Fetal endocrine and metabolic adaptations to hypoxia: the role of the hypothalamic-pituitary-adrenal axis

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Newby EA, Myers DA, Ducsay CA. Fetal endocrine and metabolic adaptations to hypoxia: the role of the hypothalamic-pituitary-adrenal axis. Am J Physiol Endocrinol Metab 309: E429–E439, 2015. First published July 14, 2015; doi:10.1152/ajpendo.00126.2015.—In utero, hypoxia is a significant yet common stress that perturbs homeostasis and can occur due to preeclampsia, preterm labor, maternal smoking, heart or lung disease, obesity, and high altitude. The fetus has the extraordinary capacity to respond to stress during development. This is mediated in part by the hypothalamic-pituitary-adrenal (HPA) axis and more recently explored changes in perirenal adipose tissue (PAT) in response to hypoxia. Obvious ethical considerations limit studies of the human fetus, and fetal studies in the rodent model are limited due to size considerations and major differences in developmental landmarks. The sheep is a common model that has been used extensively to study the effects of both acute and chronic hypoxia on fetal development. In response to high-altitude-induced, moderate long-term hypoxia (LTH), both the HPA axis and PAT adapt to preserve normal fetal growth and development while allowing for responses to acute stress. Although these adaptations appear beneficial during fetal development, they may become deleterious postnatally and into adulthood. The goal of this review is to examine the role of the HPA axis in the convergence of endocrine and metabolic adaptive responses to hypoxia in the fetus.

fetus; cortisol; adipose; hypoxia

MAMMALIAN FETUSES, and in particular fetuses from long-gestational length pregnancies such as humans (primates) and ruminants, have the ability to respond to and/or adapt to stress during gestation to survive the potentially harsh intrauterine environment and continue to term. Postbirth, both the hypothalamic-pituitary-adrenal (HPA) axis via cortisol and the adrenomedullary/sympathetic nervous system via catecholamines serve as homeostatic regulators in response to acute and chronic stress. In larger mammalian fetuses, these systems exhibit maturation in late gestation and serve similar roles, providing the fetus with the means to respond to intrauterine stressors. Activation of these two mechanisms leads to rapid glucose, renal, and cardiovascular changes (35, 62, 88, 180) as well as slower adaptive modifications of gene expression to combat potential long-term effects (3, 4, 51, 120, 181). Although these responses to hypoxic stress may often be beneficial acutely, they have the potential to be deleterious, especially under sustained periods of hypoxic stress.

The influence of hypoxia during fetal development is of particular importance due to its potential to induce or “program” alterations in endocrinology and metabolism long after birth. It has been recognized for more than two decades that an “adverse intrauterine environment” could lead to offspring predisposed to a variety of related disorders, including cardiovascular disorder, metabolic disorder, and obesity as adults. This so-called programming, also referred to as the “fetal origins of adult disease hypothesis,” describes how an adverse intrauterine environment can trigger adaptive or maladaptive changes in the developing fetus to overcome the hostile conditions and survive (9, 12, 13, 15, 147). This adverse environment imprinted during development can increase the susceptibility of the fetus to acquire cardiovascular and metabolic pathologies later in life (10, 15–17, 45, 46, 97, 111). Although the original hypothesis was derived largely from observations of offspring from malnourished or undernourished pregnancies, later studies have expanded to include the impact of a variety of so-called intrauterine stressors. This has been reviewed in more detail by Godfrey and Barker (69) and Calkins and Devaskar (30).

Fetal hypoxia is a common stressor that occurs during pregnancy as the result of a variety of situations, including maternal under- or malnutrition, preeclampsia, preterm labor, smoking, heart or lung disease, obesity, and exposure to high altitude (14, 40, 48, 73, 91, 125, 150). Therefore, due to its prevalence, hypoxia likely plays a key role in the influence of an adverse intrauterine environment on the developing fetus. The impact of hypoxia on the fetus is dependent on a wide range of variables, including gestational age, severity, and duration of hypoxia, as well as confounders such as acidemia and hypercapnia.

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When considering changes in response to hypoxic stress, the HPA axis is key due to its role in growth and maturation of the fetus. The HPA axis, through regulation of glucocorticoid biosynthesis (138, 157), dictates differentiation and maturation of key organ systems, including lung, liver, and kidney, and regulation of metabolism, including lipolysis, glycogenolysis, and protein catabolism (37, 108, 115). Acutely, activation of the HPA axis leads to a significant increase in cortisol (2, 23, 24, 80, 87), a glucocorticoid that plays a critical role in governing metabolism by influencing plasma glucose, lipid, and protein concentrations as well as immune regulation, inflammation, and cardiovascular function. Under chronic stress conditions, cortisol production is associated with hyperglycemia, immune suppression, excess adipose deposition, bone loss, and hypertension (38, 148, 169). Therefore, the ability of the fetal HPA axis to adapt to limit cortisol production under conditions of chronic stress is crucial for maintaining normal development during gestation. The HPA axis must mature to permit the normal ontogenic rise in cortisol in preparation for birth (19, 76, 105, 126, 138, 157) while allowing for an acute response to a secondary stessor.

Another key regulatory mediator influenced by hypoxia in the fetus is perirenal adipose tissue (PAT). In sheep, ~80% of fetal adipose tissue deposition occurs in the perirenal-abdominal region (165). During late gestation, fetal mass expands and adipose tissue develops and responds to hormonal and nutritional perturbations that can alter lipid storage and release as well as induce secretion of leptin (140). Early changes in adipose function in response to hypoxia may play a role in fetal programming due to the influence of leptin and gene expression on metabolic processes and the possible overlap between leptin and cortisol regulation.

One of the biggest roadblocks to advancement of our understanding of fetal adaptive responses to hypoxia is an appropriate model. Because of obvious ethical considerations, there are little data on the effect of hypoxia on endocrine and metabolic alterations in human fetuses. Additionally, although there are programming studies of the effects of hypoxia in rodents, due to the small size and developmental maturity of the fetus, they are not ideal for fetal endocrine and metabolic studies. Fetal studies have also been conducted in nonhuman primates, but they are limited due to the tremendous cost and lack of availability of animals. The sheep has become a major animal model for studying the impact of hypoxia on the developing fetus due to its relatively long gestational period, similarity of endocrine and physiological systems, and relative ease of fetal and maternal instrumentation.

Throughout this review, we will highlight key findings in relation to the impact of hypoxia on the complex interactions between the endocrine and metabolic responses of the fetus in the fetal HPA axis and PAT. Although as described previously the majority of information has been derived from studies utilizing the ovine fetus, wherever possible we will draw correlates from human and nonhuman primate studies.

**Acute Hypoxia**

As described above, from a clinical perspective, fetal hypoxia can occur as a result of a wide range of maternal conditions. In an effort to mimic some of these conditions, multiple models of hypoxia have been developed, varying in degree and duration. Acute hypoxia can be induced through maternal hypoxia (6, 42), blood flow restriction (ischemia) (177), or umbilical cord occlusion (UCO; asphyxia) (63, 70, 172) for a duration of a few minutes to several hours. Differential responses may occur in response to varying degrees of altered PO2, and hypoxia ischemia may be associated with metabolic changes; however, the key activator of the fetal stress response is the decrease in PO2. In response to acute hypoxia, there is a rapid release of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) from the hypothalamus, which triggers adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary, followed by glucocorticoid production in the fetal adrenal cortex, proportional to the degree and duration of hypoxia. This swift response of the HPA axis to acute stress emphasizes the critical role glucocorticoids play in limiting the physiological impact of stress and maintaining fetal homeostasis.

Several studies conducted in the fetal sheep examined the effects of acute hypoxemia induced by reduction in maternal oxygen or by restriction of uteroplacental blood flow by UCO. Akagi and Challis (6) showed that moderate maternal hypoxia (PO2 reduced by 8.4 mmHg) for 1 h increased fetal plasma AVP and ACTH in 106- to 117-days gestation (dG) fetuses. In later-gestation fetuses (131 dG), Unno et al. (172) observed increased fetal plasma ACTH and cortisol concentrations following a 50% reduction in blood flow by UCO. Furthermore, several studies found that acute episodes (1–48 h) of fetal hypoxemia (induced by maternal hypoxia or UCO) resulted in increased CRH mRNA in the fetal hypothalamus, propiomelanocortin (POMC) mRNA in the fetal pituitary, and increased circulating AVP, ACTH, and cortisol concentrations in fetal plasma (7, 24, 26, 36, 87, 106, 129, 149, 161). Although the response of the HPA axis to stress is best seen in the late-gestation fetus, as the fetal HPA has matured and become fully responsive (58), changes in fetal plasma cortisol concentrations in response to acute hypoxemia have been reported in the ovine fetus as early as 120 dG (23). Together, the results of these studies exemplify the integrated response of the HPA axis to acute hypoxic stress.

While the response to an acute hypoxic insult results in upregulation of the HPA, prolonged elevated cortisol levels lead to fetal growth restriction and, in ruminants, activation of the parturition cascade and early birth of the small fetus (66, 154). To further examine the effects of hypoxia as a fetal stress, the response of the fetal HPA axis to repeated hypoxic perturbations or prolonged hypoxia over the course of several days has been investigated. Unno et al. (172) found that after repeated UCOs, fetal anterior pituitary responsiveness was maintained with increased levels of plasma ACTH released after each UCO, but adrenocortical responsiveness was blunted; despite elevated ACTH, cortisol levels remained similar to basal levels by the 12th UCO. Green et al. (70) subjected 112- to 116-dG fetal sheep to repeated UCOs and saw increased plasma ACTH and cortisol concentrations, but this response was attenuated after 4 days. These studies show that whereas fetal CRH/AVP and ACTH remained elevated in fetal plasma, cortisol returned to basal levels by the end of the hypoxic insult.

As term approaches, glucose production increases as cortisol and catecholamine concentrations increase in the fetus (57). In its role as a glucocorticoid, cortisol regulates metabolism by influencing plasma glucose concentrations. Along with cortisol, plasma glucose levels and fetal growth...
and development are also regulated by insulin, a hormone secreted from pancreatic β-cells in response to increased plasma glucose concentrations that stimulates cellular uptake of glucose (56). Insulin secretion is tightly coupled to plasma glucose concentration, maintaining a relatively constant insulin-to-glucose ratio. However, in response to an acute hypoxic challenge, several studies found that the fetus had decreased insulin secretion (18, 84, 182) accompanied by increased norepinephrine and epinephrine secretion (18, 41) and increased cortisol and corticosterone secretion (83). Further studies showed that hypoxic stress acts through an α2-adrenergic mechanism to induce inhibition of insulin secretion (82, 84, 98, 158). Jackson et al. (82) showed that acute hypoxemia (2 h, 126–128 dG) in the sheep fetus resulted in increased catecholamine secretion and reduced insulin concentrations, suggesting that fetal insulin production is mediated in part through sympathoadrenal stimulation. Together these studies suggest that glucocorticoids and catecholamines play a role in regulating insulin and glucose in response to hypoxia. Limesand et al. (100) observed that the increase in glucocorticoid elevated plasma glucose and circulating catecholamines prevented hyperinsulinemia, but together they resulted in hyperlactacemia and hypoparacort, showing a direct impact on fetal metabolism. In response to hypoxia, however, gluconeogenesis was initiated, and the excess lactate generated was used as a substrate for hepatic glucose production (100). This sympathoadrenal suppression of insulin secretion may act as a mechanism to conserve glucose and oxygen for essential organs such as the brain and heart (28, 64) but if sustained could result in reduced birth weight (82). In the human fetus, Zamudio et al. (187) found that women living at high altitude experienced chronic hypoxia that resulted in intrauterine growth restriction (IUGR), which was potentially initiated by fetal hypoglycemia; there were decreased circulating fetal glucose concentrations and consumption. This suggests altered placental metabolism that spares oxygen for fetal use but limits glucose availability for fetal growth. IUGR as a result of altered glucose metabolism has also been reported in a rat model of hypoxia by Lueder et al. (104). They observed that maternal exposure to 5 days of 10% ambient oxygen in the third trimester resulted in similar fetal plasma glucose concentrations between hypoxic and control but increased relative glucose utilization of hypoxic fetal tissues accompanied by acidosis, suggesting anaerobic metabolism and increased glycolysis in the hypoxic fetus.

In response to changes in metabolism, cortisol works to restore homeostasis to allow for the continued growth and development of the fetus. In the case of recurring acute hypoxic stress, continuous bursts of cortisol can become detrimental to fetal development, as excess glucocorticoids have been shown to lead to a growth-restricted fetus that is often delivered preterm (20, 66, 113, 127, 141, 154, 172). From these results, the ovine fetus has demonstrated an adaptation in the HPA axis, where there is dissociation in the response between the hypothalamic-pituitary axis and the adrenocortical response to brief repeated hypoxic stress or prolonged hypoxia over several days. Whereas CRH/AVP and ACTH levels remain elevated, cortisol returns to basal levels to allow for normal growth and development of the fetus.

### Chronic Hypoxia

Experimentally, chronic hypoxia (over days to weeks or even months) can be initiated early or late in gestation and can be induced through placental embolization (29, 61), placental restriction, secondary to nutrient restriction (53, 143), or by high altitude resulting in moderate continuous hypoxia with normal pregnancy duration and no accompanying growth restriction (2, 75, 80, 89). Because the HPA axis matures in the latter third of gestation and increases in responsiveness as the fetus nears term (32, 95, 96, 124, 132, 134, 146, 173), studies often measure the effects of hypoxia in late gestation.

Gagnon et al. (61) and Murotsuki et al. (116) examined the effects of fetal placental embolization (30% reduction in arterial PO2) for 10 days in 122-dG sheep. It resulted in progressive hypoxemia with reduced fetal plasma ACTH but increased prostaglandin E2 (PGE2) and maintained cortisol (61, 116). Infusions of PGE2 have been shown to increase cortisol levels in fetal sheep (78, 99, 103, 167, 168, 183, 184), and PGE2-induced cortisol production was not affected by hypophysectomy (99). This suggests that PGE2 may be involved in an adaptation to maintain basal fetal cortisol levels when ACTH is reduced and indicates that additional factors other than ACTH play a role in regulating cortisol production in the ovine fetus.

Another method of experimentally induced chronic hypoxia is through placental restriction (PR) via caruncletomy (143, 145). Phillips et al. (135) performed caruncle tomies prior to mating to reduce the number of placentomes formed in ewes. This resulted in gestational hypoxia, with fetal arterial PO2 reduced by 30%. This highly successful model by Phillips et al. (135) allows for hypoxia throughout the entire course of gestation. However, the hypoxia is accompanied by nutrient restriction and IUGR. Due to PR-induced hypoxia, there was decreased POMC mRNA in the fetal pituitary and higher cortisol levels compared with control despite similar levels of plasma ACTH at 140 dG (135). From these results, they hypothesized that the HPA axis adapts to operate at a new set point in the growth-restricted fetus in response to nutrient restriction.

The fetal response to stress includes changes in cardiovascular and metabolic elements regulated by catecholamines (3, 43, 130, 136). Regulation of phenylethanolamine N-methyltransferase (PNMT), the enzyme responsible for catecholamine production, is classically thought to be affected by glucocorticoids in most species (21, 166, 179), with increases in glucocorticoid stimulating PNMT expression in adrenomedullary cells (105, 159, 174). However, in growth restriction models of hypoxia via caruncle tomies, Coulter et al. (44) showed that dopamine β-hydroxylase (DBH) and encephalin-containing peptide immunostaining was decreased, and Adams et al. (4) observed decreased PNMT expression. In high-altitude induced long-term hypoxia (LTH), fetal plasma epinephrine concentrations were shown to be attenuated in response to superimposed acute hypoxia compared with normoxic controls despite similar basal plasma catecholamine levels, with no changes in norepinephrine (90). Glucocorticoid receptor expression was unaffected by LTH in the adrenal medulla; however, there were deficits in catecholamine biosynthesis and decreased expression of PNMT, tyrosine hydroxylase, and DBH that may be due to decreased nicotinic receptor subunit expression in the LTH fetal adrenal (51). These changes in

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catecholamine regulation indicate reduced sensitivity to glucocorticoids in the LTH fetus and could affect the transition from fetus to newborn, impacting both metabolism and cardiovascular function.

The effects of chronic hypoxia have also been investigated in a high-altitude-induced LTH ovine model, where ewes are maintained at 3,820 m beginning at approximately day 40 of gestation and continuing through to near-term (139–141 dG, term is ~145 days, hypoxic fetal Po2 is ~18 mmHg, and normoxic is ~23 mmHg). In this model, the fetus has adapted to hypoxia such that pregnancies are of normal duration, fetuses are not growth restricted, and there is no accompanying acidosis (75, 89). Initial studies examining the effects of LTH on the ovine fetus showed that basal immunoreactive ACTH and cortisol concentrations were similar to normoxic control fetuses (75, 118). However, subsequent studies revealed that LTH stimulated hypothalamic drive, which enhanced expression of POMC and processing to ACTH with increased concentrations of ACTH1–39 and key POMC precursors (POMC and 22-kDa ACTH) in plasma (118). Despite higher basal levels of ACTH1–39, cortisol concentrations were not increased above normoxic controls in near-term fetuses.

This apparent discordance between elevated ACTH and normal basal cortisol levels became even more interesting in response to a superimposed acute secondary stressor. Surprisingly, in the LTH fetuses in response to hypotension or UCO, both ACTH and cortisol increased, but the cortisol response was greater compared with the response in normoxic fetuses (2, 80, 117). Further studies by Myers et al. (120) demonstrated reduced expression of ACTH receptor (ACTH-R), CYP17, and CYP11A1 with no changes in CYP21 or steroidogenic acute regulator (StAR) in the late-gestation LTH fetal adrenal cortex compared with normoxic controls. This suggests that reduced steroidogenic capacity in the LTH fetus may play a role in the apparent disconnect between basal ACTH and basal cortisol levels. However, mechanisms must exist to allow for a height-ened cortisol response to acute stress despite the lowered expression of these key steroidogenic enzymes. The fetus has developed such that, despite elevated basal plasma ACTH, normal ontogenic maturation of cortisol production is maintained and the prepartum exponential rise is preserved (75), as well as the capacity to respond to an acute secondary stress (2). These adaptations indicate that the hypothalamic-pituitary portion of the axis responds to hypoxia as a stress by increasing the synthesis and release of ACTH secretagogues and activating the stress response. However, adaptive responses at the level of the adrenal cortex suppress excess stimulation under basal conditions.

As described above, in the LTH fetal adrenal cortex, there is decreased expression of CYP11A1 and CYP17, two key enzymes mediating cortisol synthesis, as well as decreased ACTH receptor expression (120). The reduction of these factors would result in attenuated adrenal responsiveness to ACTH and limited cortisol production. Along with these changes, however, there is an increase in the spent form of StAR protein (30 kDa), indicating increased transport of cholesterol into the inner mitochondrial membrane for the first step in cortisol biosynthesis. This could balance the adaptations of elevated basal plasma ACTH1–39 but reduce adrenal responsiveness to maintain basal levels of plasma cortisol similar to those observed in normoxic fetuses. In the LTH adrenal cortex, there are no changes in splicing factor-1 (SF-1) or DAX-1 expression, key transcription factors for ACTH-R, CYP11A1, and CYP17. This suggests that activation of these transcription factors is altered possibly via phosphorylation state or increased recruitment of corepressors (155).

The mechanisms involved in these adaptations have not been fully elucidated; however, nitric oxide (NO) may play a major role in regulating cortisol production intracellularly in the LTH fetal adrenal cortex. Tsubaki et al. (170) examined adrenal tissue and observed increased expression of endothelial NO synthase (eNOS) in adrenal tissue that colocalizes with CYP17 in LTH fetuses, suggesting that NO plays a role in regulation of adrenal steroidogenesis. Monau et al. (109) also showed that eNOS is the dominant NOS isoform in the ovine fetal adrenal cortex, and eNOS mRNA and protein expression are increased in the LTH adrenal primarily in CYP17-expressing cells in the cortisol-producing zona fasciculate. Subsequent studies by Monau et al. (110) showed that NO reduced ACTH-mediated cortisol production in LTH fetal adrenocortical cells (FACs) in vitro, whereas inhibition of NOS activity increased cortisol production in LTH cells, with no effect on normoxic cells. Furthermore, ACTH reduced eNOS activation via phosphorylation in LTH FACs (123) and NO-dependent inhibition of ACTH-induced cortisol production, which further supports the role of NO in regulating cortisol production in the LTH fetal adrenal (171). This may be possible by NO competing with the oxygen-binding site of CYP11A1 and CYP17 (74, 170), disrupting the heme-oxygen complex attack by the enzyme on the steroid substrate. The increased release of NO under basal conditions would limit cortisol synthesis, whereas elevated ACTH release and signaling due to a secondary stress would inhibit NOS activity and remove NO inhibition, resulting in enhanced cortisol production in the LTH fetus. This provides a mechanism (NO) for the capability of the LTH fetus to overcome the reduced steroidogenic enzyme gene expression and mount an enhanced cortisol response to acute stressors. However, the question remains as to what factor(s) is involved in the decreased expression of the key steroidogenic machinery in response to LTH. Contributing to the changes in eNOS expression and activation, intracellular mechanisms, like those mediating endothelial and vascular responses to hypoxia such as VEGF or NF-κB as well as intracellular upstream factors such as hypoxia-inducible factor-1α (HIF-1α), may play a role in regulating the fetal adrenal response to LTH (22, 27, 33, 60, 131, 142, 156, 175). However, these mechanisms are relatively unexplored in the fetal adrenal, and further investigations into the mechanisms involved in regulating eNOS activity and NO interactions with cortisol production are needed.

**PAT and Leptin**

One factor that may play a role is leptin. This 16-kDa protein derived from adipose tissue is most widely recognized for its role in appetite regulation in the adult (5, 86). However, leptin has also been clearly demonstrated to regulate adrenal steroid biosynthesis. In adult bovine adrenocortical cells, leptin suppressed cortisol output in response to ACTH stimulation, and this effect was mediated through a reduction in CYP17 and CYP11A1 expression (25, 93). Furthermore, leptin is a hypoxia-inducible gene (102). This adipocyte-derived hormone, like in human fetuses (101), circulates in the fetal sheep and increases...
in abundance in perirenal adipocytes as gestation progresses (185, 186). As in other metabolic tissues, maternal conditions and intrauterine stressors, such as hypoxia, influence fetal PAT.

In sheep, similar to the human, ~80% of fetal adipose tissue deposition occurs in the perirenal-abdominal region (137, 139, 140, 164). Fetal PAT differentiation is initiated in midgestation and expands during late gestation with a concomitant increase in hormone receptor populations (140). Importantly, this adipose tissue depot works as an endocrine organ to produce leptin (140). As PAT begins to develop, it also responds to hormonal and nutritional perturbations in the fetus, which in turn affects lipid storage and release.

The extracellular regulation of cortisol and the fetal response to hypoxia may be regulated by leptin along with the intracellular regulation of cortisol production by NO in the fetal adrenal, as described above. When infused into the late-gestation ovine fetus, leptin attenuated the prepartum increase in fetal plasma ACTH and cortisol (79, 107, 186). Ducsay, et al. (50) found that plasma leptin was elevated in the LTH fetus compared with normoxic controls, with PAT and placenta expressing higher levels of leptin mRNA. Also, OB-Ra (the inactive short isoform) leptin receptor expression was reduced in the LTH hypothalamus, whereas OB-Rb (the active long-form isoform) expression was increased in the adrenal (50), suggesting the potential for enhanced leptin activity in the fetal adrenal. Thus, leptin appears to be a hypoxia-inducible gene in the ovine fetus with the capacity to inhibit cortisol biosynthesis at the adrenocortical level.

Subsequent studies showed that StAR, ACTH-R, CYP11A1, and CYP17 expression were lower in the LTH fetus (49) and that a 96-h leptin infusion into late-gestation spontaneously hypoxemic fetal sheep downregulated CYP21 mRNA and ACTH-R and StAR mRNA and protein (160), indicating reduced adrenal responsiveness and a reduced capacity to produce cortisol. A 4-day infusion of a leptin receptor antagonist restored expression of CYP11A1 and CYP17 in the LTH fetus to levels similar to normoxic but did not affect fetal plasma ACTH or cortisol (49), demonstrating that LTH regulation of leptin can influence adrenal steroidogenic enzyme expression.

Although leptin plays a role in regulating the response of the HPA and adipose tissue to chronic stress, it appears to work alongside cortisol and the adrenal to facilitate the fetal adaptation to hypoxia. Understanding the role of leptin in the intrauterine environment and the influence it has on the fetal HPA axis will help determine the long-term metabolic consequences of early life events and may include the ability of leptin to influence the development of obesity and its comorbidities.

Metabolic Gene Expression

Along with the production of leptin, other factors in adipose tissue are affected by hypoxia and may have a metabolic impact on the fetus. In the fetal sheep, as well as in the human, PAT has classically been considered a brown fat deposit [brown adipose tissue (BAT)]. It expresses uncoupling protein 1 (UCP1) (34, 39, 47), which increases proton conduction of the inner mitochondrial membrane and catalyzes adaptive thermogenesis (31, 163). This enables the rapid generation of a significant amount of heat, and expression is most abundant in the newborn (163).

The fetal perirenal adipose depot in the LTH fetus, however, has been characterized with an unusual brown fat phenotype; there are mixed populations of multilocular deposits typical of white fat and unilocular fat deposits that are more common in brown fat. Leptin expression is more typical of white fat, and it is equally distributed in unilocular and multilocular adipocytes, with UCP1 staining distributed throughout the PAT. This unique phenotype has been termed “beige” fat, with white adipose tissue (WAT; myf5 +5 lineage) expressed as BAT (myf5 −5) (34, 47, 71, 81, 133, 151). Within PAT, Myers et al. (119) showed upregulation of UCP1, deiodinase 2 (DIO2), 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1), peroxisome proliferator-activated receptor (PPAR)γ, and PPAR coactivator-1α (PGC-1α) mRNA. LTH appears to enhance brown fat functionality through upregulation of these hallmarks of the brown fat phenotype. Increased 11β-HSD1 and DIO2 would allow adipose tissue to increase the BAT phenotype without systemic increases in cortisol or triiodothyronine (T3), preventing deleterious effects on fetal growth and organ function. Along with upregulated brown fat gene expression, Myers et al. (121) found increased mRNA of transcription factors that regulate expression of NRF2 and mtTFA, genes that govern mitochondrial function, further indicating a BAT phenotype. Fibroblast growth factor 21 (FGF21) has also been shown to enhance the beige/BAT phenotype of adipose tissue (55), and LTH enhanced hepatic FGF21 expression coupled with enhanced expression of FGF21 receptors in PAT (Myers DA and Ducsay CA, unpublished observations). This could serve as another mechanism of LTH-induced enhancement of changes in PAT.

The fetal adaptation to LTH in adipose tissue appears to involve increased leptin production and regulation of basal cortisol, as described above, as well as enhanced activation of adipose tissue. In the newborn, abdominal adipose is important for nonshivering thermogenesis and is regulated by UCP1 (31, 163). By increasing UCP1 expression, the fetus ensures adequate thermogenesis in the event of birth into oxygen-limited conditions. UCP1 expression is regulated by cortisol and T3 (67, 114), and increases in 11β-HSD1 and DIO2 indicate increased capacity for local synthesis and regulation by these hormones in the adipose tissue. This enhanced brown fat phenotype in anticipation of birth into a potentially hostile environment creates a balance between the upregulation of the hypothalamic-pituitary axis while downregulating adrenal responsiveness to maintain basal cortisol levels.

These changes in the LTH fetus, however, are not maintained postnatally. After birth, LTH lambs lose their brown fat phenotype; Ducsay et al. (52) and Symonds et al. (163) showed that expression of UCP1, PGC-1α, and PR domain-containing protein 16 (PRDM16) decrease postbirth, implying a lineage derived from WAT and not BAT. Although the beige fat phenotype is initially protective of adiposity, decreases in UCP1, PGC-1α, and PRDM16 suggest a predisposition of the lamb to fat deposition. In the transition from fetus to neonate, there is a shift toward an enhanced white fat phenotype that may result in greater adiposity as the newborn matures; decreased BAT has been shown to result in obesity and related metabolic disorders that develop later in life (77, 81, 152).
The combined increased PAT expression and release of leptin, increased adrenocortical leptin receptor (OB-Rb) expression, and increased zona fasciculata-specific eNOS expression and activity (NO release) would limit the ability of elevated fetal plasma ACTH to stimulate cortisol production under basal conditions. Overcoming these mechanisms may allow for increased synthesis and release of cortisol in response to an acute secondary stressor.

Conclusions

The influence of hypoxia on the developing fetus has clearly been shown in the HPA axis and adipose tissue in the ovine model. A variety of other studies have shown changes in response to hypoxia in the macaque as well as the human. Hypoxia in the human fetus has been associated with both maternal and fetal conditions, including high altitude, maternal heart disease or pulmonary hypertension, preeclampsia, and placental insufficiency. These conditions often result in IUGR, preterm delivery, or stillbirth (1, 65, 72, 73, 85, 94, 112, 122). Maternal smoking also leads to hypoxia in the human and has been associated with IUGR (8, 54, 92, 147, 178), and low birth weight is a significant risk factor for the development of obesity, hypertension, and type 2 diabetes (11, 14, 68, 69, 128, 153). Studies in a nonhuman primate model, Japanese macaques, show that a high-fat diet reduces uterine volume blood flow, resulting in undernourished fetuses and an increased incidence of stillbirth (59). These studies show a dramatic effect of hypoxia on the growth potential of the fetus by either preventing full development or predisposing the fetus to numerous detrimental disorders.

The sheep has emerged as a major model for studying the effects of hypoxia on the fetus. When challenged with an acute stress, the fetal HPA axis is activated to release cortisol to counteract the perturbation and return the fetus to homeostasis. Sympathoadrenal inhibition of insulin secretion in response to hypoxia ensures adequate glucose for essential functions to restore homeostasis. In the case of a chronic stress such as long-term hypoxia, several studies have shown the remarkable ability of the fetus to adapt to circumvent growth restriction and preterm birth.

Hypoxia is a potent stressor that commonly affects the developing fetus and can result in adaptations in both the HPA axis as well as the adipose tissue. This complex interplay is summarized in Fig. 1, which illustrates the effects of both acute hypoxia and LTH. In the LTH fetus, the HPA adapts such that despite the upregulation of hypothalamic CRH/AVP and pituitary ACTH under basal conditions, adrenal production of cortisol is maintained at normoxic levels. However, in response to an acute secondary stressor, the production of cortisol is enhanced beyond the stress response in normoxic controls. This proposes an adaptation of the system that maintains cortisol levels required for growth and development but is combined with a programmed heightened response to acute stress. This mechanism may be mediated by NO production in adrenal cortical cells as well as by leptin production in fetal PAT.

Fig. 1. Adaptive responses of the fetal hypothalamic-pituitary-adrenal (HPA) axis to hypoxia. The diagram summarizes endocrine and metabolic adaptations to both acute (AH) and long-term hypoxia (LTH). The solid-line arrows illustrate stimulatory effects, whereas the dashed-line arrows denote inhibitory influences. CRH, corticotropin-releasing hormone; AVP, arginine vasopressin.

Abbreviations
catecholamines (CAts)
cytochrome P450 11A1 (CYP11A1)
cytochrome P450 17 (CYP17)
dopamine beta hydroxylase (DBH)
endothelial nitric oxide synthase (eNOS)
fibroblast growth factor 21 (FGF21)
hydroxysteroid (11-beta) dehydrogenase 1 (11β-HSD1)
nitric oxide (NO)
paraventricular nucleus (PVN)
phenylethanolamine N-methyltransferase (PNMT)
pituitary (PIT)
proopiomelanocortin (POMC)
tyrosine hydroxylase (TH)
uncoupling protein 1 (UCP1)
In this review, we have described the novel roles of NO and leptin in regulating cortisol biosynthesis in the LTH fetal adrenal. As described above, both NO and leptin appear to play significant roles in regulating the fetal adaptation to chronic hypoxia. NO seems to work via intra-adrenal regulation, potentially inhibiting steroidogenic enzyme activity via competition at the heme-oxygen binding site of CYP11A1 and CYP17 (74, 170) to inhibit cortisol biosynthesis. This inhibition is overcome by dramatically elevated stress levels of ACTH, resulting in reduced NOS activity and enhanced cortisol production (110). Leptin may function via extra-adrenal regulation, reducing steroidogenic enzyme expression (25, 93) and possibly inhibiting glucocorticoid secretion (186). Together, these factors work at the level of the adrenal to facilitate appropriate cortisol responses both in the normal ontogenic rise near term and in response to a secondary stress. Both NO and leptin are capable of inhibiting cortisol synthesis; however, the exact mechanisms are still undetermined. Further studies investigating the mechanisms responsible for these changes in the fetal HPA axis in response to chronic hypoxia and the coordination of NO and leptin to allow for normal fetal growth and development will help determine how the fetus survives and continues to term.

In adipose tissue, there is a unique beige phenotype developed in response to LTH. There is an upregulation of expression of the BAT phenotypic genes UCP1, DIO2, 11β-HSD1, PPARG, and PGC-1α that would ensure adequate nonshivering thermogenesis and indicate reduced adiposity. These genes, however, become downregulated after birth, shifting toward a WAT phenotype and predisposing the newborn to fat deposition. If the fetus was born into a hypoxic environment, this adaptation would be beneficial, but in a normoxic environment this could have a significant detrimental lifelong impact resulting in a variety of metabolic disorders, including obesity and diabetes.

The interactive adaptive responses at the level of the HPA axis and adipose tissue play a key role in the immediate response to hypoxia. It will be important to determine whether these systems are a unique response to LTH or are invoked as a general adaptive response to other intrauterine stressors that aid in fetal survival. More critically, however, will be our ability to determine the mechanisms involved in the adaptive responses to LTH. Although such adaptations are critical to survival under conditions of the chronic stress, they may have “unintended consequences” from the standpoint of fetal programming of adipose tissue and HPA axis function. The challenge for the future will be to further elucidate the mechanisms responsible for this shift in phenotype induced by LTH. Understanding epigenetic changes in adipose tissue induced by LTH may lead to new treatment modalities to reverse the untoward effects on adipose tissue. Suppression of 11β-HSD1 in adipose tissue or selective treatment with FGF21 to enhance the expression of the metabolically active beige adipose tissue coupled with new imaging techniques like thermal imaging to assess adipose tissue function (162) will not only enhance our knowledge of the role of LTH on programming but also help to ameliorate long-term effects.

DISCLOSURES
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AUTHOR CONTRIBUTIONS

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