Long-term exposure to high-altitude chronic hypoxia during gestation induces neonatal pulmonary hypertension at sea level

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Long-term exposure to high-altitude chronic hypoxia during gestation induces neonatal pulmonary hypertension at sea level. Am J Physiol Regul Integr Comp Physiol 299: R1676–R1684, 2010. —We determined whether postnatal pulmonary hypertension induced by 70% of pregnancy at high altitude (HA) persists once the offspring return to sea level and investigated pulmonary vascular mechanisms operating under these circumstances. Pregnant ewes were divided into two groups: conception, pregnancy, and delivery at low altitude (580 m, LLL) and conception at low altitude, pregnancy at HA (3,600 m) from 30% of gestation until delivery, and return to lowland (LHL). Pulmonary arterial pressure (PAP) was measured in vivo. Vascular reactivity and morphometry were assessed in small pulmonary arteries (SPA). Protein expression of vascular mediators was determined. LHL lambs had higher basal PAP and a greater increment in PAP after N(′)-nitro-L-arginine methyl ester (20.9 ± 1.1 vs. 13.7 ± 0.5 mmHg; 39.9 ± 5.0 vs. 18.3 ± 1.3 mmHg, respectively). SPA from LHL had a greater maximal contraction to K⁺ (1.34 ± 0.05 vs. 1.16 ± 0.05 N/m), higher sensitivity to endothelin-1 and nitroprusside, and persistence of dilation following blockade of soluble guanylate cyclase. The heart ratio of the right ventricle-to-left ventricle plus septum was higher in the LHL relative to LLL. The muscle area of SPA (29.3 ± 2.9 vs. 21.1 ± 1.7%) and the protein expression of endothelial nitric oxide synthase (1.7 ± 0.1 vs. 1.1 ± 0.2), phosphodiesterase (1.4 ± 0.1 vs. 0.7 ± 0.1), and Ca²⁺-activated K⁺ channel (0.76 ± 0.16 vs. 0.30 ± 0.01) were greater in LHL compared with LLL lambs. In contrast, LHL had decreased heme oxygenase-1 expression (0.82 ± 0.26 vs. 2.22 ± 0.44) and carbon monoxide production (all P < 0.05). Postnatal pulmonary hypertension induced by 70% of pregnancy at HA promotes cardiopulmonary remodeling that persists at sea level.

pulmonary hypoxic vasoconstriction; pulmonary vascular reactivity; nitric oxide; pulmonary vasodilators; pulmonary vasoconstrictors

THE ETIOLOGY OF PULMONARY hypertension in the postnatal period is complex and not completely understood (2, 44, 48). Proposed mechanisms underlying the physiology mediating elevations in postnatal pulmonary arterial pressure include impaired endothelial function promoting an increase in pulmonary vascular resistance (1, 2, 44). Pulmonary hypertension in the postnatal period is associated with high mortality, and children who survive may have decreased postnatal growth and devastating neurological, respiratory, and cardiac complications that often persist into childhood (9, 44). One condition that may lead to elevations in pulmonary arterial pressure in the postnatal period is sustained fetal hypoxia (1, 44). In humans and animals, a common form of sustained fetal hypoxia is pregnancy at high altitude (16, 23). Pulmonary hypertension in the postnatal period due to this condition is an important problem, since currently nearly 140 million people reside at over 2,500 meters above sea level, being permanently exposed to chronic hypoxic conditions (34, 41). Sustained or partial exposure to high altitude of pregnant women, either permanently resident at high altitude or native to low altitude, is therefore a current problem.

Several mediators act upon the pulmonary vasculature, triggering alterations in vascular tone and structure. A potent vasoconstrictor is endothelin-1 (ET-1), which acts via the ETA receptor to stimulate both contraction and remodeling of the pulmonary vascular bed. ET-1 therefore plays an important role in the regulation of pulmonary vascular resistance (3, 4, 26). Interestingly, it has been reported that ET-1 function is increased in neonatal pulmonary hypertension (3). Nitric oxide (NO) is also another important modulator of the pulmonary circulation, and its vasodilator actions are mediated via several mechanisms, including the activation and opening of Ca²⁺-activated K⁺ channels (BKCa) and the balance between the synthesis of cGMP through the activation of soluble guanylate cyclase (sGC) and its degradation by the isoenzyme phosphodiesterase 5 (PDE5) (2, 42). The impairment of NO-dependent dilatation has also been closely related to pulmonary hypertension in the postnatal period (1, 44). In addition, the endogenous gas carbon monoxide (CO) is a dilator in the pulmonary vascular bed, and it protects against pulmonary vascular remodeling (31, 37, 48). In newborn llamas, augmented pulmonary CO, rather than pulmonary NO, helps to prevent pulmonary hypertension in the newborn period at high altitude (25).

Using ovine pregnancy at high altitude as an experimental model, we have previously reported that pregnancy and delivery at high altitude yields offspring with pulmonary hypertension, coupled with increased constrictor reactivity of isolated pulmonary vessels despite enhanced pulmonary NO function (23, 24, 25). In those studies, the in vivo and in vitro measurements were performed at high altitude. It remains unknown whether long-term exposure of the pregnancy to high altitude...
results in altered pulmonary vascular function and anatomy in offspring, even following return to sea level. Therefore, this study tested the hypothesis that long-term exposure of the pregnancy to high altitude results in postnatal pulmonary hypertension even following return to sea level and that this is associated with cardiopulmonary remodeling and alterations in the pulmonary vascular function. We used an integrative approach at the whole animal, isolated organ, and molecular level to determine the effects of 70% of gestation at high altitude on: 1) in vivo pulmonary arterial pressure under basal and acute hypoxic conditions, both before and after NO blockade; 2) the reactivity of isolated small pulmonary arteries to KCl, ET-1, and to sodium nitroprusside (SNP) before and after treatment with the sGC inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ); 3) the mRNA and protein expression of endothelial NO synthase (eNOS), and protein expression of sGC, BKCa, PDE5, heme oxygenase-1 (HO-1), and CO production for 24 h at 4°C and embedded in paraffin; van Giesson staining was performed continously via a data acquisition system (Powerlab/SP System and Chart v4.1.2 Software; ADInstruments, New South Wales, Australia) connected to a computer. Cardiac output was determined at set intervals by the thermodilution method by the injection of 3 ml of chilled (0°C) 0.9% NaCl in the pulmonary artery through the Swan-Ganz catheter connected to a cardiac output computer (COM-2 model; Baxter, Irvine, CA). Pulmonary vascular resistance was calculated as described previously (23). The production of CO by the pulmonary circulation was calculated as follows: cardiac output multiplied by the difference in the concentration of CO between the aorta and the pulmonary artery (percent of carboxyhemoglobin; IL-Synthesis 25) (25).

**Ex Vivo and In Vitro Experiments**

The remaining uninstrumented lambs (LLL, n = 6; LHL, n = 5) underwent euthanasia with an overdose of sodium thiopentone (100 mg/kg iv) and were studied ex vivo. *Wire myography.* The left lung was removed by dissection and immediately immersed in cold saline. Fourth-generation pulmonary arteries (counting from the pulmonary artery trunk, id LLL: 410 ± 20 μm and LHL: 365 ± 22 μm) were dissected from the caudal lobe of the left lung. Isolated arteries were mounted in a wire myograph, maintained at 37°C, and aerated with 95% O2-5% CO2. Concentration-response curves (CRCs) were analyzed in terms of sensitivity and maximal or minimal responses by fitting experimental data to the Boltzmann equation (Prism 5.0; GraphPad Software, La Jolla, CA). Contractile responses were expressed in terms of tension (N/m) and contraction or relaxation responses as a percentage of increase or reduction of 125 mM K+–induced contraction or tension (N/m). Sensitivity was calculated as pD2, where pD2 = -log(EC50), with EC50 being the concentration at which 50% of the maximal response was obtained (22). CRCs were constructed for KCl, ET-1, and for the NO donor SNP following precontraction with 125 mM K+. CRCs to SNP were repeated following blockade of sGC with 50 μM of ODQ (10-5 M) incubation for 10 min.

**RT-PCR and Western blot.** Total RNA purification from lung tissue, cDNA synthesis, and PCR amplification was performed as described previously (11). Primers for amplification of partial DNA sequences from eNOS (forward 5’-AGCTTGAGACCCTCAGT-3’) and reverse 5’-GTCTCCAGCTTGGACGTCG-3’, accession no. DQ015701) and 18S rRNA (forward 5’-TCAAGAAC-3’, reverse 5’-GGACATCTAAGGGCATCACA-3’, the housekeeping gene, accession no. BK00096) were derived from the corresponding sheep and mouse genes, respectively. All of the PCR products were sequenced to verify their identity. The PCR products were visualized under ultraviolet light and quantified by densitometry. Protein expression of eNOS, HO-1, sGC, BKCa, PDE5, and β-actin was determined in total lung lysates by immunoblot with specific anti-eNOS monoclonal antibody (Transduction Laboratories), anti-HO-1 monoclonal antibody (Research Diagnostic), anti-sGC polyclonal antibody (Cayman Laboratories), anti-BKCa polyclonal antibody (Alomone laboratories), anti-PDE5 polyclonal antibody (BD Transduction Laboratories), and anti-β-actin monoclonal antibody (Sigma) as described elsewhere (25). The signals obtained on immunoblot or RT-PCR determinations were quantified by densitometric analysis using the Scion Image Software (Scion Image Beta 4.02 Win; Scion).

**Heart and lung biometry.** The neonatal heart was obtained, and the free wall of the right ventricle, the left ventricle, and the septum were dissected. The ratio of the weights of the right ventricle to the left ventricle and septum was calculated (20). In addition, the lungs were removed and weighed. Lung weight-to-body weight ratio was calculated.

**Histology.** We isolated and perfused the right lung with 4% paraformaldehyde. Excised lungs were fixed in 4% paraformaldehyde for 24 h at 4°C and embedded in paraffin; van Giessen staining was...
performed on 10-μm slides. At least four arteries (100–200 μm diameter) per lung were chosen, and an average of four measurements from each artery was recorded. Images of parenchymal arterioles were acquired using a workstation (Olympus trinocular microscope-BX51 plus digital camera QimaginGO3) linked to Image Pro software 6.3, and vascular areas were calculated using an image analysis program.

The wall-to-vessel area ratio was calculated and expressed as a percentage, as previously described (24, 33). Briefly, the percent wall thickness was calculated as follows: wall thickness (%) = external area – internal area/external area × 100, where external area and internal area are the area bounded by external and internal elastic laminae, respectively. In addition, the area of vascular smooth muscle was calculated as follows: muscle area (%) = external muscle area – internal area/external muscle area × 100, where the external muscle area and the internal area are the external and internal boundaries of the tunica media, respectively.

Statistical Analysis

Data are expressed as means ± SE. Groups were compared by two-way ANOVA and the post hoc Newman-Keuls test, or the Student’s t-test for unpaired data, as appropriate. We used the Fisher Exact Test to compare survival between groups. For all comparisons, differences were considered statistically significant when \( P < 0.05 \) (18).

RESULTS

Survival and Weight

In marked contrast to sea level pregnancies (LLL) with 100% survival, pregnancies after 70% exposure to high altitude (LHL) had increased mortality, with 21% abortions and 14% stillbirths (\( P < 0.05 \)). No lambs died after birth in either group. Surviving LHL lambs were much lighter than LLL lambs \( (3.8 ± 0.3 \text{ kg}, n = 9 \text{ vs. } 7.0 ± 0.4 \text{ kg}, n = 14, P < 0.001, \) weights at the time of experimentation between 6 and 11 days of age).

In Vivo Experiments

Because of differences in survival, eight LLL and four LHL lambs were studied in vivo. Values for basal \( \text{pH}, \text{PaO}_2, \text{PaCO}_2, \text{SaO}_2, \text{and } [\text{Hb}] \) were similar in both groups of lambs (Table 1). During acute hypoxia on a background of saline infusion, a similar fall in \( \text{PaO}_2 \) and \( \text{SaO}_2 \) occurred in both groups of lambs, without any alteration to \( \text{PaCO}_2 \) from baseline (Table 1). During recovery, all variables returned toward basal values in both groups. However, values for \( \text{PaCO}_2 \) were significantly depressed from baseline in LLL lambs (Table 1). Treatment with l-NAME had no significant effect on arterial blood gas and acid base status either during basal or acute hypoxic conditions (Table 1).

Basal values for pulmonary arterial pressure, pulmonary vascular resistance, and cardiac output were significantly greater in LHL than LLL lambs (Fig. 1 and Table 1). Basal values for heart rate were similar between the groups (Table 1). Basal systemic arterial pressure was similar in LHL and LLL lambs \( (87 ± 3 \text{ vs. } 82 ± 1 \text{ mmHg, respectively}) \).

During acute hypoxia on a background of saline infusion, pulmonary arterial pressure, pulmonary vascular resistance, cardiac output, and heart rate increased significantly in both groups of lambs. However, in LHL relative to LLL lambs, values for pulmonary arterial pressure and cardiac output reached significantly greater values during the acute hypoxic challenge (Fig. 1 and Table 1). No changes in systemic arterial pressure were seen in either of the experimental groups during hypoxia. During recovery, pulmonary arterial pressure, and cardiac output remained significantly elevated from baseline, but heart rate and pulmonary vascular resistance returned toward basal values in LHL lambs. In contrast, all variables returned toward basal values in LLL lambs (Fig. 1 and Table 1).

Treatment of the lambs with l-NAME during the basal period led to an increase in pulmonary arterial pressure and pulmonary vascular resistance and a decrease in heart rate and cardiac output in both groups of lambs. Although the fall in heart rate and cardiac output was similar between the groups, the increment in pulmonary arterial pressure and in pulmonary vascular resistance was significantly greater in LHL than in LLL lambs (Fig. 1 and Table 1).

At the time of surgery up until the time of study, there was no evidence of a patent ductus arteriosus. At the time of dissection, after the last study, the ductus arteriosus was examined, and no lumen was visible in any of the studied animals. Additional evidence for the closure of the ductus arteriosus is provided by the similarities of oxygen saturation and \( \text{PO}_2 \) in samples obtained from the ascending and descending aorta (data not shown).

Ex Vivo Experiments

Isolated small pulmonary arteries from LHL relative to LLL lambs showed a greater maximal contraction to KCl (\( K_{\text{max}}: 1.34 ± 0.05 \text{ vs. } 1.16 ± 0.05 \text{ N/m, } P < 0.05 \)) with similar sensitivity (EC\(_{50}: 28.54 ± 2.48 \text{ vs. } 31.53 ± 4.44; \text{Fig. 2A})

In contrast, the maximal contraction with ET-1 was similar in the two groups, although the sensitivity to the contraction elicited by ET-1 was significantly greater in LHL than in LLL lambs (PD\(_2: 8.08 ± 0.13 \text{ vs. } 7.22 ± 0.28, P < 0.05; \text{Fig. 2B})). The NO donor SNP evoked a similar maximal relaxation in pulmonary vessels from LLL and LHL lambs (%\( K_{\text{max}}: 98.1 ± 3.0 \text{ vs. } 100.0 ± 2.3%; \text{Fig. 3A})

However, the relaxant sensitivity of the pulmonary vessels to SNP was significantly greater in LHL than in LLL lambs (PD\(_2: 7.31 ± 0.12 \text{ vs. } 5.77 ± 0.07, P < 0.05; \text{Fig. 3A})). This SNP-induced vasorelaxation in the pulmonary vasculature was completely abolished by blocking sGC with ODQ in LLL lambs (Fig. 3B).

In marked contrast, SNP-induced vasorelaxation in vessels isolated from LHL lambs persisted following treatment with ODQ, but the maximal relaxation and sensitivity were significantly diminished (before ODQ, %\( K_{\text{max}}: 98.1 ± 3.0\% \text{, PD}_2: 7.31 ± 0.12; \text{after ODQ, } %K_{\text{max}}: 37.7 ± 3.2\%, \text{PD}_2: 4.97 ± 0.13, P < 0.05; \text{Fig. 3B})).

Western Blot

The expression of eNOS mRNA and protein in lung tissue was significantly greater in LHL lambs than in LLL lambs (Figs. 4 and 5). This was associated with a significantly
greater protein expression of pulmonary BKCα, and PDE5 but not sGC in LHL than in LLL lambs (Fig. 5). Furthermore, protein expression of pulmonary HO-1 and the production of CO by the pulmonary circulation were both diminished in LHL compared with LLL lambs (Fig. 6).

**Heart and Lung Biometry**

The ratio of the weight of the right ventricle to the left ventricle plus septum was augmented in the LHL compared with the LLL group (0.370 ± 0.007 vs. 0.328 ± 0.009, P < 0.005).

### Table 1. Cardiorespiratory variables in LLL and LHL lambs

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Basal + I</th>
<th>Hypoxia + I</th>
<th>Recovery</th>
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<td><strong>pH</strong></td>
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<tr>
<td>LHL</td>
<td>NaCl (0.9%)</td>
<td>7.412 ± 0.014</td>
<td>7.407 ± 0.013</td>
<td>7.391 ± 0.019</td>
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<td></td>
<td>l-NAME</td>
<td>7.459 ± 0.007</td>
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<td>LHL</td>
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<td></td>
<td>l-NAME</td>
<td>7.464 ± 0.005</td>
<td>7.450 ± 0.011</td>
<td>7.417 ± 0.032</td>
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<tr>
<td><strong>PaO₂, mmHg</strong></td>
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<tr>
<td>LHL</td>
<td>NaCl (0.9%)</td>
<td>97.5 ± 1.9</td>
<td>77.7 ± 3.4</td>
<td>30.9 ± 0.6*</td>
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<td></td>
<td>l-NAME</td>
<td>78.3 ± 2.8</td>
<td>82.6 ± 5.5</td>
<td>32.0 ± 0.7*</td>
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<tr>
<td><strong>PaCO₂, mmHg</strong></td>
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<tr>
<td>LHL</td>
<td>NaCl (0.9%)</td>
<td>85.0 ± 4.4</td>
<td>83.7 ± 3.3</td>
<td>29.2 ± 0.1*</td>
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<tr>
<td></td>
<td>l-NAME</td>
<td>86.8 ± 1.8</td>
<td>81.5 ± 5.3</td>
<td>30.2 ± 0.8*</td>
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<td><strong>SaO₂, %</strong></td>
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<tr>
<td>LHL</td>
<td>NaCl (0.9%)</td>
<td>94.7 ± 0.7</td>
<td>93.5 ± 1.2</td>
<td>52.7 ± 3.1*</td>
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<tr>
<td></td>
<td>l-NAME</td>
<td>94.9 ± 1.1</td>
<td>93.9 ± 1.6</td>
<td>55.9 ± 5.6*</td>
</tr>
<tr>
<td>LHL</td>
<td>NaCl (0.9%)</td>
<td>95.1 ± 1.6</td>
<td>93.1 ± 1.7</td>
<td>55.4 ± 5.7*</td>
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<tr>
<td></td>
<td>l-NAME</td>
<td>93.8 ± 1.2</td>
<td>91.3 ± 1.1</td>
<td>47.0 ± 2.3*</td>
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<tr>
<td><strong>[Hb], g/dl</strong></td>
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<tr>
<td>LHL</td>
<td>NaCl (0.9%)</td>
<td>10.9 ± 0.5</td>
<td>11.0 ± 0.5</td>
<td>11.3 ± 0.4</td>
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<td>9.7 ± 0.7</td>
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<tr>
<td>LHL</td>
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<td>11.9 ± 0.7</td>
<td>12.6 ± 1.3</td>
<td>12.3 ± 1.1</td>
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<td></td>
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<td>12.2 ± 1.3</td>
<td>12.6 ± 1.2</td>
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<tr>
<td>Heart rate, min⁻¹</td>
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<tr>
<td>LHL</td>
<td>NaCl (0.9%)</td>
<td>198.2 ± 8.0</td>
<td>192.8 ± 7.6</td>
<td>267.9 ± 13.2*</td>
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<tr>
<td></td>
<td>l-NAME</td>
<td>173.4 ± 11.6</td>
<td>132.1 ± 8.0†</td>
<td>203.8 ± 12.2†</td>
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<tr>
<td>LHL</td>
<td>NaCl (0.9%)</td>
<td>188.5 ± 7.4</td>
<td>188.9 ± 4.9</td>
<td>265.1 ± 13.4*</td>
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<tr>
<td></td>
<td>l-NAME</td>
<td>167.3 ± 7.4</td>
<td>148.0 ± 8.3*†</td>
<td>196.1 ± 19.3§</td>
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<td>Cardiac output, ml·min⁻¹·kg⁻¹</td>
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<tr>
<td>LHL</td>
<td>NaCl (0.9%)</td>
<td>281.0 ± 13.3</td>
<td>276.0 ± 13.0</td>
<td>394.0 ± 15.3*</td>
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<tr>
<td></td>
<td>l-NAME</td>
<td>298.8 ± 14.6</td>
<td>194.1 ± 9.1†</td>
<td>261.2 ± 12.6†</td>
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<tr>
<td><strong>PVR, mmHg·min⁻¹·kg⁻¹</strong></td>
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<tr>
<td>LHL</td>
<td>NaCl (0.9%)</td>
<td>373.2 ± 13.0†</td>
<td>326.8 ± 19.1†</td>
<td>474.5 ± 7.9†</td>
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<td>l-NAME</td>
<td>373.5 ± 41.8†</td>
<td>216.2 ± 16.8‡</td>
<td>308.4 ± 49.8</td>
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Values are the means ± SE, in units, for arterial pH (pHₐ), partial pressure of oxygen (PaO₂), partial pressure of carbon dioxide (PaCO₂), saturation of hemoglobin with oxygen (SaO₂), hemoglobin concentration ([Hb]), heart rate, cardiac output, and pulmonary vascular resistance (PVR). LLL, conception, pregnancy, and delivery at low altitude (580 m); LHL, pregnancy at high altitude (3,600 m) from 30% of gestation until delivery, and return to lowland; N⁵-nitro-l-arginine methyl ester, l-NAME. Blood samples and cardiovascular variables were taken, measured, and calculated during preinfusion baseline (Basal), during infusion of saline or l-NAME (Basal + I), during acute hypoxia (Hypoxia + I), and during recovery. Significant differences (P < 0.05) are as follows: vs. basal (*), LHL vs. LLL (†), and l-NAME vs. 0.9% NaCl (‡).
The ratio of the lung weight to the body weight was similar in LHL compared with LLL lambs (0.0200 vs. 0.0180, not significant).

**Histology**

Morphometric analysis of the pulmonary vasculature revealed no significant difference in vascular wall thickness between LLL and LHL lambs (48.79 ± 3.59 vs. 55.03 ± 4.35%, respectively, P = 0.31, n = 5 for each group). However, there was a significant increase in the area of vascular smooth muscle in LHL compared with LLL lambs (29.28 ± 2.96 vs. 21.09 ± 1.73%; P < 0.05; Fig. 7).

**DISCUSSION**

These studies show that 70% exposure to high-altitude chronic hypoxia during gestation yields postnatal lambs with basal pulmonary hypertension and an increased pulmonary vascular response to an episode of acute hypoxia even following return to sea level. These findings persist despite evidence of enhanced pulmonary NO function obtained through in vivo, isolated organ and molecular approaches. Furthermore, the results show a decrease in pulmonary CO function and an increase in the vascular reactivity of constrictors associated with cardiopulmonary remodeling processes. Combined, the data support the hypothesis tested and provide a mechanistic explanation for the persistence of neonatal pulmonary hypertension at sea level induced by high-altitude pregnancy.

A striking difference between the groups of lambs in the present study was the much greater mortality and pronounced growth restriction in lambs born from pregnancies after prolonged exposure to high altitude. Pregnancy at high altitude induces maternal hypobaric hypoxia, and we have previously reported lower maternal and fetal arterial Po2 in a separate cohort of animals exposed to the same altitude during the whole pregnancy (12). A similar effect on fetal growth restriction and mortality during development at high altitude has been reported in highland human populations (16, 30, 34, 38) and in chick embryos following highland incubation (17, 43). Malnutrition during early gestation in high-altitude cattle also resulted in a higher incidence of elevated pulmonary arterial pressure and right ventricular hypertrophy compared with controls when measured in the offspring at 15 mo. This was associated with differential gene expression in the right ventricle, but the resulting interaction between undernutrition and high-altitude hypoxia is unclear (21). In our study, both groups of lambs received the same nutrition, so the changes observed in pulmonary arterial pressure and growth restriction appear to be independent of nutrition. Accordingly, the effects on fetal growth restriction and mortality of developmental hypoxia at high altitude have been shown to be independent of the maternal nutritional status and of highland hypobaria in other species, since fetal growth restriction persists in ewes undergoing pregnancy at high altitude with food intake values similar to those as sea level pregnancies (23, 39). These effects have also been shown in the chick embryo, where incubation at high altitude of sea level eggs with oxygen supplementation completely prevented the high altitude-induced fetal growth restriction and mortality (17). The present study extends these...
findings and reports that 70% rather than 100% exposure to high altitude during fetal development can also have dramatic effects on the maintenance of pregnancy, on fetal growth, and on fetal mortality (abortion and stillbirth). In contrast, we did not have neonatal mortality.

Lambs born from pregnancies after 70% exposure to high altitude had a greater basal cardiac output, pulmonary vascular resistance, and pulmonary arterial pressure, even when their PaO2 had recovered to normoxic levels, and also showed a greater pulmonary pressor response to L-NAME and to acute hypoxia. The greater basal cardiac output in the highland group is independent of differences in basal heart rate, suggesting a greater resting stroke volume in lambs from pregnancies after long exposure to high altitude. The differences in basal pulmonary arterial pressure and vascular resistance between the groups may be explained, in part, by the larger area of vascular smooth muscle, the greater pulmonary vessel maximal constriction response to KCl, and the increased sensitivity to ET-1 in the LHL lambs. ET-1 is induced by chronic hypoxia and is a potent pulmonary vasoconstrictor and a mitogen, leading to smooth muscle cell proliferation (3). Previous studies have correlated an increased vascular response with greater smooth muscle cell remodeling (29, 45), conditions that were both

Fig. 3. Vasodilator function of small pulmonary vessels isolated from LLL (○) and LHL (●) lambs. Values are the means ± SE for the vascular response to sodium nitroprusside (SNP, A) and to SNP in the presence of 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10⁻⁵ M, B). Emax and sensitivity (EC50 or pD2) were calculated (see text). Significant differences (P < 0.05) are as follows: LHL vs. LLL for Emax (†) and LHL vs. LLL for sensitivity (‡).

Fig. 4. mRNA expression of endothelial nitric oxide synthase (eNOS) in LLL (open bar) and LHL (filled bar) lambs. Values are the means ± SE for the mRNA expression of eNOS. Individual immunoblots are shown. Significant differences (P < 0.05) are LHL vs. LLL (†).

Fig. 5. Components of the NO signaling pathway in LLL (open bars) and LHL (filled bars) lambs. Values are the means ± SE for the protein expression of eNOS (A), soluble guanylate cyclase (sGC, B), phosphodiesterase 5 (PDE5, C), and Ca²⁺-activated K⁺ channels (BKca, D). E: individual immunoblots. Significant differences (P < 0.05) are LHL vs. LLL (†).

Lambs born from pregnancies after 70% exposure to high altitude had a greater basal cardiac output, pulmonary vascular resistance, and pulmonary arterial pressure, even when their PaO2 had recovered to normoxic levels, and also showed a greater pulmonary pressor response to L-NAME and to acute hypoxia. The greater basal cardiac output in the highland group is independent of differences in basal heart rate, suggesting a greater resting stroke volume in lambs from pregnancies after long exposure to high altitude. The differences in basal pulmonary arterial pressure and vascular resistance between the groups may be explained, in part, by the larger area of vascular smooth muscle, the greater pulmonary vessel maximal constriction response to KCl, and the increased sensitivity to ET-1 in the LHL lambs. ET-1 is induced by chronic hypoxia and is a potent pulmonary vasoconstrictor and a mitogen, leading to smooth muscle cell proliferation (3). Previous studies have correlated an increased vascular response with greater smooth muscle cell remodeling (29, 45), conditions that were both
observed in LHL lambs in our study. Moreover, it has been suggested that the longer the exposure to high altitude, the greater the vascular smooth muscle remodeling (29, 40, 45). Dissociation between changes in vascular wall area and in wall thickness is a common finding with established explanations. Elegant studies by Baumbach and Heistad (5, 6) and by Mulvany (35, 36) have made it clear that an increase in the ratio of the vascular wall to lumen may be achieved by at least two very different situations. For instance, the ratio may be increased by a reduction in lumenal diameter without a change in medial volume. There is thus rearrangement of the same volume of vessel wall around a smaller-diameter lumen, what is now termed inward eutrophic vascular remodeling. Conversely, an increase in the vascular wall-to-lumen ratio may be achieved by an increase in wall material with or without a change in lumen diameter, what has been termed outward hypertrophic vascular growth. An increase in wall material with an increase in lumen diameter is what is occurring in the LHL vessels. Interestingly, the main driving forces that promote this type of vascular remodeling are increased flow and pressure (36), both of which are present in the pulmonary bed of LHL lambs.

The present study also reports an increase in right ventricular mass in LHL neonates. This is a common finding in humans and animals that have suffered arterial pulmonary hypertension (1, 15, 46, 47).

Other components contributing to basal pulmonary hypertension in lambs from pregnancies after prolonged exposure to high altitude may include alterations in the tonic balance between dilator and constrictor influences on the pulmonary vascular bed. For instance, we have previously reported in highland lambs reduced synthesis of dilators, such as CO (25). In this set of experiments, it was also found that LHL lambs had an important decrease in the production of CO by the pulmonary circulation concordant with the reduced HO-1 protein expression. Interestingly, a study in fetal lambs showed them to be unresponsive to CO (19). However, this was performed in ventilated (hypoxic, <10% FIO2) fetuses rather than normally oxygenated postnatal lambs. Lambs native to high altitude do not increase HO as do llamas, which suggests that they are insensitive to endogenous CO, although they may be responsive to induced CO production (19). CO is a dilator via activation of sGC (13, 27, 32) and via hyperpolarizing the vascular smooth muscle secondary to activation of BKCa channels (7, 10, 50). CO can also diminish the vasoconstrictor responses to phenylephrine and 20-hydroxyeicosatetraenoic acid while reducing the synthesis and release of ET (28, 51). The diminished production of CO by the pulmonary circulation determined in this study may play a putative role in the maintenance of persistent pulmonary hypertension of the newborn at sea level. In addition, chronic developmental hypoxia is known to result in lung hypoplasia and immaturity, pulmonary edema, and altered endothelial function (2, 20, 39, 46). Alterations in the synthesis and function of vasoconstrictors such as ET-1, as reported in this paper, thromboxane, IGF,
serotonin, and leukotriene C₄/D₄ have also been implicated in the pulmonary hypertensive phenotype during chronic hypoxia (29, 45).

In the present study, the greater pulmonary hypertension under basal and stimulated conditions in lambs from pregnancies after 70% exposure to high altitude occurred despite evidence of enhanced NO-dependent dilator function in the pulmonary vascular bed. The greater pressor response to treatment with l-NAME, the increased expression of eNOS mRNA and protein, and the enhanced isolated vessel dilator response to SNP all strongly support enhanced NO function in the pulmonary vasculature of lambs from pregnancies after long-term exposure to high altitude. PDE is an enzyme that breaks down cGMP and thus halts the NO vasodilator cascade (42). In this study, LHL also showed greater pulmonary protein expression of PDE5, findings similar to those reported in hypertensive lambs and lambs native to high altitude (22, 24). Although a greater protein expression of pulmonary PDE5 may itself favor constriction in the pulmonary vascular bed, it is likely that the increased expression of PDE5 occurs to match all other components of the enhanced NO cascade, and it does not underlie a cause but is likely a consequence of the pulmonary hypertension in lambs from pregnancies exposed to high altitude. In the present study, blockade of sGC with ODQ completely prevented the pulmonary dilator response to the NO donor SNP in control lambs but not in lambs from pregnancies after prolonged exposure to high altitude. In the latter group, the dilator response to SNP persisted, albeit at a reduced level. This suggests that long-term exposure to high altitude during pregnancy may trigger an enhancement of NO dilatation pathways in addition to the activation of sGC in vascular smooth muscle. One possibility is the direct action of NO on the activation of K⁺ channels, as has already been described for the BKCa channel (8). Accordingly, in the present study, LHL lambs showed a significantly greater pulmonary BKCa protein expression. What is important to highlight is that, despite evidence of enhanced pulmonary NO function via at least two different signaling cascades, this adaptive response is insufficient to offset pulmonary hypertension and vascular remodeling in lambs even following return to sea level.

In conclusion, postnatal pulmonary hypertension induced by long-term exposure of the pregnancy to high altitude persists at sea level, despite enhanced pulmonary NO function. This condition is associated with a decrease in the production of pulmonary CO coupled with an increase in the vascular reactivity of constrictors associated with cardiopulmonary remodeling processes.

Perspectives and Significance

During acute episodes of hypoxia, the pulmonary vascular bed undergoes constriction to match the reduced oxygenation with reduced perfusion. During sustained hypoxia, this initial homeostatic response becomes maladaptive, triggering sustained increases in pulmonary vascular resistance, leading to the establishment of pulmonary hypertension. Our studies show that this maladaptive pulmonary constrictor response to hypoxia can be triggered in the newborn lamb following pregnancy at high altitude, when the measurements are performed at high altitude (23, 24, 25) and, even, following return to sea level. Sustained pulmonary hypertension and remodeling of the pulmonary vasculature suggest possible persistence of this maladaptive response until adulthood. The implications of these findings are not only relevant to women of reproductive age native to sea level countries, considering trips or work at high altitude, but also to the developmental programming of pulmonary hypertension in adulthood by prenatal hypoxia (14, 34).

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DISCLOSURES

No conflicts of interest are declared by the authors.

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