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Adrenocortical Suppression in Highland Chick Embryos Is Restored during Incubation at Sea Level

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Abstract

Salinas, Carlo E., Mercedes Villena, Carlos E. Blanco, and Dino A. Giusssani. Adrenocortical suppression in highland chick embryos is restored during incubation at sea level. *High Alt. Med. Biol.* 12:79–87, 2011.—By combining the chick embryo model with incubation at high altitude, this study tested the hypothesis that development at high altitude is related to a fetal origin of adrenocortical but not adrenomedullary suppression and that hypoxia is the mechanism underlying the relationship. Fertilized eggs from sea-level or high altitude hens were incubated at sea level or high altitude. Fertilized eggs from sea-level hens were also incubated at altitude with oxygen supplementation. At day 20 of incubation, embryonic blood was taken for measurement of plasma corticotropin, corticosterone, and Po_2. Following biometry, the adrenal glands were collected and frozen for measurement of catecholamine content. Development of chick embryos at high altitude led to pronounced adrenocortical blunting, but an increase in adrenal catecholamine content. These effects were similar whether the fertilized eggs were laid by sea-level or high altitude hens. The effects of high altitude on the stress axes were completely prevented by incubation at high altitude with oxygen supplementation. When chick embryos from high altitude hens were incubated at sea level, plasma hormones and adrenal catecholamine content were partially restored toward levels measured in sea-level chick embryos. There was a significant correlation between adrenocortical blunting and elevated adrenal catecholamine content with both asymmetric growth restriction and fetal hypoxia. The data support the hypothesis tested and provide evidence to isolate the direct contribution of developmental hypoxia to alterations in the stress system.

Key Words: corticotropin; cortisol; hypoxia; high altitude; chick embryo

Introduction

In the prenatal period, one of the most common forms of stress is fetal hypoxia (Giussani et al., 2001; Thakor and Giussani, 2009). In the late- gestation fetus, hypoxic episodes elicit an integrated defense response that facilitates fetal survival and the protection of hypoxia-sensitive tissues during the period of reduced oxygen availability (Giussani et al., 1994). Increases in fetal plasma catecholamines contribute to the fetal glucogenic (Fowden et al., 1998) and cardiovascular (Giussani et al., 1993) defenses against acute hypoxic stress. Increased fetal plasma concentrations of glucocorticoid amplify the actions of the sympathetic nervous system, contributing also to the fetal metabolic (Fletcher et al., 2000) and cardiovascular (Fletcher et al., 2003) responses to acute stress. However, prolonged elevations in fetal plasma glucocorticoid can reduce fetal growth (Ikegami et al., 1997), trigger preterm birth (Nathanielsz et al., 1988), and program cardiovascular and metabolic defects in later life (Seckl et al., 1999). Therefore, switching the pituitary–adrenocortical axis (HPA) off during prolonged stress is just as important as switching it on during short-term stress (Ducsay, 1998). For instance, in ovine pregnancy, if the fetal exposure to hypoxia lasts from hours to days, dissociation in the plasma corticotropin and cortisol responses occurs, such that glucocorticoid is still maintained high despite plasma corticotropin concentrations returning to basal levels (Murotsuki et al., 1996). However, if the fetal exposure to hypoxia is more prolonged, lasting months, such as during pregnancy at high altitude (>2500 m), plasma corticotropin remains high while plasma cortisol concentrations return to basal levels (Ducsay, 1998). Blunting of fetal basal adrenocortical output may therefore be an appropriate

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homeostatic response to prolonged periods of hypoxic stress to protect sensitive tissues from inappropriate and sustained elevations in plasma glucocorticoid levels during fetal development (Ducsay, 1998).

In human pregnancy, several studies have reported that newborn infants from pregnancies complicated by placental insufficiency are very low birth weight and show adrenocortical suppression (Kajantie et al., 2003; Watterberg et al., 2004). However, because placental insufficiency decreases the delivery of both nutrients and oxygen to the fetus, the extent to which the effects on the developing stress axes are owing to fetal hypoxia or underoxygenation is uncertain. Because most high altitude populations are also impoverished, with a high prevalence of maternal undernutrition (Julian et al., 2009), and because chronic maternal hypoxia in experimental animals also reduces maternal food intake (de Grauw et al., 1986), whether the associated adrenocortical blunting is owing to fetal underoxygenation or undernutrition again remains uncertain.

By combining the chick embryo model with incubation at high altitude, we have previously been able to isolate the direct effects of chronic hypoxia in the control of fetal growth and on fetal cardiovascular development, independent of changes in maternal nutrition or other confounders, such as the maternal and placental physiology (Giussani et al., 2007; Salinas et al., 2010). Since the stress axis is functional before hatching in chicks (Jenkins and Porter, 2004), the present study tested the hypothesis that high altitude is related to fetal adrenocortical but not adrenomedullay suppression. The hypothesis was tested in three ways:

1. By investigating the effects on the stress axes of incubation at high altitude of fertilized eggs laid by sea level-hens
2. By investigating whether alterations in the stress axes induced by development at high altitude could be prevented by incubation at sea level of fertilized eggs laid by high altitude hens
3. By investigating whether alterations in the stress axes induced by development at high altitude could be prevented by incubation at high altitude of sea-level eggs with oxygen supplementation

We were also interested in whether generational high altitude residence altered the effects of chronic hypoxia on the stress system and whether pituitary–adrenal hormone concentration correlated with fetal biometry and/or arterial Po_2.

Materials and Methods

The study was done in Bolivia, in the high altitude city of La Paz (3600 m, 494 mmHg, approximate ambient dry Po_2 100 mmHg) and in the sea-level city of Santa Cruz (420 m, 760 mmHg, approximate ambient dry Po_2 160 mmHg). The incubation procedures have been previously published in detail (Giussani et al., 2007; Salinas et al., 2010). In brief, fertilized eggs were obtained from Black Leghorn chickens that had been reared at the sea-level city of Santa Cruz or at the high altitude city of La Paz for at least six generations. Egg storage is commonly practiced in the artificial incubation of domestic birds. If the storage temperature for freshly laid chicken eggs is kept below the physiological zero (25° to 27°C), dormancy of the embryo can be maintained and fertile eggs can be stored for 1 to 3 weeks. In this study, fertilized eggs from Santa Cruz and La Paz were accumulated, maintained, and transported over 2 to 3 days at 14°C to arrest and synchronize development prior to incubation. This permitted incubation at different altitudes to start at day 1 of embryonic age (Giussani et al., 2007). Fertilized eggs from sea-level hens, laid at sea level, were randomly divided and incubated either at sea level (SLSL, $n = 35$) or high altitude (SLHA, $n = 24$). Eggs from high altitude hens, laid at high altitude, were randomly divided and incubated either at high altitude (HAHA, $n = 36$) or sea level (HASL, $n = 31$). SLHA embryos were also incubated with oxygen supplementation ($SLHA + O_2$, $n = 28$) at rates to maintain sea-level oxygen partial pressures according to Dalton's law (West, 1999).

All incubations (Polyhatch, Brinsea Products Ltd., Sanford, North Somerset, UK) were carried out under conditions to optimize development, with controlled temperature (38°C) and humidity (60%) and appropriate egg rotation. On day 20 of the 21-day incubation period, the egg was weighed, the air cell was exposed, and chorioallantoic venous blood was drawn into a 1-mL syringe for analysis of Po_2 (ABL 500, Radiometer, Copenhagen, Denmark), whenever possible in duplicate. At the same time, as much blood as possible (1 to 2 mL) was collected from chorioallantoic arterial vessels for subsequent measurement of plasma corticotropin and corticosterone. These samples were collected under sterile conditions into chilled EDTA tubes (K^+/EDTA, LIP Ltd., Shipley, West Yorkshire, UK); they were then centrifuged at 4000 rpm for 4 min at 4°C. The plasma obtained was then dispensed into prelabeled tubes, and the samples were stored at −80°C until analysis.

Following euthanasia by spinal transection, the embryo was removed from the eggshell, the adrenal glands were isolated, and embryonic body weight and the combined adrenal weight were recorded. The adrenal glands were snap frozen in liquid nitrogen for subsequent analysis of catecholamine content, because insufficient blood was available from any one embryo for analysis of circulating plasma concentrations of catecholamines.

Measurements for plasma corticotropin, corticosterone, and adrenal catecholamine content were performed in a subset of animals for each group within 2 months of sample collection, as previously described in detail, (Gardner et al., 2001; Fletcher et al., 2006). Plasma corticotropin and corticosterone concentrations were determined by radioimmunoassay (RIA) using commercially available kits (corticotropin: DiaSorin Inc., Stillwater, Minnesota, USA; corticosterone: ICN Biomedicals, Irvine, CA, USA). For corticotropin, the lower limit of detection for the assay was between 10 and 25 pg/mL^{-1}. The intraassay coefficients of variation for two plasma pools (37 and 150 pg/mL^{-1}) were 3.6% and 4.1%, respectively. The interassay coefficient of variation was 8.4%. For corticosterone, the assay sensitivity was 25 pg/mL^{-1}, and the intra- and interassay coefficients of variation were 5.8% and 7.5%, respectively. Noradrenaline and adrenaline concentrations were measured in both adrenal glands per chick embryo by high-pressure liquid chromatography (HPLC) using electrochemical detection. The samples were prepared by absorption of 250 μL onto acid-washed alumina, and 20 μL aliquots of the 100-μL perchloric acid elutes was injected onto the column. Dihydroxybenzylamine was added as the internal standard to each sample before absorption. Recovery ranged from 63% to 97%, and all catecholamine values were corrected for their respective recovery. The interassay coeffi-

cients of variation for adrenaline and noradrenaline were 7.3% and 6.2%, respectively. Measurements were made in duplicate on two dilutions of each purified sample, and the data were expressed as $\mu g/mg^{-1}$ for tissue content.

All procedures were approved by the local ethics committee of the Bolivian Institute for High Altitude Biology (Consejo Técnico, IBBA, Universidad Mayor de San Andrés, La Paz, Bolivia). Values for Po_2, embryonic and adrenal weights, and endocrine variables are expressed as mean ± SEM. Comparisons between groups were assessed statistically using one-way ANOVA with the Student Newman–Keuls post hoc test (Sigma-Stat, SPSS Inc., Chicago, IL, USA). The relationship between parallel measurements of plasma concentrations of corticotropin and corticosterone in all individual chick embryos was assessed using the Pearson product moment correlation. A comparison between the slopes and intercepts of regression lines was conducted according to Armitage and Berry (1994). For all comparisons, statistical significance was accepted when $p < 0.05$.

Results

Oxygenation and biometry

Embryonic systemic hypoxia and growth restriction occurred during incubation at high altitude (SLHA and HAHA) (Table 1). When weight was expressed as a percentage of the initial egg mass, embryos from high altitude hens (HAHA) relative to those from sea-level hens (SLHA) showed a diminished reduction in growth during high altitude incubation. Further, when this group was incubated at sea level (HASL), the relative body weight was greater than for any other group. The absolute combined adrenal weight was also reduced during incubation at high altitude (SLHA and HAHA); however, this effect no longer occurred when the combined adrenal weight was expressed relative to the embryonic body weight. Reductions in body and adrenal weights no longer occurred during incubation at high altitude with oxygen supplementation ($SLHA + O_2$).

Plasma corticotropin and corticosterone

Although plasma concentrations of corticotropin were significantly elevated, plasma concentrations of corticosterone were significantly depressed in embryos incubated at high altitude (SLHA and HAHA) relative to sea-level embryos (SLSL; Fig. 1). The magnitudes of the increment in plasma corticotropin and of the decrement in plasma corticosterone following incubation at high altitude were similar in embryos from hens native to sea level (SLHA) or to high altitude (HAHA). Plasma corticotropin in high altitude embryos incubated at sea level (HASL) was no longer different from sea-level embryos (SLSL). Plasma corticosterone concentrations in these embryos (HASL) were significantly greater than in SLHA and HAHA embryos, but still significantly depressed relative to SLSL embryos. Incubation of sea-level embryos at high altitude with oxygen supplementation ($SLHA + O_2$) prevented the high altitude-induced increase in plasma corticotropin and the high altitude-induced decrease in plasma corticosterone.

Correlation analysis, using the Pearson product moment test of paired plasma corticotropin and corticosterone values for all individual chick embryos (Fig. 2), revealed significant relationships for sea-level embryos incubated at sea level (SLSL, $r = 0.9$, $n = 10$, $p < 0.001$) and for sea-level embryos incubated at high altitude with oxygen supplementation ($SLHA + O_2$, $r = 0.9$, $n = 9$, $p < 0.003$). However, no significant relationship between plasma corticotropin and corticosterone was found in sea-level embryos incubated at high altitude (SLHA, $r = 0.7$, $n = 7$, $p = 0.07$), in high altitude embryos incubated at high altitude (HAHA, $r = 0.1$, $n = 7$, $p = 0.90$), or in high altitude embryos incubated at sea level (HASL, $r = 0.1$, $n = 9$, $p = 0.88$).

A comparison of slopes of the linear regressions (Fig. 2) also revealed that the slopes of sea-level chick embryos incubated at high altitude (SLHA, $y = 0.0101x + 2.4$), of high altitude embryos incubated at high altitude (HAHA, $y = 0.0007x + 5.3$), and of high altitude embryos incubated at sea level (HASL, $y = 0.0017x + 10.5$) were significantly depressed ($p < 0.05$) relative to sea-level embryos incubated at sea level (SLSL, $y = 0.1103x - 3.4$) or sea-level embryos incubated at high altitude with oxygen supplementation ($SLHA + O_2$, $y = 0.0838x + 11.0$).

Adrenal catecholamine content

Adrenal concentrations of noradrenaline and adrenaline were significantly elevated in embryos incubated at high altitude (SLHA and HAHA) relative to sea-level embryos (SLSL; Fig. 3). The magnitudes of these increments in adrenal catecholamine content following incubation at high altitude were similar in embryos from hens native to both sea level (SLHA) and high altitude (HAHA). Adrenal catecholamine content in high altitude embryos incubated at sea level (HASL) were significantly depressed relative to SLHA and HAHA embryos, but still significantly elevated relative to SLSL embryos. Incubation of sea-level embryos at high altitude with oxygen

Table 1. Oxygenation and Biometry in the Chick Embryo

	PO_2 (mmHg)	*Embryonic body weight (%)*	*Adrenal weight (mg)*	*Relative adrenal weight (%)*
SLSL	57 ± 3 (12)[a]	41 ± 1 (35)[a]	5.5 ± 0.3 (35)[a]	0.020 ± 0.001 (35)
SLHA	34 ± 2 (14)[b]	21 ± 1 (24)[b]	3.6 ± 0.3 (24)[b]	0.025 ± 0.002 (24)
HAHA	35 ± 1 (13)[b]	29 ± 1 (36)[c]	3.7 ± 0.3 (36)[b]	0.024 ± 0.002 (36)
HASL	59 ± 5 (9)[a]	54 ± 2 (31)[d]	5.2 ± 0.3 (31)[a]	0.021 ± 0.002 (31)
$SLHA + O_2$	60 ± 4 (11)[a]	46 ± 1 (28)[a]	5.9 ± 0.4 (28)[a]	0.020 ± 0.002 (28)

Values are mean ± SEM for the partial pressure of oxygen in chorioallantoic venous blood, the embryonic weight expressed as a percentage of the initial egg mass, and the absolute combined adrenal weight and the combined adrenal weight expressed as a percentage of the embryonic body weight. Groups are sea-level chick embryos incubated either at sea level (SLSL) or at high altitude (SLHA), high altitude embryos incubated at high altitude (HAHA) or at sea level (HASL), and sea-level chick embryos incubated at high altitude with oxygen supplementation ($SLHA + O_2$). Numbers in brackets refer to n. Values within columns that have different letters as superscripts are significantly different from each other (one-way ANOVA with Student Newman–Keuls test; $p < 0.05$).

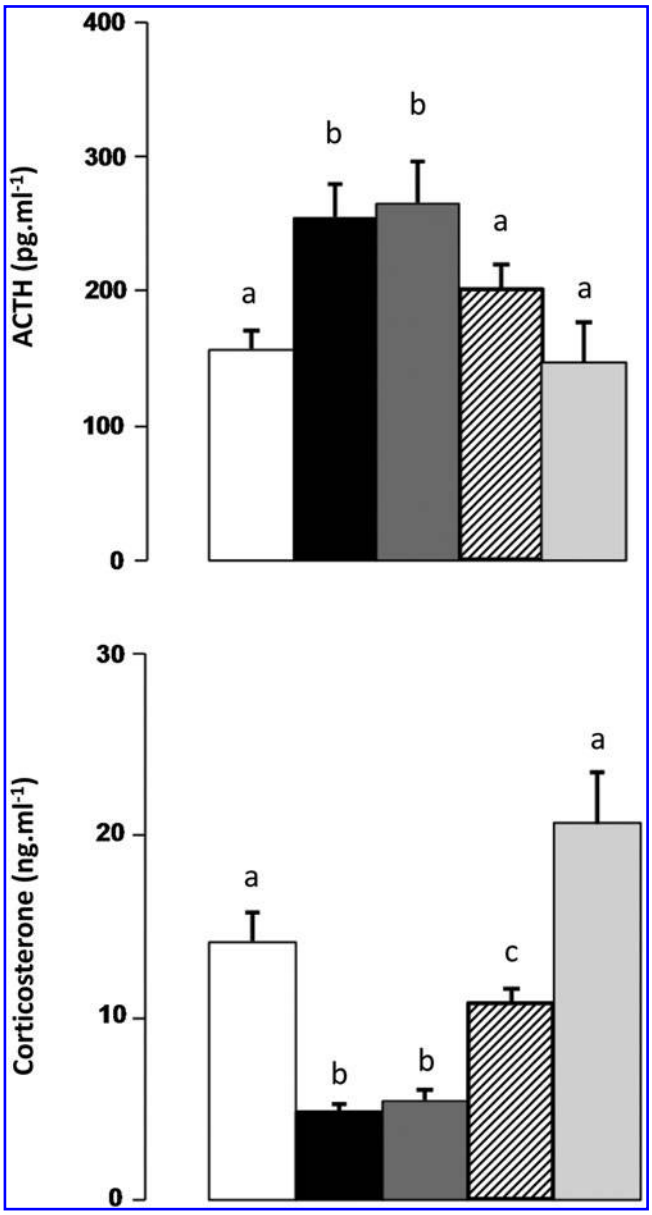

FIG. 1. Plasma corticotropin and corticosterone in the chick embryo. Values are mean ± SEM for the plasma concentrations of corticotropin and corticosterone in chorioallantoic arterial blood at day 20 of the incubation period. Groups are sea-level chick embryos incubated either at sea level (SLSL, $n = 9$, □) or at high altitude (SLHA, $n = 7$, ■), high altitude embryos incubated at high altitude (HAHA, $n = 9$, ■) or at sea level (HASL, $n = 7$, ▨), and sea-level chick embryos incubated at high altitude with oxygen supplementation (SLHA ± O_2, $n = 10$, ■). Values within columns that have different letters as superscripts are significantly different from each other (one-way ANOVA with Student Newman–Keuls test, $p < 0.05$).

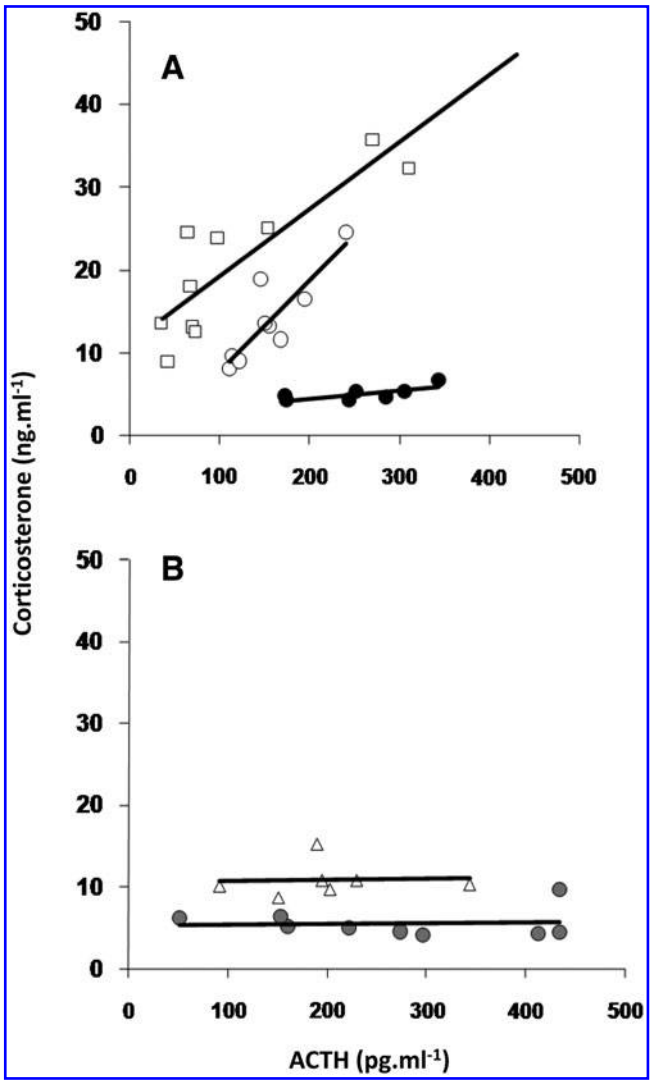

FIG. 2. Relation between plasma corticotropin and corticosterone in the chick embryo.Values are the paired plasma corticotropin and corticosterone concentrations in chorioallantoic blood at day 20 of the incubation period for all individual samples in chick embryos. (**A**) Sea-level chick embryos incubated either at sea level (SLSL, $n = 9$, ○), or at high altitude (SLHA, $n = 7$ ●), or at high altitude with oxygen supplementation (SLHA ± O_2, $n = 10$, □). (**B**) High altitude embryos incubated either at high altitude (HAHA, $n = 9$, ●) or at sea level (HASL, $n = 7$, △).

supplementation (SLHA + O_2) prevented the high altitude-induced increase in adrenal catecholamine content.

Relation between adrenal function and embryonic size or Po_2

Correlation analysis revealed that the plasma corticosterone–corticotropin ratio was positively related to the embryonic body weight and to Po_2, but negatively related to the head diameter–body weight ratio in all groups, independent of treatment (Fig. 4). Conversely, the adrenal catecholamine content was negatively related to embryonic body weight and to Po_2, but positively related to the head diameter–body weight ratio in all groups, independent of treatment (Fig. 4).

Discussion

The data show that the development of chick embryos at high altitude leads to pronounced adrenocortical blunting, but an increase in adrenal catecholamine content, by the end of the incubation period. These effects of high altitude incubation are similar whether the fertilized eggs were laid by sea-level or high altitude hens. The effects of high altitude on the stress axes are completely prevented by incubation at high

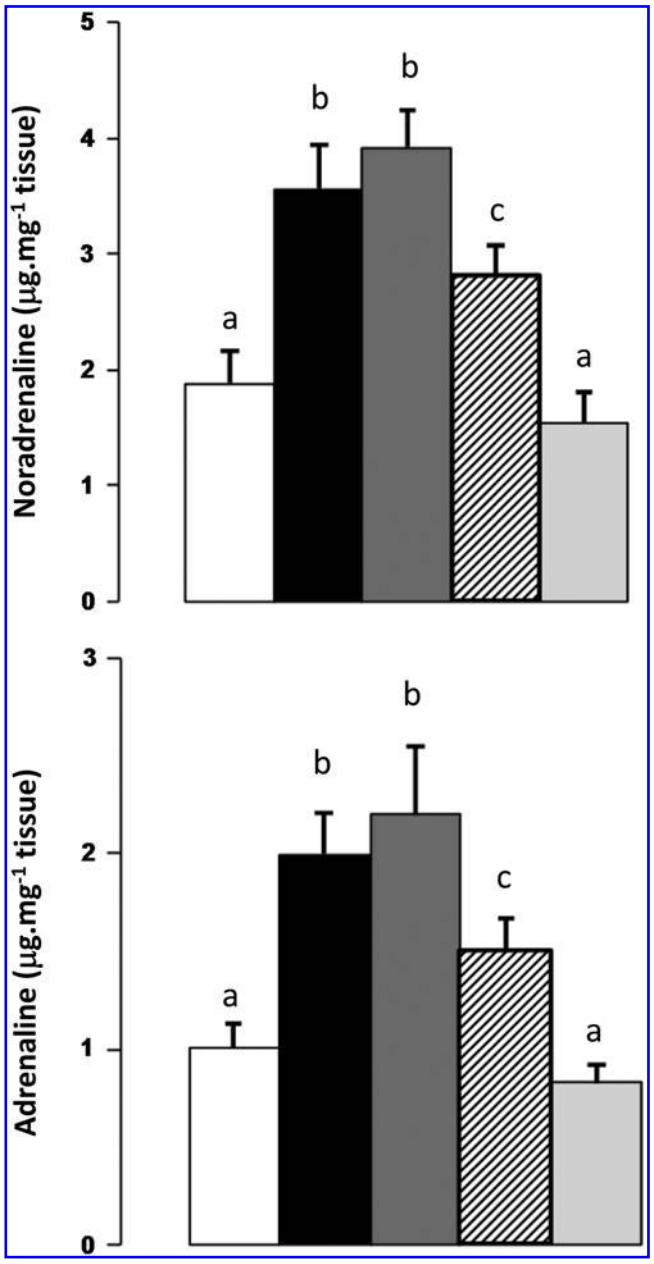

FIG. 3. Adrenal catecholamines in the chick embryo. Values are mean ± SEM for the content noradrenaline and adrenaline expressed per milligram of tissue at day 20 of the incubation period. Groups are sea-level chick embryos incubated either at sea level (SLSL, $n = 10$, □) or at high altitude (SLHA, $n = 10$, ■), high altitude embryos incubated at high altitude (HAHA, $n = 10$, ■) or at sea level (HASL, $n = 9$, ▨), and sea-level chick embryos incubated at high altitude with oxygen supplementation (SLHA ± O_2, $n = 10$, ■). Values within columns that have different letters as superscripts are significantly different from each other (one-way ANOVA with Student Newman–Keuls test, $p < 0.05$).

altitude with oxygen supplementation. When chick embryos from high altitude hens are incubated at sea level, plasma corticosterone and adrenal catecholamines by the end of incubation are partially restored toward levels measured in sea-level chick embryos. Adrenal cortical blunting and elevated catecholamine concentrations were significantly related to both growth restriction and fetal hypoxia.

Basal plasma concentrations of corticosterone in embryos from sea-level hens incubated at sea level were within the range of those reported by others for chick embryos at this stage of incubation or at hatching (Marie, 1981; Scott et al., 1981). Although several studies have reported that the adrenal cortex of the chick embryo is functionally responsive to corticotropin by the end of the incubation period (Woods et al., 1971; Wise and Frye, 1973), to our knowledge, this is the first report of circulating levels of corticotropin in the chick embryo during development at both at sea level and high altitude. One previous study has published the adrenaline content of the chick embryo adrenal gland during development (Wassermann and Bernard, 1970). In the present study, the levels of adrenaline content in the adrenal gland of embryos from sea-level hens incubated at sea level were similar to those reported by Wasserman and Bernard (1970) for chick embryos at this stage of incubation.

The physiology underlying the suppression of fetal adrenocortical function during development at high altitude has been studied extensively in a series of elegant contributions by Ducsay and Myers. Using the long-term hypoxemic (LTH) ovine model, whereby pregnant ewes are maintained at 3820 m above sea level from day 30 of gestation (term is ~150 days), they reported that the ability of the late-gestation sheep fetus to respond to a corticotropin challenge is markedly suppressed (Harvey et al., 1993). In addition, LTH sheep fetuses have significantly enhanced anterior pituitary processing of proopiomelanocortin (POMC) to corticotropin (Myers et al., 2005a) and an enhanced pituitary responsiveness to arginine vasopressin (Ducsay et al., 2009), both of which result in greater basal corticotropin concentrations, but in similar concentrations of cortisol (Ducsay, 1998). These findings are consistent with blunting of fetal basal adrenocortical output, which may be an appropriate adaptive fetal response to prolonged stress, to protect itself against the deleterious effects on fetal growth and the development of inappropriate and sustained elevations in plasma glucocorticoid levels (Ducsay, 1998). Reduced expression of adrenal corticotropin receptor and key steroidogenic enzymes (Myers et al., 2005b), as well as increased NO-mediated inhibition of steroidogenesis (Monau et al., 2009), may contribute to the physiology underlying adrenocortical blunting in the high altitude fetus. The data in the present article showed that adrenocortical blunting could be induced in chick embryos incubated at high altitude. Indeed, incubation at high altitude of fertilized eggs from sea-level hens produced severely growth restricted embryos with basal plasma corticosterone concentrations substantially lower than sea-level embryos. Adrenocortical blunting could be prevented by high altitude incubation with oxygen supplementation, and it could be reversed by incubating chick embryos from high altitude hens at sea level. Therefore, the present data extend the findings of Ducsay and Myers to confirm, by using a three-prong approach, that it is the direct effect of hypoxia during development at high altitude, rather than maternal or placental factors and/or hypobaria, that is responsible for adrenocortical blunting in the fetus. Of interest, our data also show that there is a strong positive relationship between adrenocortical blunting and intrauterine growth retardation (IUGR) and a strong negative relationship between adrenocortical blunting and arterial Po_2 in the chick embryo. These significant relationships further support the concepts that development under chronic hypoxia promotes IUGR (Giussani et al., 2007;

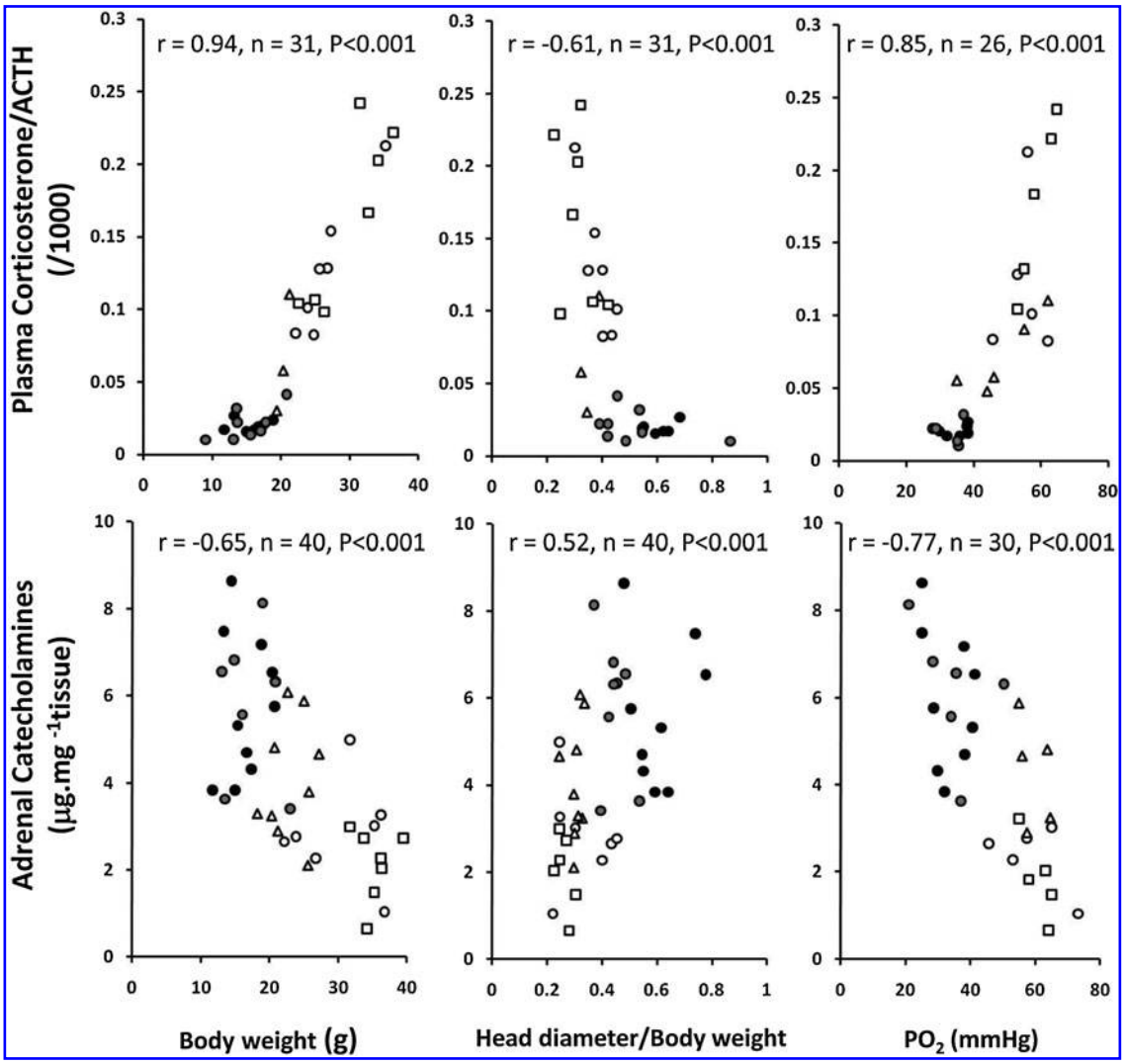

FIG. 4. Relationship between adrenal function and embryonic size or Po_2. Body weight, the head diameter to body weight ratio, and chorioallantoic venous Po_2 at the end of the incubation period were related to the ratio of corticosterone–corticotropin in plasma and the adrenal combined catecholamine content in all embryos independent of treatment. *r*, Pearson product moment correlation coefficient; *n*, number of observations. SLSL (○), SLHA (●), HAHA (◉), HASL (△), and SLHA ± O_2 (□).

Copeland and Dzialowski, 2009; Tintu et al., 2009) and that hypoxia-sensitive genes are involved in adrenocortical blunting.

Although blunting of basal adrenocortical output during long-term hypoxic development may protect the fetus against the deleterious effects of sustained elevations in glucocorticoid, it also renders the mammalian fetus susceptible to an inappropriate adrenocortical defense to a secondary acute stressor, such as the intrapartum hypoxia at the end of pregnancy. Our group (Riquelme et al., 1998; Riquelme et al., 2002) and Ducsay and Myers (Adachi et al., 2004; Imamura et al., 2004) have hypothesized that possible mechanisms exist to override suppression of fetal adrenocortical function during superimposed stress during development at high altitude. One strategy may be to increase acutely the gain of neural influences on adrenocortical function, which are triggered by a carotid chemoreflex (Riquelme et al., 1998; Riquelme et al, 2002) and mediated by splanchnic innervation to the adrenal gland (Myers et al., 1990). Compared to sheep, such neural influences on adrenocortical ouput during acute stress in the fetus are sensitized in the llama (Riquelme et al., 1998; Riquelme, 2002), a species adapted to the chronic hypoxia of life at high altitude for generational times (Giussani et al., 1999). Ducsay and Myers have recently reported (Vargas et al., 2010) that the enhanced plasma cortisol response to a second stressor in the LTH sheep fetus may involve enhanced adrenocortical intracellular signaling downstream from cAMP-dependent protein kinase A (PKA). Data in the present article also show that, when chick embryos from hens native to high altitude are incubated at sea level, plasma corticosterone by the end of incubation is partially restored toward the concentrations measured in sea-level chick embryos. However, the mechanism promoting this increase appears independent of corticotropin, because the relation between corticosterone and corticotropin in this group is still blunted relative to sea-level chick embryos. It is possible that the mechanisms employed by the highland chick embryo to restore adrenocortical output during incubation at sea level are similar to those

employed by the mammalian fetus to unleash an adrenocortical response of appropriate magnitude in response to a second stressor, despite basal adrenocortical blunting.

In contrast to the present study, Hassanzadeh and colleagues (2004) reported significantly higher plasma corticosterone concentrations in chick embryos following highland incubation compared to incubation at sea level. However, that study has several important differences. They incubated chick embryos at 2000-m altitude relative to ~4000- m altitude as in the present study. Also, their blood samples were obtained by cardiac puncture, rather than by chorioallantoic arterial blood sampling. It is likely that the difference in the effect of high altitude incubation on plasma corticosterone in the chick embryo between their and our studies is owing to the actual level of fetal hypoxia experienced. Blood sampling by cardiac puncture is stressful. Therefore, the significantly higher plasma corticosterone concentrations measured in highland chick embryos in the study of Hassanzadeh and colleagues (2004) may represent a greater capacity of the adrenal cortex to respond to acute stress, rather than basal concentrations during chronic exposure to high altitude. Therefore, the finding is in keeping with the hypothesis that possible mechanisms exist to override suppression of fetal adrenocortical function during superimposed stress following development at high altitude (Riquelme et al., 1998; Riquelme et al., 2002; Adachi et al., 2004; Imamura et al., 2004).

By late gestation in the developing ovine fetus, stress activates the sympathetic nervous system, eliciting the release of both noradrenaline and adrenaline. These catecholamines play an essential role in regulating fetal cardiovascular and metabolic responses to a wide variety of stresses and are fundamental in the adaptation of the neonate to the environment after birth (Giussani et al., 1993; Fowden et al., 1998; Olver et al., 2004). Several investigators have reported that fetal exposure to chronic hypoxia leads to upregulation of the sympathoadrenomedullary system. For instance, chronically hypoxemic fetal sheep (Gardner et al., 2002) and fetal llamas (Llanos et al., 2003) have higher resting plasma concentrations of noradrenaline than normoxic fetal sheep at equivalent stages of gestation. Studies by Ruijtenbeck and colleagues (2000) have reported that development of chick embryos under chronic isobaric hypoxia from 0.3 to 0.9 of the incubation period leads to elevations in the vascular noradrenaline content of peripheral circulations. Of interest, Camm and colleagues (2004) reported that the increase in catecholamine as a consequence of chronic hypoxia during development induced impaired memory in chicken. It has also been reported that both llama fetuses (Giussani et al., 1999) and chronically hypoxemic, growth-retarded fetal sheep (Creasy et al., 1973) have a greater dependence on α-adrenergic mechanisms to survive episodes of acute hypoxic stress than do control fetal sheep. Data in the present study show that in the chick embryo (1) adrenal catecholamine content is enhanced by development at high altitude, (2) this effect could be prevented by high altitude incubation with oxygen supplementation and could be reversed by incubating chick embryos from high altitude hens at sea level, and (3) there is a strong negative relationship between arterial Po_2 and adrenal catecholamine content and a strong positive relationship between adrenal catecholamine content and IUGR. Therefore, the present data also extend previous findings to confirm that it is the direct effect of reductions in oxygenation during chronic hypoxic development that leads to upregulation of the sympathoadrenomedullary system, of the type which is associated with IUGR.

In conclusion, the combined data in the present study provide strong evidence to support the hypothesis tested. High altitude is related to fetal adrenocortical but not to adrenomedullary suppression, and hypoxia is the mechanism underlying the relationship. Blunting of fetal basal adrenal cortical but not medullary function may be an appropriate homeostatic response to prolonged periods of hypoxia to protect sensitive tissues from sustained elevations of plasma cortisol levels while maintaining appropriate glucogenic capacity during fetal development. The biological trade-off may yield newborns with adrenocortical suppression.

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Disclosures

The authors have no conflicts of interest or financial ties to disclose.

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