Long-term enhancement of pulmonary gas exchange after high-altitude residence during maturation

Paul McDonough, D. Merrill Dane, Connie C. W. Hsia, Cuneyt Yilmaz, and Robert L. Johnson, Jr.

Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas

Submitted 1 September 2005; accepted in final form 20 September 2005

McDonough, Paul, D. Merrill Dane, Connie C. W. Hsia, Cuneyt Yilmaz, and Robert L. Johnson, Jr. Long-term enhancement of pulmonary gas exchange after high-altitude residence during maturation. J Appl Physiol 100: 474–480, 2006. First published October 6, 2005; doi:10.1152/japplphysiol.01069.2005.—In a previous study, our laboratory showed that young dogs born at sea level (SL) and raised from 2.5 mo of age to beyond somatic maturity at a high altitude (HA) of 3,100 m showed enhanced resting lung function (Johnson RL Jr, Cassidy SS, Grover RF, Schutte JE, and Epstein RH. J Appl Physiol 59: 1773–1782, 1985). To examine whether HA-induced adaptation improves pulmonary gas exchange during exercise and whether adaptation is reversible when animals return to SL before somatic maturity, we raised 2.5-mo-old foxhounds at HA (3,800 m) for 5 mo (to age 7.5 mo) before returning them to SL. Lung function was measured under anesthesia 1 mo and 2 yr after return to SL and during exercise ~1 yr after return. In animals exposed to HA relative to simultaneous litter-matched SL controls, resting circulating blood and erythrocyte volumes, lung volumes, septal volume estimated by a rebreathing technique, and lung tissue volume estimated by high-resolution computed tomography scan were persistently higher. Lung diffusing capacity, membrane diffusing capacity, and pulmonary capillary blood volume estimated at a given cardiac output were significantly higher in animals exposed to HA, whereas maximal oxygen uptake and hematocrit were similar between groups. We conclude that relatively short exposure to HA during somatic maturation improves long-term lung function into adulthood.

chronic hypoxia; canine; somatic maturation; lung volume; pulmonary diffusing capacity; membrane diffusing capacity; pulmonary capillary blood volume; erythrocyte volume; exercise

NATIVE HIGHLANDERS EXHIBIT a larger vital capacity and lung volume than lowlanders of a similar ethnic background (3, 8–11, 13, 23). Pulmonary gas exchange and lung diffusing capacity for CO (DLCO) are also enhanced in native highlanders compared with lowlanders (6, 8, 29). It is not clear whether these changes are attributable to genetic predisposition or induced by chronic high-altitude (HA) exposure. Johnson et al. (12, 21, 22) exposed 2.5-mo-old young beagles born at sea level (SL) to a moderate HA (3,100 m) for 14 mo, i.e., beyond somatic maturity. Three months after return to SL, these animals exhibited significantly higher lung volumes and resting DLCO compared with size- and age-matched control animals raised simultaneously at SL; lung tissue volume and alveolar surface area were also higher. A lower station of the diaphragm accommodated the larger lungs in HA-raised animals, whereas indexes of somatic or bony growth were not altered and thoracic distensibility remained unchanged. These data support HA residence as a stimulus for postnatal lung growth. Dogs raised at HA also developed persistent pulmonary arterial hypertension of a severity comparable to that observed in humans acclimatized to HA (12); these animals provide a suitable model for studying the extent and mechanisms of adaptation at HA. In the study by Johnson et al. (12, 21, 22), HA exposure was prolonged beyond maturity and functional assessment was performed only under anesthesia. We reasoned that if growing animals adapt readily to HA exposure, they should also adapt with equal vigor to the withdrawal of the HA stimulus. If so, we expect HA-induced adaptation to regress when animals return to SL before reaching maturity. Therefore, we conducted the present studies to determine 1) whether adaptation during maturation at HA improves long-term pulmonary gas exchange at rest and exercise and 2) whether HA-induced adaptive changes are reversible after return to SL before somatic maturity is reached. To address these issues, we raised young foxhounds (2.5 mo of age) at a well-tolerated altitude of 3,800 m for 5 mo and then returned them to SL at 7.5 mo of age. Resting lung function was assessed 1 mo and 2 yr after return to SL and during exercise 1–2 yr after return to SL. Results from animals raised at HA were compared with litter-matched controls raised simultaneously at SL.

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 both approved the protocols (a timetable is shown in Fig. 1). 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 at the Barcroft Laboratory (altitude 3,800 m, barometric pressure 485 mmHg) of the University of California White Mountain Research Station and remained there for 5 mo (Table 1). Their male littermates (n = 6) were raised simultaneously at SL in Dallas, TX (altitude ~156 m, barometric pressure 750 mmHg). Animals were fed similar diet and given water ad libitum. Body weight was measured each week. At 7.5 mo of age, animals residing at HA returned to SL in Dallas. Physiological studies of lung function and circulating blood volume were measured at rest 1 mo later. Then, the animals were trained to run on a motorized treadmill according to an established exercise program (39). A customized leak-free respiratory mask was made for each animal to allow for ventilatory and pulmonary gas-exchange measurements during exercise. Bilateral carotid artery loops were surgically constructed to allow arterial catheterization. Lung function during exercise was measured ~1 yr after return to SL. Circulating blood volume and resting lung function measurements were repeated ~2 yr after returning to SL.
Blood Volume Measurements

With the animal standing at rest, Evans blue dye (2 ml) (34) was injected intravenously via a peripheral catheter. Serial blood samples were withdrawn 2, 5, 10, and 15 min later from a separate venous catheter placed in the contralateral forelimb. The blood samples were centrifuged for 15 min; the plasma was decanted and analyzed using a spectrophotometer (Beckman DU 640 B, Beckman Coulter, Fullerton, CA) at 620- and 740-nm absorbance. Hemocrit was measured using a microcapillary centrifuge and a microhematocrit capillary tube reader (Oxford Labware, St. Louis, MO). Hemoglobin concentration was measured by a hemoximeter (model OSM3, Radiometer, Copenhagen, Denmark). Plasma volume was calculated from the dye-dilution curve. Total blood volume was obtained from plasma volume and the systemic hemocrit. Erythrocyte volume was determined by subtracting plasma volume from total blood volume.

Lung Function Measurement at Rest

The animal was fasted overnight and premedicated (acepromazine 0.2 mg/kg im and glycopyrrolate 0.01 mg/kg sc). Anesthesia was induced with an intravenous bolus of ketamine (6 mg/kg) and diazepam (0.3 mg/kg) and maintained by an infusion of the same medication (0.3% CO, 0.3% CH4, 0.6% C2H2, and 40 parts/million NO in a balance of either 21 or 99% O2. Before each measurement using the gas mixture containing 99% O2, the animal was ventilated with 100% O2 for 1–3 min until end-tidal PO2 stabilized. The lungs were hyperinflated with three tidal breaths and allowed to deflate to EELV. A predetermined volume of the rebreathing gas mixture (30 or 45 ml/kg) was drawn into a calibrated syringe, delivered via the endotracheal tube, and manually rebreathed between the lung and the syringe at 30 breaths/min for a period of ~18 s. Duplicate measurements were obtained using each gas mixture at each lung volume. Gas concentrations were monitored at the mouth via a mass spectrometer for O2 and CO2 (model MGA-1100, Perkin-Elmer, Wellesley, MA); infrared analyzer for CO, CH4, and C2H2 (Sensors, Saline, MI); and a chemiluminescence NO analyzer (model 280, Sievers Instruments, Boulder, CO). All signals were digitized at 100 Hz.

Lung volume (BTPS) was determined from methane dilution. DLCO and lung diffusing capacity for NO (DLNO) and cardiac output were calculated from end-tidal points selected from the log-linear portion of the CO, NO, and acetylene disappearance curves, respectively, with respect to methane. Septal volume was estimated from the extrapolated intercept of the acetylene disappearance curve to time 0 (15, 31, 32).

Table 1. Resting data

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>SL exposure group</th>
<th>HA exposure group</th>
<th>SL exposure group</th>
<th>HA exposure group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mo After Return to SL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>24.3±1.4</td>
<td>23.9±0.8</td>
<td>24.8±1.2</td>
<td>25.3±0.4</td>
</tr>
<tr>
<td>0.8</td>
<td>41.5±0.9</td>
<td>44.0±1.6</td>
<td>48.3±1.3</td>
<td>47.8±1.2</td>
</tr>
<tr>
<td>0.3</td>
<td>12.3±0.3</td>
<td>12.0±0.3</td>
<td>11.4±0.4</td>
<td>10.9±0.3</td>
</tr>
<tr>
<td>0.1</td>
<td>26±1</td>
<td>29±1†</td>
<td>31±1</td>
<td>31±1</td>
</tr>
<tr>
<td>0.2</td>
<td>77±1</td>
<td>81±1</td>
<td>85±1</td>
<td>87±1</td>
</tr>
<tr>
<td>0.4</td>
<td>98±4</td>
<td>123±58</td>
<td>127±4</td>
<td>145±6†</td>
</tr>
<tr>
<td>0.5</td>
<td>152±2</td>
<td>135±28</td>
<td>125±2</td>
<td>130±3</td>
</tr>
<tr>
<td>0.6</td>
<td>22.6±0.04</td>
<td>2.53±0.05</td>
<td>2.23±0.05</td>
<td>2.45±0.09†</td>
</tr>
<tr>
<td>0.7</td>
<td>0.58±0.01</td>
<td>0.73±0.025</td>
<td>0.69±0.02</td>
<td>0.85±0.03§</td>
</tr>
<tr>
<td>0.8</td>
<td>1.13±0.03</td>
<td>1.33±0.045</td>
<td>1.16±0.05</td>
<td>1.42±0.07§</td>
</tr>
<tr>
<td>0.9</td>
<td>1.79±0.05</td>
<td>2.40±0.07§</td>
<td>2.22±0.09</td>
<td>2.55±0.10†</td>
</tr>
<tr>
<td>1.0</td>
<td>5.30±0.29</td>
<td>6.70±0.275</td>
<td>4.76±0.45</td>
<td>6.02±0.36§</td>
</tr>
</tbody>
</table>

Values are means ± SE. SL, sea level; HA, high altitude; DLNO, lung diffusing capacity for NO; DLCO, lung diffusing capacity for CO; DMCO, membrane diffusing capacity for CO; Vc, capillary volume. DLCO is expressed at a standard alveolar O2 tension (P(a)O2) of 120 Torr and hemoglobin concentration of 14.6 g/dl. *Measured at 45 ml/kg above end-expiratory lung volume. HA vs. SL. †P < 0.05; ‡P < 0.01; §P < 0.001.

J Appl Physiol • VOL 100 • FEBRUARY 2006 • www.jap.org
were calculated by the Roughton-Forster technique (30).

\[
\frac{1}{DL_{CO}} = \frac{1}{DM_{CO}} + \frac{1}{\Theta_{CO} \cdot Vc}
\]

where \( \Theta_{CO} \) is the rate of CO uptake in whole blood at 37°C [ml (STPD)·min⁻¹·mmHg⁻¹] estimated from the average alveolar O₂ tension (PₐO₂; in mmHg) during rebreathing and the measured hemoglobin concentration ([Hb]; in g/dl).

\[
\frac{1}{\Theta_{CO}} = (\alpha + \beta \cdot P_{ACO}) \cdot \frac{14.6}{[Hb]}
\]

where \( \alpha = 0.929 \) and \( \beta \) varies with rectal temperature (14, 19). Estimates of DMCO and Vc were used to standardize DLCO (DLCO-std) to a constant PₐO₂ (120 Torr) and hemoglobin concentration (14.6 g/dl).

**Backpressure of CO.** The backpressure of CO was determined by a rebreathing maneuver three times (before, halfway point, and at the end of each study). The calibrated syringe was filled with 100% O₂ (~45 ml/kg), and the rebreathing duration was ~90 s. Expired CO concentration was monitored continuously and the backpressure estimated from the exponential washout curve. The interpolated CO backpressure with respect to time was used to correct the end-tidal CO concentration measured during rebreathing.

**Lung Function Measurement During Exercise**

**Rebreathing measurements.** A 3-liter anesthetic bag was prefilled with the rebreathing gas mixture to the animal’s average tidal volume at a given workload plus 200 ml (STPD). The test gas mixture consisted of 0.3% C¹⁸O, 0.6% C₂H₂, 8–9% He in a balance of either 90% O₂ (STPD) or 90% N₂ (STPD) to achieve 80% of the animal's maximal O₂ uptake for 3 min. At a selected end-expiratory point, a pneumatic valve was automatically switched to allow the animal to breathe in and out of the anesthetic bag for 8–12 s. Gas concentrations at the mouth were recorded continuously during rebreathing. Before each measurement using the gas mixture containing 90% O₂, the animal breathed 100% O₂ for 30–60 s to ensure equilibration of O₂ concentration between the test mixture and resident air in the lung.

**High-Resolution Computed Tomography**

Spiral computed tomography (CT) scan was performed using a GE high-speed CTI scanner at 3 × 3-mm collimation as described previously (28). Animals were fasted overnight, premedicated, anesthetized, intubated, placed supine on the CT table, and mechanically ventilated to eliminate spontaneous breathing effort. Airway pressure was monitored. Before each imaging sequence, the lungs were hyper-inflated for three tidal breaths, followed by passive expiration to EELV. A calibrated syringe was used to deliver a volume of air that had been previously determined in each animal to inflate the lungs to a transpulmonary pressure of 20 cmH₂O (1-mo follow-up). In later studies (2-yr follow-up), an esophageal catheter was also inserted to directly verify transpulmonary pressure. The breath was held for 30–40 s while CT images were obtained, after which the animal was reconnected to the respirator.

Images were reconstructed at consecutive 1-mm intervals and analyzed using Object-Image version 1.6.2 (public domain software). The area occupied by lung was outlined on each image using density thresholding, which excluded conducting blood vessels larger than ~1-mm diameter. The trachea and next three generations of large conducting airways were excluded manually by marking them with the background color. Lung volume in each image is equal to the product of its area and thickness (1 mm); total lung volume was the sum of the volume of all images. The CT density (in Hounsfield units) of tracheal air and of skeletal muscle were measured as estimates of air and tissue density, respectively, and used to partition total lung volume into air and tissue volumes by established equations (28). Owing to the resolution limit of high-resolution CT (HRCT), these estimates of tissue volume include the volume of alveolar septa as well as small extraseptal airways and blood vessels.

**Data Analysis**

Results were normalized by body weight, expressed as means ± SE, and compared between groups by one-way analysis of variance and unpaired or paired t-test (StatView version 5.0, SAS Institute, Cary, NC). Static pressure-volume relationships of the lung were analyzed by the method of Salazar and Knowles (33); the volume at a given transpulmonary pressure (in 10-cmH₂O increments from 0 to 50 cmH₂O) were compared between groups by repeated-measures ANOVA. DLCO, DMCO, and Vc were plotted with respect to cardiac output; slopes and intercepts of the individual regression lines were compared by the method of Zar (43). A P value of <0.05 was considered significant.

**RESULTS**

**Measurements at Rest**

Body weight, hematocrit, and hemoglobin concentration did not differ between HA and SL exposure groups at 1 mo or 2 yr after return to SL (Table 1). Compared with the corresponding SL controls, circulating blood volume was higher in the HA group at both time points due to a larger erythrocyte volume; plasma volume was also higher, reaching statistical significance at 2 yr (Fig. 2). The passive EELV was higher in HA-raised animals 1 mo after return to SL but were no longer different 2 yr later (Table 1). Static lung volume was similar between groups at transpulmonary pressures below 30 cmH₂O, but the maximum static lung volume was significantly higher in HA-raised animals at both 1 mo and 2 yr after return to SL (Fig. 3). Resting cardiac output, DLCO, DMCO, Vc, DLNO, and alveolar sepal volume were significantly higher in the HA-raised group at both time points (Table 1).

**Measurements From HRCT**

Air volume of the lung per kilogram body weight declined similarly during the 2-yr interval after return to SL and did not differ between groups. Lung tissue volume per kilogram body weight did not change during the 2-yr interval and was significantly higher in the HA group at both time points (Fig. 4).

**Measurements During Exercise**

At the peak exercise workload, O₂ uptake, CO₂ output, minute ventilation, hematocrit, hemoglobin concentration, heart rate, respiratory rate, and rectal temperature were not different between HA and SL groups (Table 2). End-expiratory and end-inspiratory lung volumes and sepal volume were significantly higher in HA-raised animals, whereas the mean PₐO₂ was similar. Absolute DLCO, DMCO, and Vc measured at peak exercise were not significantly different between groups; however, when expressed at any given cardiac output, DLCO, DMCO, and Vc were significantly higher in HA-raised animals than in SL controls (Fig. 5).
**DISCUSSION**

**Summary of Results**

Our study provides the first longitudinal data to characterize the progression of HA-induced adaptation in pulmonary gas exchange during exercise. Compared with matched controls raised at SL, growing dogs exposed to HA (3,800 m) for 5 mo and then returned to SL before somatic maturation demonstrate significantly higher lung volumes, septal volume, lung tissue volume estimated from CT scan, and circulating erythrocyte and blood volumes. At a given cardiac output, lung diffusing capacity was significantly higher due to increases in both membrane diffusing capacity and pulmonary capillary blood volume. The functional enhancement persists even when measured 1–2 yr after return to SL. Somatic growth was not adversely affected by this severity of HA exposure. Thus a relatively short period of HA exposure during somatic maturation significantly improves long-term lung function in the adult animal. These findings of functional improvement suggest the development of structural adaptation, which may be permanent.

**Critique of the Methods**

As already mentioned, in vivo lung tissue volume estimated from HRCT density gradient includes the tissue and blood of gas-exchange units as well as small extraseptal conducting structures. On the other hand, the tissue volume estimated from acetylene uptake includes only the tissue and blood that participate in gas exchange. Hence, these two quantities are not the same; the HRCT tissue volume is systematically larger than...
either the acetylene tissue volume or the gold standard of alveolar septal tissue volume estimated by morphometry, although there are strong correlations between HRCT estimates and those by the other methods (present data and Ref. 28). One potential advantage of HRCT is the ability to noninvasively monitor longitudinal and regional changes during growth. Future studies should examine whether the precision of HRCT estimates might be improved by repeat scanning before and after the injection of an intravenous contrast agent to obtain blood-free lung tissue volume.

**Previous Literature on Chronic HA Exposure**

**Small animals.** Rodent lungs respond vigorously to hypoxia. Bartlett and Remmers (2) reported in young rats exposed to simulated 4,200-m altitude [inspired $O_2$ tension ($P_{O_2}$) = 95 Torr] an initial period of mild pulmonary edema followed by accelerated alveolar development over 3 wk, resulting in increased lung volumes and alveolar surface areas than in control normoxic animals. Burri and Weibel (4, 5) noted in growing rats that ambient hypoxia ($P_{O_2}$ = 100 Torr) over 3 wk increased lung volume and morphometric estimates of $O_2$ diffusing capacity, whereas ambient hyperoxia ($P_{O_2}$ = 290 Torr) decreased these indexes compared with control normoxic animals. Later, Sekhon and Thurlbeck (36, 37) reported that severe normobaric or hypobaric hypoxia ($P_{O_2}$ = 80 Torr) increased lung weight as well as RNA, DNA, and protein contents in growing rats independent of nutrition status or change in body weight, whereas exposure to hypobaric normoxia had a comparatively minor effect.

Guinea pigs show more mature lung development at birth than rats but adapt equally vigorously to HA exposure. In weanling guinea pigs, Lechner and Banchero (24) observed rapid increases in lung volume and alveolar surface area within 3 wk of exposure to severe simulated HA (5,100 m, $P_{O_2}$ = 80 Torr). However, the rate of increase in body weight and lung indexes progressively slowed with exposure duration up to 14 wk, suggesting that whereas severe HA exposure accelerates lung development it may actually stunt overall growth, thus limiting final lung dimensions at maturity. Extending HA exposure even longer at a more tolerable altitude that does not blunt somatic growth, Hsia et al. (18) raised separate cohorts of weanling guinea pigs at 3,800 m ($P_{O_2}$ = 100 Torr) for 1, 3, and 6 mo compared with matched controls simultaneously raised at a lower altitude (1,200 m, inspired $P_{O_2}$ = 130 Torr). In HA animals, lung volume and alveolar surface area were significantly elevated at all time points. Alveolar-capillary blood volume was initially higher after 1 mo at HA but normalized by 3 and 6 mo. Prolonged (3 and 6 mo) HA exposure was associated with larger alveolar septal tissue volume and surface areas, which were further augmented by smaller alveolar duct volume and smaller mean harmonic diffusion-barrier thickness. These structural changes induce a higher oxygen diffusing capacity estimated by a morphometric method, and they suggest a progressive response to chronic HA exposure in actively growing animals where cellular activities (proliferation and/or hypertrophy) and generation of additional alveolar surfaces occur early, whereas acinar and septal remodeling becomes evident later.

**Table 2. Rebreathing data at heavy exercise**

<table>
<thead>
<tr>
<th></th>
<th>SL Exposure Group</th>
<th>HA Exposure Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>$26.5 \pm 0.5$</td>
<td>$25.6 \pm 0.3$</td>
</tr>
<tr>
<td>Ventilation, l/min $^{-1} \cdot$ kg $^{-1}$</td>
<td>$7.0 \pm 0.2$</td>
<td>$7.8 \pm 0.3$</td>
</tr>
<tr>
<td>Oxygen uptake, ml/min $^{-1} \cdot$ kg $^{-1}$</td>
<td>$137.7 \pm 8.6$</td>
<td>$139.6 \pm 8.5$</td>
</tr>
<tr>
<td>CO$_2$ output, ml/min $^{-1} \cdot$ kg $^{-1}$</td>
<td>$127.9 \pm 14.6$</td>
<td>$130.8 \pm 9.6$</td>
</tr>
<tr>
<td>Tidal volume, ml/kg $^{-1}$</td>
<td>$49.1 \pm 2.5$</td>
<td>$56.3 \pm 2.8$</td>
</tr>
<tr>
<td>Rectal temperature, °C</td>
<td>$40.8 \pm 0.1$</td>
<td>$40.3 \pm 0.2$</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>$52.0 \pm 0.4$</td>
<td>$51.1 \pm 1.3$</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>$18.1 \pm 0.6$</td>
<td>$17.3 \pm 0.7$</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>$286 \pm 9$</td>
<td>$288 \pm 7$</td>
</tr>
<tr>
<td>End-expiratory lung volume, ml/kg</td>
<td>$63 \pm 2$</td>
<td>$70 \pm 2^*$</td>
</tr>
<tr>
<td>End-inspiratory lung volume, ml/kg</td>
<td>$128 \pm 4$</td>
<td>$158 \pm 9^*$</td>
</tr>
<tr>
<td>Mean $P_{O_2}$ during rebreathing, Torr</td>
<td>$110 \pm 2$</td>
<td>$112 \pm 2$</td>
</tr>
<tr>
<td>Cardiac output, ml/min $^{-1} \cdot$ kg $^{-1}$</td>
<td>$77 \pm 45$</td>
<td>$685 \pm 62$</td>
</tr>
<tr>
<td>$DL_{CO}$, ml/min $^{-1} \cdot$ Torr $^{-1} \cdot$ kg $^{-1}$</td>
<td>$1.48 \pm 0.10$</td>
<td>$1.76 \pm 0.09$</td>
</tr>
<tr>
<td>$D_{MCO}$, ml/min $^{-1} \cdot$ Torr $^{-1} \cdot$ kg $^{-1}$</td>
<td>$2.42 \pm 0.16$</td>
<td>$3.16 \pm 0.48$</td>
</tr>
<tr>
<td>$V_c$, ml/kg</td>
<td>$8.96 \pm 0.99$</td>
<td>$10.74 \pm 0.49$</td>
</tr>
<tr>
<td>Septal volume, ml/kg</td>
<td>$19.78 \pm 1.82$</td>
<td>$30.29 \pm 3.07^*$</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 HA vs. SL. $DL_{CO}$ is expressed at a standard $P_{O_2}$ of 120 Torr and hemoglobin concentration of 14.6 g/dl.

**Fig. 4. Lung air and tissue volumes estimated from high-resolution computed tomography scan at 1 mo and 2 yr after return to SL.** Air volume was not significantly different (NS) between groups. Lung tissue volume was significantly higher in HA-raised animals at both time points. Values are means ± SE. P values indicate HA vs. SL by paired t-test.
Collectively, these data from small animals show that, as long as the capacity for normal somatic and thoracic growth is preserved, chronic exposure to moderate HA during maturation enhances the structural capacity of the growing lung and improves long-term alveolar function. However, these benefits are probably lost at extreme HA where thoracic growth becomes blunted and the size of the lung is restricted by the size of the thorax.

Large animals. As mentioned in the introduction, young beagles raised from 2.5 to 16 mo of age at moderate HA (3,100 m) show elevated lung volume and resting DLCO measured physiologically as well as elevated lung tissue volume and alveolar surface area measured postmortem compared with control animals raised at SL (21, 22). In contrast, adult beagles residing at 3,100-m altitude for 3 yr showed no increase in lung volume, alveolar surface area, or resting lung function compared with corresponding adult control animals kept at SL (21, 22). Thus HA exposure readily elicits adaptation in actively growing lungs, whereas the same signals are insufficient for eliciting adaptation in adult lungs. The maturity-dependent difference in adaptive intensity mirrors that seen after pneumonectomy (16, 17, 39) and illustrates the susceptibility of active pathways of lung development to experimental manipulation, whereas quiescent pathways in the adult animal are noticeably less plastic. The maturity-dependent responses also highlight reciprocal mechanical thorax-lung interactions governing the intensity and upper limit of lung growth (1). Before epiphyseal union, reciprocal mechanical forces acting on the lung and thorax dictate their more or less matched growth rate to accommodate the growing lung without distorting normal anatomic relationships. After epiphyseal union, thoracic dimensions are fixed and mechanical forces on the lung and the thorax diminish. Additional space for alveolar growth is provided mainly by depression of the diaphragm, but this mechanism incurs a loss of mechanical advantage and could compromise diaphragm function. Within a confined thoracic space, additional alveolar growth would cause septal crowding, which in turn may impair gas-exchange efficiency. Accordingly, compensatory growth of alveolar tissue in adult lungs is elicited only when the adaptive signals are very intense and advantageous only when space is available to accommodate a larger lung (as after right pneumonectomy).

Reversibility of HA-Induced Adaptation

The present study in foxhounds differs from that by Johnson et al. (22) in beagles in several respects. 1) The present animals were exposed to a higher though easily tolerated altitude (3,800 m) that does not adversely affect somatic growth. 2) The present animals returned to SL before reaching somatic maturity. We reasoned that if actively growing lungs adapt vigorously to HA exposure, they should also adapt vigorously in the opposite direction on withdrawal of the HA stimulus. The previous finding of persistent HA-induced adaptation by Johnson et al. could have been related to the prolonged HA exposure; their animals did not return to SL until well after somatic maturity when adaptive potential has greatly diminished. 3) We assessed lung function at rest as well as during exercise, and physical fitness was standardized by the use of a training program, whereas in the study by Johnson et al. the animals were untrained and studied only at rest.
Many of the physiological changes induced by HA residence, including polycythemia, compartmental fluid shift, hyperventilation, reduced maximal cardiac output (42), pulmonary arterial hypertension, and pulmonary vascular hyperreactivity (12), are reversible within a few weeks after return to SL, whereas structural changes such as increased lung tissue volume and alveolar-capillary surface area are considered irreversible (21). We found that after 5 mo of HA residence, minute ventilation and hematocrit normalized and cardiac output showed a rebound increase as expected 1 mo after return to SL. However, blood volume and its components (erythrocyte and plasma volumes) as well as DLCO and its components (DmCO and Vc) were significantly elevated 1 mo after return to SL and remained elevated up to 2 yr later (Tables 1 and 2, Figs. 2 and 5).

Two factors may be responsible for the unexpected persistent elevation of blood volumes. First, exercise training at SL increases resting circulating blood volume by 13–16% in dogs and human subjects (26, 38). Exercise training and HA exposure are known to synergistically augment hemoglobin mass and blood volume in human athletes (25, 35). Although we did not institute exercise training during HA exposure, training, after return to SL may have contributed to the long-term maintenance of a higher erythrocyte volume in animals exposed to HA. We did not study untrained animals after HA exposure. Direct comparison of trained and untrained groups will be needed to quantify the extent to which exercise training contributes to the maintenance of HA-induced adaptation.

Second, the spleen functions as a major reservoir of erythrocytes in athletic species to augment hematocrit and blood volume by contracting in response to sympathetic stimulation (41). It is possible that polycythemia induced by chronic HA residence permanently enlarged splenic structural capacity for erythrocyte storage even after animals returned to SL. Exposure to severe HA has been associated with a higher spleen-to-body weight ratio in rats (20, 27, 40); the reversibility of this response has not been examined. In the present animals, average spleen wet weight, obtained postmortem after clamping the splenic vessels, was slightly but not significantly higher in the HA animals compared with SL controls [13.5 ± 2.5 and 8.6 ± 1.5 (SE) g/kg, respectively; P > 0.05]; spleen dry weight was also slightly but not significantly higher (4.1 ± 0.8 and 2.7 ± 0.5 g/kg for HA and SL groups, respectively; P > 0.05). Although these data do not unequivocally support structural enlargement of the splenic reservoir, splenic volume is highly variable, and further examination of in vivo splenic volume and splenic histology will be needed to adequately address this issue. Because erythrocytes are the ultimate sink for diffusive alveolar oxygen uptake, the persistently higher circulating blood and erythrocyte volumes after HA exposure contribute at least partly to the observed improvement in DLCO, DmCO, and Vc as well as gas-exchange efficiency during exercise.

In addition to the hematologic adaptation, the expected gain in alveolar tissue volume and surface area due to accelerated lung growth and/or remodeling in HA-raised animals (18, 22) may not regress after return to SL, thereby contributing to long-term enhancement of lung diffusing capacity. We are currently examining the histology of lungs from these animals to determine the extent to which alveolar structural or ultrastructural changes were responsible for the observed enhancement in gas exchange.

We conclude that, in actively growing animals, a relatively short period (5 mo) of HA residence that is discontinued before somatic maturation is sufficient to enhance long-term pulmonary gas exchange at SL after animals reach adulthood. The functional enhancement could have resulted from a combination of persistently elevated blood volume that is maintained by exercise training after return to SL and/or by permanent HA-induced structural lung growth or remodeling. Further studies will be needed to clarify the interaction between exercise training and HA exposure to determine whether exercise training augments ongoing adaptation to HA in growing animals or induces adaptation to HA in adult animals.

ACKNOWLEDGMENTS

The authors thank Dr. Frank L. Powell, Dr. Phil Richter, and the staff of the University of California White Mountain Research Station for assistance in animal care and transport that made this study possible. We also thank Richard Hogg, Deborah Hogg, Daryn Clyburn, Myresa Hurst, and Jennifer Fehmuel for technical assistance as well as the staff of the Animal Resources Center at University of Texas Southwestern Medical Center for veterinary assistance.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grants R01 HL-54060, HL-40070, HL-45716, and HL-62873.

DISCLOSURES

The contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of the National Heart, Lung, and Blood Institute or of the National Institutes of Health.

REFERENCES


