal PAT (11). However, in the current study, the magnitude of change in PAT leptin mRNA abundance observed with gestational age and manipulation of glucocorticoid and thyroid hormone concentrations was disproportionately greater than the coincident change in plasma leptin concentration. These findings may be attributable to smaller subsequent effects on leptin protein synthesis and/or secretion from PAT, the relative clearance of leptin from the fetal circulation, or reductions in leptin synthesis by other fetal and placental tissues. In the sheep fetus near term, 85–95% of adipose tissue is perirenal (26), and leptin mRNA abundance is greater in PAT than in other adipose depots (5). Therefore, PAT appears to be an important source of circulating leptin in the ovine fetus during late gestation. Indeed, as demonstrated in the present and previous studies (5, 27), the ovine placenta does not express significant amounts of leptin mRNA and is unlikely to make a major contribution to leptin secretion in utero.

Before birth, glucocorticoids stimulated leptin gene expression in fetal PAT. These findings extend those that previously showed glucocorticoid-induced changes in plasma leptin in utero (13) but contrast with an earlier investigation, using tissues collected by our laboratory, that showed no change in PAT leptin mRNA abundance in sheep fetuses.
toward term or after glucocorticoid manipulation (28). Differences between these studies may be attributable to the more sensitive method of measurement of gene expression used in the current study. Glucocorticoids are likely to act directly on leptin gene transcription in utero because a glucocorticoid response element has been identified in the promoter region of the human leptin gene (29). In addition, the pronounced leptin response seen in the dexamethasone-exposed fetuses may have been partly caused by a coincident rise in plasma insulin concentration that is a known stimulant of leptin production in adipose tissue (30, 31). The actions of dexamethasone in utero are consistent with previous studies that show higher umbilical leptin concentration in preterm human infants whose mothers were treated with antenatal glucocorticoids (32). In the pregnant ewe, however, the lack of response to dexamethasone contrasts with previous observations in adult nonpregnant rats and human...

**TABLE 2.** Mean ± SEM values of body weight and absolute PAT and relative PAT weights, at delivery in the fetuses of the different treatment groups

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of fetuses</th>
<th>Gestational age (d)</th>
<th>Fetal body weight (g)</th>
<th>Absolute PAT weight (g)</th>
<th>Relative PAT weight (% of body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact/saline infusion</td>
<td>7</td>
<td>130</td>
<td>3104 ± 168</td>
<td>10.4 ± 1.3</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>Intact</td>
<td>4</td>
<td>144</td>
<td>4226 ± 696</td>
<td>9.8 ± 0.8</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>AX</td>
<td>3</td>
<td>144</td>
<td>4667 ± 203</td>
<td>11.8 ± 0.5</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>TX</td>
<td>5</td>
<td>144</td>
<td>2839 ± 166a</td>
<td>10.9 ± 1.5</td>
<td>0.41 ± 0.06</td>
</tr>
<tr>
<td>Saline infusion</td>
<td>5</td>
<td>130</td>
<td>3028 ± 223</td>
<td>10.9 ± 1.7</td>
<td>0.36 ± 0.04</td>
</tr>
<tr>
<td>Cortisol infusion</td>
<td>5</td>
<td>130</td>
<td>2894 ± 207</td>
<td>9.9 ± 0.6</td>
<td>0.34 ± 0.02</td>
</tr>
<tr>
<td>T3 infusion</td>
<td>5</td>
<td>130</td>
<td>2657 ± 100</td>
<td>8.8 ± 0.6</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>Maternal saline</td>
<td>5</td>
<td>127</td>
<td>3130 ± 155</td>
<td>10.6 ± 1.0</td>
<td>0.34 ± 0.02</td>
</tr>
<tr>
<td>Maternal dexamethasone</td>
<td>5</td>
<td>127</td>
<td>3105 ± 62</td>
<td>8.5 ± 0.8</td>
<td>0.27 ± 0.03</td>
</tr>
</tbody>
</table>

* P < 0.05, significantly different from intact fetuses at 144 d (t test).

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FIG. 3. Mean ± SEM values of plasma leptin concentration (A) and adipose leptin mRNA (B) in the saline-, cortisol-, and T3-infused fetuses. For plasma leptin, within each day, columns with different letters are significantly different from each other (P < 0.05), and, within each treatment group, a indicates a significant difference from preinfusion baseline (P < 0.05). For adipose leptin mRNA on the fifth day of infusion, † indicates a significant difference from saline-infused fetuses (P < 0.05).

FIG. 4. Mean ± SEM values of plasma leptin and adipose leptin mRNA in fetuses (A) and ewes (B) sampled at 10 h after the second daily injection of either saline or dexamethasone. *, P < 0.05, significant difference from the saline-treated animals.
subjects in which dexamethasone administration, at higher and lower doses to the present study, increased adipose leptin mRNA and plasma leptin levels (33, 34).

The stimulatory effects of glucocorticoids on plasma leptin and PAT leptin mRNA levels in utero did not appear to be mediated by changes in thyroid hormone concentration, unlike that seen in other physiological systems in the fetus (14, 15). In contrast, the thyroid hormones had an inhibitory effect on PAT leptin mRNA abundance and plasma leptin concentration in the sheep fetus near term. Fetal thyroidectomy, which lowered plasma T3 concentration to an undetectable level and prevented the prepartum T3 surge, caused elevations in both PAT leptin gene expression and circulating leptin concentration. Previous studies have demonstrated an inverse relationship between plasma concentrations of leptin and T3 in rats after experimental manipulation of thyroid hormone status and in human subjects with thyroid dysfunction (35–37).

There are a number of mechanisms by which thyroid hormones in utero may influence leptin synthesis and secretion by fetal adipose tissue. To date, a thyroid hormone response element has not been identified in the leptin gene, although T3 causes dose-dependent inhibition of leptin gene expression and protein secretion in brown and white adipocytes cultured from adult rats (38). There may also be a direct action of the high TSH concentration associated with thyroidectomy, because TSH increases leptin secretion from human adipose tissue in vitro (39). In addition, some of the effects of the thyroid hormones on leptin synthesis and secretion in utero may be induced indirectly by changes in tissue sensitivity to sympathetic stimulation. In adult animals, β3-adrenergic stimulation inhibits leptin gene expression in adipose tissue and suppresses circulating leptin concentration (40, 41). Furthermore, adipose β3-adrenergic receptor mRNA abundance has been shown to depend on thyroid hormones in adult rats (42). In sheep fetuses, noradrenergic-induced cellular respiration is lower in PAT obtained from TX fetuses compared with TX fetuses infused with a replacement dose of T3 (43), which suggests that thyroid hormones have an important role in determining adrenergic sensitivity in fetal adipose tissue. Hypothyroidism in the ovine fetus is also known to alter oxygen consumption (23), and this, in turn, may have had consequences for the control of leptin synthesis in PAT. Although arterial blood oxygen tension remains unchanged in TX fetuses (23), chronic hypoxia in utero increases both leptin gene expression in periadrenal fat and circulating leptin concentration (44). Last, part of the rise in circulating leptin concentration seen in the TX fetuses may have been attributable to greater relative PAT weight. The TX fetuses were growth retarded compared with intact fetuses at the same gestational age, but absolute PAT weight was maintained. Previous studies in fetal sheep have shown that plasma leptin concentration correlates with PAT mass and specifically with the relative amount and cell size of unilocular fat (26). However, the cellular structure of PAT was not investigated in the fetuses of the present study. Therefore, there may be both direct and indirect effects of thyroid hormones on leptin synthesis in utero. In the present study, although thyroid hormone deficiency upregulated leptin synthesis in the sheep fetus near term, an exogenous infusion of T3 between 125 and 130 d of gestation had no effect on PAT leptin mRNA abundance or plasma leptin concentration. This may have been attributable to a relative lack of thyroid hormone receptors and/or postreceptor sensitivity in fetal PAT at this gestational age. Furthermore, indirect mechanisms of thyroid hormone action on leptin synthesis may be influenced by gestational age and/or the experimental method used to manipulate thyroid hormone concentration in utero. These include PAT sensitivity to catecholamines and the control of TSH, sympathetic output, oxygen consumption, and relative PAT mass.

Developmental and glucocorticoid-induced increments in plasma leptin concentration may have an important role in the control of fetal maturation near term. Indeed, some of the maturational effects of endogenous and synthetic glucocorticoids in the ovine fetus may be mediated by the coincident rise in circulating leptin. Leptin receptors have been identified in a variety of fetal and placental tissues in several animal species (6, 7). For example, leptin receptor mRNA and protein are expressed in the lungs of baboon fetuses (45), and leptin has been shown to stimulate surfactant production in type II pneumocytes cultured from fetal rats and rabbits (46, 47). At birth, thyroid hormone and catecholamine release in response to labor and delivery into a cold environment may contribute to the fall in plasma leptin seen in human and ovine neonates (13, 48). This may be an important drive to suckling in the immediate postnatal period. Furthermore, the role of perinatal and endocrine-induced changes in leptin production in the long-term control of food intake and energy balance remains to be established. Exposure of the fetus to leptin at critical periods of development may have important consequences for the formation and activity of hypothalamic neural networks responsible for appetite regulation and, hence, obesity in adult life (1, 2).

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