LONG TERM HYPOXIA ALTERS OVINE FETAL ENDOCRINE AND PHYSIOLOGIC RESPONSES TO HYPOTENSION

Keiichi Adachi¹, Hikaru Umezaki¹, Kanchan M. Kaushal¹ and Charles A. Ducsay¹,²

Center for Perinatal Biology, Departments of Physiology/Pharmacology¹ and Pediatrics²,
School of Medicine, Loma Linda University, Loma Linda, California

Running title: Fetal hypotension and long term hypoxia

Address all correspondence to:
Charles A. Ducsay, Ph.D.
Center for Perinatal Biology, School of Medicine,
Loma Linda University
Loma Linda, California 92350
909-558-4325 Office
909-558-4029 Fax
cducsay@som.llu.edu
ABSTRACT

Exposure to long-term hypoxia (LTH) results in altered cortisol responses in the ovine fetus. The present study was designed to test the hypothesis that LTH alters adrenal responsiveness to fetal hypotension. Pregnant ewes were maintained at high altitude (3,820 m) from day 30 of gestation. Normoxic control and LTH fetuses were catheterized on day 132 of gestation. In the LTH group, maternal PO₂ was maintained comparable to that observed at altitude (~60 mmHg) by nitrogen infusion through a tracheal catheter. On day 137, fetuses received a 5 h saline infusion followed by infusion of sodium nitroprusside to reduce fetal arterial pressure by 30-35% for 10 min. The study was repeated on day 139 of gestation with a continuous cortisol infusion (10 µg/min). Hypothalamic and pituitary tissues were collected from additional fetuses for assessment of glucocorticoid receptors. During the saline infusion in response to hypotension, plasma ACTH increased over pre-infusion mean values in both groups (p < 0.05). Plasma cortisol concentrations increased in both groups concomitant with increased ACTH secretion. However, peak values in the LTH fetuses were significantly higher compared with controls (p<0.05). During the cortisol infusion, the ACTH response was eliminated in both groups with ACTH levels significantly lower in the LTH group (p<0.05). Glucocorticoid receptor binding was not different between groups. These results demonstrate an enhanced cortisol response to hypotension in LTH fetuses which does not appear to be the result of an increase in negative feedback sensitivity of the hypothalamic-pituitary-adrenal axis.

Keywords: ACTH, cortisol, nitroprusside.
INTRODUCTION

The ovine fetus responds to an acute stressor with a dramatic elevation in plasma ACTH and cortisol. Acute hypoxia (1; 4; 16) as well as hypotension (26; 32; 35), and hemorrhage (25; 35) all have profound stimulatory effects on the fetal hypothalamic-pituitary-adrenal axis (HPA). Short term reductions in uterine blood flow also enhance cortisol secretion in the ovine fetus (3; 27). Placental embolization to induce fetal hypoxia for up to 3 weeks (20) has also been shown to result in elevated fetal ACTH and cortisol concentrations. The response of the fetal HPA to stressors lasting more than a few weeks however is far less clear. Phillips et al., (23) utilized surgical removal of caruncles to subject the fetus to sustained hypoxia throughout gestation and found that plasma cortisol concentrations were elevated despite ACTH levels similar to controls. The ability of the fetus to respond to a secondary stressor, following exposure to chronic hypoxic or hypotensive stress is less clearly defined. Although a few studies have examined the response of the fetal HPA to a superimposed secondary stressor following hypoxia lasting up to a few weeks (9; 22), there are no data on the effects of such a challenge following fetal hypoxia for more than 3 to 4 weeks.

Our laboratory has focused on the effects of chronic, long term hypoxia (LTH) with a model in which the ewe is maintained at 3,800 m from day 30 of gestation to near term (13;14). During this time, the maternal arterial PO$_2$ was approximately 60 mmHg and fetal arterial PO$_2$ was approximately 17-19 mmHg. Basal fetal ACTH and cortisol concentrations were similar to values in normoxic fetus while the cortisol response to exogenously administered ACTH was blunted (14). However, in response to an episode of severe asphyxia induced by 5 minutes of complete cord occlusion, the cortisol response was actually enhanced in the LTH ovine fetus compared with normoxic controls (15).

We designed the present study to test the hypothesis that a less severe secondary stressor like fetal hypotension also results in enhanced cortisol secretion in LTH fetuses compared with
normoxic controls. By infusing cortisol prior to hypotension and measuring ACTH and cortisol responses, we also tested the hypothesis that LTH alters cortisol negative feedback sensitivity.

METHODS

*Pre-surgical procedures.* Time-dated pregnant ewes of mixed Western breed (Nebeker Farms, Lancaster, CA) were housed in outdoor sheltered pens at the Barcroft Laboratory White Mountain Research Station (elevation 3,820 m) from day 30 to 123-125 of gestation. The ewes were allowed alfalfa pellets, mineral supplements, and water *ad libitum.*

*Surgical procedures.* Between days 123-125 of gestation, the LTH ewes were transported from the White Mountain research station to Loma Linda University Medical Center Animal Research Facility (elevation: 346m) where they were implanted with a non-occlusive tracheal catheter (4.0 mm OD) and an arterial catheter. Maternal PO₂ for LTH group was maintained at ~60 mmHg (mean PO₂ measured in animals at altitude) throughout the studies by adjusting humidified nitrogen (N₂) gas flow through the tracheal catheter. Normoxic, age-matched pregnant ewes were maintained near sea level (~300m) throughout the study.

On day 132 of gestation, surgeries were performed on normoxic control and LTH fetuses (n=6 for each group) under halothane general anesthesia, induced with intravenous thiopental as previously described (14; 15). The uterus was exposed through a midline vertical laparotomy, and the fetal head was delivered. We placed Tygon catheters (2.28 mm OD) in the fetal carotid artery and jugular vein and advanced them to the ascending aorta and superior vena cava (SVC), respectively. A third catheter (1.78 mm OD) was placed in the tibial vein and advanced to the inferior vena cava (IVC). An amniotic fluid catheter was anchored to the fetal hind limb to measure amniotic fluid pressure by methodology previously described in detail (14; 15). All catheters were tunneled under the ewe’s skin, exteriorized through the left flank, and stored in a
nylon pouch sutured to the skin. All procedures were approved by the Institutional Animal Care and Use Committee, and the animals were maintained in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

Postoperative care. After surgery, the ewes were maintained in a metabolic cart with food and water provided *ad libitum*. The ewes received antibiotics (3cc Crystiben, im (450,000 units penicillin G procaine and 450,000 units penicillin G bensathine; Solvay Animal Health, Inc., Mendota Heights, MN) once a day for the first 3 days after surgery. The fetus received antibiotics (40 mg Tobramycin, iv; Bristol-Myers Squibb Co. Princeton, NJ, 150 mg Clindamycin, iv; Abbott Labs and 1 g Mezlocillin, in the amniotic fluid; Miles Inc. West Haven, CT) twice daily for the first 3 days after surgery. Afterward, 500 mg Mezlocillin was administered into the amniotic fluid twice daily until the start of the experiment. All vascular catheters were flushed twice daily with heparinized saline.

Hemodynamic measurements. The ewes were monitored while standing undisturbed. Fetal mean arterial pressure, heart rate and amniotic fluid pressure were monitored using an eight-channel recorder (Gould 2800S; Gould Inc., Cleveland, OH). Analog signals were converted to digital input by a microcomputer (IBM PC-AT; IBM, Armonk, NY) and processed with real time data acquisition software developed in our laboratory (5). Fetal mean arterial pressure was corrected for changes in amniotic fluid pressure.

Experimental Protocol. On day 137, we administered 5% ethanol saline (vehicle) into the SVC at the rate of 2.5 ml/h for 7 h by an infusion pump (Model 944; Harvard Apparatus, S. Natick, MA). Fetal arterial blood samples were collected at -30, -15 min, before the start of the saline infusion then 1, 2, 3, 4 and 5 h after the infusion was started. Blood was collected into chilled potassium-EDTA syringes (Monovette; Sarstedt, Nümbrecht, Germany) and centrifuged at 4,000 rpm for 10 min at 4°C. Plasma was then separated and stored at -70°C until analyzed. Fetal
erythrocytes were reconstituted with sterile saline and returned to the fetal circulation following withdrawal of the next sample. Five hours after the start of saline infusion, sodium nitroprusside (Nitropress, Abbott Labs) in 5% dextrose was infused into the IVC for 10 min at a rate (ranging from 17-50 µg/min) sufficient to reduce fetal arterial pressure by 30-35% of baseline. Additional blood samples for ACTH and cortisol were collected during and after the start of the nitroprusside infusion (0, 5, 10, 15, 30, 60 and 120 min). Fetal arterial blood samples were also drawn at selected time intervals for determination of fetal blood gas using an automated blood gas analyzer (ABL300; Radiometer, Copenhagen, Denmark).

To examine the potential effects of cortisol negative feedback on pituitary ACTH secretion, the study was repeated on day 139 with a continuous cortisol infusion (Hydrocortisone; Sigma, St. Louis, MO) (10 µg/min, in 5% ethanol saline). A 10 µg/min dose of cortisol was chosen, as doses in this range have been shown to effectively inhibit ACTH secretion in the near term ovine fetus (33).

Hormonal assays. Plasma immunoreactive ACTH concentrations were determined by radioimmunoassay (DiaSorin Corp., Stillwater, MN) which has been previously described and validated in our laboratory for use in the ovine fetus (2; 14). The intra-assay and inter-assay coefficients of variation were 7% and 11%, respectively. Assay sensitivity was 1 pg/ml.

Plasma cortisol concentrations were measured by radioimmunoassay as previously described and validated. The intra-assay and inter-assay coefficients of variation were 8 and 11%, respectively while the assay sensitivity was 0.2 ng/ml (2; 14).

Glucocorticoid receptor quantification. To examine a potential mechanism of differences in glucocorticoid negative feedback sensitivity, we collected hypothalamic and pituitary tissue from additional control and LTH fetuses (n=10 for each group). Between days 139 and 141 of gestation, ewes were c-sectioned, the fetuses delivered and euthanized. The fetal brains were immediately removed and the hypothalamus was dissected out and the pituitary removed from
the sella turcica. The tissues were snap frozen in liquid nitrogen and stored at –70 C until analyzed.

The methodology for the binding assay was similar to that previously described (24). Briefly, tissues were homogenized in cold TEGT buffer (20mM Tris, 10mM thioglycerol, 1 mM EDTA, 10% glycerol, at pH 7.5). This was followed by centrifugation at 100,000 g for 30 min at 4 C. The protein concentration was determined by the Lowry method and the cytosolic prep was diluted with cold buffer for a final protein concentration of 2 mg/ml. The binding assays were performed using 100 µl of cytosol with 20 nM of [3H] triamcinolone acetonide (TA) (Amershan, 26 Ci/mmol) with and without a 100-fold excess of cortisol incubated overnight. Following the incubation, the reaction was stopped with the addition of 200 µl of dextran-coated charcoal slurry (1% charcoal, 0.5% dextran in TEGT buffer). After centrifugation for 15 min at 2220 rpm (800 g), 150 µl aliquots in duplicate were added to 4 ml of scintillation cocktail and counted in a Packard 1900CA analytic scintillation counter. Scatchard analysis for determination of Bmax and Kd was performed using GraphPad Prism analytical software (GraphPad Software, San Diego CA).

Statistical analyses. Differences over time and between control and LTH fetuses were analyzed using two-way analysis of variance with repeated measures and Bonferroni’s post-hoc test where appropriate. All values were expressed as means ± SEM and p < 0.05 was considered statistically significant. Statistical comparisons of glucocorticoid receptor binding parameters were performed using a Students t-test.

RESULTS

Maternal blood gases. Nitrogen flow was maintained through the maternal tracheal catheter at a rate sufficient to maintain maternal arterial blood gas values at a level similar to that observed at 3,820m at the Barcroft Laboratory (14). As expected, mean arterial PO2 in the LTH ewes was
significantly lower than in the normoxic controls (57.5 ± 1.5 vs. 102.5 ± 2.0 mmHg, p < 0.01, LTH vs. control). Mean maternal pH values (7.43 ± 0.01; LTH and 7.42 ± 0.01; control) and arterial PCO2 (31.6 ± 0.5; LTH and 32.2 ± 0.8, control) did not differ between the groups. Maternal blood gas values were unaffected by fetal nitroprusside or cortisol infusion (data not shown).

Effects of hypotension during vehicle infusion

Fetal arterial pressure and heart rate. Saline vehicle infusion had no effect on blood pressure or heart rate in either control or LTH fetuses (Figure 1). The sodium nitroprusside infusion reduced mean arterial pressure in both groups of fetuses to a similar extent, a decrease of approximately 30-35% from baseline. Following the termination of the infusion, mean arterial pressure returned to pre-hypotension levels within approximately 10 min. Fetal heart rate was unaffected by hypotension and remained unchanged throughout the study in both control and LTH fetuses (Figure 1). Mean arterial pressure in the LTH group was higher than control over the course of the study (p<0.01).

Fetal blood gases. Mean basal arterial PO2 in the LTH fetuses during the saline infusion was significantly lower than in the normoxic controls (18.6 ± 0.4 vs. 22.6 ± 0.4 mmHg, p < 0.01) (Figure 2, upper panel). Mean arterial PCO2 was also significantly lower in the LTH group compared with the normoxic controls (43.8 ± 0.6 vs. 49.9 ± 0.5 mmHg, p < 0.01) (Figure 2, middle panel) while arterial pH was higher in the LTH fetuses compared to control (7.35 ± 0.01 vs. 7.31 ± 0.01, p<0.01) (Figure 2, lower panel). However, PO2, PCO2 and pH values did not change significantly in response to hypotension in either treatment group.

ACTH and cortisol responses to hypotension. During the saline infusion, basal ACTH concentrations were similar in the both groups (24.9±4.2 control vs. 24.4±3.2 LTH, pg/ml) and
levels were unaffected by the saline infusion (Figure 3, upper panel) Hypotension significantly increased plasma ACTH concentrations in both groups and the time course of the plasma ACTH rise in response to hypotension was similar in both groups. Peak plasma ACTH concentrations were attained at the end of the nitroprusside infusion and were similar in both groups (92.9±17.0 control vs. 119±22.1 LTH pg/ml, Figure 3, upper panel). Following termination of the nitroprusside infusion, plasma ACTH gradually returned to near pre-infusion values in both groups. No differences in ACTH concentrations were observed between LTH and control fetuses.

Basal cortisol values were also similar in both groups (10.3±1.4 control vs. 12.7±2.8 LTH ng/ml) and saline infusion had no effect on basal plasma cortisol concentrations in either group (Figure 3, lower panel). Following hypotension, cortisol concentrations increased significantly over baseline values (p<0.05) in both control and LTH fetuses. However, cortisol concentrations were significantly higher in the LTH fetuses (p<0.05, compared with control) at 15, 30 and 60 min after the start of hypotension. By the end of the study period, mean plasma cortisol concentrations returned to pre-infusion values in both groups.

Effects of hypotension during cortisol infusion

Fetal arterial pressure and heart rate. During the cortisol infusion, there was a gradual increase in mean arterial pressure in the LTH fetuses. Immediately prior to the start of the nitroprusside treatment, blood pressure was significantly higher in the LTH fetuses compared with control (p<0.05, Figure 4, upper panel). Since the nitroprusside infusion rate was adjusted to reduce mean arterial pressure by 30-35%, the nadir in mean arterial pressure did not reach the same level in the LTH and control groups. Following the end of the hypotensive episode, mean arterial pressure remained higher in the LTH fetuses (p<0.01, compared with control). Fetal heart rate
was significantly higher in the LTH fetuses compared with control following hypotension (p<0.01) (Figure 4, lower panel).

*Fetal blood gases.* Cortisol infusion had no effect on any of the parameters measured (Figure 5). As observed in the fetuses during the saline infusion, mean basal arterial PO2 values were lower in the LTH group compared to control (18.1 ± 1.0 vs. 23.8 ± 0.8 mmHg, p < 0.01). Following hypotension, there was a trend towards a decrease in arterial PO2 in the LTH, but the change did not reach statistical significance. Mean arterial PCO2 values were consistently lower in the LTH fetuses (44.4 ± 1.1 vs. 50.1 ± 0.7 mmHg, p < 0.01, compared to control) and were unchanged by hypotension. Mean basal arterial pH values were higher in the LTH group compared to control (7.33 ± 0.01 vs. 7.30 ± 0.01, p<0.01) but were unaffected by hypotension.

*ACTH and cortisol responses to hypotension.* Prior to the start of the cortisol infusion, basal levels of ACTH were similar in both groups (27.0±5.8 control vs. 29.0±5.3 LTH, pg/ml, Figure 6, upper panel). Following the start of the cortisol infusion, there was a significant effect of time, with a decline in plasma ACTH concentrations in both control and LTH fetuses during the course of the study (p<0.01). Hypotension had no effect on ACTH, however following the end of the hypotensive period, ACTH levels in the LTH group remained lower than values for the control group for the duration of the experiment (p<0.01, Figure 6, upper panel).

As expected, during the cortisol infusion, plasma cortisol concentrations were significantly higher than pre-infusion values in both groups (p<0.01, Figure 6, lower panel). Hypotension had no further effect on plasma cortisol levels in either group. However, despite the same rate of cortisol infusion and no difference in fetal weight (3.56±0.19 vs. 3.77±0.29 kg, control vs. LTH), cortisol concentrations were significantly higher in the LTH group compared to control (p<0.01) during the course of the cortisol infusion. Plasma clearance of cortisol was estimated based on the equilibrium values of cortisol attained during the cortisol infusion. Although there was a
trend towards reduced cortisol clearance in the LTH fetuses compared to controls, the difference did not reach statistical significance (2.99±0.40 vs 2.31±0.15 l/kg/h in control vs. LTH, respectively, p>0.05).

Glucocorticoid receptor binding. Chronic hypoxia had no effect on glucocorticoid receptor density in the fetal hypothalamus or pituitary (Figure 7). Likewise, the Kd did not differ between the two treatment groups in either region.

DISCUSSION

There is a wealth of information regarding responsiveness of the ovine fetal HPA to a wide range of acute physiological stressors ranging from hypoxia (1; 4; 16) to hypotension (26; 32; 35). Efforts to study more long term hypoxia have centered on the use of placental embolization (9) or oxytocin-induced uterine contractures (22) and also showed that these forms of stress stimulated ACTH and cortisol levels in the ovine fetus. Phillips et al., (23) utilized surgical removal of caruncles to subject the fetus to sustained hypoxia throughout gestation and found that plasma cortisol concentrations were elevated despite ACTH levels similar to controls. The ability of the fetus to respond to a secondary stressor, following exposure to a long-term, chronic stress is not clearly defined. A small number of studies have examined the response of the fetal HPA to a superimposed secondary stressor following hypoxia lasting up to a few weeks (9; 11; 22). However, there are no data on the effects of such a challenge following fetal hypoxia lasting through the course of gestation. This underscores the fact that the magnitude of the HPA response may not only depend on the stage of development but also on the duration and the degree of the hypoxic stress. Further, such factors may alter the ability of the fetus to respond to a secondary stressor (10).

In our model of hypoxia lasting the course of gestation, we previously showed that LTH fetuses had an attenuated cortisol response to exogenously administered ACTH (14). In marked
contrast, umbilical cord occlusion resulted in an enhanced cortisol response in LTH fetuses compared with normoxic controls, despite a similar ACTH response (15). The present study was designed to determine if a less severe secondary stressor like hypotension also resulted in a differential cortisol response in LTH fetuses. In this study, we demonstrated that ACTH response of LTH ovine fetuses was similar to normoxic controls. However, the cortisol response was enhanced in the LTH group in response to the secondary stressor.

The issue of what adaptive value enhanced cortisol secretion may have for the LTH fetus is unclear at present. In addition to the role of cortisol in the initiation of labor in the sheep, the stimulation of glucocorticoid secretion in response to stress is a central adaptive mechanism among mammals (18). Acute elevations in cortisol result in a number of catabolic effects including glycogenolysis, increased lipolysis and protein catabolism with a resultant elevation of blood glucose levels (19). We have not yet determined potential differences in energy substrate between control and LTH fetuses in response to a secondary stressor. Enhanced glucose availability could be viewed as an adaptive advantage during an acute stressor.

It is interesting to note that the cortisol response was greater in LTH fetuses compared to control during saline infusion despite similar ACTH responses. A number of factors may contribute to the enhanced circulating cortisol levels in the LTH fetuses. ACTH clearly stimulates short term cortisol output. However, neuronal input to the adrenal may also play an important role in the regulation of cortisol secretion. Splanchnic nerve section (21) in the near term ovine fetus attenuated the cortisol response to hypotension while peak ACTH levels were unaffected. Carotid denervation had a similar effect on the fetal response to acute hypoxemia (12) An additional study found that sinoaortic denervation attenuates reflex responses to hypotension (32). In the LTH animals in the present study, one could therefore hypothesize an enhanced neuroendocrine reflex arc.
A difference in cortisol clearance could also potentially explain the differential cortisol response. Despite similar fetal weights between groups and the same cortisol infusion rate, the overall levels of cortisol attained during the infusion was significantly higher in the LTH fetuses. This potential difference in cortisol clearance could result in higher cortisol levels observed in response to hypotension. This possibility is further strengthened by the fact that cortisol levels remained elevated for a longer period of time in the LTH group during the saline infusion. Although not significant, estimates of plasma clearance from the present study suggest that a reduction in cortisol clearance may indeed play a role in the observed higher cortisol concentrations in the LTH fetuses. Metabolic clearance studies using dual-labeled cortisol may be helpful to more accurately assess the potential effects of LTH on cortisol clearance. One could also suggest that perhaps adrenal blood flow is enhanced in the LTH fetuses. However previous studies from our group demonstrated no differences in total adrenal blood flow in response to a superimposed hypoxia between LTH and normoxic fetuses (17).

It is also of interest that the enhanced cortisol response to hypotension in the LTH fetuses compared to normoxic controls is similar to the change observed following a 5 min, complete umbilical cord occlusion (15). It is interesting to note however that the maximal ACTH response following cord occlusion was 530 ± 90 pg/ml, whereas in the present study, peak values only reached 119±22 pg/ml. These data suggest that a much milder stressor like hypotension, is sufficient to elicit a maximal cortisol response and that baroreceptor, like chemoreceptor stimulation of the cortisol response is enhanced in the LTH fetus.

Unlike the observed baroreceptor stimulation of the cortisol response to hypotension, the lack of baroreflex heart rate response to hypotension in both the control and LTH fetuses in the present study is puzzling. Pervious studies using a similar protocol indeed showed the typical bradycardia associated with hypotension in the fetus. There were however 2 key differences between these studies and the protocol in the present study. First, the percent ethanol in the
vehicle solution was less than our study. Secondly and more importantly, the vehicle infusion was stopped for 1 h prior to the start of the hypotension. One could speculate that ethanol may have played a role in blunting the heart rate response to hypotension. Although to our knowledge, there are no studies in the literature examining the effect of ethanol on fetal baroreceptor function, there are abundant data in adult animals and humans to suggest that ethanol can attenuate baroreflex-mediated heart rate function (7; 29). The apparent dissociation between baroreflex mediation of endocrine and cardiovascular responses in the fetus observed in the present study will serve as an area of future study.

The principal focus of the present study was to determine the effects of LTH on ACTH and cortisol responses to hypotension. However, we secondarily studied potential differences in cortisol negative feedback following LTH. Previous studies demonstrated that the fetal sheep between 117 and 131 days of gestation is very sensitive to the negative feedback effects of cortisol and the ACTH response to nitroprusside-induced hypotension was completely inhibited following cortisol infusion.(6; 30). In near-term fetuses however, although basal ACTH levels are suppressed by cortisol (33) the ACTH response to hypotension was unaffected by cortisol infusion (31). In the present study, the ACTH response to hypotension was blunted in both control and LTH fetuses (Figure 6). Although the exact reason for the difference between the two studies is unknown, there are a number of potential explanations. In the present study, the cortisol infusion was maintained during the nitroprusside infusion whereas in the study by Wood et al.,(31), the cortisol infusion was terminated one hour before the start of nitroprusside. Also, all of the fetuses in the present study underwent a previous hypotensive episode at day 137 which could have potential effects on cortisol negative feedback. Previous studies have clearly shown that exposure to a repetitive stressor can alter HPA function (13; 22).

Regardless of the causes, in our experimental design, there was no increase in fetal ACTH concentrations in response to hypotension in either control or LTH fetuses. However, following
hypotension, the degree of suppression of ACTH was greater in the LTH fetuses compared with
the control group. Alone, these data suggest that there may be enhanced negative feedback
sensitivity to cortisol in the LTH fetuses. However, the overall cortisol levels attained during the
cortisol infusion were also higher in the LTH fetuses. A higher level of cortisol would be
expected to produce a greater suppression of ACTH. Further there was no difference in either
hypothalamic or pituitary glucocorticoid receptor density in the LTH fetuses compared to
normoxic controls. Together, these data suggest that there in no change in cortisol negative
feedback sensitivity following LTH.

It is interesting to note the effects of cortisol on fetal mean arterial pressure. Although saline
infusion had no effect on blood pressure, cortisol infusion caused a significant increase in mean
arterial pressure in the LTH group. There was a trend toward elevated pressure in the control
animals over the first 3 hours of the infusion but the change did not reach statistical significance.
Previous studies using different age fetuses and different cortisol infusion protocols have
demonstrated an increase in blood pressure following cortisol infusion (8; 28; 34) and suggested
that the hypertensive effect of cortisol is through activation of the renin-angiotensin system,
increasing vascular sensitivity to angiotensin-II (8; 28). These data lead to the speculation that
the apparent increased sensitivity of the LTH fetuses to the hypertensive effects of cortisol may
be the result of LTH on the renin-angiotensin system. Future studies utilizing angiotensin-II and
angiotensin type 1-specific antagonists will be necessary to elucidate the effects of LTH.

Fetal PO2 values are typical of what we have previously reported for chronically catheterized
LTH animals (14; 15). PCO2 and pH are also in the range of values previously observed in these
animals. Although all three blood gas parameters were different between control and LTH
fetuses, neither hypotension nor cortisol infusion had an effect on these values. Previous studies
also failed to demonstrate an effect of hypotension on fetal blood gasses (26).
To our knowledge, this is the first study to describe the effects of exposure to a long-term (longer than a few weeks) stressor on the response to hypotension in the ovine fetus. Taken together, the findings from the present study indicate that LTH enhances the fetal cortisol response to hypotension compared to normoxic controls without differences in ACTH output. Further, the LTH fetus appears to be more sensitive to the effects of cortisol infusion at the level of the cardiovascular system. These findings suggest an alteration or resetting of HPA to maintain hormone levels within a normal physiologic range despite long-term hypoxia, as well as respond to a secondary stressor in a robust manner. Such information may have important clinical implications.

**GRANTS**

This work was supported by NIH grant HD 31226.
REFERENCES


23. **Phillips ID, Simonetta G, Owens JA, Robinson JS, Clarke IJ and McMillen C.**


FIGURE LEGENDS

Figure 1. Fetal mean arterial pressure and heart rate in response to 10 min nitroprusside-induced hypotension during saline infusion. Overall mean arterial pressure was significantly different between control and LTH fetuses during the course of the experiment (p<0.01) while heart rates did not differ. The 10 min period of hypotension is represented by the shaded area and all values represent mean ± SEM in this and subsequent figures.

Figure 2. Fetal blood gas parameters in control and LTH fetuses during saline infusion. All three parameters were different between control and LTH fetuses (p<0.01) but were unaffected by saline infusion or hypotension.

Figure 3. Fetal plasma ACTH and cortisol concentrations in control and LTH fetuses in response to 10 min nitroprusside-induced hypotension during saline infusion. (*p<0.05, compared to control).

Figure 4. Fetal mean arterial pressure and heart rate in response to 10 min nitroprusside-induced hypotension during cortisol infusion. Mean arterial pressure and heart rate were significantly different between control and LTH fetuses during the course of the experiment (p<0.01).

Figure 5. Fetal blood gas parameters in control and LTH fetuses during cortisol infusion. All three parameters were different between control and LTH fetuses (p<0.01) but were unaffected by cortisol infusion or hypotension.

Figure 6. Fetal plasma ACTH and cortisol concentrations in control and LTH fetuses in response to 10 min nitroprusside-induced hypotension during cortisol infusion. Both ACTH and cortisol
values were significantly different between control and LTH fetuses during the course of the experiment (p<0.01). Note scale changes compared to Figure 3.

**Figure 7.** Hypothalamic and pituitary glucocorticoid receptor values in near-term control and LTH fetuses. Bmax values are fmol/mg protein and Kd values are listed inside each histogram.
Figure 1
Figure 2.
Figure 3.
Figure 5.
Figure 6.
HYPOTHALAMUS

PITUITARY

B_{max} (fmol/mg protein)

CONTROL  LTH

HYPOTHALAMUS

0.52±0.05  0.59±0.06

PITUITARY

0.59±0.03  0.61±0.05