Differential effects of leptin administration on the abundance of UCP2 and glucocorticoid action during neonatal development

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LEPTIN IS A 16-kDA POLYPEPTIDE HORMONE that is principally synthesized and secreted by adipose tissue and acts to regulate energy homeostasis and a range of neuroendocrine and reproductive functions in the adult (1, 23, 62). The role of leptin in the neonate has yet to be fully determined. Plasma leptin concentrations increase with gestational age in conjunction with an increase in fetal adipose tissue deposition, leptin mRNA abundance, and plasma cortisol (10, 76). In the newborn sheep, plasma leptin concentrations decline during the immediate 6 h after birth and then increase up to 7 days of age, whereas plasma cortisol continues to decrease (5). These temporal changes in leptin and cortisol coincide with maturation of the hypothalamic-pituitary-adrenal axis and the rapid activation of uncoupling protein (UCP)1, which is unique to brown adipose tissue (BAT) (13, 57) and is followed by the gradual loss of UCP1 (11). UCP1 has a defined role in nonshivering thermogenesis at birth by its avoidance of adenosine triphosphate (ATP) synthase and its allowance of proton reentry into the mitochondrial matrix, thus creating the proton electrochemical gradient to be dissipated as heat (25). In the neonatal sheep, both acute (1-day) and chronic (6-day) leptin administration result in reduced UCP1 mRNA and protein abundance, in conjunction with maintained colonic temperature and plasma nonesterified fatty acid (NEFA) concentration, therefore not affecting thermogenic potential (43). Similar assessments of leptin administration on physiological function and mitochondrial protein abundance in the lung have not previously been undertaken, even though fetal and adult lungs are leptin responsive (16, 33, 71), and the survival of the neonate is dependent on the extraterine adaptation of the fetal lung and the establishment of independent ventilation.

The peak in UCP1 abundance at birth is accompanied by parallel increases in other mitochondrial proteins including voltage-dependent anion channel (VDAC), located within the inner mitochondrial membrane, and cytochrome c, present within the intermembrane space (46). VDAC is a component of the mitochondrial permeability pore, which regulates the supply of mitochondrial adenosine diphosphate and ATP and is proposed to have a role in apoptosis (15, 31). Cytochrome c is an essential component of the mitochondrial respiratory chain and is a mobile electron transporter involved in the electron transfer from complex III to complex IV (34, 40). In the lung, the peak in VDAC abundance at 7 days of postnatal age coincides with the maximal abundance of UCP2 protein, which, although undetectable in the fetal lung, follows the peak in UCP2 mRNA at 6 h of age before declining rapidly in postnatal life (29, 46). UCP2, a recently discovered member of the inner mitochondrial membrane carrier subfamily, is highly abundant in the lung (29, 53) and has postulated roles in energy regulation (7, 9), reactive oxygen species production (36, 50), and apoptosis in conjunction with VDAC and cytochrome c proteins (37, 73). UCP2 has also been genetically linked to obesity (22) and may have a limited role in thermogenesis (21). Adult rodent studies have shown that administration of leptin to
ob/ob mice, which do not produce leptin and are thus hypothermic, hyperphagic, and obese, restores a normal body temperature despite a 50% reduction in food intake. These changes in body temperature have been linked to increased abundance of UCP1 (58) and UCP2 (30) in BAT by some, but not all, studies (42). Moreover, these changes appear to be unique to rodents, as leptin treatment of large mammals, such as sheep and pigs, has been found to have a more limited role in thermogenesis (38, 43). No study to date has previously investigated the effect of leptin administration on the development of neonatal BAT and lung with respect to the abundance of UCP2, VDAC, and cytochrome c proteins that change dramatically over this period (29).

The interaction and increase in plasma concentrations of catecholamines, thyroid hormones, prolactin, cortisol, and leptin are crucial in fetal and neonatal BAT development (67). However, the impact of acute and chronic leptin administration on local glucocorticoid action, as determined by the expression of glucocorticoid receptor (GR, type 2) and isoforms of 11β-hydroxysteroid dehydrogenase (11β-HSD), within neonatal BAT have not been previously examined. In the lung, for example, the peripartum peak in GR and 11β-HSD1 mRNA peak at 140 days gestation (term ~147 days), in conjunction with UCP2, has emphasized the developmental link between UCP2 and cortisol and may aid in the extraterrestrial adaptation of the fetal lung and in the establishment of independent breathing (29). 11β-HSD1 behaves predominantly as an 11-oxoreductase, catalyzing the conversion of inactive cortisone to active cortisol, thereby amplifying activation of intracellular GR, whereas 11β-HSD2 behaves as an 11-dehydrogenase, catalyzing the inactivation of cortisol to inert cortisone (4, 63).

In the developing lung and BAT of the fetus, UCP2, GR, 11β-HSD1, and 11β-HSD2 mRNA are significantly correlated with fetal plasma cortisol and norepinephrine (28). Leptin is a potent stimulator of the sympathetic nervous system (32, 59) and inhibitor of adrenocorticotropic hormone-stimulated glucocorticoid secretion by the adrenal gland (55). A further aim of our study was to examine the effect of leptin administration on local glucocorticoid action within neonatal BAT over the neonatal period, in which there are rapid changes in both UCP1 and cortisol (5, 11).

The aims of this study were thus to determine 1) the effect of acute and chronic leptin administration on the abundance of UCP2, GR, 11β-HSD1, and 11β-HSD2 mRNA in neonatal sheep BAT at 1 and 7 days postnatal age; 2) the effect of chronic leptin administration on the abundance of UCP2, VDAC, and cytochrome c proteins in the neonatal lung as measured at 7 days postnatal age, when maximal mitochondrial protein abundance normally occurs (46); and 3) significant associations between plasma leptin and NEFA concentrations with physiological and molecular variables measured during the study period.

**MATERIALS AND METHODS**

**Animals**

Seventeen pairs of triplet lambs, born normally at term to Blue-faced Leicester × Swaledale ewes, were entered into the study. The first eight pairs of lambs were used to determine the effect of acute administration of ovine recombinant leptin (26) on thermoregulation and to establish the required dose of leptin necessary to obtain a 10-fold rise in plasma concentration of leptin. During the course of this study, it was observed that plasma leptin was higher in female than in male lambs (43), thus only female lambs were used in a subsequent study in which the effects of chronic treatments of leptin were examined in nine pairs of lambs. For each ewe, one untreated triplet remained with its mother throughout the study. Full details of materials and methods have been previously published (43). All operative procedures and experimental protocols had the required Home Office approval as designated by the Animals (Scientific Procedures) Act of 1986.

**Acute Study**

Each lamb remained with its mother until 16 ± 2.5 h after birth, during which time all lambs obtained adequate amounts of colostrum. Pairs of lambs were selected on the basis of matched body weight (±10%) and were then placed in a constant temperature room of 15 (±1)°C, which represents a cool challenge to neonatal lambs (65). A jugular vein catheter was inserted into each animal under local anesthetic (2% xylocaine) to enable vehicle or leptin to be given and blood samples to be taken. In the acute study, one lamb from each pair was randomly assigned (irrespective of sex) to receive recombinant ovine leptin (26). After a 1-h acclimatization period in the calorimeter box, lambs were initially injected intravenously with vehicle (1 ml of sterile water), and 5-ml blood samples were taken ~40–60 min after each injection while the lambs were sleeping. Subsequently, six males and two females were treated with leptin to be compared with four males and four females treated with vehicle alone. One lamb was then injected with 10 µg of leptin in 1 ml of sterile water, and its sibling received water alone. Water rather than saline was used for intravenous injection, since leptin does not dissolve with saline. This procedure was then repeated twice, with the modification that treated lambs were injected with 100 µg of leptin. The rationale for the incremental leptin dosages was to determine a dose-response curve because there were no comparable studies previously undertaken in sheep. Finally, between 70 and 90 min after the final injection, each lamb was humanely euthanized by intravenous administration of barbiturate [100 mg/kg pentobarbital sodium (Euthatal); RMB Animal Health, Essex, UK]. Perirenal adipose tissue depots and all major organs were rapidly removed, placed in liquid nitrogen, and stored at ~70°C until subsequent laboratory analysis.

**Chronic Study**

Pairs of lambs were selected on the basis of matched body weight (±10%), and a jugular vein catheter was inserted into each animal under local anesthetic (2% xylocaine) to enable vehicle or leptin administration and blood sampling. Lambs were entered into the study on day 1 of life and injected daily for 6 days at ~9:30 AM with 100 µg of either leptin or vehicle (sterile water). Body weight was measured daily, and blood samples were taken on each study day before treatment. On day 7, each lamb was humanely euthanized, and tissue was sampled as described previously.

**Laboratory Analyses**

**Protein detection.** Mitochondria were prepared from 1 g of frozen lung and BAT (specifically perirenal adipose tissue, which constitutes ~80% of adipose tissue in a newborn sheep) (66), and protein contents of each preparation were determined by the Lowry method (39). Western blotting was utilized to measure the abundance of each protein. Exactly the same amount of protein was loaded onto each gel for every sample. After electroblotting of the polyacrylamide gel onto a nitrocellulose membrane, Ponceau red staining was used to visually confirm that similar amounts of protein had been transferred before subjecting the membranes to immunodetection (46). Abundance of cytochrome c was determined on 10 mg of mitochondrial protein by using an antibody (Santa Cruz, CA) at a dilution of 1:1,000. VDAC
abundance was determined using an antibody raised in rabbits to ovine VDAC, purified from the kidney of a newborn lamb as described by Mostyn et al. (46) and used at a dilution of 1:2,000. UCP1 content was measured as described by Schermer et al. (60). Abundance of UCP2 protein was determined using the same antibody as described by Pecqueur et al. (53) at a dilution of 1:10,000, which was raised against human UCP2. A single band was detected at the same molecular mass as the UCP2 peptide in the postnatal tissues (46, 53). Densitometric analysis was performed on each gel, and all values were expressed in densitometric units. Specificity of detection was confirmed using nonimmune rabbit serum. A range of molecular-mass markers was included on all gels. Densitometric analysis was performed using AIDA (version 2.0, Raytest Isotopenmeßgeräte) on each membrane after image detection, using a Fujifilm LAS-1000 cooled charge-coupled device (CCD) camera (Fuji Photo Film, Tokyo, Japan), and all values are expressed in densitometric units. Specificity of detection was confirmed using nonimmune rabbit serum. A range (10–68 kDa) of molecular-mass markers was included on all gels. All gels were run in duplicate, and a reference sample (an appropriate ovine mitochondrial sample) was included on each to allow comparison between gels.

**Messenger RNA detection.** Total RNA was isolated from BAT using Tri-Reagent (Sigma, Poole, UK) and the expression of UCP2, GR, 11β-HSD1, and 11β-HSD2 mRNA determined by reverse transcriptase-polymerase chain reaction (RT-PCR), as previously described in detail by Gnanalingham and colleagues (28, 29). Briefly, the PCR analysis 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 annealing temperature and cycle number of each primer pair were optimized and used in the linear range for each tissue. Agrose 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 CCD camera, and UCP2, GR, 11β-HSD types 1 and 2, and 18S mRNA 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 and a range of molecular-mass markers. The resultant PCR product was extracted (QIAquick gel extraction kit, Qiagen, catalogue no. 28704) and sequenced, and results were cross-referenced against the GenBank website to determine specificity of the target gene.

**Plasma leptin and NEFA determinations.** Plasma concentrations of NEFA were measured enzymatically (12) and cortisol by radioimmunoassay, as described by Bispham et al. (6). Plasma leptin concentration was determined using a validated double-antibody radioimmunoassay, as described by Delavaud et al. (19). Plasma concentrations of leptin were assayed in duplicate 200-μl samples with a rabbit anti-ovine leptin primary antibody, iodinated ovine leptin, and sheep anti-rabbit secondary antibody. The leptin intra- and interassay coefficients of variation were 4.2 and 9.1% (n = 5), respectively.

**Statistical Analyses.**

All data are presented as means ± SE. Tests of normality as determined by the Kolmogorov-Smirnoff test revealed that the data were nonparametric. Statistically significant (P < 0.05) differences between values obtained from vehicle-control and leptin-treated groups were determined by Mann-Whitney U-test. Significant correlations (P < 0.05) between fetal plasma leptin concentration, physiological, and molecular indexes were undertaken independently by a two-tailed Spearman’s rank order test (SPSS v. 11.0, SPSS) in vehicle and leptin groups on day 7, after 6 days of intravenous vehicle or leptin (100 μg/day), because this was the last time point when all of the measured parameters were available.

**RESULTS**

**Effect of Acute and Chronic Leptin Administration on Plasma Cortisol Concentration in Neonatal Sheep**

There was no significant difference in plasma cortisol concentration between vehicle-control and leptin-treated groups following acute leptin administration (data not shown). There was a decline in plasma cortisol concentration between day 1 and day 6 of postnatal life in both vehicle-control and leptin-treated groups (Fig. 1). By day 6 of chronic leptin administration, plasma cortisol concentration was significantly lower in the leptin-treated group compared with vehicle-control (Fig. 1). In the vehicle group, plasma cortisol concentration on day 6 was negatively correlated (R² = 0.44, P = 0.003) to the mean colonic temperature over 7 days.

**Effect of Acute and Chronic Leptin Administration on UCP2, GR, and 11β-HSD type 1 and 2 mRNA Abundance in BAT**

The abundance of UCP2, GR, and 11β-HSD2 mRNA was decreased (P < 0.01) between 1 and 7 days of postnatal life in BAT, a pattern that was reversed for the abundance of 11β-HSD1 mRNA (Figs. 2 and 3). Acute leptin administration decreased (P < 0.01) the abundance of UCP2, GR, and 11β-HSD1 mRNA and increased (P < 0.01) the abundance of 11β-HSD2 mRNA. This pattern of abundance was reversed with chronic leptin administration. There were no effects of sex on any of the measurements made during the acute leptin study.

A number of significant relationships were observed between plasma leptin and NEFA concentration, for which leptin was persistently raised [e.g., leptin day 6: vehicle, 2.1 ± 0.2; leptin, 17.6 ± 2.7 μg/ml (P < 0.05)], and for physiological and molecular indexes measured during the chronic leptin study in BAT of the vehicle-control and leptin-treated groups at 7 days postnatal life, as outlined in Table 1. UCP2 and GR mRNA were positively correlated with each other in both groups and to 11β-HSD1 mRNA in BAT after leptin administration. In the leptin group, UCP2 mRNA was positively correlated with cytochrome c protein and with plasma leptin and NEFA concentration on day 7. In this group, UCP2 mRNA was also negatively correlated with the mean colonic temperature over the 7-day study period and UCP1 protein as previously published (43). Plasma leptin concentration on day 7 was positively correlated with GR mRNA in the leptin group and with
guanosine diphosphate (GDP) binding in the vehicle group, which was also positively correlated with GR in the vehicle group and with 11β-HSD2 mRNA in the leptin group. In the leptin group, 11β-HSD1 mRNA was positively correlated with plasma leptin on day 7 and negatively correlated with 11β-HSD2 mRNA. Similarly, in the leptin group, 11β-HSD2 mRNA was positively correlated with UCP1 mRNA and protein and with the mean colonic temperature on day 7, as previously published (43). In this group, VDAC was positively correlated with the mean colonic temperature over the 7-day study period. Plasma cortisol on day 6 was positively correlated with GR and 11β-HSD1 mRNA and negatively correlated to UCP1 protein and GDP binding in the leptin group.

**Effect of Chronic Leptin Administration on Mitochondrial Protein Abundance in Neonatal Lung**

Chronic leptin administration decreased ($P < 0.01$) UCP2 protein abundance in the lung (Fig. 4), whereas VDAC and cytochrome c protein abundance were unaffected (data not shown). A number of significant relationships were observed between plasma leptin and NEFA concentration and physiological and molecular indexes measured, as outlined in Table 2. In this regard, mean plasma leptin concentration over 7 days was positively correlated to UCP2 and total lung per kilogram body weight in the leptin group. Plasma leptin on day 7 was negatively correlated to UCP2 in the lungs of controls. Cytochrome c was positively correlated with VDAC protein and with total lung per kilogram body weight in the lungs of the leptin group.

**DISCUSSION**

The exact function and regulation of UCP2 in newborns remain unclear, and this study has highlighted that leptin differentially regulates UCP2 within neonatal BAT and lung, which may be important in the transition from fetal to neonatal life. The observed changes in UCP2 after leptin administration occurred during the decline in plasma cortisol concentration (5) and the establishment of independent ventilation and thermoregulation in the newborn (11). Our findings suggest that leptin has a role in the regulation of UCP2 within neonatal BAT and lung, which may be important in the regulation of lung function in the newborn (29). Clearly, additional studies are warranted to clarify how the present observations directly impact on physiological function and/or regulation.

**Leptin and UCP2 in Neonatal BAT**

We have shown for the first time that chronic but not acute leptin administration upregulates UCP2 mRNA abundance in neonatal BAT and that this effect may be mediated by an
Fig. 4. Effect of chronic (6 days) administration of leptin (100 μg/day) on abundance of UCP2 protein in the lung of neonatal sheep sampled at 7 days postnatal age. Examples of protein expression are given. Values are means ± SE (n = 8–9 per group). *P < 0.05, mean value significantly different from vehicle-treated lambs.

Table 1. Significant relationships in brown adipose tissue between plasma leptin concentration and physiological and molecular indexes measured in sheep neonate subjected to intravenous leptin (100 μg/day) or vehicle for 6 days

<table>
<thead>
<tr>
<th>Group</th>
<th>x-Axis</th>
<th>y-Axis</th>
<th>R²</th>
<th>n</th>
<th>P Value</th>
<th>Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>GR mRNA</td>
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<td>0.40</td>
<td>9</td>
<td>0.013</td>
<td>+</td>
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<td>9</td>
<td>0.020</td>
<td>+</td>
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<td>11β-HSD1 mRNA</td>
<td>UCP2 mRNA</td>
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<td>9</td>
<td>0.005</td>
<td>+</td>
</tr>
<tr>
<td>Leptin</td>
<td>UCP2 mRNA</td>
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<td>0.32</td>
<td>8</td>
<td>0.019</td>
<td>+</td>
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<tr>
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<td>8</td>
<td>0.028</td>
<td>+</td>
</tr>
<tr>
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<td>9</td>
<td>0.005</td>
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<td>9</td>
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<td>Leptin</td>
<td>Mean colonic temperature over 7 days</td>
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<td>9</td>
<td>0.050</td>
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<td>0.010</td>
<td>+</td>
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<td>Mean colonic temperature over 7 days</td>
<td>VDAC</td>
<td>0.32</td>
<td>6</td>
<td>0.037</td>
<td>+</td>
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</table>

Values are means ± SE. UCP, uncoupling protein; GR, glucocorticoid receptor; 11β-HSD, 11β-hydroxysteroid dehydrogenase; VDAC, voltage-dependent anion channel; GDP, guanosine diphosphate; NEFA, nonesterified fatty acids.

increase in glucocorticoid action and lipolysis. Importantly, these responses were observed in the absence of any effect of leptin on food intake or behavior (43). An increase in UCP2 mRNA in neonatal BAT following chronic leptin administration has not been demonstrated previously. Earlier studies have established for UCP1 (13, 48). However, UCP2-null mice (11). The cellular mechanisms by which leptin promotes UCP2 mRNA abundance, but loss of UCP1 protein in neonatal BAT, remains to be elucidated but might involve apoptosis, which has been linked to the transformation of brown to white adipocytes in the adult mouse (35).

It has been shown that intracerebroventricular leptin administration promotes catabolism of adipocytes by stimulating apoptosis in rats, which is characterized by internucleosomal fragmentation of genomic DNA, elevated levels of DNA strand breaks, and a reduction in total DNA content and cellular volume (56). These findings have led to the “adipocyte apoptosis hypothesis,” whereby leptin, acting via brain receptors, causes a hormonal and/or neural signal to be sent to adipose tissue depots (20). Enhanced apoptosis, potentially acting through UCP2, and increased production of reactive oxygen species then lead to increased transcription of endonucleases, proteases, and phospholipases, which ultimately results in apoptotic cell death (20). In vitro studies support a role for UCP2 in adipocyte apoptosis, demonstrating an increased expression of several caspases in preadipocytes overexpressing UCP2 (64). The positive association between UCP2 mRNA and cytochrome c protein noted with raised leptin suggests augmented apoptosis in neonatal BAT following leptin administration (37, 73).

Although the regulation and function of the BAT-specific UCP1 has been well established (13, 44), this is less clear for UCP2 (47). By the ability of UCP2 to uncouple mitochondrial respiration, several rodent studies have implied a role for UCP2 in nonshivering thermogenesis (22, 30), a role well established for UCP1 (13, 48). However, UCP2-null mice maintain their body temperature in a cold environment, in
contrast to UCP1-null mice (47, 48). Moreover, we have shown that BAT UCP2 mRNA is negatively correlated with mean colonic temperature over 7 days and with UCP1 protein in the leptin group, supporting studies that dismiss a direct thermogenic role for UCP2 (47, 48). We have also shown that GR and both plasma leptin and NEFA appear to regulate UCP2 mRNA in neonatal BAT, thereby supporting a role for glucocorticoids in regulating UCP2 (29). Although the activity of UCP1 is tightly regulated by plasma NEFA concentration (24), this has not been shown for UCP2, with in vivo physiological and pathological states associated with a two- to threefold elevation in plasma NEFA not affecting UCP2 mRNA abundance in rodent BAT (69). In rodent and human preadipocyte cell lines, however, unsaturated fatty acids have markedly elevated 11α-HSD1 mRNA and decreasing 11β-HSD2 mRNA. Surprisingly, this pattern was reversed after 6 days of leptin administration, as the relative mRNA abundance changed with age. One reason for the change in response to increasing age is the gradual decline in plasma cortisol that follows the postpartum surge (5). In the present study, we have shown that chronic leptin administration promotes local glucocorticoid action by increasing GR and 11β-HSD1 mRNA and decreasing 11β-HSD2 mRNA, suggesting that these peripheral leptin effects may be independent of any central effects that leptin potentially has on neonatal BAT. Indeed, these adaptations are accompanied by lower plasma cortisol by 6 days of age. Although there are no comparable in vivo studies in the neonatal period, in vitro studies utilizing adipose tissue biopsies from idiopathic obesity patients showed that leptin mRNA was positively correlated with 11β-HSD1 expression and activity. These were both associated with indexes of obesity such as body mass index and fasting insulin (74). Leptin also increases 11β-HSD1 expression and activity in human adipose stromal cells (70). In addition, transgenic mice overexpressing 11β-HSD1 selectively in adipose tissue are hyperleptinemic (41), paralleling the positive association between 11β-HSD1 mRNA and plasma leptin on day 7 in the present study. Interestingly, we found that 11β-HSD2 mRNA, which was reduced by chronic leptin administration, appeared to have a potential role in neonatal BAT heat production, being positively correlated with UCP1, GDP binding, and the mean colonic temperature on day 7 in the leptin group alone. These associations might be important in maintaining thermoregulation, especially when UCP1 abundance is diminished by chronic leptin administration (43). Although these significant correlations do not necessarily indicate clinically significant effects, they do, however, indicate an association between the parameters examined, suggesting potential means of regulation. Additional studies are needed to determine whether these observations underlie potential clinical effects and physiological regulation, as well as functional changes.

**Leptin and UCP2 in the Neonatal Lung**

Chronic leptin administration decreased UCP2 protein in the lung, in contrast with the increase in UCP2 mRNA in BAT, for which we were unable to confirm UCP2 protein in BAT because the antibody raised against UCP2 cross-reacts with UCP1 (53). Although, UCP2 is abundant in the neonatal lung (29, 53), its exact role and function have yet to be determined. Cumulative evidence identifies both fetal and adult lungs as leptin responsive (16, 33, 71), and respiratory anomalies that are common with the obese phenotype (tachypnea, decreased lung compliance, and aberrant respiratory muscle adaptations) are attenuated after prolonged leptin administration in the ob/ob mouse (68). This is the first time that the impact of chronic leptin administration on the abundance of UCP2 in the neonatal lung has been examined. UCP2-deficient mice are resistant to infection with *Toxoplasma gondii*, and their macrophages generate 80% more reactive oxygen species than wild-type mice and have fivefold greater toxoplasmaidal activity in vitro, which is absent in the presence of a quencher of reactive oxygen species (2). This proposed role for UCP2 in macrophage-mediated immunity and limitation of reactive oxygen species has been supported by others (36, 50). It is conceivable that the decreased abundance of UCP2 protein in the lung with chronic leptin administration could promote reactive oxygen species production and maintain host immunity through augmentation of alveolar macrophage phagocytosis and leukotriene synthesis. The impact of a precocious decrease in UCP2 with leptin administration on later lung function has yet to be examined.

Mean plasma leptin over 7 days was positively associated with total lung per kilogram body weight in the leptin group alone, suggesting a potential role for leptin in lung growth. This finding accords with leptin receptor-deficient mice (71) that exhibit a 75% decreased rate of tracheal epithelial proliferation compared with wild-type littermates, emphasizing a potential role for leptin in pulmonary growth. Other studies further suggest a significant impact of leptin on general pulmonary health in rodents both as a growth factor and as a neurohumoral modulator of central respiration (51), as well as on specific respiratory disorders in humans including obesity.
hypventilation syndrome and obstructive sleep apnea (52). Although we did not observe a significant association between UCP2 mRNA and NEFA in the leptin group, Xiao et al. (75) proposed that NEFA regulate lung UCP2 mRNA in both neonatal and adult rodents. In this study, caloric restriction caused a rapid increase in NEFA, and lung UCP2 mRNA was increased by NEFA administration to fed animals. In the sheep, we have not found any evidence of a close relationship among maternal nutrient restriction, plasma NEFA, and acute or chronic changes in UCP2 (29), indicating that other mechanisms are involved in UCP2 regulation in large mammals. In addition, the positive association between the apoptotic mitochondrial proteins VDAC and cytochrome c (37, 73) with raised plasma leptin and total lung per kilogram body weight supports a role for apoptosis in neonatal lung development. In accord with the possible leptin-induced apoptotic increase in UCP2 mRNA within BAT as discussed above, apoptotic activity has been observed during all six (embryonic, pseudoglandular, canalicular, saccular, alveolar, and microvascular) stages of fetal lung development, suggesting its important role during this highly orchestrated process (18). After birth, apoptosis also emerges as an important process after extensive proliferation and subsequent transformation of primary sacules into functional alveoli (8, 61) that could be leptin mediated.

In conclusion, we have shown for the first time that leptin administration to the neonate has differential effects on the abundance of UCP2 in BAT and lung. These effects may be important in the development of these tissues, thereby optimizing lung function and fat growth.

GRANTS

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