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Persistent Structural Adaptation in the Lungs of Guinea Pigs Raised at High Altitude

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Abstract

Laboratory guinea pigs raised at high altitude (HA, 3,800m) for up to 6mo exhibit enhanced alveolar growth and remodeling. To determine whether initial HA-induced structural enhancement persists following return to intermediate altitude (IA), we raised weanling guinea pigs at a) HA for 11-12mo, b) IA (1,200m) for 11-12mo, and c) HA for 4 mo followed by IA for 7-8mo (HA-to-IA). Morphometric analysis was performed under light and electron microscopy. Body weight and lung volume were similar among groups. Prolonged HA residence increased alveolar epithelium and interstitium volumes while reducing alveolar-capillary blood volume. The HA-induced gains in type-1 epithelium volume and alveolar surface area were no longer present following return to IA whereas volume increases in type-2 epithelium and interstitium and the reduction in alveolar duct volume persisted. Results demonstrate persistent augmentation of some but not all aspects of lung structure throughout prolonged HA residence, with partial reversibility following re-acclimatization to IA.

Keywords

Chronic hypoxia; lung growth; alveolar surface area; alveolar tissue volume; alveolar duct; blood-gas diffusion barrier; gas exchange

Introduction

Native highlanders exhibit elevated lung volumes and diffusing capacity (DL_{CO}) compared to their ethnic counterparts residing at sea level (SL) (Brody et al., 1977, Cerny et al., 1973, DeGraff et al., 1970, Droma et al., 1991, Lahiri et al., 1976, Remmers and Mithoefer, 1969, Frisano, 2013); the observations suggest that chronic hypoxia at high altitude (HA) enhances developmental lung growth and remodeling, although structural adaptation to HA residence has not been studied in human lungs. Ambient hypoxia is a primitive and universal

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stimulus for the hypertrophy and remodeling of gas exchange organs across animal phyla (Burggren and Mwalukoma, 1983, Bond, 1960, Sollid et al., 2003), including the mammalian placenta (de Grauw et al., 1986) and lung (Burri and Weibel, 1971a) as a compensatory mechanism for augmenting oxygen uptake. While extreme hypoxia suppresses metabolism and causes cell death, chronic exposure to moderate hypoxia accelerates postnatal lung growth and remodeling in mice and rats (Burri and Weibel, 1971b, Reinke et al., 2011, Burri and Weibel, 1971a, Sekhon and Thurlbeck, 1996b), guinea pigs (Hsia et al., 2005) and dogs (Johnson et al., 1985). Chronic hypoxia induces VEGF expression (Tuder et al., 1995) and Rho-kinase dependent angiogenesis in rat lung (Hyvelin et al., 2005, Howell et al., 2003), indicating a highly plastic alveolar microvasculature. The mechanisms underlying hypoxia-induced lung growth and remodeling are not fully understood but likely involve signaling through the hypoxia-inducible factor pathways (Asikainen et al., 2006, Zhang et al., 2006).

In an earlier study (Lechner and Banchemo, 1980), weanling guinea pigs exposed to chronic hypoxia equivalent to 5,100m HA showed initially accelerated gain in lung volume and alveolar surface area compared to control animals exposed to intermediate hypoxia. Inter-group differences progressively diminished with exposure duration and converged after 14 wk where the final lung volume and surface area were no longer different. The investigators concluded that chronic hypoxia accelerates lung maturation towards but does not extend normal adult dimensions. In contrast, (Johnson et al., 1985) reported persistently elevated lung tissue volume and DL_{CO} in young (2 mo old) dogs raised for 14 mo at a moderate HA (3,100m) and then re-acclimatized to SL for 3 mo, suggesting that chronic moderate hypoxia during maturation enhances long-term lung growth and function in adulthood. Several factors could explain the discrepancy between these two studies: 1) Extreme hypoxia in the guinea pig study (Lechner and Banchemo, 1980) may have limited rib cage growth and secondarily restricted lung size. 2) The guinea pig study (Lechner and Banchemo, 1980) ended before somatic maturation, i.e., bony epiphyseal closure, which begins at ~6 mo of age (Zuck, 1938) whereas the canine study (Johnson et al., 1985) extended beyond somatic maturation (~10-12 mo of age).

To address the time course of hypoxia accelerated lung growth, we previously raised weanling guinea pigs at moderate HA (3,800m) for 1, 3 and 6 mo compared to littermates raised simultaneously at intermediate altitude (IA, 1,200m). We found elevated lung volume, alveolar-capillary surface area, and extra-vascular alveolar septal tissue volume as well as a smaller alveolar duct volume and a lower mean harmonic thickness of the diffusion barrier (τ_{hb}), indicating accelerated lung growth, reduced diffusive resistance and septal crowding, respectively (Hsia et al., 2005). Structural changes are associated with significant increases in DL_{CO} (Yilmaz et al., 2007). Structural adaptation beyond 6 mo have not been reported.

Hypoxia-induced DNA and protein synthesis in rat lung returns immediately to normal levels after removal of the hypoxic stimulus (Sekhon and Thurlbeck, 1996b), and circulating hematocrit normalizes in a few weeks. Young dogs raised to maturity at 3,100m show complete reversal of pulmonary arterial hypertension following return to SL (Grover et al., 1988). However, it is not known whether structural lung growth induced by HA residence is

permanent. We hypothesized that HA-induced structural adaptation in distal lung persists following re-acclimatization to a lower altitude. To test this hypothesis and extend the time course of adaptation beyond 6 mo, we raised 3 groups of male weanling guinea pigs at a) 3,800 m HA for 12 mo, b) 1,200 m IA for 12 mo, and c) HA for 4 mo and then returned to IA for 8 mo (HA-to-IA). At the end of exposure, the lung was fixed for detailed morphometric analysis under light and electron microscopy. Results were compared among groups and with earlier cohorts that had been exposed to the same altitudes for shorter periods (1, 3, and 6 mo) and studied by the same methods (Hsia et al., 2005). Our results show that some but not all features of HA-induced structural adaptation persist into adulthood or following return to a lower altitude.

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 (WMRS) approved the protocols. This study is part of a series conducted over 7 years (1999 to 2006) in which different cohorts were exposed to different altitudes for up to 12 mo. Structural analysis from earlier cohorts exposed to HA and IA for 1 mo (HA n=9, IA n=10), 3 mo (HA n=10, IA n=10), and 6 mo (HA n=11, IA n=12) has been published (Hsia et al., 2005). The groups reported here were studied in 2003-2004. Three separate groups of male weanling Hartley guinea pigs (*Cavia porcellus*, Charles River Laboratory, Wilmington, MA) born at sea level were raised from about 3 weeks of age at: 1) HA (3800 m, barometric pressure P_B =485 mmHg, n=9) for 11-12 mo at the Barcroft Laboratory of WMRS, 2) IA (1,250 m, P_B =660 mmHg, n=8) for 11-12 mo at the Owens Valley Laboratory of WMRS, or 3) HA for 4 mo followed by IA for 7-8 mo (HA-to-IA, n=8). All animals were given food and water *ad libitum*. Ambient temperature, diet, light cycle and care were matched. Body weight and crown-rump length were measured weekly.

Lung fixation and processing

Following established procedures (Hsia et al., 2010, Hsia et al., 2005), the animals were deeply anesthetized with an intra-muscular injection of ketamine (87 mg/kg), xylazine (13 mg/kg) and acepromazine (1 mg/kg); a flexible cannula was inserted and tied securely to the trachea. Lung function was measured and reported separately. The chest was opened, and the left lung removed for separate studies. An overdose of pentobarbital and phenytoin was administered to stop the heart. Simultaneously the right lung was fixed *in situ* by tracheal instillation of 2.5% buffered glutaraldehyde at a constant airway pressure (25 cmH₂O) above the sternum. Following instillation the tracheal tube was closed while maintaining airway pressure. The fixed right lung was removed and immersed in 2.5% buffered glutaraldehyde for 3 weeks before further processing.

Morphometric analysis

The volume of intact lung was measured by saline immersion (Yan et al., 2003). Then the lung was sectioned serially at 3 mm intervals, and each cut surface was photographed using a digital camera (Nikon Coolpix). Volume of the sectioned lung was estimated from the

photographs using the Cavalieri principle (Yan et al., 2003), i.e., measuring the slice area by point counting, multiplying by slice thickness, and summing over all slices. The lung volume estimated by the Cavalieri principle when the tissue was free from tension was used in subsequent morphometric calculations. From the serial sections, 4 tissue blocks were systematically sampled (2 from upper and 2 from lower half of the lung) for microscopic analysis. A previously established 3-level stratified analytical scheme (low and high power light microscopy and electron microscopy) was employed (Hsia et al., 2010, Hsia et al., 2005).

For low-power microscopy each block was embedded in glycol methacrylate, sectioned at 4- μm thickness and stained with toluidine blue. One section per block was overlaid with a test grid. From a random start, twenty non-overlapping fields per section were systematically examined at 275X, for a total of 80 fields per animal. Using point counting, the volume-to-volume ratio of fine parenchyma to lung, $V_v(\text{fp}, \text{L})$, was estimated by excluding the points falling on non-parenchyma (airways or blood structures $\geq 20 \mu\text{m}$ in diameter) relative to the total number of points falling on lung tissue.

For high power light microscopy and electron microscopy, tissue blocks were post-fixed with 1% osmium tetroxide in 0.1 M cacodylate buffer, treated with 2% uranyl acetate, dehydrated through graded alcohol and embedded in SpurrTM. Each block was sectioned at 1 μm thickness; stained with toluidine blue and examined at 550X. Fifteen non-overlapping fields per section were systematically sampled from a random start, for a total of 60 fields per animal. Using the same test grid, the volume ratio of alveolar septa per unit volume of fine parenchyma, $V_v(\text{s}, \text{fp})$, was estimated from the number of points falling on alveolar septa relative to the total number of points falling on fine parenchyma. In addition, alveolar ducts and alveolar sacs were identified and their volumes relative to fine parenchyma estimated separately.

For transmission electron microscopy, each block was sectioned at 70 nm, mounted on a copper grid and examined at approximately 20,000X (JEOL EXII). Thirty non-overlapping fields per grid were systematically sampled from a random start, for a total of 120 fields per animal. The volume-to-volume ratios of alveolar epithelium, interstitium, endothelium, capillary blood and erythrocytes were determined by point counting with alveolar septum as the reference space. The surface-to-volume ratios of alveolar epithelial and endothelial surface area to septal volume were determined by intersection counting. Harmonic mean thickness of the air-blood barrier (τ_{hb}), a measure of alveolar septal resistance to diffusion, was measured from the length of all intercepts extending from the air-epithelial interface to the nearest erythrocyte membrane.

In each animal the data from 4 blocks were averaged. 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 lung volume measured by the Cavalieri Principle (Yan et al., 2003).

Data analysis

Results were expressed as mean $\pm$ SD. The present dataset (residence at HA, IA or HA-to-IA for 12 mo) were compared by factorial ANOVA. In addition, data from the two groups residing at HA or IA for 12 mo were combined with data from previously reported groups (residence at HA or IA for 1, 3, and 6 mo) (Hsia et al., 2005) and compared by factorial ANOVA. Post-hoc analysis used Fisher's Least Significance Difference test. A p value of 0.05 or less was considered significant.

Results

Data in HA, IA and HA-to-IA groups after ~12 mo of exposure (~13 mo of age) are shown in **Tables 1-3**. To demonstrate the time course of adaptation, the present data are also compared to the previously published morphometric data from cumulative cohorts of guinea pigs raised continuously at HA or IA for 1, 3 or 6 mo (Hsia et al., 2005) (**Figures 2-6**). Body weight was transiently higher in the HA groups after 3 and 6 mo of exposure (Hsia et al., 2005) but similar among groups (HA, IA and HA-to-IA) at 12 mo (**Table 1**). As discussed previously (Hsia et al., 2005), the transiently higher body weight was likely related to differences in activity level between HA and IA groups as there were no differences in the indices of skeletal growth. Circulating hematocrit was higher throughout HA exposure but in the HA-to-IA group declined to the expected IA level following return to IA (**Table 1**). Wet weights of the right ventricle did not differ among groups (data not shown). Lung morphology shows appreciably smaller alveolar ducts in the HA group at 12 mo compared to IA; the change persisted in the HA-to-IA group (**Figure 1**). Lung volume was consistently higher (8-30%) in animals raised at HA at all time points, reaching statistical significance at 1 and 6 mo (**Figure 2**); the average alveolar duct volume was significantly lower while the average alveolar sac volume was higher in HA and HA-to-IA groups relative to IA controls (**Figure 2** and **Table 2**). After 12 mo at HA, mean arithmetic thickness of the septum was lower while thickness of the septal extra-vascular tissue layer was higher compared to IA controls (**Table 1**). Mean arithmetic thickness of extravascular septal tissue remained higher in HA-to-IA group compared to IA controls (**Table 1**). The τ_{hb} normally declines during early postnatal maturation as a result of septal remodeling; the normal decline was accentuated by HA exposure so that after 3 mo of HA exposure, τ_{hb} was 11% lower compared to IA controls. However, by 12 mo of exposure τ_{hb} was no longer different among groups (**Table 1, Figure 3**).

The volume and surface densities of alveolar septal components (expressed as ratios with respect to lung volume) after 12 mo of exposure at IA, HA or HA-to-IA, are shown in **Table 2**. These ratios were multiplied by lung volume to obtain absolute volumes (in ml) and surface areas (in m²) of the major septal components, shown in **Table 3**. These results are plotted together with cumulative results obtained in previous cohorts exposed continuously at HA or IA for 1, 3 or 6 mo. The volume of alveolar ducts was transiently 27% higher after 1 mo at HA compared to IA, then became significantly (18-35%) lower at later time points; the reduction (28%) persisted in the HA-to-IA group (**Figure 2**). Alveolar septal tissue volume was not different between groups at 1 mo, but became significantly higher (30-36%) in the HA group at later time points; the increase (32%) persisted in the HA-to-IA group

(**Figure 3**). Total epithelial cell volume was higher (22-51%) in animals raised at HA, reaching statistical significance at all time points after 1 mo; the increase (49%) persisted in the HA-to-IA group (**Figure 4**). Both alveolar type-1 (AT1) and alveolar type-2 (AT2) epithelial cell volumes were higher; however, in the HA-to-IA group the increase in AT2 cell volume persisted while the increase in AT1 cell volume regressed (**Figure 4**). Volume of total interstitium became progressively elevated with the duration of HA exposure due to increases in interstitial cells and matrix as well as collagen fiber volumes; the increase in cells/matrix but not collagen fibers remained significant in the HA-to-IA group (**Figure 5**). Endothelial cell volume was transiently higher after 6 mo of HA exposure but not significantly different from IA at other time points (**Figure 6**). Alveolar capillary blood volume increased modestly early during HA exposure, then reached a plateau by 6 mo and ultimately was lower in HA and HA-to-IA groups compared to IA animals at 12 mo (**Figure 6**). Alveolar surface area was significantly higher (23-28%) in the HA group throughout HA residence, but the increase reversed in the HA-to-IA group after return to IA (**Figure 7**). Capillary surface area was significantly higher in the HA group compared to IA group at 3 and 6 mo but not at 12 mo or in the HA-to-IA group compared to the corresponding IA control group (**Figure 7**).

Discussion

Summary of findings

We examined the persistence of HA-induced acinar adaptation in growing guinea pigs during prolonged HA exposure and following re-acclimatization to IA. The animals were exposed to a habitable altitude, equivalent to inspiring ~13% O₂, that did not adversely affect skeletal growth or cause right ventricular hypertrophy, thus avoiding the pitfalls in data interpretation that plagued earlier studies that exposed rodents to severe hypoxia. Many of the changes observed during shorter (1 to 6 mo) HA exposure (Hsia et al., 2005) persisted to 12 mo, including the smaller alveolar duct volume, the larger alveolar sac volume, the higher volumes of AT2 alveolar epithelium and interstitial components, and the increased alveolar surface area. Other HA-induced changes, including a mildly lower τ_{hb} , and the transiently elevated endothelium volume and capillary blood volume, were no longer evident after 12 mo of exposure. Thus, with prolonged HA residence up to young adulthood, alveolar epithelium and interstitium dimensions but not endothelium or capillary dimensions were persistently enhanced. Long-term barrier resistance to diffusion indexed by τ_{hb} was similar.

In the HA-to-IA group compared to IA group, volume of the fixed lung was similar. Alveolar duct volume was lower and alveolar sac volume higher, indicating persistent acinar remodeling. Volumes of AT2 cells, the interstitium, and the septal extravascular tissue, remained elevated, while AT1 cell volume and alveolar surface area normalized. Thus, some but not all aspects of HA-induced structural adaptation persist after return to IA.

Critique of methods

Guinea pigs are phylogenetically distinct from rodents (D'Erchia et al., 1996). Unlike rodents where the bony epiphyses never fully close and somatic growth continues through

life (Dawson, 1934), epiphyseal closure occurs in guinea pigs (Zuck, 1938) as in larger mammals effectively limiting thoracic size in adulthood (~1 year of age). For manpower and logistic reasons, the cohorts undergoing different exposure were studied in separate years. The Barcroft Laboratory (3,800m) is accessible by regular vehicle for ~4 mo each year; longer residence required special winter arrangement for animal care. While we do not have morphometric analysis from simultaneous control animals after 4 mo of HA exposure, the cumulative data show a robust pattern of structural enhancement during continued HA exposure up to early adulthood.

Comparison to earlier studies

Our findings in guinea pigs agree with data in rodents. Mice native to HA exhibit a higher fractional lung tissue volume due to hypertrophy of AT1, AT2 and endothelial cells compared to control animals living at SL (Pearson and Pearson, 1976); epithelial, endothelial, and erythrocyte surface areas per gram body weight are also elevated. Hypoxia accelerates lung growth and alveolization in rats (Bartlett and Remmers, 1971, Burri and Weibel, 1971a, Cunningham et al., 1974, Sekhon and Thurlbeck, 1996a, Sekhon and Thurlbeck, 1996b); within 3 wk of exposure lung volume becomes 20% larger than in normoxic control animals; subsequently the rate of lung growth returns to normal, although the relative increase in volume is retained.

The effects of chronic hypoxia on the pulmonary microvasculature are complex. Chronic hypoxia promotes angiogenesis in the lung (Heil et al., 2006, Marti and Risau, 1998, Howell et al., 2003). Yet, progressive pulmonary vascular pruning and remodeling also develops in response to hypoxia-induced pulmonary arterial hypertension (Hislop and Reid, 1976). The increases in endothelium and capillary blood volumes after 1 to 6 mo of HA residence (**Figure 6**) are consistent with pro-angiogenesis. By 12 mo, capillary blood volume reaches a plateau and was lower than the IA group while endothelium volume was not different from the IA group. In the HA-to-IA group, capillary blood volume was also lower than that in IA but not different from HA group. These findings suggest that the accelerated increase in alveolar microvascular capacity reached an upper limit after ~6 mo of HA exposure.

Persistence of structural adaptation

In rodents, cellular proliferation is stimulated early during normobaric hypoxia exposure. RNA/DNA ratio increased on day 1 and protein/DNA ratio on day 3 of hypoxia exposure; the increased cellular proliferation reverts immediately to normal after removal of HA stimulus (Sekhon and Thurlbeck, 1996b). Pulmonary arterial hypertension partially reverses while muscularization of pulmonary acinar arteries persists following recovery in normoxia (Rabinovitch et al., 1981). In our animals, the gains in AT2 cell and interstitium volumes are maintained throughout exposure, and persist after return to IA. In contrast, the elevated AT1 cell volume completely regresses following return to IA. Since AT1 cells cover most of the alveolar surfaces, the gain in alveolar surface area during HA exposure also regresses. Thus, AT1 cell stimulation at HA permits an increase in gas exchange surface area, which is no longer needed after return to IA. Consistent with this interpretation, the HA-induced increase in septal collagen content needed to support gas exchange surfaces also regresses following return to IA (**Tables 2 and 3**).

Two experimental series examined long-term pulmonary adaptation to HA residence in canines. The first series (Johnson, 1994, Johnson et al., 1985) reports that HA exposure (3,100m) in young (2.5 mo old) beagles throughout maturation (14 mo exposure) permanently enhances lung function, lung volumes and alveolar surface area in adulthood. In contrast, adult beagles kept at 3,100m for 3 years do not show enhancement of lung structure or function. However, alveolar surface area was measured under light microscopy and septal ultrastructure was not examined. In a subsequent series, young foxhounds born at SL and raised at HA (3,800 m) for 5 mo exhibit enhanced pulmonary gas exchange at rest and exercise more than 2 years after return to SL (Hsia et al., 2007, McDonough et al., 2006). Total erythrocyte and blood volumes also remain elevated. Three years after return to SL, lung volumes remained higher due to enlarged alveolar ducts and sacs (37% and 13%, respectively) with a mildly (~10%) lower τ_{hb} while septal cell and tissue volumes and surface areas measured under electron microscopy are not different from control animals raised at SL (Ravikumar et al., 2009). Thus, in canines HA-induced enhancement of hematological capacity, lung expansion and alveolar remodeling persist into adulthood, leading to long-term reduction of the resistance to O₂ diffusion without persistent enhancement of alveolar tissue growth.

Both guinea pigs and dogs exposed to HA exhibit long-term physiologic enhancement of physiologic lung volume and diffusing capacities (Yilmaz et al., 2007, McDonough et al., 2006). Because control guinea pigs resided at 1,250m and control dogs at 160m, the modestly higher altitude in guinea pigs may have helped preserve prior HA-induced changes. Structural dimensions were measured in guinea pigs 8 mo after return to IA (age ~13 mo), and in dogs 3 years after return to SL (age ~34 mo) (Ravikumar et al., 2009). Since the dogs had a longer period of de-acclimatization, there was more time for regression of HA-induced changes. Conversely, the persistent AT2 cell and interstitial volume enhancement in our guinea pigs may continue to regress following longer residence at IA.

A large canine spleen (~3% of body weight) sequesters and releases on demand concentrated erythrocytes (hematocrit 80-90%) into the circulation, a highly efficient form of reversible blood doping that augments both convective and diffusive O₂ transport by 30-40% while avoiding the adverse effects of chronic polycythemia (Dane et al., 2006). Following return to SL and normalization of hematocrit, hematological capacity remains persistently elevated associated with a slightly larger spleen (Hsia et al., 2007, McDonough et al., 2006, Ravikumar et al., 2009). The canine splenic contribution for O₂ transport in chronic hypoxia mitigates the need for alveolar tissue growth, which incurs a higher metabolic cost. In our guinea pigs there is no consistent difference in spleen weight between groups up to 6 mo of exposure except for a modest elevation in HA group at 3 mo (**Figure 8**). By 12 mo of exposure spleen weight in HA group was lower than in the other two groups, reaching statistical significance compared to HA-to-IA group. Results suggest greater splenic contraction during prolonged HA residence. Given the small guinea pig spleen (~0.3% of body weight), their ability to augment hematological O₂ carrying capacity is limited. Consequently, guinea pigs like other small animals rely heavily on structural lung growth for gas exchange compensation at HA. In the absence of sustained alveolar capillary expansion, septal cellular hyperplasia/hypertrophy and connective tissue deposition tend to

increase the barrier resistance to diffusion even as the larger alveolar sacs keep the alveolar surfaces unfolded to minimize resistance; these opposing effects may explain the lack of change in τ_{hb} in guinea pigs exposed to prolonged HA residence.

The species difference in compensatory mechanisms is also evident in air space size, which may be differentially regulated by contractile elements such as smooth muscle cells in distal airways and parenchyma myofibroblast-like cells in alveolar ductal tissue that contain α -smooth muscle actin (Oldmixon et al., 2001). In guinea pigs raised at HA with persistently enhanced alveolar tissue growth and unchanged total lung volume, the smaller alveolar ducts help accommodate extra alveolar tissue while allowing unfolding of a larger alveolar surface. In contrast, in dogs raised at HA total lung volume increases without permanent increases in alveolar tissue volume or surface area, thus allowing air space enlargement (Ravikumar et al., 2009).

In summary, HA residence in young guinea pigs stimulates distal lung growth and remodeling, leading to sustained augmentation of most but not all alveolar structural dimensions up to maturity. A shorter period (4 mo) of HA residence followed by re-acclimatization to IA leads to persistently and selectively elevated volumes of alveolar epithelium and interstitium. In spite of significant plasticity in response to HA stimuli, the patterns and mechanisms of compensation in guinea pigs are dissimilar from that observed in young dogs raised at HA and then returned to SL before somatic maturity. Differences reflect species disparity in alveolar microvascular reserves as well as whole body hematological reserves. The fact that HA exposure enhances lung growth in young but not adult animals suggest that hypoxia adds to developmental signals but does not re-initiate compensatory growth *de novo*. The developmental signals depend to a large extent on mechanical interactions between the enlarging thorax and the lung; these interactions diminish with age. Consequently, the magnitude of HA enhanced lung growth also diminishes with age. Our findings of a converging total lung volume with persistently larger alveolar tissue volume and surface area, and the smaller alveolar ducts and larger sacs, indicate airspace architectural remodeling to accommodate the extra alveolar tissue within a finite volume, suggesting that long-term enhancement of lung growth in guinea pigs is ultimately limited by thoracic size.

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Highlights

- High altitude (HA) residence up to 12 month enhances lung growth in guinea pigs.
- Gains in type-1 epithelium volume and surface reverse after return to low altitude
- Gains in type-2 epithelium and interstitium volumes do not reverse.
- HA-induced alveolar growth and remodeling are partially reversible.
- Results indicate significant structural plasticity in the distal lung.

Figure 1.

Representative lung micrographs stained with toluidine blue are shown from one animal each raised at IA for 11-12 mo (*left*), HA for 11-12 mo (*middle*) and HA for 4 mo followed by returned to IA for 7-8 mo (*right*). Scale bar = 100 μ m.

Figure 2.

Lung volume after fixation (*left*), volume of alveolar ducts (*middle*) and alveolar sacs (*right*) are shown with respect to the duration of exposure at HA, IA or HA-to-IA. Data at 1, 3, and 6 mo exposure are from (Hsia et al., 2005). Number of animals: 1 mo (HA 9, IA 10), 3 mo (HA 10, IA 10), 6 mo (HA 11, IA 12), 11-12 mo (HA 9, IA 8, HA-to-IA 8). Mean $\pm$ SD, ^a p 0.05; ^b p 0.01; ^c p 0.001; ^d p 0.0001 vs. IA; ^e p 0.05; ^f p 0.01; ^g p 0.001; ^h p 0.0001 vs. HA by factorial ANOVA.

Figure 3.

Alveolar septal tissue volume (*left*) and mean harmonic thickness of the air-blood diffusion barrier (τ_{hb}) (*right*) are shown with respect to the duration of exposure at HA, IA or HA-to-IA. Data from 1, 3, and 6 mo exposure are from (Hsia et al., 2005). Number of animals: 1 mo (HA 9, IA 10), 3 mo (HA 10, IA 10), 6 mo (HA 11, IA 12), 11-12 mo (HA 9, IA 8, HA-to-IA 8). Mean $\pm$ SD, ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$; ^d $p < 0.0001$ vs. IA; ^e $p < 0.05$; ^f $p < 0.01$; ^g $p < 0.001$; ^h $p < 0.0001$ vs. HA by factorial ANOVA.

Figure 4.

Total epithelial (*left*), AT1 (*middle*) and AT2 (*right*) cell volumes are shown with respect to the duration of exposure at HA, IA or HA-to-IA. Data from 1, 3, and 6 mo exposure are from (Hsia et al., 2005). Number of animals: 1 mo (HA 9, IA 10), 3 mo (HA 10, IA 10), 6 mo (HA 11, IA 12), 11-12 mo (HA 9, IA 8, HA-to-IA 8). Mean $\pm$ SD, ^a p 0.05; ^b p 0.01; ^c p 0.001; ^d p 0.0001 vs. IA; ^e p 0.05; ^f p 0.01; ^g p 0.001; ^h p 0.0001 vs. HA by factorial ANOVA.

Figure 5.

Volumes of total interstitium (*left*), interstitial cells and matrix (*middle*), and collagen fibers (*right*) are shown with respect to the duration of exposure at HA, IA or HA-to-IA. Data from 1, 3, and 6 mo exposure are from (Hsia et al., 2005). Number of animals: 1 mo (HA 9, IA 10), 3 mo (HA 10, IA 10), 6 mo (HA 11, IA 12), 11-12 mo (HA 9, IA 8, HA-to-IA 8). Mean $\pm$ SD, ^a p 0.05; ^b p 0.01; ^c p 0.001; ^d p 0.0001 vs. IA; ^e p 0.05; ^f p 0.01; ^g p 0.001; ^h p 0.0001 vs. HA by factorial ANOVA.

Figure 6.

Volumes of endothelial cells (*left*) and alveolar capillary blood (*right*) are shown with respect to the duration of exposure at HA, IA or HA-to-IA. Data from 1, 3, and 6 mo exposure are from (Hsia et al., 2005). Number of animals: 1 mo (HA 9, IA 10), 3 mo (HA 10, IA 10), 6 mo (HA 11, IA 12), 11-12 mo (HA 9, IA 8, HA-to-IA 8). Mean $\pm$ SD, ^a p 0.05; ^b p 0.01; ^c p 0.001; ^d p 0.0001 vs. IA; ^e p 0.05; ^f p 0.01; ^g p 0.001; ^h p 0.0001 vs. HA by factorial ANOVA.

Figure 7.

Alveolar (*left*) and capillary (*right*) surface areas are shown with respect to the duration of exposure at HA, IA or HA-to-IA. Data from 1, 3, and 6 mo exposure are from (Hsia et al., 2005). Number of animals: 1 mo (HA 9, IA 10), 3 mo (HA 10, IA 10), 6 mo (HA 11, IA 12), 11-12 mo (HA 9, IA 8, HA-to-IA 8). Mean $\pm$ SD, ^a p 0.05; ^b p 0.01; ^c p 0.001; ^d p 0.0001 vs. IA; ^e p 0.05; ^f p 0.01; ^g p 0.001; ^h p 0.0001 vs. HA by factorial ANOVA.

Figure 8.

Spleen weights are shown with respect to the duration of exposure at HA, IA or HA-to-IA. Data from 1, 3, and 6 mo exposure are from (Hsia et al., 2005). Number of animals: 1 mo (HA 9, IA 10), 3 mo (HA 10, IA 10), 6 mo (HA 11, IA 12), 11-12 mo (HA 9, IA 8, HA-to-IA 8). Mean $\pm$ SD, ^a p 0.05; ^b p 0.01; ^c p 0.001; ^d p 0.0001 vs. IA; ^e p 0.05; ^f p 0.01; ^g p 0.001; ^h p 0.0001 vs. HA by factorial ANOVA.

Table 1

Baseline data

Altitude	11-12 mo Exposure			P value		
	IA	HA	HA-to-IA	HA vs. IA	HA-to-IA vs. IA	HA-to-IA vs. HA
Number of animals	8	9	8			
Body weight (g)	1,293±95	1,277±95	1,279±42	0.7244	0.7336	0.9511
Circulating hematocrit (%)	53.9±2.6	57.1±3.8 ^c	53.2±5.0	0.0008	0.6295	0.5937
Right lung volume (ml)						
Intact (immersion method)	20.9±3.3	22.6±2.0	22.9±2.4	0.2032	0.14	0.8345
Sectioned (Cavalieri method)	18.8±2.5	20.3±1.8	20.7±2.3	0.1658	0.0977	0.7256
Mean arithmetic thickness of septum (μm)	5.91±0.37	4.73±0.29 ^d	5.83±0.70 ^h	<0.0001	0.7424	<0.0001
Mean arithmetic thickness of septal tissue (μm)	0.99±0.08	1.12±0.07 ^b	1.19±0.16 ^b	0.0017	0.0011	0.2278
Mean harmonic thickness of blood–gas barrier (τ _{hb} , μm)	0.64±0.04	0.63±0.02	0.65±0.03	0.6640	0.4045	0.1966

Mean±SD

^a p 0.05;^b p 0.01;^c p 0.001;^d p 0.0001 vs. IA;^e p 0.05;^f p 0.01;^g p 0.001;^h p 0.0001 vs. HA by factorial ANOVA.

Table 2

Volume and surface densities of alveolar structures

	11-12 mo Exposure			P value		
Altitude	IA	HA	HA-to-IA	HA vs. IA	HA-to-IA vs. IA	HA-to-IA vs. HA
Volume density per unit lung volume						
Fine parenchyma	0.9050±0.0170	0.9300±0.0090 ^b	0.9340±0.0080 ^d	0.0016	<0.0001	0.555
Alveolar duct	0.1600±0.0110	0.0970±0.0160 ^d	0.1040±0.0120 ^d	<0.0001	<0.0001	0.307
Alveolar sac	0.6040±0.0245	0.7041±0.0189 ^a	0.6915±0.0131 ^a	0.0262	0.0311	0.2884
Septum	0.1420±0.0120	0.1290±0.0110 ^a	0.1380±0.0140	0.0385	0.5661	0.1398
Epithelium	0.0203±0.0021	0.0267±0.0039 ^c	0.0262±0.0036 ^c	0.0008	0.0007	0.9275
Type 1 cells	0.0110±0.0010	0.0121±0.0018	0.0110±0.0020	0.0847	0.5279	0.3056
Type 2 cells	0.0094±0.0016	0.0146±0.0022 ^d	0.0152±0.0021 ^d	<0.0001	<0.0001	0.4694
Interstitium	0.0216±0.0023	0.0252±0.0044 ^a	0.0235±0.0028	0.0345	0.197	0.2561
Collagen fibers	0.0169±0.0024	0.0201±0.0040 ^a	0.0178±0.0023	0.0444	0.464	0.1096
Cells and matrix	0.0046±0.0009	0.0051±0.0010	0.0057±0.0009 ^a	0.2050	0.0244	0.2726
Endothelium	0.0116±0.0027	0.0118±0.0020	0.0130±0.0020	0.8350	0.3614	0.4667
Septal tissue	0.0543±0.0018	0.0637±0.0095 ^a	0.0618±0.0069 ^a	0.0107	0.0145	0.7376
Capillary blood	0.0907±0.0078	0.0650±0.0047 ^d	0.0732±0.0074 ^{bf}	<0.0001	0.0015	0.0096
Surface density per unit lung volume (cm ⁻¹)						
Alveolar surface	493±24	548±71 ^a	460±86 ^e	0.034	0.9777	0.0451
Capillary surface	595±55	588±79	565±88	0.7637	0.801	0.9941

Mean±SD

^a_p 0.05;^b_p 0.01;^c_p 0.001;^d_p 0.0001 vs. IA;^e_p 0.05;^f_p 0.01;^g_p 0.001;^h_p 0.0001 vs. HA by factorial ANOVA.

Table 3

Absolute volumes and surface areas of septal structures

Altitude	11-12 mo Exposure			P value		
	IA	HA	HA-to-IA	HA vs. IA	HA-to-IA vs. IA	HA-to-IA vs. HA
Absolute volume (ml)						
Fine Parenchyma	16.99±2.25	18.87±1.67	19.28±2.07 ^a	0.0676	0.0314	0.6715
Alveolar duct	2.99±0.41	1.97±0.35 ^d	2.14±0.26 ^d	<0.0001	<0.0001	0.3192
Alveolar sac	11.34±1.58	14.28±1.35 ^c	14.29±1.61 ^c	0.0006	0.0008	0.9945
Septum	2.66±0.42	2.61±0.33	2.85±0.46	0.8106	0.342	0.2333
Epithelium	0.38±0.08	0.54±0.10 ^c	0.53±0.07 ^b	0.0009	0.0038	0.6824
Type 1 cells	0.20±0.03	0.25±0.05 ^a	0.22±0.04	0.0501	0.4501	0.2357
Type 2 cells	0.18±0.05	0.30±0.05 ^d	0.30±0.04 ^d	<0.0001	<0.0001	0.7294
Interstitial	0.39±0.06	0.51±0.10 ^b	0.48±0.05 ^a	0.0085	0.0289	0.3539
Collagen fibers	0.31±0.05	0.41±0.09 ^b	0.36±0.05	0.0107	0.1056	0.1651
Cells and matrix	0.08±0.02	0.10±0.02	0.11±0.01 ^b	0.0556	0.0012	0.1607
Endothelium	0.23±0.05	0.24±0.05	0.24±0.04	0.6902	0.616	0.8932
Septal tissue	0.98±0.17	1.30±0.23 ^b	1.29±0.19 ^b	0.006	0.0049	0.9489
Capillary blood	1.69±0.29	1.32±0.15 ^b	1.47±0.15 ^a	0.0013	0.0504	0.1435
Absolute surface area (cm ²)						
Alveolar surface	9,132±1,029	11,139±1,918 ^a	9,282±1,906 ^e	0.0293	0.8697	0.0422
Capillary surface	11,011±1,363	11,966±2,120	11,396±1,975	0.3253	0.7054	0.5541

Mean±SD

^a_p 0.05;^b_p 0.01;^c_p 0.001;^d_p 0.0001 vs. IA;^e_p 0.05;^f_p 0.01;^g_p 0.001;^h_p 0.0001 vs. HA by factorial ANOVA.