Extracellular signal-regulated kinases (ERK1/2) signaling pathway plays a role in cortisol secretion in the long-term hypoxic ovine fetal adrenal near term

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Vargas VE, Kaushal KM, Monau TR, Myers DA, Ducsay CA. Extracellular signal-regulated kinases (ERK1/2) signaling pathway plays a role in cortisol secretion in the long-term hypoxic ovine fetal adrenal near term. Am J Physiol Regul Integr Comp Physiol 304: R636–R643, 2013. First published February 20, 2013; doi:10.1152/ajpregu.00318.2012.—This study assessed the role of the extracellular signal-regulated kinase (ERK) signaling pathway on the previously observed enhanced cortisol secretion in response to adrenocorticotropic hormone (ACTH) treatment in fetal adrenocortical cells (FACs) from near-term hypoxic (LTH) ovine fetuses. Ewes were maintained at high altitude (3,820 m) from ~40 to 138–141 days gestation when FACs were collected and challenged with either ACTH (10 nM) or 8-bromoadenosine 3′,5′-cyclic monophosphate (8-bromo-cAMP, 10 mM) in the presence or absence of the mitogen-activated protein kinase/extracellular signal-regulated protein kinase (MEK)/ERK inhibitor UO126 (10 μM). FACs from age-matched normoxic fetuses served as controls. Media and FACs were collected at selected time intervals after ACTH or 8-bromo-cAMP stimulation for cortisol measurement and Western analysis of ERK1/2 and phospho-ERK1/2 (-pERK1/2). After ACTH or 8-bromo-cAMP treatment, cortisol production was greater in the LTH group compared with control (P < 0.05). UO126 reduced ACTH and 8-bromo-cAMP-mediated cortisol output in both groups (P < 0.01 vs. ACTH or 8-bromo-cAMP alone). Under basal conditions, ERK1/2 and pERK1/2 were not different between LTH and normoxic fetuses. In response to ACTH or 8-bromo-cAMP treatment, ERK1/2 were not different between groups; however, pERK1/2 were elevated in the LTH FACs compared with normoxic control FACs. ERK1/2 phosphorylation declined following ACTH treatment in the control group, but UO126 had no effect on ERK1/2 compared with untreated levels. Both ACTH and 8-bromo-cAMP treatment resulted in a decline of protein levels. UO126 pretreatment virtually eliminated pERK1/2 expression. We conclude that basal ERK signaling in FACs is necessary for normal cortisol production and sustained pERK in LTH adrenals enhances cortisol production.

ACTH; UO126; 8-bromo-cAMP; sheep; fetus

The classical adrenocorticotrophic hormone (ACTH) signaling pathway via 3,5-cAMP is generally accepted as the major mechanism regulating cortisol biosynthesis in the adrenal cortex (10, 36). The late gestation increase in fetal plasma cortisol resulting from adrenocortical maturation is critical for optimal fetal organ maturation, and in sheep, it plays an essential role in parturition (24, 25). Hypoxia has clearly been shown to play a key role in the regulation of the fetal hypothalamic-pituitary-adrenal axis (3, 17, 11). Importantly, from a clinical perspective, not only women that live at high altitude, but also women that smoke, have heart/lung disease, or are anemic during pregnancy expose the fetus to hypoxia (41, 22, 31).

Our laboratory has developed an ovine model of high altitude-induced long-term hypoxia (LTH). We have found that development under conditions of LTH has profound effects on the function of the fetal hypothalamic-pituitary-adrenal (HPA) axis at all levels, the hypothalamic paraventricular nucleus, anterior pituitary, and adrenal gland (2, 21, 28, 29). Basal plasma concentrations of cortisol are maintained in the LTH fetus at levels similar to levels observed in normoxic fetuses (29, 27) despite elevated basal plasma ACTH concentrations (23). The ability of the LTH adrenal to maintain normal ontogenic basal plasma cortisol likely reflects the noted decreased expression of two key steroidogenic enzymes, cytochrome P-450c11A1 (CYP11A1) and P-450c17 (CYP17), as well as the ACTH receptor. While the LTH fetus maintains seemingly normal basal plasma cortisol concentrations, paradoxically, basal ACTH and acute secondary stress-induced cortisol biosynthesis is enhanced (2, 21, 28). We recently demonstrated that the enhanced cortisol production observed in the LTH fetal adrenocortical cells (FACs) compared with normoxic FACs in response to ACTH was not the result of enhanced cAMP production and/or protein kinase A (PKA) stimulation (40).

Extracellular signal-regulated kinases (ERKs) have been suggested to play a regulatory role in ACTH-induced steroidogenesis (33, 34). In general, ERK1/2 are activated via phosphorylation by mitogen-activated protein kinase/extracellular signal-regulated protein kinase (MEK)1/2 (4, 9, 6), which itself is activated by the proto-oncogene serine/threonine-protein kinase, RAF. A variety of extracellular signals converge at RAF to stimulate ERK signaling (8, 5, 13, 16, 43, 38, 39). Relative to adrenal cortisol biosynthesis, studies by Ferreira et al. (15) recently demonstrated a major role for ERK1/2 activation in chronic ACTH-induced steroidogenesis in the rat adrenal gland. Additionally, studies by Poderoso et al. (34) showed that ERK1/2 are implicated in phosphorylation of steroidogenic acute regulatory protein (StAR). StAR is essential for transport of cholesterol to the inner mitochondrial membrane, the site of the CYP11A1 conversion of cholesterol to pregnenolone, the rate-limiting step for cortisol synthesis. Thus regulation of StAR activity by ERK would have significant impact on cortisol production. Considering that cAMP production and protein kinase A activation are not different in the LTH compared with normoxic FACs, we designed the present study to examine the role of ERK1/2 signaling in the enhanced ACTH-stimulated cortisol production observed in
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LTH FACs. Specifically, we hypothesized that the enhanced cortisol production noted in the LTH FAC in response to ACTH is mediated by ERK.

METHODS

Animals Procedures

All procedures were conducted with approval of the Institutional Animal Care and Use Committees (Loma Linda University School of Medicine, Loma Linda, CA). Pregnant sheep were transported to Barcroft Laboratory White Mountain Research Station in Bishop, California at an elevation of 3,820 m at ~day 40 of gestation, where they were maintained until near term (term = 146 days), at which time they were transferred to Loma Linda University. Upon arrival, hypoxia was maintained in the ewes by nitrogen infusion through a maternal tracheal catheter to maintain maternal arterial PO2 levels. Hypoxia was maintained in the ewes by nitrogen infusion through a maternal tracheal catheter to maintain maternal arterial PO2 levels that are similar to that observed at high altitude (~60 mmHg) as previously described (2, 21). On days 138–141 of gestation, both LTH and normoxic ewes were sedated with pentobarbital sodium, intubated, and maintained under general anesthesia with 1.5–2% halothane in oxygen. Fetuses were then delivered through a midline laparotomy, and the fetal adrenal glands were collected in ice-cold media M-199 (Sigma-Aldrich, St. Louis, MO), containing 2.2 g sodium bicarbonate, 2.0 g bovine serum albumin, and 0.1 g 1-glutamine as we have previously described (40).

Adrenocortical Cell Dispersion

The adrenal cortex was separated from the medulla, enzymatically dispersed with 40 mg collagenase Type II (Worthington Biomedical, Lakewood, NJ), 40 mg of Poly pep bovine protein digest (Sigma-Aldrich), and 100 μl of DNase I (Type IV) (Sigma-Aldrich) dissolved in 10 ml of sodium Krebs buffer (0.4% collagenase). The resulting monodispersed FACs were aliquoted in duplicate (2.5 × 10^6 cells) into individual tubes with media (M-199) and allowed to equilibrate for 2 h before initiation of each study. Cell viability was confirmed by Trypan blue exclusion. All experiments were conducted on FACs at 37°C. All procedures were performed by methods that we have previously described and validated (40).

Experimental Protocol

Fetal adrenal cells (2.5 × 10^6 cells/tube; in duplicate) from control and LTH FACs were treated with ACTH (10 nm) or 8-bromo-cAMP (10 μM) for 5, 10, 15, 30, and 60 min, and cells and media were collected separately at each time point. Untreated cells were collected at the start of each experiment for basal controls. To assess the effects of MEK/ERK inhibition on cortisol production, additional FACs were pretreated with the MEK inhibitor U0126 (10 μM) for 1 h before ACTH or 8-bromo-cAMP treatment. At the time of collection, media and cells (in lysis buffer containing 1 mM sodium orthovanadate) were snap frozen in liquid nitrogen and stored at −80°C. The media was analyzed for cortisol in our laboratory.

Cortisol Assay

Cortisol was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) cortisol kit (Oxford, MI) that has been previously described and validated for use in our laboratory (11, 27).

Western Blot Analysis for ERK1 and ERK2 and phospho-ERK1 and pERK2

ERK1/2 and phospho-ERK1/2 were analyzed from FACs collected at 0 min (baseline), 5, 10, 15, 30, and 60 min for both LTH and normoxic control groups as described above. FACs were lysed in 150 μl of lysis buffer [97.9% prelysis buffer (150 mM NaCl, 50 mM Tris-HCl, 10 mM EDTA, 0.01% Tween-20, 0.01% β-mercaptoethanol), 1.67% PMSF, 0.2% leupeptin, 0.1% DTT, 0.04% aprotinin, 1 mM sodium orthovanadate (Sigma-Aldrich, St. Louis, MO)]. Protein concentration was determined using the BCA protein assay (Pierce Bioreagents, Rockford, IL) with bovine serum albumin as the standard. Absorbance was measured at 562 nm on a Synergy HT Multi-Detection Microplate reader (BioTek, Winooski, VT) using Gen5 software. Levels of ERK1/2 protein were determined by Western blotting using methods, which we have previously described and validated (28, 29, 40). Briefly, protein samples were denatured by boiling for 5 min, and 3 μg (ERK1/2) or 4 μg (pERK1/2) of protein were loaded per lane. Proteins were subjected to SDS-PAGE using 10% polyacrylamide gels (200 volts for 35 min, Bio-Rad, Hercules, CA). Proteins were transferred to nitrocellulose membranes using a Transblot cell apparatus (4°C, 270 mA for 2 h). Subsequently, membranes were incubated with ERK1/2 (1:1,000) and pERK1/2 (1:2,000 dilution; Cell Signaling, Danvers, MA) primary antiserum overnight (4°C) in Tris-buffered saline containing 0.1% Tween 20 (TTBS) and 5% BSA. Membranes were then washed (3 × 10 min) with TTBS washing buffer and incubated with secondary DyLight goat anti-rabbit antibody (1:10,000) solution (Thermo Scientific, Rockford, IL) at room temperature. After secondary antibody incubation, the membranes were washed (3 × 10 min) with TTBS washing buffer. The membranes were analyzed for bands using Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE). An internal positive standard prepared from fetal adrenal cells was used to normalize ERK1/2 and pERK1/2 protein.

Statistical Analysis

Differences between LTH and normoxic control FACs were determined using analysis of variance (ANOVA) with Bonferroni post-hoc tests where appropriate, and P < 0.05 was considered significant. Cortisol data are expressed as means ± SE (in ng·ml⁻¹·250,000 cells⁻¹). Data for protein expression are expressed as percentage of basal (untreated for each group).

RESULTS

Cortisol Responses to ACTH and 8-Bromo-cAMP

Basal cortisol secretion (Fig. 1A) was similar in both groups. In response to ACTH, there was a significant increase in cortisol biosynthesis in both control and LTH FACs (Fig. 1B). However, in response to ACTH, cortisol production in the LTH FACs was significantly greater compared with control FACs (P < 0.01) at both 30 min (8.10 ± 0.14 vs. 3.19 ± 0.80) and 60 min (12.43 ± 2.31 vs. 4.76 ± 1.13). After 8-bromo-cAMP treatment there was a significant increase in cortisol biosynthesis in both normoxic control and LTH FACs (Fig. 1C). Similar to the response to ACTH, cortisol production was greater in the LTH group compared with control in response to 8-bromo-cAMP but only at 60 min (7.90 ± 0.28 vs. 3.89 ± 0.34, P < 0.01). A similar trend was noted at 30 min but did not reach statistical significance.

Interestingly, in the LTH FACs, the total cortisol production in response to ACTH (as measured by area under the curve from 0 through 60 min) was significantly greater than the response to 8-bromo-cAMP (P < 0.05; Fig. 2). In marked contrast, the total cortisol output in control FACs was similar for both stimuli.
60 min following stimulation with either ACTH or 8-bromo-cortisol production in the LTH FACs that became significant at cortisol production. However, there was a gradual increase in In the control FACs, UO126 pretreatment completely blocked P 8-bromo-cAMP treatment alone (Fig. 1, C) and 8-bromo-cAMP-stimulated cortisol secretion in both groups compared with ACTH and 8-bromo-cAMP alone, respectively (P < 0.05). Pretreatment with UO126 significantly inhibited ACTH (D) and 8-bromo-cAMP-stimulated (E) cortisol secretion in both groups (Fig. 1, P 0.05). Pretreatment with UO126 significantly inhibited ACTH (D) and 8-bromo-cAMP-stimulated ACTH and 8-Bromo-cAMP-Stimulated Cortisol Production

Pretreatment with UO126 significantly (P < 0.01) inhibited both ACTH and 8-bromo-cAMP-stimulated cortisol production in both groups (Fig. 1, D and E) compared with ACTH or 8-bromo-cAMP treatment alone (P < 0.01) (Fig. 1, B and C). In the control FACs, UO126 pretreatment completely blocked cortisol production. However, there was a gradual increase in cortisol production in the LTH FACs that became significant at 60 min following stimulation with either ACTH or 8-bromo-cAMP.

Effect of U0126 on ACTH and 8-Bromo-cAMP Stimulated Cortisol Production

Basal treatment. Under basal, untreated conditions, there were no differences in either basal ERK1/2 or pERK1/2 protein in control and LTH FACs (data not shown). Furthermore, since there were no differences between control and LTH FACs at time 0, values over the time course for the rest of the experimental treatment are expressed as percentage of time 0 (Figs. 3 and 5).

ACTH treatment. Neither ERK1 (Fig. 3B) nor ERK2 (Fig. 3C) expression changed in response to ACTH in control or LTH FACs. In marked contrast, both pERK1 (Fig. 3E) and pERK2 (Fig. 3F) decreased in response to ACTH over time in the normoxic control FACs but not LTH FACs (P < 0.05).

UO126 and ACTH treatment. One hour of preincubation of FACs with UO126 (time 0 min, Fig. 4, B and C; expressed as % of basal) had no effect on either ERK1 or -2 compared with untreated levels. However, after the initiation of ACTH treatment, there was a significant decline (P < 0.05) in both ERK1 and ERK2 throughout the remainder of the study (Fig. 4, B and C). As expected, U0126 virtually eliminated phosphorylation of ERK1 and ERK2 in both control and LTH FACs (Fig. 4D).

ERK1/2 and pERK1/2 Expression in Response to 8-Bromo-cAMP and UO126 Treatment

8-Bromo-cAMP treatment. Similar to ACTH, 8-bromo-cAMP treatment had no effect on ERK1 or -2 protein in either control or LTH FACs (Fig. 5, B and C). However, unlike the effects of ACTH on pERK1/2, there was no effect of 8-bromo-cAMP on either treatment group.

Fig. 1. Cortisol production in control and long-term hypoxia (LTH) fetal adrenocortical cells (FACs). Under basal conditions no differences were observed between groups (A). Both adrenocorticotropic hormone (ACTH, 10 nM; B) and 8-bromoadenosine 3’,5’-cyclic monophosphate (8-bromo-cAMP, 10 mM; C) significantly stimulated cortisol biosynthesis compared with basal conditions (P < 0.01). The responses were greater in the LTH (n = 8) group compared with control (n = 7; *P < 0.05). Pretreatment with UO126 significantly inhibited ACTH (D) and 8-bromo-cAMP-stimulated (E) cortisol secretion in both groups compared with ACTH and 8-bromo-cAMP alone, respectively (P < 0.05).

Fig. 2. Total cortisol production during the experiment (area under the curve, AUC) in response to ACTH and 8-bromo-cAMP in FACs from control and LTH fetuses.
UO126 and 8-bromo-cAMP treatment. One hour of preincubation of FACs with UO126 (time 0 min, Fig. 6, B and C) had no effect on ERK1 or ERK2 compared with untreated levels. Unlike ACTH, UO126 pretreatment did not result in a significant change in either ERK1 or ERK2 in response to 8-bromo-cAMP during the experiment in either control or LTH FACs (expressed as % of time 0 under basal conditions). As in the ACTH study above, UO126 eliminated phosphorylation of ERK1 and ERK2 (Fig. 6D).

DISCUSSION

We have previously demonstrated that the ovine fetus mounts a significant adaptive endocrine strategy under the potentially adverse conditions of LTH (see Ref. 30 for review). Despite higher basal plasma ACTH concentrations, the normal ontogeny of basal plasma cortisol is maintained (2, 21, 28). However, the LTH fetus exhibits enhanced cortisol biosynthesis in response to an acute secondary stressor, such as umbilical cord occlusion or hypotension (2, 21). There are several key adaptive changes at the level of the fetal adrenal that likely contribute to these LTH fetuses being able to maintain normal basal plasma cortisol concentrations. For instance, the observed decrease in expression of CYP17, CYP11A1, and the ACTH receptor (29) would limit the capacity to produce cortisol in the face of elevated plasma ACTH. However, these differences did not fully define the mechanism(s) that would allow for an enhanced production of cortisol in the face of a superimposed acute stressor.

In an effort to elucidate the mechanism(s) responsible for the enhanced cortisol biosynthesis in response to an acute stress (or ACTH) in the LTH fetus, we recently investigated the effect of LTH on key elements of the ACTH/cAMP signaling pathway that regulate cortisol biosynthesis in fetal FACs (40). We found no apparent differences in cAMP production or subsequent PKA activation that could explain the observed difference in cortisol production between control and LTH FACs. In the present study, we addressed the capacity for cAMP to stimulate cortisol production in the LTH FAC. Consistent with ACTH, 8-bromo-cAMP elicited a greater production of cortisol in LTH FACs compared with those from normoxic control fetuses. In control FACs, cortisol production in response to 8-bromo-cAMP was equal to ACTH-induced cortisol production, consistent with cAMP-PKA being the primary pathway regulating cortisol production in the ovine fetal adrenal cortex. However, production of cortisol in response to ACTH was greater than that elicited by 8-bromo-cAMP in the LTH FACs, suggesting that LTH activates alternative signaling mechanisms via which ACTH stimulates acute cortisol production.

The role of the MEK-ERK signaling pathway in regulating cortisol biosynthesis remains largely unexplored and somewhat
controversial. Peterson et al. (32) clearly demonstrated the presence of ERK1/2 in the adult ovine adrenal cortex. In adult bovine adrenocortical cells, ERK1/2 activation inhibits ACTH-induced steroidogenesis (7), and a negative effect of ACTH on ERK activity was also observed by Watanabe et al. (42). In contrast, in Y-1 adrenocortical cells and ovarian granulosa cells, ERK1/2 promoted hormone-induced steroidogenesis (18, 23). In adult rats, in vivo chronic treatment with ACTH increased pERK1/2 in the adrenal cortex and as expected, corticosterone production, whereas MEK inhibition prevented both ACTH-induced ERK activation and the increase in corticosterone (14, 15). Fassnacht et al. (12) noted that NH2-terminal proopiomelanocortin (POMC) 1–28 stimulated mitosis in adrenocortical tumor cells (Y-1, NCI-h295) and primary cultures of bovine adrenocortical cells via an ERK-dependent pathway that was associated with decreased steroidogenesis. Elevated levels of NH2-terminal POMC-derived peptides may contribute to the enhanced level of phosphorylation. In response to UO126 inhibition of ERK1/2 activation,

as it identifies a novel pathway, previously unrecognized, for the regulation of fetal cortisol biosynthesis and the key role that LTH plays in this process.

In the present study we observed no differences in either ERK1 or ERK2 between LTH and normoxic control FACs under basal conditions. Surprisingly, we noted robust basal phosphorylation of both ERK1 and ERK2 in both LTH and control FACs, perhaps reflecting the mitotically active nature of the FACs. Elevated levels of growth factors observed during development (1, 20, 26) or NH2-terminal POMC-derived peptides may contribute to the enhanced level of phosphorylation. In response to UO126 inhibition of ERK1/2 we noted a profound inhibitory effect on both ACTH and 8-bromo-cAMP stimulated cortisol biosynthesis in the ovine fetal adrenal cortex acting at least partially at a level post-cAMP production. However, the similar levels of ERK1/2 phosphorylation in the LTH and control FACs would indicate that the enhanced production of cortisol in response to ACTH and 8-bromo-cAMP in the LTH FAC is not due to enhanced ERK1/2 expression or activation.

Based on the UO126 inhibition of cortisol production, we examined the effect of both ACTH and 8-bromo-cAMP on ERK1/2 activation. Seemingly contradictory to the noted in-
hibitory effects of U0126 on cortisol production in both groups, which implicates ERK1/2 as facilitating ACTH-induced cortisol biosynthesis, we observed that ACTH decreased pERK1/2 in control FACs but not in LTH FACs. However, it should be noted that, albeit significant, the effect of ACTH on pERK1/2 was relatively minor (~30 to 40% reduction in levels) and occurred at 5–10 min post-ACTH and then remained stable throughout the 60-min experiment. This is different from U0126 pretreatment, where ERK1/2 phosphorylation is eliminated before the addition of ACTH. Thus these studies support an essential role for ERK1/2 activation in ACTH-induced cortisol synthesis in the ovine fetal adrenal cortex. We postulate that the ACTH-induced decrease in pERK1/2 provides a feedback mechanism limiting ACTH-stimulated cortisol production. The decreased ERK phosphorylation in response to ACTH is in agreement with data from Sewer and Waterman (37) indicating that ACTH stimulates dual-specific phosphatases PP1 and PP2 that are capable of deactivating ERK1/2. Furthermore, the inability of ACTH to alter ERK1/2 phosphorylation in the LTH FACs provides a potential mechanism for the observed increased cortisol production noted in these cells compared with control, perhaps by limiting the decrease in pERK1/2. Clearly, future studies will need to be conducted to determine whether differences exist in protein phosphatase expression or activation in the LTH compared with control FACs.

As noted above, similar to ACTH, 8-bromo-cAMP elicited a greater production of cortisol in LTH compared with control FACs. Also similar to ACTH, U0126 prevented or severely blunted 8-bromo-cAMP-induced cortisol production in FACs from both groups supporting the concept that the ERK contribution to cortisol production is dependent on ACTH-induced cAMP generation. However, unlike ACTH actions on pERK1/2 in the control FACs, 8-bromo-cAMP had no effect on ERK1/2 phosphorylation. However, the sustained signaling from the diesterase-resistant cAMP analog versus increased cAMP in response to ACTH may impact cortisol biosynthesis by different mechanisms. Indeed, the cortisol response to ACTH is considerably more rapid compared with 8-bromo-cAMP in FACs, possibly reflecting the time needed for intracellular 8-bromo-cAMP to reach stimulatory levels. The lack of effect of 8-bromo-cAMP on phospho-ERK1/2 seemingly indicates that ACTH-induced dephosphorylation of ERK1/2 is not cAMP dependent and rather that alternative mechanisms...
induce phosphatase activity. The significantly lower cortisol production in the LTH FACs in response to 8-bromo-cAMP compared with ACTH likely indicates that alternative signaling mechanisms in addition to pERK are invoked in the LTH FACs.

**Perspective and Significance**

In summary, based on our present and previous findings, we propose that intracellular signaling via the ERKs is fundamental for basal cortisol production in ovine FACs. Furthermore, ACTH-induced ERK dephosphorylation appears to provide an intracellular feedback mechanism limiting cortisol production in response to ACTH (stress). The inability of ACTH to dephosphorylate ERK in LTH FACs provides a potential mechanism for the enhanced cortisol production in response to 8-bromo-cAMP that we have observed in the LTH FACs. In addition to the classical cAMP/PKA pathway, ERK signaling is involved in part in ACTH-induced steroidogenesis possibly by playing a more permissive role of integrating information from extracellular signals. The enhanced cortisol response coupled with the enhanced effect of ERK inhibition in the LTH group suggests that downstream signaling pathways are upregulated in response to LTH. The modulation of ERK signaling in the adrenal cortex has broad implications for other physiological systems in fetuses developing under conditions of sustained moderate hypoxia.

While cortisol is clearly essential for the late gestation maturation of fetal organ systems in preparation for birth in mammals, including humans, premature activation of glucocorticoid production, both the ontogenic late gestation rise in fetal cortisol production as well as in response to fetal stressors induces fetal growth restriction, including negative impacts on glucocorticoid responsive fetal organs. We have found that the LTH fetus invokes multiple mechanisms to prevent an early and potentially deleterious rise in fetal plasma cortisol, with the present findings on the role of ERK showing an intracellular mechanism that works in concert with extracellular (systemic) adaptations to limit cortisol production during this critical window of gestation. Our findings that ERK is essential for basal and ACTH-initiated cortisol production is a novel finding that the ERK signaling system is fundamentally been altered by the LTH conditions represents a novel adaption to LTH.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

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