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# Long-term hypoxia modulates expression of key genes regulating adipose function in the late-gestation ovine fetus

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**Myers DA, Hanson K, Mlynarczyk M, Kaushal KM, Ducsay CA.** Long-term hypoxia modulates expression of key genes regulating adipose function in the late-gestation ovine fetus. *Am J Physiol Regul Integr Comp Physiol* 294: R1312–R1318, 2008. First published February 20, 2008; doi:10.1152/ajpregu.00004.2008.—A major function of abdominal adipose in the newborn is nonshivering thermogenesis. Uncoupling protein (UCP) UCP1 and UCP2 play major roles in thermogenesis. The present study tested the hypothesis that long-term hypoxia (LTH) modulates expression of UCP1 and UCP2, and key genes regulating expression of these genes in the late-gestation ovine fetus. Ewes were maintained at high altitude (3,820 m) from 30 to 138 days gestation (dG); perirenal adipose tissue was collected from LTH and age-matched, normoxic control fetuses at 139–141 dG. Quantitative real-time PCR was used to analyze mRNA for UCP1, UCP2, 11β hydroxysteroid dehydrogenase type 1 (HSD11B1) and 2 (HSD11B2), glucocorticoid receptor (GR), β3 adrenergic receptor (β3AR), deiodinase type 1 (DIO1) and DIO2, peroxisome proliferator activated receptor (PPAR) α and γ and PPARγ coactivator 1 (PGC1α). Concentrations of mRNA for UCP1, HSD11B1, PPARγ, PGC1, DIO1, and DIO2 were significantly higher in perirenal adipose of LTH compared with control fetuses, while mRNA for HSD11B2, GR, or PPARα in perirenal adipose did not differ between control and LTH fetuses. The increased expression of UCP1 is likely an adaptive response to LTH, assuring adequate thermogenesis in the event of birth under oxygen-limiting conditions. Because both glucocorticoids and thyroid hormone regulate UCP1 expression, the increase in HSD11B1, DIO1, and DIO2 implicate increased adipose capacity for local synthesis of these hormones. PPARγ and its coactivator may provide an underlying mechanism via which LTH alters development of the fetal adipocyte. These findings have important implications regarding fetal/neonatal adipose tissue function in response to LTH.

uncoupling protein; cortisol

THERE IS A SIGNIFICANT DEPOSITION of adipose tissue during the final third of gestation in both sheep and human fetuses, particularly perirenal/abdominal fat (40). Fetal adipose tissue has characteristics of both brown and white fat (9, 13, 15), with brown fat accumulation being maximal at birth (11, 12). A major function of fetal adipose tissue is to provide for effective thermoregulation in response to potential cold exposure in the extrauterine environment postbirth to prevent hypothermia (13).

Mitochondrial uncoupling protein (UCP) 1, a gene highly expressed in brown adipocytes, plays a primary role in adipose-generated thermogenesis in the newborn. Fetal adipose tissue expresses both UCP1, as well as its ortholog, UCP2 (see Ref. 31 for a review). UCP1 catalyzes adaptive thermogenesis in mammalian brown adipose tissue by greatly increasing the proton conductance of the mitochondrial inner membrane (7). Unlike UCP1, the physiological role of UCP2 is less clear, but UCP2 may attenuate mitochondrial production of free radicals and protect against oxidative damage, mediate mitochondrial export of fatty acids and fatty acid peroxides, and may be involved in activated thermogenesis (6). Expression of UCP1 and UCP2 increases dramatically during the final week of gestation in fetal adipose (12, 18, 19, 35), and both UCP1 and UCP2 are highly responsive to endocrine regulation. Administration of the synthetic glucocorticoid, dexamethasone, to pregnant ewes during the week before birth increases UCP1 expression in fetal perirenal adipose (10). Similarly, infusion of cortisol to the ovine fetus during late gestation increases while fetal adrenalectomy decreases UCP1 and UCP2 expression in fetal perirenal adipose tissue (18, 30). These studies implicate a major role for fetal adrenocortical maturation and the concomitant increase in fetal plasma cortisol during the final weeks of gestation in the increase in adipose UCP1 and UCP2 expression. However, because thyroid hormones have been implicated in regulating both UCP1 and UCP2 expression (18) and glucocorticoids induce T3 synthesis, glucocorticoids may exert their maturational actions on fetal adipose thermogenic maturation indirectly via the known effects of these steroids on $T_3$ production in the late-gestation ovine fetus. Sympathetic innervation of adipose during late gestation, coupled with increased $\beta_3$-adrenoreceptor density in adipose, are also likely involved in the increased expression of UCP in the near-term fetus and thermogenesis postbirth (18, 40).

Hypoxia presents a major threat to the well being of the developing fetus. A fetus can experience varying degrees and durations of hypoxia, and chronic, sustained hypoxia is not uncommon during pregnancy. We have developed a model of long-term hypoxia (LTH), in which pregnant ewes are maintained at high altitude (3,820 m) for approximately the last 100 days of gestation (dG) (1, 23). This model of LTH results in a decrease in mean maternal $P_{O_2}$ to ~60 mmHg (mean $P_{O_2}$ measured in animals at altitude) and a concomitant decline in fetal $P_{O_2}$ by approximately 4–5 mmHg without accompanying acidosis or fetal growth retardation. We found that this model of LTH induces significant adaptive changes in the fetal hypothalamo-pituitary-adrenocortical (HPA) axis, resulting in enhanced cortisol production in response to a secondary stressor

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without altering basal cortisol concentrations (1, 24, 34). We also found that LTH alters the capacity of the adrenal medulla to produce catecholamines, in particular, epinephrine (26). Cortisol and catecholamines are major endocrine regulators of fetal organ maturation and adaptive changes enhancing the transition to ex-utero life. We have also observed another adaptive response to LTH: increased fetal plasma leptin concentrations coupled with enhanced adipose leptin expression (16). Therefore, as in adults (25, 36, 41), it appears that leptin expression can be induced by hypoxia in the fetus.

Hypoxia also plays a role in modifying glucocorticoid synthesis. Gnanalingham et al. (17) recently observed that transient hypoxia induced by a sustained (3 day) umbilical cord compression in the late-gestation ovine fetus resulted in an induction of HSD11B2, glucocorticoid receptor (GR), and UCP2 mRNA in fetal adipose tissue. They also noted a decrease in HSD11B1 mRNA in fetal adipose tissue. Thus, the transient hypoxia, associated with acidemia in this model, was capable of modifying potential function of fetal adipose tissue related to thermogenic activity, as well as glucocorticoid regulation. This short-term umbilical cord compression also induces a transient increase in basal fetal cortisol and catecholamine concentrations, unlike our model of LTH.

Considering the capacity of short-term hypoxia to modify expression of key genes in the fetal adipocyte, and our prior observation that LTH can alter fetal adipocyte expression of leptin, we examined the effect of LTH on ovine fetal adipose expression of UCP1, UCP2, HSD11B1, HSD11B2, GR, and deiodinases (DIO) 1, 2, and 3. To explore potential effects of LTH on expression of adipogenic genes, we examined expression of peroxisome proliferator-activated receptor (PPAR) $\alpha$, $\gamma$ and the PPAR$\gamma$ coactivator PGC1$\alpha$. Because LTH also alters fetal adrenomedullary catecholamine biosynthesis, we also examined $\beta_3$-adrenoreceptor expression in fetal perirenal adipose tissue.

## METHODS

*Animals.* All procedures were conducted with the approval of the Institutional Animal Care and Use Committee (Loma Linda University School of Medicine). Pregnant ewes were maintained at the Barcroft Laboratory White Mountain Research Station (elevation 3,820 m) beginning at approximately *day 30* of gestation until 137–138 days of gestation (term = 146 days). Immediately after arrival at Loma Linda University Medical Center Animal Research Facility (elevation: 346 m), they were implanted with an arterial and nonocclusive tracheal catheter, as previously described (1, 23). The maternal $Po_2$ for LTH group was maintained at ~60 mmHg by adjusting humidified nitrogen ($N_2$) gas flow through a maternal tracheal catheter. Normoxic control ewes were maintained near sea level (~300 m) throughout gestation.

Between days 139 and 141 days of gestation, both control and LTH ewes were sedated with pentobarbital, intubated, and maintained under general anesthesia with 1.5–2% halothane in oxygen. Fetuses were then delivered through a midline laparoptomy and immediately euthanized by exsanguination. Perirenal adipose tissue was collected from the anterior pole of the kidney during the process of dissecting the adrenal glands for another study. Immediately after collection, the adipose tissue was frozen rapidly in liquid nitrogen and stored at −80°C until analyzed.

*Quantitative reverse-transcriptase PCR.* Messenger RNAs for UCP1, UCP2, HSD11B1, HSD11B2, GR, iodotyrosine deiodinases DIO1, DIO2, DIO3, PPAR$\alpha$ and $\gamma$, PPAR$\gamma$ coactivator 1 (PGC1$\alpha$) and the $\beta_3$-adrenoreceptor were quantified using quantitative reverse-transcriptase PCR (qRT-PCR); we have previously described and validated the methods for qRT-PCR for a variety of genes in our laboratory (16, 33, 34). Total RNA was prepared from perirenal adipose tissue ($n = 6$ per group for control and LTH fetal sheep) with an RNA preparation kit as per the manufacturer's instructions (Qiagen). Prior to reverse transcription, residual genomic DNA was removed from total RNA with DNase I (1 Unit, 60 min at 37°C; Ambion). The DNase I was subsequently removed from the RNA samples via PCR clean-up columns (Qiagen). An initial denaturation step was performed for 5 min at 95 C prior to first-strand synthesis at 42 C for 50 min. Reverse transcription was then performed using 1 $\mu$g total RNA, with oligo dT as the primer, and Superscript II as reverse transcriptase; the reaction was terminated by heating to 70°C for 15 min.

Real-time PCR was performed using 50 to 100 ng of cDNA (equal to input RNA) per PCR. All PCR were performed in triplicate. Initial qRT-PCRs were performed using serial dilutions of cDNA ranging from 250 to 15.625 ng (250, 125, 62.5, 31.25, 15.625 ng) to determine that the quantity of cDNA used for analysis of each specific mRNA was within the linear range of amplification for each primer. For each mRNA, the starting amount of cDNA for qRT-PCR used was within the linear amplification range. For each primer set, the amplicon was directly sequenced by Sanger dideoxysequencing (Oklahoma Medical Research Foundation Sequencing Core, Oklahoma City, OK) to confirm amplicon identity. SYBR Green (1 × SYBR Green master mix; Bio-Rad) was used as the fluorophore, and PCR was performed using a Bio-Rad iCycler equipped with the real-time optical fluorescent detection system. The primer sequences were derived from ovine or bovine cDNA sequences obtained from the National Center for Biotechnology Information (NCBI). The NCBI accession numbers used are listed in Table 1. A three-step PCR was used: 95°C for 45 s, annealing (primer specific ranging between 55 and 60°C) for 30 s and 72°C extension for 30 s. A total of 35 cycles was performed. A melt curve analysis was conducted on each sample after the final cycle to ensure that a single product was obtained, and agarose gel electrophoresis confirmed that the single PCR product was of the expected size. We used cyclophilin as a "housekeeping" mRNA; the identical first-strand cDNA was used for quantification of specific mRNAs of interest to circumvent any between-run variation. As previously reported (16, 33, 34), we found that cyclophilin and GAPDH are equally efficacious when used as internal housekeeping mRNAs in our real-time PCR application and, unlike GAPDH, cyclophilin is not subject to glucocorticoid regulation. Control PCR for each primer pair and RNA source included *1*) elimination of reverse transcriptase during first-strand cDNA synthesis (ensures that the PCR product depends upon RNA) and *2*) no RNA/cDNA in reverse-transcription reaction (ensures that no amplicon contamination has occurred). Primers were used that provided *1*) a single PCR product (identity confirmed by sequencing), *2*) dilution curve of cDNA exhibited a slope of 100% ± 10% "efficiency," where 100% = $\Delta$3 Ct/log cDNA input (Ct is the threshold PCR cycle at which fluorescence is detected above baseline), and *3*) the melt curve analysis post-PCR must demonstrate one product. For quantification purposes, a synthetic single-stranded DNA standard was used to generate a standard curve (100, 10, 1, 0.1, 0.01, and 0.001 picogram of standard DNA) for extrapolation of starting cDNA concentrations per reaction. Each standard point was run in duplicate and in the same PCR block as the unknowns. Linear regression was used to quantify starting RNA (cDNA) based on Ct values as extrapolated from the standard curve. The efficiency of the standard and primers was 100% based on the above criteria.

*Statistical analysis.* Differences between normoxic control and LTH fetuses in mRNA for each gene of interest were compared using Student's two-tailed *t*-test (GraphPad/Prism Software, ver. 4.0; GraphPad Software). Significance was set at the 0.05 level; all data are presented as means ± SE.

Table 1. *Primer sequences and accession numbers for genes used*

| Gene | | Primer Sequence | NCBI Accession No. |
|---|---|---|---|
| CYCLO | FW RV | 5 -CCATCGTGTGTCAAGGACTTCAT-3 5 -CTTGCCATCTAGCCAGGGTCTT-3 | BT020966 (Bt) |
| UCP1 | FW RV | 5′-CAGTGAAACTCTACAGTGGGCTGC-3′ 5′-TGGTGAAGAACTCCTGGACAGTATC-3′ | XM 616977 (Bt) |
| UCP2 | FW RV | 5′-TTGCTGACCTCATCACCTTTCCCC-3′ 5′-GTAGAACTGCTTGACGGAGTCGTAG-3′ | NM_001033611 (Bt) |
| HSD11B1 | FW RV | 5′-GGGAATCGGAAGAGAAATGGC-3′ 5′-GTAGTGGATGTGGTTGAGAATGAGC-3 | NM_001009395 (Oa) |
| HSD11B2 | FW RV | 5′-TGTGACTCTGGTTTTGGCAACG-3′ 5′-AGACGAGAAGAACAGCAGGCAC-3′ | NM_174642 (Bt) |
| GR | FW RV | 5′-CCCAAGGAAGGCTTGAAGAGTC-3′ 5′-CTCTGGGAATTCAATACTCAT-3′ | EU371026 (Oa) |
| DIO1 | FW RV | 5′-GGTTCCGTAGCAGATTTTCTCATC-3′ 5′-GTTCCAAGGACCAGGTTTACCC-3′ | XM_001252654 (Bt) |
| DIO2 | FW RV | 5′-GACTCGGTCATTCTGCTCAAGC-3′ 5′-TGCCACTGTTGTCACCTCCTTC-3′ | NM_001010992 (Bt) |
| DIO3 | FW RV | 5′-TCATCCAGAGTGGCACCATTATG-3′ 5′-ACGTGTCCATCGGTTGTTTACAC-3′ | AY656759 (Oa) |
| PPARγ | FW RV | 5′-AGGAGAACGATTCGGCTGAAGC-3′ 5′-AAAGGCGGGGTTGTTGTTGGTC-3′ | NM_181024 (Bt) |
| PPARα | FW RV | 5′-TTGACACAGAGATGCCGTTTTG-3′ 5′-TGACGCTTTATCCCCACAGACC-3′ | AF229356 (Bt) |
| PGC1α | FW RV | 5′-GAGATGTGACCACCGAGAATGAG-3′ 5′-GCTGTTGACAAATGCTCTTCGC-3′ | NM_177945 (Bt) |
| $\beta_3$ Adrenergic | FW RV | 5′-TGCTGGTTGCCTTTCTTTGTG-3′ 5′-GCAGTAGATGAGCGGGTTGAAG-3′ | AF109928 (Oa) |

FW, forward; RV, reverse; CYCLO, cyclophilin; UCP, uncoupling protein; HSD11B, 11β hydroxysteroid dehydrogenase; DIO, deiodinase, PPAR, peroxisome proliferator-activated receptor; PGC, PPARγ coactivator; Bt, *Bos taurus*; Oa, *Ovis aries*.

## RESULTS

Development under conditions of LTH resulted in a significant elevation in mRNA concentrations for UCP1 compared with control fetuses (Fig. 1*A*); there was no effect of LTH on concentrations of mRNA for UCP2 (Fig. 1*B*). On the basis of qRT-PCR analysis, UCP1 mRNA was ~10-fold more abundant than UCP2 in fetal perirenal adipose under control normoxic conditions and ~25-fold more abundant compared with UCP2 under conditions of LTH. Using several sets of primers, we noted that mRNA levels for UCP3 were very low compared with either UCP1 or UCP2 (data not shown). Therefore, we did not further quantify mRNA for this gene by real-time PCR. As we have previously reported for other tissues (16, 33, 34), LTH had no effect on cyclophilin mRNA in adipose tissue compared with controls (Fig. 1*C*).

Concentrations of mRNA for HSD11B1 were significantly elevated in LTH compared with control perirenal adipose (Fig. 2*A*). Unlike HSD11B1, perirenal adipose levels of mRNA for HSD11B2 were not altered in response to LTH (Fig. 2*B*). Messenger RNA for HSD11B1 was approximately four-fold greater compared with HSD11B2 in perirenal adipose from control normoxic fetuses and ~15-fold greater than HSD11B2 in adipose from LTH fetal sheep. Glucocorticoid receptor mRNA concentrations in adipose tissue did not differ between LTH and control fetal sheep (Fig. 2*C*).

During our initial RT-PCR for the iodothyronine deiodinases, we found that fetal adipose expressed both DIO1 and DIO2, consistent with previous studies measuring enzymatic activity of the two deiodinases in perirenal adipose obtained from neonatal lambs (12). However, levels of DIO3 mRNA were very low (orders of magnitude) compared with DIO1 and DIO2. As a control for potential primer failure, we performed RT-PCR from two tissues known to express all three deiodinases (hypothalamus and pituitary) (5) and a tissue not known for expressing high levels of deiodinase (adrenal). As predicted, all three deiodinases were detected in both the hypothalamus and anterior pituitary, while the adrenal expressed only very low levels of DIO3 (Fig. 3*A*). Using qRT-PCR, we observed that mRNA for DIO1 and DIO2 was significantly elevated in perirenal adipose of LTH fetuses compared with controls (Fig. 3, *B* and *C*). In control fetal sheep, the levels of mRNA for DIO1 in perirenal adipose were approximately twofold higher compared with DIO2, and this ratio increased to ~10-fold higher in response to LTH.

The effects of LTH on mRNA levels for the PPARγ, PPARα, and PGC1α in fetal perirenal adipose tissue are illustrated in Fig. 4. LTH significantly elevated mRNA concentrations for fetal adipose PPARγ ($P < 0.05$) and PGC1α (Fig. 4, *A* and *C*). In marked contrast, PPARα mRNA was unaffected by LTH (Fig. 4*B*), and overall expression in fetal perirenal adipose was approximately five-fold (control) to 20-fold (LTH) lower compared with PPARγ. A significant increase in $\beta_3$-adrenoreceptor mRNA was also observed in perirenal adipose obtained from LTH compared with control fetuses (Fig. 5).

## DISCUSSION

We previously reported that LTH significantly elevated both fetal plasma leptin concentrations and leptin mRNA levels in adipose tissue in the late gestation ovine fetus (16), indicating that development under conditions of sustained moderate hypoxia, not associated with acidemia or fetal growth restriction, is capable of altering adipose function. In the present study, we extend these observations and show that LTH up-regulates UCP1, a mitochondrial protein integral in nonshivering thermogenesis in the newborn. Further, we demonstrate that expression of both HSD11B1 and DIO1 and DIO2 are enhanced in fetal adipose in response to LTH, thus increasing potential for local glucocorticoid and thyroid hormone regulation of adipocyte function. We also show that mRNA for PPARγ and its coactivator, PGC1α, are increased in fetal adipose in response to LTH, providing a potential mechanism via which LTH alters expression of a cassette of genes governing the differentiation and function of this important tissue.

To our knowledge, only one previous study has examined the effect of hypoxia on gene expression in adipose tissue in the late-gestation ovine fetus (17). In that study, Gnanalingham and coworkers (17) used umbilical cord occlusion (30% reduction in umbilical blood flow for 3 days) to induce fetal hypoxemia. In addition to hypoxemia, umbilical cord occlusion induced fetal acidemia and also activated the fetal HPA axis, resulting in a transient but significant elevation in fetal ACTH and cortisol concentrations. They noted that umbilical cord occlusion induced an increase in adipose UCP1, UCP2, GR,

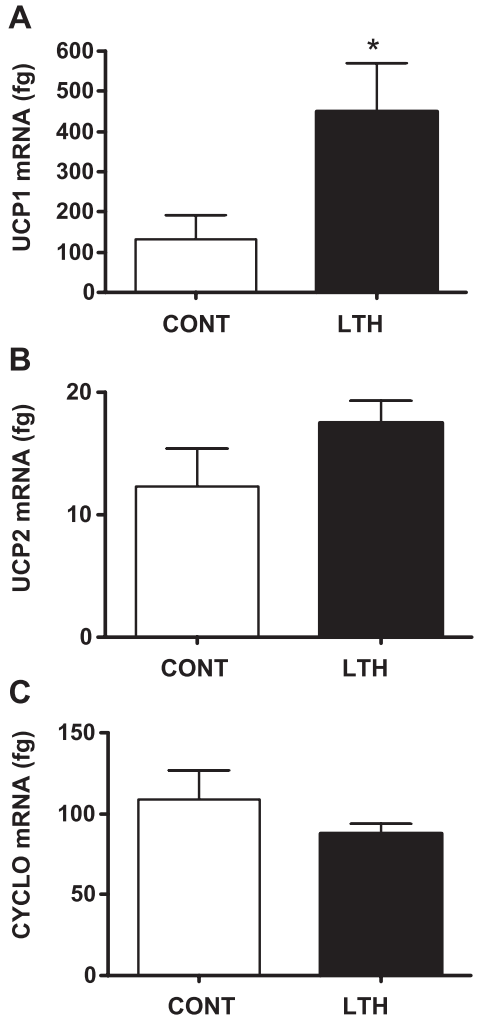


Fig. 1. Messenger RNA concentrations (fg mRNA per 50 ng total RNA) for Uncoupling Protein 1 (UCP1) (*A*), UCP2 (*B*), and cyclophilin (CYCLO; *C*) in adipose tissue from long-term hypoxia (LTH) and control fetal sheep. LTH significantly increased UCP1 (*$P > 0.05$) but not UCP2 or cyclophilin mRNA concentrations. UCP1 mRNA concentrations were ~10-fold more abundant than UCP2 in control adipose tissue and ~25-fold more than UCP2 in the LTH adipose ($n = 6$ per group).

and HSD11B2 expression, while decreasing expression of HSD11B1. The expression of these genes was significantly correlated to fetal plasma cortisol concentrations. In accordance with previous observations that the late-gestation increase in fetal plasma cortisol, as well as synthetic glucocorticoids, enhance UCP expression and thermogenic potential of fetal adipose tissue (10, 18, 30), these authors attributed changes in expression of these key genes in fetal adipose in response to cord occlusion to activation of the endocrine stress axis rather than hypoxia per se.

In contrast to the Gnanalingham et al. study (17), our model of LTH results in development under conditions of a moderate sustained hypoxia not associated with increases in basal concentrations of either fetal plasma cortisol or catecholamines (1, 26). However, LTH does alter HPA axis function, since cortisol production is enhanced in response to a secondary stressor, while the epinephrine levels achieved in response to a secondary stressor are severely attenuated (23, 26). Thus, it is possible that the LTH fetus, over time, may be subjected to short episodes of increased cortisol in response to occasional stresses such as transient, positional occlusion of the umbilical cord as occurs during pregnancy. However, the lack of growth retardation of the LTH fetus and normal timing of birth (a fetal cortisol-driven event in sheep) support that the fetus is not subject to deleterious levels of cortisol in this model of LTH. Thus, the changes that we observed in the expression of key genes in adipose tissue are likely dependent, at least initially, on the hypoxic environment. That said, the selective increase in HSD11B1 compared with HSD11B2, in adipose tissue would provide a mechanism for the local production of cortisol and subsequent activation of the GR without having to expose the entire fetus to the maturational/growth-retarding effects of this glucocorticoid.

In addition to the increased potential for local synthesis of cortisol, we noted an increase in expression of DIO1 and DIO2. DIO2 is the major enzyme converting $T_4$ to $T_3$. Although the kinetically inefficient enzyme DIO1 ($K_m$ of $\sim 1 \times 10^{-6-7}$ vs.

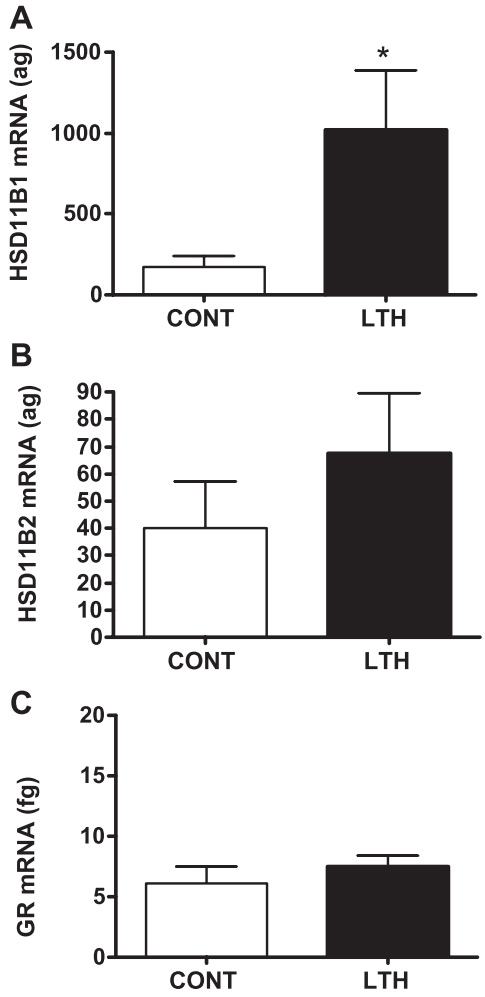


Fig. 2. Messenger RNA concentrations for 11β hydroxysteroid dehydrogenase type 1 (HSD11B1; *A*) and type 2 (HSD11B2; *B*) (ag mRNA per 50 ng total RNA) and glucocorticoid receptor (GR; *C*) (fg mRNA per 50 ng total RNA) in perirenal adipose tissue from LTH and control fetal sheep. LTH significantly increased HSD11B1 (*$P < 0.05$) but not HSD11B2 mRNA concentrations. HSD11B1 mRNA was approximately four-fold more abundant compared with HSD11B2 in fat from control fetuses and ~15-fold more abundant compared with HSD11B2 in LTH adipose ($n = 6$ per group).

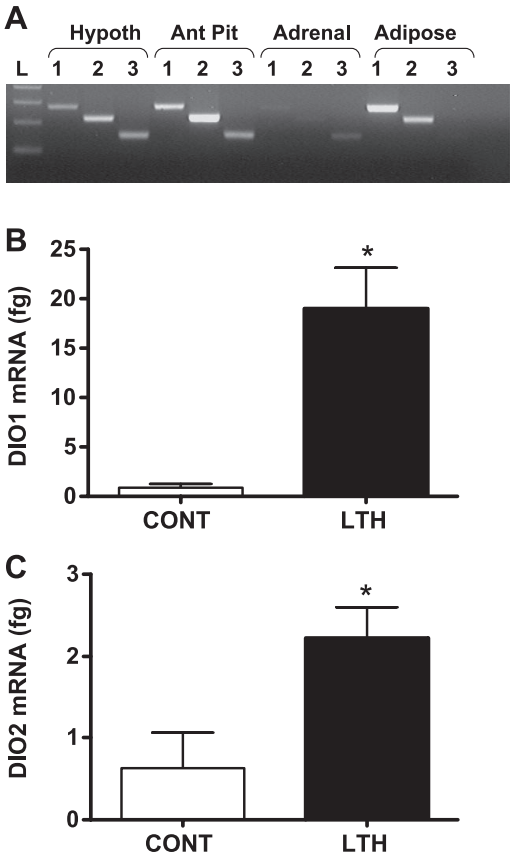


Fig. 3. *A*: representative ethidium bromide stained agarose gel of RT-PCR for iodothyronine deiodinase type 1 (DIO1; *lane 1*), DIO2 (*lane 2*) and DIO3 (*lane 3*) using total RNA (50 ng/reaction) from fetal hypothalamus (Hypoth), anterior pituitary (Ant Pit), adrenal gland (Adrenal) and adipose (L = DNA ladder). *B*: messenger RNA levels for DIO1 in adipose tissue from LTH and control fetal sheep (fg mRNA per 50 ng total RNA). LTH significantly increased DIO1 mRNA concentrations ($*P < 0.01$) compared with control fetuses. *C*: messenger RNA levels for DIO2 in adipose tissue from LTH and control fetal sheep (fg mRNA per 50 ng total RNA). LTH significantly increased DIO2 mRNA concentrations ($*P < 0.02$) compared with control fetuses ($n = 6$ per group).

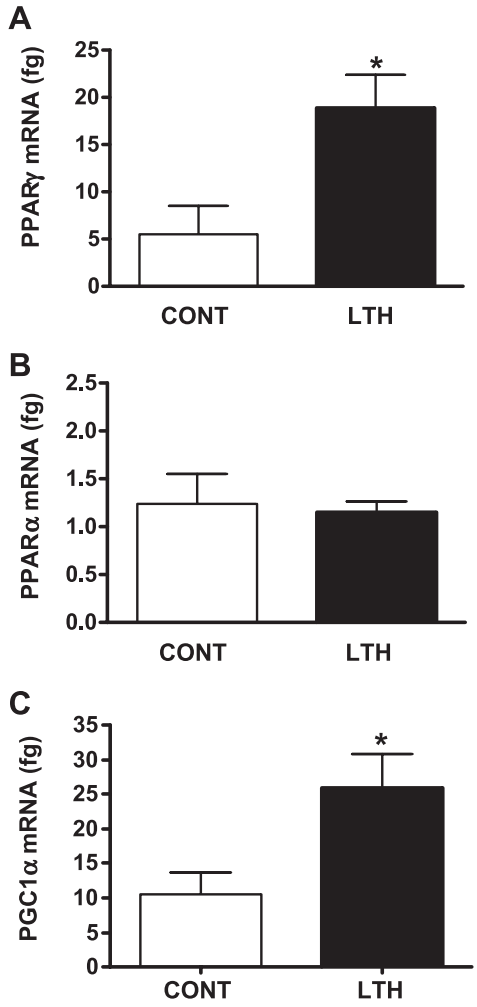


Fig. 4. Messenger RNA concentrations (fg mRNA per 50 ng total RNA) for PPARγ (*A*), PPARα (*B*), and PGC1 (*C*) in perirenal adipose tissue from LTH and control fetal sheep. LTH significantly elevated mRNA concentrations for the PPARγ ($P < 0.05$) and PGC1α ($*P < 0.03$) in perirenal adipose tissue compared with control fetuses ($n = 6$ per group).

$\sim 1 \times 10^{-9}$ M) can activate or inactivate T4 on an equimolar basis, the physiological role of this enzyme in tissues such as fat remains equivocal (5). Triiodothyronine has been shown to increase the expression of UCP1 via the thyroid hormone (TR) receptor response elements in the 5′-flanking region of the UCP1 gene (8, 22). Triiodothyronine also regulates numerous other aspects of mitochondrial function related to thermogenesis. Mostyn and coworkers (30) noted that cortisol administration to adrenalectomized fetal sheep resulted in increased UCP1 expression that correlated to plasma $T_3$ levels, suggesting a permissive role for thyroid hormones in glucocorticoid induced UCP1 expression. However, because glucocorticoids have been implicated in directly increasing DIO2 expression (2), the increase in DIO2 that we observed in the present study may be in response to the increase in HSD11B1 and local cortisol production rather than hypoxia per se.

As we previously noted (16), leptin expression and plasma concentrations of leptin are elevated in the late-gestation LTH ovine fetus. Leptin has also been implicated in UCP1 expression and alters the structural characteristics of fetal adipose tissue (39, 43). In adult mice, central administration of leptin leads to an induction of UCP1 expression in adipose (29); thus, this adipokine may act locally or centrally in the regulation of thermogenic potential of adipose tissue. Because leptin is a hypoxia-inducible gene (25, 36, 41), the increase in adipocyte expression of this key adipokine may represent a primary response of this tissue to the sustained hypoxic conditions. In

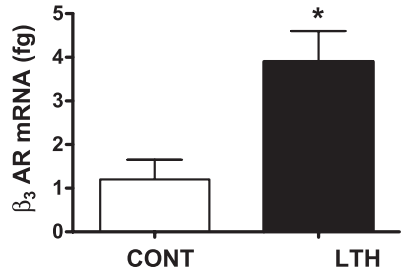


Fig. 5. Messenger RNA concentrations (fg mRNA per 50 ng total RNA) for the β3 adrenergic receptor in perirenal adipose tissue from LTH and control fetal sheep. LTH significantly ($*P < 0.05$) elevated mRNA concentrations for the β3 adrenergic receptor in adipose tissue compared with control fetuses. ($n = 6$ per group)

addition to UCP1 regulation, leptin has been implicated as an angiogenic factor for adipose tissue, ensuring adequate blood supply during adipogenesis. Glucocorticoids have also been shown to increase leptin expression both in vivo and in vitro (14); thus, the increase in HSD11B1 again may provide a local mechanism for the induction of yet another glucocorticoid-responsive gene in fetal adipose tissue in response to LTH. Clearly, future studies are warranted to decipher the roles of hypoxia vs. glucocorticoids in the changes in gene expression observed in the present study.

PPARs, particularly PPARα and PPARγ, have been demonstrated to play key roles in differentiation of both brown and white adipocytes, as well as being key regulators of UCP expression (42). Indeed, PPARγ is essential for adipocyte differentiation (38). The preferential expression of PPARα in brown vs. white adipocytes implicates this form of PPAR in regulation of UCP1 gene expression (3, 27). However, agonists of PPARα and PPARγ can induce UCP1 in brown fat (27), and PPARα-null mice exhibit normal expression of adipose UCP1 (28). Thus, PPARγ may play a major role in UCP1 regulation during development and adipocyte differentiation. In the present study, we noted a significant elevation in expression of PPARγ with no change in mRNA for PPARα. Since UCP1 was selectively elevated compared with UCP2 (a trend for being increased), this differential effect of LTH on UCP1 vs. UCP2 may be linked to the selective induction of PPARγ in comparison to PPARα. We also noted an increase in PPARγ coactivator, PGC1α, in response to LTH. PPARγ-induced transcription of UCP1 is dependent upon its interaction with PGC1α, as is the PPARγ capacity to induce brown adipocyte differentiation (37). PGC1α also serves as a coactivator of other nuclear receptors, including PPARα and the thyroid receptor in inducing the expression of all of the genes required for a complete thermogenic response in brown adipocytes. The induction of PPARγ and PGC1α in response to LTH may represent a key integrative component in the overall changes in gene expression that we have observed in the present study. A recent study demonstrating that central administration of leptin selectively increases expression of PPARγ in comparison to PPARα implicates leptin as a potential driving factor in the induction of PPARγ and its critical coactivator and reinforces the potential for hypoxic induction of leptin as the primary mediator of the changes in expression of the numerous key thermogenic genes observed in the present study.

Although PPARs play a key role in the regulation of UCP1, stimulation of UCP1 occurs through activation of $\beta_3$-adrenergic receptors, which are found primarily in both brown and white adipose tissue (21, 32). $\beta_3$-adrenergic receptor agonists have been shown to stimulate UCP1 with subsequent thermgoenesis in mice (20). Although this appears to be primarily through activation of UCP1 in brown adipose tissue, studies in knockout mice indicate that white adipose tissue is also involved (21). Of interest, on the basis of our above findings showing an increase in PGC1α mRNA, activation of $\beta_3$-adrenergic receptors increases expression and stabilize PGC1α (4). To our knowledge, the data in the present study are the first to examine $\beta_3$-adrenergic receptor expression in fetal adipose tissue. The enhanced expression of $\beta_3$-adrenergic receptors suggests an increased sensitivity to sympathetic activation of adipose tissue at birth in the LTH fetus.

*Perspectives and Significance*

On the basis of our present findings, development under conditions of long-term hypoxia in the ovine fetus results in enhanced expression of key genes regulating endocrine control of adipocyte differentiation and function and expression of an essential gene mediating nonshivering thermogenesis, UCP1. Underlying the increase in expression of these key genes, we found a significant elevation in the expression of the nuclear receptor PPARγ and its important coactivator PGC1α. PPARγ mediates the development and differentiation of brown adipose tissue, in particular, and as such, may represent a major regulator for the coordinated changes in expression of these genes in response to LTH. We speculate that the changes in adipocyte function could be beneficial to a fetus born into a hypoxic environment such as high altitude, aiding in its survival and growth in this environment. However, at lower altitudes, if these changes in gene expression persist postbirth and even to adulthood, they could impact energy balance and propensity for adiposity and perhaps contribute to dysregulation of metabolism. Clearly, future studies are warranted to delineate the mechanisms through which LTH mediates these critical changes in gene expression and to discover whether this altered gene expression persists postbirth through adulthood with the subsequent effects on adipose tissue.

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