Permanent alveolar remodeling in canine lung induced by high-altitude residence during maturation

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Young canines born at sea level (SL) and raised for 5 mo at high altitude (HA, 3,800 m), followed by return to SL before somatic maturation, showed enhanced alveolar gas exchange and diffusing capacity at rest and exercise that persisted into adulthood (McDonough P, Dane DM, Hsia CC, Yilmaz C, Johnson RL Jr. J Appl Physiol 100: 474–81, 2006; Hsia CCW, Johnson RL Jr, McDonough P, Dane DM, Hurst MD, Fehmel JL, Wagner HE, Wagner PD. J Appl Physiol 102: 1448–55, 2007). To examine the associated structural response, we quantified lung ultrastructure in male foxhounds raised at 3,800 m HA or their littermates raised at SL (n = 6 each) from 2.5 to 7.5 mo of age. Three years following return to SL, lungs were fixed for morphometric analysis. In HA-exposed animals compared with SL controls, lung volume at a given inflation pressure was higher with enlargement of alveolar ducts and sacs without significant differences in the volumes of alveolar cell components, septal tissue, or in alveolar-capillary surface areas. There was a shift toward a significantly lower harmonic mean thickness of the blood-gas diffusion barrier in HA-raised animals. As a control organ, muscle capillary length density of costal diaphragm was significantly higher in HA-raised animals, indicating parallel adaptation in oxygen transport organs. We conclude that, in actively growing animals, 5 mo of HA exposure that was discontinued before somatic maturation induced acinar remodeling that increased lung compliance and reduced the resistance of blood-gas diffusion barrier to diffusion that persisted into adulthood, but without permanent enhancement of alveolar tissue growth.

alveolar surface area; alveolar tissue volume; alveolar duct; blood-gas; diffusion barrier

AMBIENT HYPOXIA IS A PRIMITIVE and universal stimulus for the growth of gas exchange organs (gills, skin, placenta, lung) across the animal kingdom (3, 4, 7, 25). Studies in rats and mice have uniformly shown accelerated increases in alveolar cell volume, as well as gas exchange surface area, in response to simulated high-altitude (HA) exposure of <1-mo duration (5, 10, 24–26). In guinea pigs exposed to HA for 6–10 mo after birth, we have also found enhanced alveolar tissue growth, as well as remodeling of the blood-gas diffusion barrier, associated with increased lung compliance and diffusing capacity (DLCO) compared with litter-matched animals raised at a lower altitude (11, 33). In large mammals, HA-related stimuli appear to interact with developmental stimuli of lung growth. In young beagles born at sea level (SL) and then raised at moderate HA (3,100 m) for 14 mo (i.e., to beyond somatic maturity), Johnson et al. found elevated lung volume, DLCO (15), as well as alveolar tissue volume and surface area following reacclimatization to SL (16), indicating that continuous HA exposure through the period of somatic maturation enhanced lung growth and function in adulthood. In contrast, exposure of adult beagles to 3,100-m HA for 3 yr did not lead to structural or functional enhancement in the lung (15, 16), suggesting that HA-related stimuli augmented developmental signals for lung growth, but were unable to initiate lung growth in the absence of developmental stimuli.

Given the above interactions, we wondered whether HA-induced adaptation during postnatal lung development persists into adulthood when the HA stimulus is withdrawn before somatic maturity. To address this issue, we exposed young foxhounds born at SL to HA (3,800 m) for 5 mo (from 2.5 to 7.5 mo of age), while their littermates were simultaneously raised at SL. At 2 mo and 1 yr after returning to SL, resting lung function and hematological volumes were measured. All animals were trained to run on a treadmill, and lung function during exercise was measured repeatedly following return to SL. These physiological studies (14, 20) showed persistently elevated exercise capacity, lung volume, and diffusing capacities [for O2 (DLCO2) and CO (DLCO)] for at least 2.5 yr after return to SL. Both membrane diffusing capacity and pulmonary capillary blood volume remained elevated following return to SL, as were the circulating blood and erythrocyte volumes. It is not certain whether the functional enhancement into adulthood was due to structural or hematological adaptation. To determine the nature and extent of structural adaptation, we performed terminal experiment on the above animals 3 yr following return to SL. Lungs were fixed at a constant airway pressure for detailed morphometric analysis under light and electron microscopy (EM). We addressed the hypothesis that HA exposure during somatic maturation that was discontinued before reaching somatic maturity permanently enlarged alveolar structural dimensions and reduced the resistance of the blood-gas diffusion barrier. As a control organ for long-term HA-induced adaptation, we also quantified muscle capillary density of the diaphragm in these animals.

MATERIALS AND METHODS

Animals. The Institutional Animal Care and Use Committees at the University of Texas Southwestern Medical Center and the University of California White Mountain Research Station approved the protocols. Twelve purpose-bred, litter-matched male mixed-breed foxhounds born at SL were used. At 2.5 mo of age, six animals were transported to HA to reside for 5 mo at the Barcroft Laboratory (3,800 m, barometric pressure 485 mmHg) of the University of California White Mountain Research Station. Six male littermates were raised simultaneously at SL in Dallas, TX (≈156 m, barometric pressure 750 mmHg). At 7.5 mo of age, animals residing at HA returned to SL in Dallas. Cardiopulmonary function was measured at rest ~2 mo following return to SL and repeated 1 yr later. All animals were trained to exercise voluntarily on a treadmill using an established...
training protocol. Leak-free respiratory masks were constructed to permit ventilatory measurement. Bilateral carotid artery loops were constructed to permit hemodynamic measurements. Cardiopulmonary function during exercise was measured using noninvasive and invasive techniques between 1 and 2.5 yr following return to SL. These physiological studies have been published (14, 20). A timetable of studies is shown in Fig. 1.

Lung fixation. About 3 yr following returning to SL and after completion of physiological measurements, animals were deeply anesthetized with pentobarbital sodium and mechanically ventilated (tidal volume 10–12 ml/kg) at a rate necessary to suppress spontaneous respiration. Through a tracheostomy, a cuffed endotracheal tube was inserted and tied securely. The abdomen was opened via a midline incision. The ventilator was disconnected, and a rent was made through each hemidiaphragm to collapse the lungs. An overdose of Euthasol was simultaneously administered to stop the heart. The lungs were reinflated within the thorax by intratracheal instillation of glutaraldehyde in a plastic bag, floated on a water bath, and stored at 4°C until further processing. Internal organs (spleen, kidney, and heart) and skeletal muscle (gastrocnemius and diaphragm) were dissected completely, trimmed of extraneous tissue, weighed, and processed for separate analysis.

Lobar volume. Each lung was separated into lobes (left lung: 3 lobes, right lung: 4 lobes). Volume of the each lobe was measured by immersion displacement (32). Each lobe was sectioned serially at 2-cm intervals, and the cut surfaces were photographed using a digital camera (Nikon Coolpix). The volume of each sectioned lobe was estimated from the photographs using the Cavalieri principle (9, 21); this volume estimated in the tension-free state was used in subsequent morphometric calculations.

Sampling and morphometric analysis. Each lobe was sampled and analyzed separately using a previously established four-level stratified scheme (27): gross (level 1, approximately ×2), low-power light microscopy (level 2, ×275), high-power light microscopy (level 3, ×550), and EM (level 4, ×19,000). For level 1, photographs of serial sections were analyzed by point counting using standard test grids, to exclude structures larger than 1 mm in diameter, yielding an estimate of the volume density of coarse parenchyma per unit of lung volume. For level 2, four tissue blocks were selected per lobe using a systematic sampling scheme with a random start. Tissue blocks were embedded in glycol methacrylate for sectioning (5 µm) and staining with toluidine blue. One section per block was overlaid with a test grid. From a random start, at least 10 non-overlapping microscopic fields were systematically sampled at ×275. Using point counting, structures between 20 µm and 1 mm in diameter were excluded to estimate the volume density of fine parenchyma per unit volume of coarse parenchyma.

For levels 3 and 4, four blocks were sampled per lobe using a systematic random scheme, postfixed with 1% osmium tetroxide in 0.1 M cacodylate buffer, treated with 2% uranyl acetate, dehydrated through graded alcohol, and embedded in Spurr resin (Electron Microscopy Sciences, Hatfield, PA). Each block was sectioned at 1-µm thickness and stained with toluidine blue. One section per block was overlaid with a test grid at ×550. From a random start, at least 20 non-overlapping microscopic fields per block were systematically imaged (80 images per stratum) to estimate the volume density of alveolar septa per unit volume of fine parenchyma by excluding all structures exceeding 20 µm in diameter (level 3).

For EM analysis (level 4), two blocks per lobe were sectioned at 80-nm thickness and mounted on copper grids. Each grid was examined under a transmission electron microscope (JEOL EXII) at approximately ×19,000. Thirty non-overlapping EM fields per grid (60 images per stratum) were sampled systematically from a random start. Each image was captured with a charge-coupled device camera (Gatan, model C73–0200), digitized, and projected onto a high-resolution monitor. Septal cells were identified by their typical morphological characteristics. The volume densities of epithelium (types I and II), interstitium, and endothelium were estimated by point counting. The alveolar epithelial and capillary surface densities were estimated by intersection counting. At least 300 points or intersections were counted per grid, yielding a coefficient of variation <10%. The length of test lines that transect the barrier from the epithelial surface to the nearest red cell membrane were measured for calculating harmonic mean thickness of the tissue-plasma barrier (τw). Morphometric data were calculated for each lobe separately, and a volume-weighted average for the entire lung was obtained. Absolute volume and surface area of individual alveolar structures were obtained by relating the respective volume and surface densities at each level back through the cascade of levels to the measured volume of the lobe (27). The arithmetic thickness was estimated as 2 (volume of septum/alveolar surface area).

Morphometric estimate of lung diffusing capacity was calculated for O2 (DLO2) using a previously established model (6, 28, 29). The model describes the gas diffusion path from alveolar air to capillary hemoglobin binding sites as serial resistance across the combined tissue-plasma barrier and the capillary erythrocytes.

Muscle morphometry. Samples taken from the midlateral region of the right costal diaphragm were immersion fixed in 6.25% glutaraldehyde buffered in 0.1 M cacodylate buffer, treated with 2% uranyl acetate, dehydrated through graded alcohol, and embedded in Spurr resin. From each muscle sample, eight blocks were cut into 1-µm sections, four transverse sections (angle between normal to section and fiber axis, α = 0°) and four longitudinal sections (α = π/2), and stained with 0.1% toluidine blue aqueous solution. Capillary number per fiber cross-sectional area and longitudinal section area [Q₄(0) and Q₄(π/2), respectively] were measured by point counting using a 100-point square test grid; sections were examined under a light microscope at approximately ×1,500. Thirty fields were examined per sample on transverse and longitudinal sections, yielding ~500 and ~250 fiber profiles per sample, respectively. Capillary density estimates were referenced to the number of muscle fibers.

Capillary length per volume of muscle fiber [L(c,f)], the degree of capillary anisotropy (K), and the relative contribution of the anisotropic components (tortuosity and branching) to capillary length per fiber volume [c(K, 0)] were estimated using the method of Mathieu-Costello et al. (19). Briefly, L(c,f) is related to capillary numerical density in transverse [Q₄(0)] and longitudinal sections [Q₄(π/2)] by the equations

\[ J(c,f) = c(K, 0)Q₄(0) \]  

Fig. 1. Timeline of exposure. HA, high altitude; SL, sea level.

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\[ J(c, f) = c(K, \pi/2)Q_A(\pi/2) \]  
where \( c(K, 0) \) and \( c(K, \pi/2) \) are anisotropy coefficients for transverse and longitudinal sections, respectively. Combining Eqs. 1 and 2 gives:

\[ Q_A(0)/Q_A(\pi/2) = c(K, \pi/2)/c(K, 0) \]

The ratio of capillary numerical density on transverse and longitudinal sections, \( R = Q_A(0)/Q_A(\pi/2) \), is used to determine \( K \) and \( c(K, 0) \) from a table of known coefficients (18). Then \( J(c, f) \) is estimated via Eqs. 1 or 2. For straight capillaries oriented perfectly with the muscle fibers, \( K = \infty \) and \( c(K, 0) = 1 \); for randomly oriented (isotropic) capillaries, \( K = 0 \) and \( c(K, 0) = 2 \). Fiber cross-sectional area was measured by point counting on the same transverse sections used to measure capillarity.

Statistical analysis. Results were normalized by body weight and expressed as means \( \pm \) SD. Values for whole lungs were derived from volume-weight averages of individual lobes. Comparison between groups was performed by one-way analysis of variance. A P value of \( \leq 0.05 \) was considered significant.

RESULTS

Body weight and morphometric hematocrit were similar between HA and SL groups (Table 1). Lung volumes measured by immersion and Cavalieri methods were 15 and 13% higher, respectively, in animals raised at HA compared with that in SL control animals. Lung morphology also shows wider alveolar duct and sac profiles in animals raised at HA (Fig. 2). Volume densities and surface densities of alveolar components, expressed with respect to volume of the septum and to total lung volume, are shown in Table 2. Total absolute volumes and surface areas are shown in Table 3.

When expressed with respect to total lung volume, the volume density of fine parenchyma was similar between groups (Table 2). In HA-raised animals compared with SL controls, volume density of alveolar septum with respect to lung volume was 13% lower, consistent with air space enlargement, which was due to 21% higher volume densities of alveolar ducts and sacs, while volume density of respiratory bronchioles was similar between groups. Volume density of type I pneumocyte was significantly (11%) lower in HA-raised animals. Volume density of type 2 pneumocyte was 9% lower in HA-raised animals, but the difference was not statistically significant. Volume density of capillary blood was significantly (17%) lower in HA-raised animals, but the volume densities of septal tissue and interstitium did not differ significantly between groups. Alveolar and capillary surface densities per unit lung volume were significantly (19% and 15%, respectively) lower in animals raised at HA compared with SL controls.

Taking into account the difference in lung volume between groups, absolute volumes of air in alveolar ducts and sacs were 37 and 13%, respectively, higher in HA-raised animals. The absolute volumes and surface areas of alveolar septal tissue components and capillary blood volume did not differ, and the average ratio of alveolar surface to septum volume was similar between groups (Table 3).

In HA-raised animals compared with SL controls, mean arithmetic septal thickness was slightly (6%) but significantly higher, but the \( t_{n\text{sh}} \) was 11% lower, indicating greater \( O_2 \) conductance across the gas-blood barrier (Table 1). About 350 measurements per lobe of the blood-gas barrier were made from the lengths of intercepts that transect the barrier from the epithelial surface to the nearest red cell membrane. The frequency distribution of harmonic barrier thickness (1/intercept length) in the left lower lobe is shown in Fig. 3. Lungs from HA-raised animals exhibited a significantly higher frequency of thin-barrier intercepts compared with SL control lungs. However, there was no significant change in absolute alveolar and capillary surface areas or in capillary blood volume; hence, conductance of the tissue-plasma barrier was similar between groups (Table 4), and overall conductance of the lung (DLco estimated from alveolar-capillary surface areas, \( t_{n\text{sh}} \), and alveolar capillary blood volume) was not different between HA and SL groups.

Morphometric results of the diaphragm (Table 5) showed no significant differences between groups in the weights of whole diaphragm, costal or crural muscles, or the mean cross-sectional area of costal muscle fiber. \( J(c, f) \) ratio was significantly higher (19%) in the HA group compared with SL controls.

DISCUSSION

Summary of findings. The purpose of the present study was to characterize the long-term ultrastructural adaptation in the lungs of dogs that had been exposed to HA (3,800 m) for 5 mo during somatic maturation and then returned to SL before reaching maturity. Three years following return to SL, animals that had been exposed to HA demonstrated significantly higher lung volumes than control animals due to enlargement of predominantly alveolar ducts and, to a lesser extent, alveolar air sacs, with no change in the volumes or surface areas of alveolar septal tissue compartments. Although arithmetic mean septal thickness was slightly higher, the harmonic mean thickness of blood-gas diffusion barrier was lower, resulting in little net change in morphometric estimates of diffusive conductance. The frequency distribution of harmonic thicknesses of the blood-gas barrier showed a shift toward smaller values. These results are consistent with the interpretation that 5 mo of HA residence during maturation induced long-term distal air space enlargement and remodeling of acinar tissue that persisted into adulthood, but without permanent addition of new alveolar tissue. Persistent adaptation was also observed in the costal diaphragm with an elevated capillary length density.

Comparison to earlier canine study. Only one previous study has examined structural adaptation of the lung to HA exposure in large mammals. Johnson et al. (15) exposed young beagles (2.5 mo old) born at SL to moderate HA (3,100 m) for 14 mo (beyond maturity). Three months following return to SL, these animals demonstrated higher lung volume and DLco measured by a physiological method under anesthesia. Subse-
Table 2. Volume and surface densities of septal structures

<table>
<thead>
<tr>
<th></th>
<th>SL</th>
<th>HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coarse parenchyma</td>
<td>0.88725+0.01067</td>
<td>0.89121±0.01959</td>
</tr>
<tr>
<td>Fine parenchyma</td>
<td>0.87250±0.01081</td>
<td>0.87726±0.01925</td>
</tr>
<tr>
<td>Total septum</td>
<td>0.09716±0.00727</td>
<td>0.08405±0.00590*</td>
</tr>
<tr>
<td>Total epithelium</td>
<td>0.01686±0.00164</td>
<td>0.01510±0.00106</td>
</tr>
<tr>
<td>Type 1 epithelium</td>
<td>0.00950±0.00093</td>
<td>0.00836±0.00047*</td>
</tr>
<tr>
<td>Type 2 epithelium</td>
<td>0.00737±0.00076</td>
<td>0.00674±0.00081</td>
</tr>
<tr>
<td>Interstitium</td>
<td>0.01368±0.00096</td>
<td>0.01280±0.00119</td>
</tr>
<tr>
<td>Collagen fibers</td>
<td>0.01078±0.00090</td>
<td>0.00986±0.00088</td>
</tr>
<tr>
<td>Cells and matrix</td>
<td>0.00290±0.00015</td>
<td>0.00295±0.00033</td>
</tr>
<tr>
<td>Endothelium</td>
<td>0.00997±0.00071</td>
<td>0.00935±0.00070</td>
</tr>
<tr>
<td>Septal tissue</td>
<td>0.04052±0.00318</td>
<td>0.03726±0.00245</td>
</tr>
<tr>
<td>Capillary blood</td>
<td>0.05664±0.000426</td>
<td>0.04680±0.000389*</td>
</tr>
<tr>
<td>Alveolar sac</td>
<td>0.67242±0.00939</td>
<td>0.67525±0.01643</td>
</tr>
<tr>
<td>Alveolar duct</td>
<td>0.07101±0.00887</td>
<td>0.08629±0.00314*</td>
</tr>
<tr>
<td>Respiratory bronchioles</td>
<td>0.03191±0.00212</td>
<td>0.03167±0.00258</td>
</tr>
</tbody>
</table>

Surface density per unit lung volume, cm⁻¹

<table>
<thead>
<tr>
<th></th>
<th>SL</th>
<th>HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar surface</td>
<td>327±37</td>
<td>265±21*</td>
</tr>
<tr>
<td>Capillary surface</td>
<td>345±38</td>
<td>294±24*</td>
</tr>
<tr>
<td>Erythrocyte surface</td>
<td>529±67</td>
<td>443±24*</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P ≤ 0.05.

Our present study differs from that of Johnson and colleagues (8, 15, 16) in several aspects. 1) The present animals resided at a higher altitude (3,800 m) for a shorter period (5 mo) and returned to SL before reaching somatic maturity, compared with the study of Johnson and colleagues, where animals resided at 3,100 m for 14 mo and returned to SL as fully grown adults. In both of these studies, somatic growth was not adversely affected by HA exposure; polycythemia developed and lung volume and function were enhanced. Therefore, adaptation occurred in response to both regimens of HA exposure. 2) In the present study, the lungs were fixed 3 yr after animals returned to SL, whereas, in the earlier study of Johnson and colleagues, the lungs were fixed ~1 yr after return to SL. Both periods should have been long enough for reacclimatization to SL. 3) Our assessment of alveolar tissue volume and surface area used a detailed stratified analytic scheme under EM, whereas, in the earlier study, morphometric assessment was limited to alveolar tissue volumes and surface areas measured under light microscopy. The estimation of...
alveolar surface area is magnification dependent; air space size and composition of septal components were not assessed in the earlier study. 4) Unlike the earlier study, where the animals remained sedentary, the present animals underwent exercise training following return to SL to permit detailed assessment of aerobic capacity and cardiopulmonary function. Continued exercise training may possibly help maintain the adaptive changes induced by prior HA exposure, particularly in the circulating blood and red cell volumes (17).

Persistent physiological adaptation. In the present animals, 5 mo of HA exposure during somatic maturation clearly induced long-term augmentation of hematological volumes, lung volumes, and diffusing capacities that facilitated exercise performance under hypoxic conditions in adulthood (14, 20). These published results demonstrate significantly and persistently higher peak $O_2$ uptake during hypoxic exercise, as well as higher circulating blood and erythrocyte volumes for $>2$ yr following return to SL (20). Diffusing capacity of the lung and membrane for carbon monoxide and pulmonary capillary blood volume estimated at a given cardiac output by a noninvasive following return to SL (20). Diffusing capacity estimated in conjunction with volume estimated at a given cardiac output by a noninvasive following return to SL (20). Diffusing capacity of the lung and architectural remodeling persisted, with permanent enlargement of distal air spaces (alveolar ducts and sacs, but not respiratory bronchioles) without a net gain in alveolar-capillary surface areas. Air space enlargement was most prominent in alveolar ducts, which increased 37% in volume, while the alveolar sacs increased 13%. This finding is in keeping with the role of alveolar ducts as force-bearing structure, especially during lung inflation (31). Enlargement of alveolar ducts is not expected to augment alveolar surface area. The larger air space volume at a given distending pressure during fixation (25 cmH$_2$O) agrees with antemortem static pressure-volume curves that showed a higher lung volume near TLC (30-cmH$_2$O transpulmonary pressure) in HA-exposed animals compared with SL controls (20). Thus both physiological and morphometric data indicate a higher lung compliance as a result of acinar remodeling. Hypoxia exposure has been reported to suppress elastin gene expression (1) and increase desmosine and hydroxyproline content in rodent lungs (23), but these changes, if they also occurred in the canine lung, did not lead to discernable morphological differences in collagen and elastin contents (Fig. 2).

Enlargement of alveolar air spaces can increase diffusion resistance in the gas phase. Our laboratory previously reported an increase in gas phase diffusion resistance in dogs following pneumonectomy, where the remaining lung doubles in size (13). However, the effect of gas phase resistance on diffusing capacity is modest and detectable only during exercise (12) or by increasing the density of inspired gas (13). This is because the diffusive resistance of tissue and blood is far greater than that of air. Thus a modest 15% increase in lung volume in this cohort is not expected to contribute significantly to gas phase resistance.

The notable reduction in harmonic mean thickness of the gas-blood barrier and the shift in its frequency distribution suggest redistribution of tissue and blood components within the alveolar septa in such a way that minimized diffusive resistance in the expanded lung. Gas conductance is inversely proportional to the local barrier thickness; a small decrease in barrier thickness leads to a large increase in gas conductance. The harmonic mean thickness of the barrier is several fold smaller than the arithmetic mean thickness (Table 1). This is because the barrier needs to maintain a minimal mass to ensure its integrity, reflected by the arithmetic mean barrier thickness, but the mass must be distributed in a way that interferes as little as possible with gas exchange, reflected by the harmonic mean thickness. Uneven distribution of barrier thickness is an advantage because it allows the diffusion-effective (harmonic) mean thickness to be much smaller, and the gas conductance to be much larger, than a barrier composed of the same total mass with uniform thickness (30).

Table 4. Morphometric estimates of diffusing capacities

<table>
<thead>
<tr>
<th>Conductance for $O_2$, ml·min$^{-1}$·mmHg$^{-1}$·kg$^{-1}$</th>
<th>SL</th>
<th>HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary blood</td>
<td>11.94±0.79</td>
<td>11.15±1.45</td>
</tr>
<tr>
<td>Tissue-plasma barrier</td>
<td>6.47±0.71</td>
<td>6.85±0.84</td>
</tr>
<tr>
<td>Lung</td>
<td>4.19±0.39</td>
<td>4.24±0.53</td>
</tr>
</tbody>
</table>

Values are means ± SD. There are no significant difference between groups.
These results are compatible with two interpretations. 1) HA exposure at 3,800 m for 5 mo was not sufficient stimulus for alveolar-capillary growth. This possibility seems unlikely, given that this degree of HA exposure induced significant alveolar structural response, as well as the expected adaptation in muscle capillary structure. HA exposure is known to increase diaphragm muscle capillary length density (2), which minimizes the mean oxygen diffusion path. In the costal diaphragm of our animals, this increase also persisted into adulthood. 2) Some aspects of HA-accelerated alveolar-capillary growth, e.g., increases in tissue and capillary volume and surface areas, in the growing lung may be transient or plastic and regress following withdrawal of the HA stimuli before reaching maturity. On the other hand, remodeled architecture of alveolar ducts and walls may be less reversible and hence persist into adulthood. Data from small-animal models support the partial reversibility of HA-induced adaptation. In rodents, maximal lung growth stimulation occurs early during simulated HA exposure, but the increased cellular activity reverts immediately to normal after removal of the HA stimulus (25). In rats exposed to one-half atmospheric pressure for 1 mo and then recovered in normoxia for up to 3 mo, pulmonary arterial hypertension and musculation of intra-acinar arteries partially regressed; residual structural changes in these vessels were more pronounced in animals exposed to hypoxia as infants than in those exposed at an older age (22). Indirect evidence from the canine model also suggests reversibility. In beagles raised at 3,100 m for at least 14 mo, the enhanced pulmonary vascular reactivity reversed almost completely within ~8 mo following return to SL, although vascular structure was not examined (8). The reversibility of alveolar structural adaptation has not been directly examined.

Persistent hematological adaptation. In both lung and diaphragm, the magnitude of persistent structural adaptation is modest, suggesting that both capillary networks rely heavily on dynamic factors, such as perfusion of extra erythrocytes, for the augmentation of convective and diffusive oxygen transport at HA. Hypoxia-stimulated erythropoiesis recruits hemoglobin mass, capillary surface, and erythrocyte membrane surface for gas exchange. The benefits of erythropoiesis are limited by the adverse effects of polycythemia, including hyperviscosity and hemodynamic impairment. Additional circulating erythrocytes may be injected by splenic contraction. In athletic mammals, such as the dog, a large spleen (nearly 3% of body weight) can sequester up to 13% of total red cell mass at a hematocrit of 80–90%. On sympathetic stimulation, splenic contraction reversibly releases stored erythrocytes and increases circulating hematocrit from ~45 to >55% and circulating blood volume by 27% (20), thereby providing a dynamic source of compensation in hypoxia while minimizing the risks of polycythemia. The efficient regulation of splenic erythrocyte storage and release in the dog may mitigate the intensity of structural compensation (e.g., growth of gas exchange tissue), which incurs greater metabolic cost. In contrast, lower vertebrates and small mammals (e.g., rodents and guinea pigs) possess a small spleen (~0.3% of body weight) and limited erythrocyte reservoir; therefore, these animals must rely on active structural adaptation of oxygen transport organs during HA exposure. The disparity in hematological capacity may explain the vigorous HA-induced lung growth in small mammals compared with the modest structural response in the dog.

We conclude that, in young dogs, 5 mo of HA exposure during somatic maturation that was discontinued before reaching adulthood induced persistent acinar architectural remodeling with redistribution of alveolar sepal constituents that led to a higher lung compliance and a modest reduction in the resistance of the gas-blood diffusion barrier without the permanent addition of new alveolar tissue. These modest structural changes in the lung, combined with enhanced muscle capillary length density and persistently elevated circulating blood and red cell volumes, are responsible for the long-term enhancement of lung diffusing capacity and exercise capacity in hypoxia observed in adulthood. Combined with previous data showing enhanced alveolar growth and function in young canines raised continuously at HA into adulthood, these results also suggest possible reversibility of structural adaptation following withdrawal of the HA stimuli.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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