Short-term hypoxic exposure at rest and during exercise reduces lung water in healthy humans

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Hypoxia and exercise increase pulmonary arterial pressure, cause pulmonary capillary recruitment, and may influence the ability of the lungs to regulate fluid. To examine the influence of hypoxia, alone and combined with exercise, on lung fluid balance, we studied 25 healthy subjects after 17-h exposure to 12.5% inspired oxygen (barometric pressure = 732 mmHg) and sequentially after exercise to exhaustion on a cycle ergometer with 12.5% inspired oxygen. We also studied subjects after a rapid saline infusion (30 ml/kg over 15 min) to demonstrate the sensitivity of our techniques to detect changes in lung water. Pulmonary capillary blood volume (Vc) and alveolar-capillary conductance (Dm) were determined by measuring the diffusing capacity of the lungs for carbon monoxide and nitric oxide. Lung tissue volume and density were assessed using computed tomodraphy. Lung water was estimated by subtracting measures of Vc from computed tomography lung tissue volume. Pulmonary function [forced vital capacity (FVC), forced expiratory volume after 1 s (FEV1), and forced expiratory flow at 50% of vital capacity (FEF50)] was also assessed. Saline infusion caused an increase in Vc (42%), tissue volume (9%), and lung water (11%), and a decrease in Dm (11%) and pulmonary function (FVC = −12 ± 9%, FEV1 = −17 ± 10%, FEF50 = −20 ± 13%). Hypoxia and hypoxic exercise resulted in increases in Vc (43 ± 19 and 51 ± 16%), Dm (7 ± 4 and 19 ± 6%), and pulmonary function (FVC = 9 ± 6 and 4 ± 3%, FEV1 = 5 ± 2 and 4 ± 3%, FEF50 = 4 ± 2 and 12 ± 5%) and decreases in lung density and lung water (−84 ± 24 and −103 ± 20 ml vs. baseline). These data suggest that 17 h of hypoxic exposure at rest or with exercise resulted in a decrease in lung water in healthy humans.

high-altitude pulmonary edema; computed tomodraphy

EXPOSURE TO LOW OXYGEN CAUSES pulmonary vasoconstriction and a rise in pulmonary arterial pressure and challenges the ability of the lungs to regulate fluid. While there is abundant evidence that hypobaric hypoxia may initiate processes associated with interstitial edema, possibly leading to lung fluid accumulation in select individuals, there is less evidence supporting the role for normobaric hypoxia in lung fluid accumulation (8, 32). In an important study in sheep, Levine et al. (32) showed that, while hypobaric hypoxia caused marked increases in lymph flow (an indicator of lung fluid changes), normobaric hypoxia did not stimulate lymph flow to the same degree, suggesting a pressure change is necessary for lung fluid changes to occur. Additional studies have suggested that short-term exposure to normobaric hypoxia has no influence on lung wet weight or may actually reduce lung water in healthy animals (1, 2, 50).

Definitive assessment of changes in lung water in humans is difficult. Previous studies have used computed tomography (CT) measures of lung tissue volume or density, while other studies have implied changes based on static lung volume measurements, closing volume, airway resistance, lung impedance, or measures of the diffusing capacity of the lungs for carbon monoxide (DLCO) and the components of DLCO, pulmonary capillary blood volume (Vc) and alveolar-capillary conductance (DM) (13, 14, 36, 43, 55, 62). Measures of CT, however, are dependent not only on changes in lung water but also on changes in thoracic blood volume. The DlCO can be influenced by changes in alveolar volume and cardiac output (Q). Additionally, changes in DM track changes in Vc, although it is likely that correcting DM for Vc (DM/Vc) should accurately estimate changes in DM and, therefore, lung water. Finally, increases or decreases in forced vital capacity (FVC) and maximal expiratory flow rates should follow changes in either thoracic blood volume or lung water.

Typically, exposure to hypoxia causes an increase in DLCO, which is caused, at least in part, by an increase in Vc (12, 13, 19, 20, 22, 62, 64). The increase in Vc with hypoxia is a result of elevations in pulmonary pressure caused from heterogenous pulmonary vasoconstriction (64, 65). The elevation in pulmonary arterial pressure has been hypothesized to play a role in the development of high-altitude pulmonary edema (HAPE) (4, 18). However, previous work has demonstrated that prolonged increases in pulmonary pressures alone may not be an adequate stimulus to induce HAPE (24, 53). Recent work by Hopkins et al. (25) has found that subjects with more heterogeneous capillary recruitment in response to hypoxia may have an increased susceptibility to pulmonary edema. These studies suggest that the heterogeneity of the response of the pulmonary capillaries to hypoxia, rather than simply elevations in pulmonary pressures, may determine the susceptibility to lung fluid accumulation.

Exercise causes an increase in Vc as a function of capillary recruitment from an increase in Q. This increase in Q also results in a rise in pulmonary arterial pressure (42). Exercise in conjunction with hypoxic exposure has been shown to cause a decrease in arterial oxygen saturation (Sao2), increases in pulmonary arterial pressure, and alterations in lung fluid balance (5, 17). Furthermore, exercise at altitude is a risk factor for the development of HAPE.

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In the present study, we sought to determine the influence of exposure to normobaric hypoxia (17 h) and normobaric hypoxic exercise (to exhaustion) on lung fluid balance in healthy humans. Changes in lung fluid were detected by measures of DLCO and the components of DLCO, Dm, and Vc, as well as CT imaging and spirometry. In addition to the hypoxic exposure, all subjects were exposed to a rapid intravenous saline infusion (which has been shown to cause mild interstitial edema in humans) to assess the sensitivity of our techniques (31). We hypothesized that hypoxic exposure and exercise with hypoxia would lead to changes in our indexes of lung water, consistent with interstitial edema, particularly with exercise.

**METHODS**

**Subjects**

The protocol was reviewed and approved by the Mayo Clinic Institutional Review Board. All participants signed informed consent before study, and all portions of the study were performed according to the Declaration of Helsinki. Twenty-five healthy nonsmokers agreed to participate in the study, had no exclusion criteria (cardiopulmonary abnormalities, pregnancy, inability to exercise), and were not taking prescription medications for cardiovascular or lung disease.

**Protocol**

The study involved three separate visits to our laboratory, including 1) a screening visit, 2) a rapid saline infusion visit, and 3) a hypoxic exposure visit, which included hypoxic exercise immediately following the 17-h resting hypoxic exposure (see Fig. 1 for a timeline of the study visits and measurements obtained). The screening tests included baseline pulmonary function testing, an incremental cycle ergometry test to exhaustion (to rule out cardiopulmonary abnormalities), a complete blood count (to rule out anemia), and, in women, a pregnancy test.

**Saline infusion.** A rapid intravenous saline infusion was used to demonstrate the sensitivity of our methods for assessing changes in lung water, because this technique has previously been shown to cause mild interstitial pulmonary edema (31). The subjects were required to report to our laboratory in a fasting state, and all saline infusions were performed between 0700 and 0900. An 18-gauge venous catheter was inserted into a prominent antecubital vein for the rapid saline infusion and for blood sampling. Baseline measures of Vc, Dm, CT-based lung density and tissue volume, and spirometry were obtained before the infusion. Saline was then infused intravenously (30 ml/kg over 15 min) while the subject was supine on the CT bed to optimize timing for postsaline CT measures. During the saline infusion, SaO2, blood pressure (BP), heart rate (HR), and physical symptoms were continuously monitored and recorded every 2 min. Catecholamines [adrenaline (ADR) and noradrenaline (NA)] were sampled pre- and immediately postsaline from the venous catheter. Following the infusion, repeat assessments of Vc, Dm, lung density, tissue volume, and spirometry were performed.

**Hypoxia.** At least 48 h following, but within 4 days of, the rapid saline infusion visit, subjects checked into our laboratory at 1300 for the resting hypoxic exposure visit. Prehypoxia measures of Vc, Dm, and spirometry were performed, and blood samples were drawn for the assessment of catecholamines along with a complete blood count. On this visit, a normoxic echocardiogram was also performed for the assessment of pulmonary arterial systolic pressure. Upon completion of the baseline room air tests, subjects entered a hypoxic tent at 12.5% O2 (Colorado Altitude Training, Boulder, CO) and remained in the tent for 17 h overnight. While in the tent, the subject’s HR and SaO2 were monitored continuously by pulse oximetry (Nellcor, Pleasanton, CA). BP, symptoms [modified Lake Louise scale (54)], and tent gases were monitored every 2 h by a nurse in the Mayo Clinic General Clinical Research Center (GCRC). Following completion of the overnight stay in the hypoxic tent (at 0700), bloods were again drawn, and a repeat echocardiogram was performed. Subjects were then connected to a mask attached to a large gas reservoir, which was connected to a portable gas tank of 12.5% O2 gas, which allowed the subjects to maintain hypoxia while in transit to the CT scanner and physiological core laboratory. The subjects then performed repeat measures of Vc, Dm, lung density, and tissue volume, as well as spirometry while maintaining 12.5% FiO2 on the CT scanner and in the laboratory.

**Hypoxic exercise.** Immediately following the measures after the overnight hypoxic exposure, and without exposure to normoxia, the subjects performed 15 min of hypoxic exercise at a workload that was initially equal to 40% of their maximal wattage performed during normoxia. If, after 5 min of exercise, the subject reported a perceived exertion rating ≤12 [Borg 6–20 rating of perceived exertion scale (RPE)], the work was increased to 50% of their normoxic maximum (10). Workload was kept constant for the next 5 min if RPE > 12. The subjects’ RPE was again assessed after 10 min of exercise, and the workload was increased for the final 5 min if the subject was ≤15 on the RPE scale but kept constant otherwise. During exercise, HR, BP,
S\textsubscript{a}O\textsubscript{2}, and symptoms were continuously monitored. Following exercise, subjects were asked to rest for 5 min (while maintaining an FIO\textsubscript{2} of 12.5%) before assessment of V\textsubscript{c}, D\textsubscript{m}, lung density and tissue volume, and spirometry.

Data collection. CATECHOLAMINES. ADR and NA were assessed according to methods developed in the Mayo Clinic GCRC immunochemical core laboratory and the methods of Sealey (56). Our laboratory intra-assay coefficients of variation are as follows: NA 4.5% and 3.3% at 224 and 429 pg/ml and ADR 12.2% and 3.6% at 13.8 and 242 pg/ml, respectively. Interassay coefficients of variation are as follows: NA 8.2% and 6.3% at 337 and 533 pg/ml and ADR 8.5% and 6.3% at 179 and 390 pg/ml, respectively.

PULMONARY VASCULAR Pressures. Pulmonary arterial pressure was calculated from the tricuspid regurgitation (TR) velocity as described previously (66), using the equation $\Delta P = \Delta v^2$, where $P$ is the pressure and $v$ is the TR velocity. The same sonographer was used for pre- and postmeasures. Venous injections of agitated saline were used as needed to enhance the signal for TR velocity. Color Doppler echocardiography was used to locate the TR jet. The maximal velocity was determined by careful application of the continuous wave sampler within and parallel to the regurgitation jet. The right atrial pressure was added to the trans-tricuspid pressure gradient as a measure of peak right ventricular systolic pressure, which is equivalent to pulmonary artery systolic pressure in the absence of pulmonary stenosis. Right atrial pressure was estimated based on an algorithm that utilizes the caliber of the inferior vena cava, the magnitude of inferior vena cava collapse with inspiration, the ratio of systolic to diastolic hepatic vein velocity measured by pulsed wave Doppler, and the peak velocity of retrograde hepatic vein flow (41, 44).

MEASURES of VC AND D\textsubscript{m}. Measurement of V\textsubscript{c} and D\textsubscript{m} has previously required the use of at least two, but preferably three, oxygen tensions. Recently, Tamhane et al. (61) have found that measuring the disappearance of nitric oxide (NO) in concert with carbon monoxide (CO) provides an accurate assessment of V\textsubscript{c} and D\textsubscript{m} using just one oxygen tension. Triplicate maneuvers of the DL\textsubscript{CO} and diffusing capacity of the lungs for nitric oxide (DL\textsubscript{NO}) were performed presaline, postsaline, prehypoxia, posthypoxia, and posthypoxic exercise.

DL\textsubscript{CO} and DL\textsubscript{NO} were assessed upright using the rebreathe technique, with gases sampled on a mass spectrometer (Perkin-Elmer, 1100) and NO analyzer (Sievers Instruments, Boulder, CO) using custom analysis software. A rebreathe bag was filled with 0.3% CO (C\textsuperscript{18}O), 40 parts per million (ppm) NO (diluted immediately before the test in the bag from an 800-ppm gas mixture), and O\textsubscript{2} (35% for the test gas, instead of the more common C\textsubscript{18}O as the test gas, because the mass molecular weights). Although a 5-liter rebreathe bag was used, the desired volume (28). The switching circuit and tank were checked before each test for accurate volumes. At the end of a normal expiration [end-expiratory lung volume (EEV)], the subjects were switched into the rebreathe bag and instructed to nearly empty the bag with each breath for 10 consecutive breaths. The respiratory rate during the rebreathe maneuver was controlled at 32 breaths/min with a metronome. For the prehypoxia measures, the subject’s alveolar O\textsubscript{2} was allowed to equilibrate to the low O\textsubscript{2} used in the test gas mixture by allowing the subjects to breathe the low O\textsubscript{2} for ~10 breaths and then immediately switching them into the rebreathe bag containing the test gas mixture, allowing us to more accurately compare pre- and posthypoxia measures. Following each diffusing capacity maneuver, the rebreathe bag was emptied with a suction device and refilled immediately before the next maneuver. For our laboratory, the coefficients of variation are 4.1% for the DL\textsubscript{CO} measures and 8.3% for the DL\textsubscript{NO} measures.

SPIROMETRY. The maximal expiratory flow-volume (MEFV) data were obtained on the same mouthpiece as the lung-diffusing capacity measures (Hans Rudolph, Kansas City, MO), as previously described (6, 29). All measures were performed in a consistent fashion, where subjects were asked to breathe quietly for six to eight breaths followed by a slow maximal inspiration to total lung capacity (TLC) and a subsequent maximal expiration to residual volume. This system was calibrated before each test using a 3-liter syringe (Hans Rudolph). Flow and volume signals were digitized at a rate of 100 samples/s and stored for later analysis. From the MEFV maneuver, FVC, forced expiratory volume after 1 s (FEV\textsubscript{1}), and maximal expiratory flow after 50% of the expired vital capacity (FEF\textsubscript{50}) were determined.

MEASURES of Q and HR. Q was assessed using a previously validated 8- to 10-breath acetylene rebreathe technique using the same anesthesia bag containing the diffusion mixtures with the addition of 0.7% C\textsubscript{18}H\textsubscript{2} and 9% He (7, 27). Gases were sampled using a mass spectrometer, which was integrated with custom analysis software for the assessment of Q. During each test, HR was assessed using either a pulse oximeter (for the saline infusion visit) or 12-lead ECG (for the hypoxia and hypoxic exercise visits).

MEASURES of CT-BASED LUNG TISSUE VOLUME and DENSITY. All CT scans were performed on the same scanner (GE LiteSpeed spiral CT scanner, GE Healthcare). Initial slices were obtained for all scans, which were 2.5 mm thick with a 1.2-mm overlap and then reconstructed to 1.25 mm with a 0.6-mm overlap. On the first visit, a scout scan was performed to determine the location and size of the lungs. Marks were placed on the skin of the subject to indicate the anatomical location of the start of the scan and the table height and field of view, and number of images obtained were recorded to ensure consistency with postscans.

Control of lung volumes during scanning. To control for the possible changes in lung volumes between baseline and our interventions, subjects were asked to breathe on a mouthpiece connected to a pneumotachometer, which was integrated with a portable computer with custom analysis software during all CT scans. This strategy provided exact lung volumes during the scans for accurate pre- and postintervention comparisons (49). During the scans, the investigators observed the breathing pattern of each subject and instructed him or her to the desired lung volume for the breath hold during scanning. The portable pneumotachometer was calibrated using a 3-liter syringe before each CT visit.

Normalization of lung volumes for pre- and postcomparisons. On the initial visit (baseline), subjects were asked to hold their breath at two lung volumes for two separate baseline scans: TLC and EELV (referred to as TLC via inspiratory capacity maneuver). All subsequent scans were performed at end-inspiratory lung volume. To obtain consistent lung density data while controlling for lung volume, the image data obtained postsaline, posthypoxia, and posthypoxic exercise were compared with values obtained by interpolating between the images obtained at TLC and EELV during baseline scanning (49). The use of a portable pneumotachometer with investigator-controlled breath holds for scanning and the interpolation of baseline scans for comparison to postintervention scans were performed to ensure accurate comparisons, because it is impossible to exactly match baseline and postintervention lung volumes. This strategy also minimized the number of scans and amount of radiation exposure to the subjects.

Lung density and tissue volume analysis using CT. The scan images were submitted to analysis using image analysis software [Pulmonary Analysis Software Suite, Physiological Imaging Laboratory, University of Iowa, Iowa City, IA]. The Pulmonary Analysis Software Suite software first segmented the images to separate lung tissue from surrounding structures and the mediastinum for analysis of parenchymal attenuation (23). In each picture element, lung density was assumed to be a linear combination of air, with an attenuation of $-1,000$ Hounsfield units (HU), and lung tissue, which has an attenuation of 0 HU. A histogram analysis of picture elements within the
lung tissue was performed to obtain mean lung density in HU and tissue volume by summation of each voxel among all elements in the lung fields. Lung tissue volume and lung density data obtained from baseline images were plotted against lung volume to be used for linear interpolation to obtain data against which other images were compared (see Fig. 4 for a graphic representation of breath-hold strategy).

Analysis of vessel area using CT. To obtain vessel area at baseline, postsaline, posthypoxia, and posthypoxic exercise, the CT scans were reconstructed with slices of 1.25 mm with 0.6-mm overlap to allow for adequate resolution to view anatomical markers and analyze the vessels. Semiautomated analysis of selected pulmonary vessels was performed using custom software (VIDA, Physiological Imaging Laboratory, University of Iowa, Iowa City, IA). Initially, three vessels were selected on the baseline scan in two separate locations (six vessels in total). To determine the location of the vessels selected in the baseline scans, the bifurcations of the airways and the larger vessels were used as anatomical guides to ensure accurate selection of the same vessels after saline, hypoxia, and hypoxic exercise. All vessels were analyzed by the same technician, who was blinded to the condition of the subjects.

Estimation of extravascular lung water. The parenchymal attenuation assessed by CT includes lung tissue, blood, and water. Saline infusion, hypoxia, and hypoxic exercise likely cause changes in lung blood volume and water volume but will minimally affect the volume of lung tissue. By combining our measures of tissue volume and Vc obtained from the lung density measuring tests, we were able to estimate changes in extravascular lung water from baseline to postsaline, posthypoxia, and posthypoxic exercise, using the following equation:

\[
\text{Lung Water} = (TV_{Pt} - Vc_{Pt}) - (TV_{P} - Vc_{P})
\]

where TV_{Pt} is the given tissue volume from the CT scan postintervention (in ml), TV_{P} is the tissue volume (in ml) from baseline CT scans, Vc is in milliliters at each time point, P is postintervention, and interpolated (IP) is baseline.

Data Analysis

All statistical comparisons were performed using the SPSS statistical software package (version 12.0, Chicago, IL). Changes in DLco, DM, Vc, pulmonary function, lung tissue volume, lung density, vessel diameter, and airway wall thickness were compared using a repeated-measures ANOVA with a Tukey post hoc honestly significant difference test to compare differences between conditions. An α of 0.05 was set to determine significance for the ANOVA and post hoc analyses. All data were found to have homogeneity of variance before the ANOVA using Levene’s test for equality of variance. All data are presented as mean ± SD, unless otherwise stated.

RESULTS

Subject Characteristics

As shown in Table 1, the subjects were primarily men, with an average age of 30 yr, range 21–44 yr, and an average peak O2 uptake of 101 ± 4% of predicted.

Table 1. Subject characteristics

| Age, yr | 30±8 |
| Gender, %female | 27 |
| Height, cm | 176±10 |
| Weight, kg | 79±14 |
| BMI, kg/m2 | 25±4 |
| VO2peak, ml/kg·min−1 | 38±8 |

Values are means ± SD; n = 25. BMI, body mass index; VO2peak, maximal oxygen consumption determined from cycle ergometry test.

Table 2. Physiological responses to saline infusion, 17-h hypoxia, and hypoxic exercise

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Fluid Challenge (Peak)</th>
<th>Hypoxia (Following 17 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>60±8</td>
<td>72±15*</td>
<td>76±11*</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td>4.3±1.1</td>
<td>5.4±1.4*</td>
<td>4.1±0.8</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>115±11</td>
<td>125±14*</td>
<td>119±13</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>79±8</td>
<td>79±11</td>
<td>71±3</td>
</tr>
<tr>
<td>O2 saturation, %</td>
<td>98±1.6</td>
<td>98±1</td>
<td>83±3*</td>
</tr>
<tr>
<td>Pulmonary artery pressures, mmHg</td>
<td>21±8</td>
<td>37±8*</td>
<td></td>
</tr>
<tr>
<td>Intervention exposure</td>
<td>2.287±522 ml</td>
<td>17±1 h</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 25. HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure. *P < 0.05 compared with baseline.

Catecholamines

Saline infusion resulted in a 267 ± 4% increase in venous ADR (range = −80–1,360%) and a 114 ± 25% increase in venous NA (range = −27–425%; mean ± SE). Hypoxia resulted in a minimal increase in venous ADR (34 ± 19%, range = −87–321%) and no change in venous NA (−5 ± 7%, range = −51–87%). Hypoxic exercise resulted in a 1,236 ± 297% increase in venous ADR (range = 93–7,300%) and a 924 ± 111% increase in venous NA (range = 22–2,267%).

Saline Infusion

With saline infusion, the subjects had increases in Q, HR, and systolic and diastolic pressures but had no changes in SaO2 (Table 2). The most common symptoms were shortness of breath and chest tightness (average 1.5 out of a possible 4 for each).

Saline infusion caused an increase in Vc (42 ± 18%) and a decrease in DM (−11 ± 2%, Table 3). Vc corrected for Q˙ was not different pre- to postsaline, suggesting that the increase in Vc during this intervention was primarily driven by blood volume and water volume but will minimally affect the volume of lung tissue.

Table 3. Changes in DLco, DM, Vc, and pulmonary function in response to saline infusion, 17-h hypoxia, and hypoxic exercise

<table>
<thead>
<tr>
<th></th>
<th>Presaline</th>
<th>Postsaline</th>
<th>Prehypoxia</th>
<th>Posthypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLco, ml/min·mmHg−1</td>
<td>26±5</td>
<td>25±7</td>
<td>28±5</td>
<td>30±7†</td>
</tr>
<tr>
<td>DM, ml/min·mmHg−1</td>
<td>35±10</td>
<td>31±10*</td>
<td>41±10</td>
<td>43±11</td>
</tr>
<tr>
<td>Vc, ml</td>
<td>79±37</td>
<td>104±79*</td>
<td>80±33</td>
<td>108±66†</td>
</tr>
<tr>
<td>FVC, liters</td>
<td>5.08±0.9</td>
<td>4.45±1.4*</td>
<td>4.81±1.0</td>
<td>5.20±0.9*</td>
</tr>
<tr>
<td>FEV1, l/s</td>
<td>4.00±0.7</td>
<td>3.34±0.6*</td>
<td>4.01±0.7</td>
<td>4.22±0.7†</td>
</tr>
<tr>
<td>FEF25, l/s</td>
<td>4.29±1.3</td>
<td>3.45±1.4*</td>
<td>4.38±1.1</td>
<td>4.69±1.4</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 25. DLco, diffusing capacity of the lungs for carbon monoxide; DM, alveolar-capillary conductance; Vc, pulmonary capillary blood volume; FVC, forced vital capacity; FEV1, forced expiratory volume at 1 s; FEF25, forced expiratory flow after 50% of the vital capacity has been expired. *P < 0.05 compared with presaline. †P < 0.05 compared with prehypoxia.
also able to calculate tissue volume changes from the apex to the base of the lung (Fig. 7); the majority of tissue volume changes in the lung with saline infusion were at the base of the lung.

Hypoxia at Rest

The subjects had a marked, sustained decrease in $S_aO_2$, with overnight exposure to hypoxia, as well as small decreases in $Q$, despite increases in HR (Table 2). During hypoxic exposure, subjects also had increases in systolic BP, a drop in diastolic BP, and an increase in pulmonary arterial systolic pressure.

Hypoxia resulted in increases in $Dl_{CO}$ (10 ± 2%), $Vc$ (35 ± 21%), and $D_M$ (6 ± 4%) ($P < 0.05$, Table 3). $Vc$ corrected for $Q$ was significantly higher posthypoxia compared with prehypoxia, suggesting that the increase in $Vc$ was proportionally larger than the increase in $Q$ (Fig. 2). The change in $D_M$ relative to $Vc$ increased with hypoxia, suggesting a loss of interstitial fluid (Fig. 3). Compared with baseline, there were improvements in pulmonary function following hypoxic exposure (FVC +9 ± 6%, $FEV_1 +5 ± 2$, $FEF_{50} +4 ± 2$, Table 3, $P < 0.05$). Exposure to 17 h of hypoxia caused decreases in lung density, tissue volume (despite an increase in $Vc$), and calculated lung water (Figs. 4–7).
Hypoxic Exercise

Hypoxic exercise resulted in increases in HR and systolic BP, and a slight drop in $S_aO_2$, compared with resting hypoxia. Following hypoxic exercise, there were further increases in $DLCO$ (17 ± 3%), $Vc$ (51 ± 18%), and $DM$ (19 ± 7%, compared with baseline, Table 3). $Vc$ corrected for $Q$ was significantly higher after hypoxic exercise, suggesting that the increase in $Vc$ was proportionally more than the increase in $Q$. $DM$ relative to $Vc$ increased further posthypoxic exercise, suggesting a further loss of interstitial fluid. Pulmonary function remained elevated following hypoxic exercise ($FVC +4 ± 3\%, \ FEV_1 +4 ± 3\%, \ FEF_50 +12 ± 5\%$). Hypoxic exercise also resulted in further decreases in lung density, tissue volume, and our estimates of lung water. Following resting hypoxia and hypoxic exercise, there was little decrease in the CT measures of tissue volume at the apex of the lung but a marked decrease in this measure at the base of the lung. This is possibly a result of a mild shift in lung blood volume toward the apex of the lung during the hypoxic challenges.

DISCUSSION

We found that exposure to 17 h of normobaric hypoxia ($FI_O_2 = 12.5\%$) caused increases in $DLCO$, $Vc$, $DM$, vital capacity, and maximal expiratory flow rates, and decreases in lung tissue volume, lung density, and estimated lung water. Hypoxic exercise to exhaustion resulted in further increases in $DLCO$, $Vc$, $DM$, and pulmonary function, and further decreases in lung tissue volume, lung density, and lung water. Importantly, hypoxia and hypoxic exercise resulted in increases in $DM$ relative to changes in $Vc$ ($DM/Vc$) following these conditions, suggesting an increase in $DM$ independent of an increase in $Vc$. These results suggest exposure to hypoxia for ~17 h and hypoxic exercise result in a loss of extravascular lung fluid in healthy individuals. While these findings are contrary to classical thinking with regard to lung fluid accumulation and normobaric hypoxia, which are largely based on assumptions from small studies on lung water with exposure to hypobaric hypoxia, they are consistent with previous work in animals, which has shown a loss of lung water following exposure to normobaric hypoxia (1, 2, 50).

Exposure to hypoxia caused a marked increase in pulmonary arterial pressure in the present study. Previous research has suggested that subjects who are susceptible to HAPE are likely to have more rapid and marked increases in pulmonary vascular pressure (33). It does appear, however, that this increase in pulmonary vascular pressure is not the sole factor that contributes to lung fluid accumulation, which is in agreement with previous research by Sartori et al. (53). Recently, Hopkins et al. (25) have demonstrated that subjects with a more heterogeneous pulmonary vasoconstriction with hypoxia are more likely to develop pulmonary edema at high altitude.

Water is dynamically regulated in the lung by passive and active forces between the capillaries, the interstitial space, and the alveoli and follow the laws described by Starling (57). When fluid leaks out of the capillaries, it goes into the interstitium of the alveolar wall and tracks through the interstitial space to the perivascular and peribronchial space within the lungs. Numerous lymphatics run in the perivascular spaces, and these help to transport the fluid to the hilar lymph nodes. The pressure in these perivascular spaces is low, thus forming a natural sump for the drainage of fluid. The earliest form of pulmonary edema (interstitial edema) is likely characterized by engorgement of the alveolar walls and the peribronchial and perivascular spaces. Although contrary to classical thinking, it is likely that fluid exchange also increases systematically near the alveolar space as well, even in healthy individuals (30). The rate of lymph flow from the lung increases considerably if the capillary hydrostatic pressure is increased. If excessive fluid crosses the alveolar epithelium into the alveolar spaces and the fluid increases at a rate that exceeds removal, the alveoli fill with fluid, compromising lung mechanics and gas exchange.

Active fluid removal from the alveoli is thought to be primarily driven by apical epithelial sodium channels (ENaC) on type II alveolar cells and basal sodium-potassium pumps driven by Na-K-ATPase (37). Of these two mechanisms, the rate-limiting step is thought to be the ENaC. Previous research...
has shown that increasing levels of cAMP lead to increases in the probability of an open ENaC or an increase in the number of ENaCs on the apical membrane of type II alveolar cells (47). Stimulation of the \( \beta_2 \)-adrenergic receptors (\( \beta_2 \)ARs) leads to increases in alveolar fluid clearance through a cAMP-dependent mechanism (40). Recent research in subjects with pseudohypoaldosteronism (a loss of function mutation of the ENaCs) provides evidence that water flows into the air spaces of the lungs, even in healthy humans under normal conditions, and that the ENaCs are important in maintaining fluid homeostasis in normal subjects (30).

Recently, several studies have suggested that hypoxia decreases the activity of the ENaCs on type II alveolar cells and alveolar fluid clearance. However, the level of hypoxia used in these studies is quite dramatic, ranging from 0 to 10%, averaging \(~8\%\) \( F_{\text{IO}_2} \) (63). Interestingly, a study by Suzuki et al. (59) demonstrated no difference in alveolar fluid clearance and an increase in the mRNA of ENaC with 15\% \( O_2 \) for 48 h or 10\% \( O_2 \) for 24 h. A study by Vivona et al. (63) showed a significant drop in alveolar liquid clearance with \( 8\% \) \( O_2 \) but also found an increase in the expression of the \( \alpha \)-subunit of the ENaC on type II alveolar cells and demonstrated a complete normalization of alveolar liquid clearance when hypoxia was coupled with the infusion of terbutaline.

Given these mechanisms of lung fluid regulation, the possible reasons for the decrease in lung fluid in the present study upon exposure to hypoxia could include the following: 1) decreased vascular hydrostatic pressure; 2) increased lymph flow, which would decrease the interstitial hydrostatic pressure; or 3) stimulation of the \( \beta_2 \)ARs, which would accelerate alveolar fluid clearance. Given the dramatic increases in pulmonary arterial pressure, it is not likely that there was a decrease in vascular hydrostatic pressure. Although not measured, there would likely have been an increase in perivascular lymph flow, which would lower the hydrostatic pressure of the interstitium. Levine et al. (32) have shown that lymph flow increased only \( 10\% \) with normobaric hypoxia in sheep, whereas Martin et al. (35) found that lymph flow was increased by \(~40\% \) in dogs when exposed to hypoxia. Increases in ventilation are thought to lead to an increase in lymph lymph flow (46). In the present study, we were able to monitor changes in ventilation for the entire night of hypoxic exposure using the LifeShirt monitoring system (Vivometrics, Ventura, CA). Although we found there was an increase in minute ventilation primarily due to an increase in tidal volume with hypoxic exposure, there was no relationship between the change in minute ventilation and change in lung fluid in these subjects (\( r^2 = 0.08, P > 0.05 \)). While it is possible that the increase in ventilation contributed to the loss of lung water, in general, it is not likely to be the primary mechanism for this reduction. Another possible mechanism that could have caused a loss of lung fluid following normobaric hypoxia is the activation of \( \beta_2 \)ARs on type II alveolar cells. On average, the subjects in the present study had a 34\% increase in venous ADR with exposure to normobaric hypoxia. It is possible, therefore, that the increase in ADR with exposure to hypoxia caused activation of the \( \beta_2 \)ARs increased in cAMP, and increased activity of the ENaCs. This would be supported with a recent study by Sartori et al. (52), who found that inhalation of Salmeterol decreased the likelihood of developing HAPE in climbers. It is likely that both an increase in lymph flow and activation of the ENaC contributed to the overall loss of lung water.

Based on previous research, the increase in \( V_c \) following exposure to hypoxia and hypoxic exercise was expected. The increase in \( V_c \) with hypoxic exposure is primarily caused by capillary recruitment, specifically at the apex of the lung, and is thought to be a result of the increase in capillary pressures caused by the pulmonary vasoconstriction, rather than by an increase in \( Q \) (13, 64, 65). The present study seems to confirm the hypothesis that the increase in \( V_c \) with exposure to hypoxia is not dependent on \( Q \), because \( V_c \) relative to \( Q \) (\( V_c/Q \)) increased with exposure to hypoxia and hypoxic exercise. On the other hand, \( V_c \) relative to \( Q \) was unchanged with rapid saline infusion, suggesting that the resulting increase in \( V_c \) was dependent on an increase in \( Q \).

Exercise during exposure to hypoxia is thought to exacerbate high-altitude illness and increase the chances for development of HAPE as a result of the dramatic increases in pulmonary pressures. Previous research has suggested that heavy exercise without hypoxic exposure may also lead to an increase in lung water (38); however, other studies have not supported this conclusion (16, 26, 34). A fall in the \( D_{LCO} \) has been used to describe an increase in lung water postexercise in normoxia and hypoxia; however, the loss in \( D_{LCO} \) postexercise appears to be due primarily to a reduction in \( V_c \), rather than \( D_M \) (26, 38). In fact, in a study by McKenzie et al. (39), the authors describe an increase in lung water after normoxic exercise in elite athletes using magnetic resonance imaging, but in this same study the subjects have a small increase in \( D_M \) and a dramatic reduction in \( V_c \), suggesting a small loss of interstitial lung water (improvement in the \( D_{M-V_c} \) ratio, i.e., an increase in \( D_M \), despite a decrease in the surface area for conductance). In the present study, all measures were performed within approximately 15 min of the completion of exercise (at a time when \( V_c \) remained elevated); therefore, it is unlikely that the reduction in lung density in the present study was due to a reduction in central blood volume. Other studies have used other imaging modalities (i.e., chest X-ray) in an attempt to describe an increase in lung water after hypoxic exercise, a technique that is less quantitative than CT scanning (3). The most obvious difference between previous studies and the present study is the use of relatively untrained individuals in our study, while the majority of the previous studies that have suggested lung fluid accumulation postexercise with normoxia and hypoxia have been performed using elite athletes, or in subjects who have HAPE. A study by Younes et al. (67) found that it takes approximately fourfold increases in \( Q \) to increase lung water, which provides evidence for the differences observed in elite athletes and relatively untrained individuals.

This is the first study to our knowledge to find that exposure to normobaric hypoxia causes decreases in lung fluid in healthy humans. This finding was unexpected, but is supported by previous work in animals. The findings in the present study are likely quite sound; however, because 1) we provide three independent measures that support the findings of fluid loss (a decrease in lung density and tissue volume from CT, an increase in \( D_M \), which was independent of changes in \( V_c \) and \( Q \), and an increase in vital capacity and maximal expiratory flow rates); and 2) we demonstrate that these measures are sensitive to a condition likely to cause mild interstitial edema, namely, rapid saline infusion.
Limitations

Although we show several independent parameters suggesting a loss of lung fluid following exposure to hypoxia and hypoxic exercise, our calculation of lung water is based on blood volume changes of the pulmonary capillaries. The calculation of Vc does not, however, include changes in vessels that are not in contact with the alveoli. For instance, a decrease in the volume of blood in these vessels would cause a drop in tissue volume and lung density using CT but would not be detected using our DLCO-derived measure.

To account for this possible mechanism that could result in a decreased calculation of lung water independent of true lung water changes, we assessed changes in the cross-sectional area of six conduit vessels before and following each intervention in each subject using CT. We found that saline infusion caused a small increase in average vessel area, whereas hypoxia resulted in no change in vessel area (normoxia = 24.3 ± 2.1 mm², saline = 27.8 ± 2.9 mm², hypoxia = 24.9 ± 1.8 mm², hypoxic exercise = 23.7 ± 1.9 mm², P > 0.05). Assuming the selected vessels represent the majority of the larger pulmonary vessels, the findings would indicate a nominal change in lung blood volume with exposure to hypoxia, suggesting that the resulting fall in tissue volume and density was not a result of a decrease in the blood volume of the conduit vessels of the lung. This is in agreement with previous studies that have suggested no change or a small increase in lung blood volume with hypoxic exposure (9, 21, 45, 48, 51). In addition, we found further decrements in our estimates of lung water following hypoxic exercise, at a time when one would expect the thoracic blood volume to be elevated. Interestingly, 5 of the 25 subjects studied did have radiographic and physiological evidence of mild lung fluid accumulation, possibly suggesting that there is a subgroup of subjects that may be HAPE-s (Fig. 8). To further support our findings, we analyzed changes in airway wall thickness in 12 of our 25 subjects, as described by King et al. (31). We found that rapid saline infusion resulted in a significant increase in airway wall thickness, whereas hypoxia and hypoxic exercise resulted in no change in airway wall thickness, possibly suggesting the greatest loss in lung water from the interstitial space, rather than from the pulmonary air space in response to hypoxia and hypoxic exercise (baseline = 1.62 ± 0.09, saline = 1.75 ± 0.15, hypoxia = 1.59 ± 0.14, hypoxic exercise = 1.62 ± 0.11; P > 0.05).

It is possible that the loss in lung water was a result of diuresis, induced by either recumbency or hypoxia, as described by Swenson et al. (60). We were able to calculate the change in plasma volume using hemoglobin and hematocrit, as described in detail previously by Dill and Costill (15). We found a nonsignificant reduction in plasma volume of 1.3%, which was not associated with the magnitude of lung water reduction. In the present study, the subjects consumed two meals, had a snack, and were encouraged by the GCRC staff to drink plenty of fluids. In addition, it is unlikely that the overnight itself (i.e., recumbency-induced diuresis) lead to a reduction in lung water as determined with CT, because the baseline CT scans and posthypoxia CT scans were taken at the same time of day (~0700).

Although the use of NO in concert with CO to determine DM and Vc is well accepted, some may argue that brief inhalation of NO may inherently alter Vc during the maneuver (because of the vasodilatory effects of this gas on the pulmonary circulation). This is highly unlikely, however, because studies that have used inhaled NO to reduce pulmonary hypertension typically use continuous breathing of at least 40-ppm NO for several minutes. In the present study, within the first breath, NO drops to <18 ppm as it dilutes in the existing volume of air in the lungs, and the amount drops off precipitously thereafter,
being negligible by the fourth to fifth breath. Moreover, Bolland et al. (11), and more recently Zavorsky and Murias (68), have specifically examined this concept and have found that inhalation of NO for over 1 min does not alter DM or Vc.

In the present study, we demonstrate a novel method for estimating changes in lung water by combining changes in Vc and DM. One possible limitation in combining these measures is that the assessment of Vc was made in the upright position, and the assessment of lung tissue volume was made in the supine position. Previous work by Stewart et al. (58), however, has suggested that body position does not play a major role in DLCO in healthy normal subjects.

Another limitation of the present study is the lack of a normoxic control for the hypoxic exercise. It is not clear, based on our findings, whether the improvement in DM and loss in lung water at this time point was a result of exercise or normobaric hypoxia. We had not anticipated a loss in lung water with hypoxic exercise and, therefore, did not include normoxic exercise control in the present study.

In conclusion, we have found that hypoxic exposure at rest and during exercise leads to increases in DLCO, Vc, and DM, and decreases in lung density, tissue volume, and estimates of lung water. This is clearly a novel finding in humans but is supported by previous work in animals. This suggests that the factors involved in lung fluid regulation (i.e., increases in pulmonary vascular pressures) are not adequate to interfere with lung fluid clearance mechanisms (i.e., increased lymph flow, activation of alveolar EnaCs). Thus either the duration of time was inadequate to challenge lung fluid regulation, or a hypobaric pressure effect is needed to cause lung fluid accumulation in the majority of healthy humans.

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