AltitudeOmics: impaired pulmonary gas exchange efficiency and blunted ventilatory acclimatization in humans with patent foramen ovale after 16 days at 5,260 m

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Alveolar ventilation, or alveolar PO2, at ALT16 compared with ALT1, 2

Elliott JE, Laurie SS, Kern JP, Beasley KM, Goodman RD, Kayser B, Subudhi AW, Roach RC, Lovering AT. AltitudeOmics: impaired pulmonary gas exchange efficiency and blunted ventilatory acclimatization in humans with patent foramen ovale after 16 days at 5,260 m. J Appl Physiol 118: 1100–1112, 2015. First published February 12, 2015; doi:10.1152/japplphysiol.00879.2014.—A patent foramen ovale (PFO), present in ~40% of the general population, is a potential source of right-to-left shunt that can impair pulmonary gas exchange efficiency [i.e., increase the alveolar-to-arterial PO2 difference (A-aDO2)]. Prior studies investigating human acclimatization to high-altitude with A-aDO2 as a key parameter have not investigated differences between subjects with (PFO+) or without a PFO (PFO−). We hypothesized that in PFO+ subjects A-aDO2 would not improve (i.e., decrease) after acclimatization to high altitude compared with PFO− subjects. Twenty-one (11 PFO+) healthy sea-level residents were studied at rest and during cycle ergometer exercise at the highest iso-workload achieved at sea level (SL), after acute transport to 5,260 m (ALT1), and again at 5,260 m after 16 days of high-altitude acclimatization (ALT16). In contrast to PFO− subjects, PFO+ subjects had 1) no improvement in A-aDO2 at rest and during exercise at ALT16 compared with ALT1, 2) no significant increase in resting alveolar ventilation, or alveolar PO2, at ALT16 compared with ALT1, and consequently had 3) an increased arterial PCO2 and decreased arterial PO2 and arterial O2 saturation at rest at ALT16. Furthermore, PFO+ subjects had an increased incidence of acute mountain sickness (AMS) at ALT1 concomitant with significantly lower peripheral O2 saturation (SpO2). These data suggest that PFO+ subjects have increased susceptibility to AMS when not taking prophylactic treatments, that right-to-left shunt through a PFO impairs pulmonary gas exchange efficiency even after acclimatization to high altitude, and that PFO+ subjects have blunted ventilatory acclimatization after 16 days at altitude compared with PFO− subjects.

shunt; high altitude; acute mountain sickness

IT IS WELL ESTABLISHED that pulmonary gas exchange efficiency, determined by the alveolar-to-arterial PO2 difference (A-aDO2) and thus incorporating contributions from pulmonary (i.e., alveolar ventilation-to-perfusion inequality, diffusion limitation, and intrapulmonary right-to-left shunt) and nonpulmonary factors (i.e., extrapulmonary right-to-left shunt), progressively worsens (i.e., the A-aDO2 increases) in a workload-dependent manner during exercise at sea level (13). This impairment in pulmonary gas exchange efficiency during exercise is exacerbated in acute hypoxia, such that for any given VO2, the A-aDO2 is greater compared with that observed during exercise while breathing room air at sea level (72). Following acclimatization to hypobaric hypoxia, pulmonary gas exchange efficiency is thought to improve compared with acute hypoxia (27, 62). Seminal work from Dempsey et al. (12) reported a trend for an increased A-aDO2 during treadmill walking after 4 days at 3,100 m compared with sea level, and a partial normalization during the same exercise protocol following 21 days at 3,100 m compared with that obtained after 4 days. Bebout et al. (4) then demonstrated that, compared with acute normobaric hypoxia, acclimatization to 3,800 m for 2 wk resulted in an ~3 mmHg reduction in the A-aDO2 during submaximal cycle ergometer exercise. Calbet et al. (9) subsequently reported that, compared with acute normobaric hypoxia, acclimatization to 5,260 m for 9-10 wk resulted in an ~9 mmHg reduction in the A-aDO2 during submaximal cycle ergometer exercise. Collectively, these data suggest that the A-aDO2 in nonacclimatized individuals decreases with acclimatization to high altitude compared with acute hypoxia.

Recently, Lovering et al. (44) explored the consequences of an intracardiac right-to-left shunt via a patent foramen ovale (PFO) in healthy humans during exercise breathing room air and in acute normobaric hypoxia at sea level. In the course of these investigations it became apparent that the presence of a PFO could be critical to the interpretation of work where pulmonary gas exchange efficiency is a key parameter, and, to our knowledge, prior work investigating human acclimatization to high altitude has not considered the effect of a PFO. The classic study by Hagen and Edwards (24) reported a PFO prevalence of 25–35% identified using a probe during autopsy (n = 965). Recent work from several research groups using saline contrast echocardiography (n = 104-1,162) reports that ~40% of adult humans have a PFO (18, 47, 81). According to the multiplication rule for conditional probability and using a 35% prevalence of PFO, there is a <5% chance that the aforementioned studies on pulmonary gas exchange efficiency after acclimatization would randomly select all subjects without PFO. Right-to-left blood flow through a PFO occurs when right atrial pressure exceeds left atrial pressure, which can occur transiently during normal respiration (21). Thus right-to-left blood flow through a PFO is likely intermittent and variable in volume; however, it does result in a measurable impact on pulmonary gas exchange efficiency at rest. Lovering
et al. (44) found that subjects with a PFO have an increased A-a\textsubscript{DO}_{2} at rest, breathing either room air at sea level or a normobaric hypoxic gas mixture (12\% \textsubscript{O}_2).

Additionally, during conditions of elevated pulmonary pressures, right-to-left intracardiac shunt across a PFO could be exacerbated because of higher right atrial pressure exceeding left atrial pressure. Exaggerated pulmonary hypertension is a hallmark of high-altitude pulmonary edema (HAPE), a potentially life-threatening complication of sojourn to high altitude, and the prevalence of PFO is \textgreater4 times higher in HAPE-susceptible than HAPE-resistant individuals (2). Moreover, systemic arterial oxygen desaturation, although unavoidable in acute hypobaria hypoxia, can be exacerbated by right-to-left intracardiac shunt, and is more pronounced in HAPE-susceptible individuals (3). Taken together, arterial hypoxemia secondary to hypobaric hypoxia may be exacerbated in individuals with PFO.

Although individuals with PFO are suggested to be at an increased risk for the development of high-altitude illnesses such as HAPE (2) and acute mountain sickness (40), very little is known regarding how the overall physiology of individuals with and without PFO differs at high altitude. Indeed, prior studies investigating human acclimatization to high altitude have not prospectively considered the effect of a PFO. Consequently, although pulmonary gas exchange efficiency and arterial hypoxemia are thought to improve following acclimatization to hypobaric hypoxia (4, 9, 12, 45) and high-altitude illness incidence and severity decreases with acclimatization (23, 58), it remains unknown if these findings are generalizable to both individuals with and without PFO.

Accordingly, the primary purpose of this study was to investigate pulmonary gas exchange efficiency at rest and during exercise in subjects with and without a PFO after acclimatization to hypobaric hypoxia. We hypothesized that in subjects with PFO, pulmonary gas exchange efficiency and arterial hypoxemia would not improve following acclimatization to high altitude and that these subjects would be more susceptible to AMS as a result of their greater arterial hypoxemia compared with PFO− subjects. To test this hypothesis, healthy male and female lowlanders, with and without a PFO, were studied at rest and during exercise at sea level, after being acutely transported to 5,260 m, and again at 5,260 m after 16 days of high-altitude acclimatization. This study was conducted as part of the AltitudeOmics project, described previously in greater detail (66).

**METHODS**

This study received approval from the University of Oregon, the University of Colorado Denver, and the U.S. Department of Defense. All subjects provided verbal and written informed consent prior to participation, and all studies were conducted in accordance with the Declaration of Helsinki.

**Subject Recruitment and Screening**

A complete description of the inclusion/exclusion criteria was published in the project overview paper of this series (66). Briefly, 21 healthy subjects (9 female) recruited from sea level (Eugene, OR, 130 m, \textsubscript{Pb} = 749 mmHg) participated in all aspects of this study and constitute the AltitudeOmics group of subjects in this report. Pertinent to the current report and not described in previous AltitudeOmics publications are the methodologies for determining adequate (\textgreater90\% predicted) pulmonary function and diffusion capacity for carbon monoxide parameters and the echocardiographic screening process each subject underwent.

**Spirometry, Diffusion Capacity, and Lung Volumes**

Baseline pulmonary function was determined using computerized spirometry (MedGraphics, Ultima CardiO2, St. Paul, MN) according to American Thoracic Society/European Respiratory Society (ATS/ERS) standards (50). Lung diffusion capacity for carbon monoxide (DL\textsubscript{CO}) was determined by the single-breath, breath hold method according to ATS/ERS standards (36, 46) using the Jones and Meade method for timing (32). Lung volumes and capacities were determined using whole body plethysmography (MedGraphics Elite Plethysmograph, St. Paul, MN) according to ATS/ERS standards (75).

**Echocardiographic Screening**

All subjects underwent a comprehensive echocardiographic screening process (Philips Sonos 5500, Eindhoven, The Netherlands) by a registered diagnostic cardiac sonographer with \textgtr25 yr of experience (R.D.G.) to ensure subjects were free of cardiac abnormalities or signs of heart disease, as previously conducted by our group (16–18, 38, 39, 52). Transthoracic saline contrast echocardiography was used to identify the presence of a PFO as described previously (42). The appearance of \textless1 microbubble(s) in the left heart in any frame during the 20 cardiac cycles following right heart opacification identified subjects as either having a PFO or the transpulmonary passage of saline contrast (22, 48, 49, 59). Delineation between these two sources of left heart contrast results from the timing of contrast appearing in the left heart following right heart opacification, in which a microbubble appearing within \textless3 cardiac cycles is consistent with PFO, while a microbubble appearing after \textgtr3 cardiac cycles is consistent with transpulmonary passage (7, 30, 37, 49, 59, 71). Saline contrast injections were performed during normal breathing as well as following the release of a Valsalva maneuver intended to transiently elevate right atrial pressure and create conditions optimal for detection of PFO. Effective Valsalva maneuvers, following a 15-s strain phase, were confirmed by a transient leftward deviation of the interatrial septum upon release, and multiple injections were performed as necessary when results were equivocal. PFO was defined by the appearance of \textless1 microbubble in the left heart within \textless3 cardiac cycles post right-heart opacification (7, 37). There were 11 (7 female) subjects with PFO (PFO+) and 10 (2 female) subjects without PFO (PFO−). Of note, we originally planned on having an equal number of PFO+ and PFO− subjects, although for reasons previously described 3 subjects were excluded (66).

**Timeline**

This report presents data collected over three experimental study visits: 1) sea level (SL: Eugene, Oregon, 130 m, \textsubscript{Pb} = 749 mmHg); 2) day 1 at high-altitude (ALT1: Mt. Chacaltaya, Bolivia, 5,260 m, \textsubscript{Pb} = 406 mmHg); and 3) after living at high-altitude for 16 days (ALT16: Mt. Chacaltaya, Bolivia, 5,260 m, \textsubscript{Pb} = 406 mmHg). A complete description of the study timeline was previously published (66). Briefly, upon landing in El Alto (4,050 m) subjects were immediately driven to Coroico (1,525 m) where they rested for 48 h prior to ascending to Mt. Chacaltaya (5,260 m) over the course of a \textless3-h drive. To provide an acute exposure to 5,260 m on ALT1, subjects breathed supplemental oxygen (2 l/min, nasal cannula or mask) during the drive up the mountain. Upon arrival at 5,260 m, the first subject immediately began the experimental protocol while the second subject rested and continued to breathe supplemental oxygen for \textless2 h until the first subject had completed the protocol. Acute mountain sickness was assessed 10–12 h after arrival to high altitude on ALT1, and on day 5 at high altitude (ALT5: Mt. Chacaltaya, Bolivia, 5,260 m, \textsubscript{Pb} = 406 mmHg) time matched to ALT1.
Subject Instrumentation and Exercise Protocol

A core temperature pill (CorTemp HQ, Palmetto, FL) was ingested ~5 h prior to the start of exercise. Subjects were instrumented with a 20-gauge radial artery catheter (Arrow International, Reading, PA) under local anesthesia (2% lidocaine), and an 18- to 22-gauge intravenous catheter was placed in the antecubital fossa. Subjects rested on an upright stationary cycle ergometer (Velotron Elite, Seattle, WA) for 10 min prior to performing standardized workloads of 70, 100, 130, and 160 W for 3 min each, followed by 15 W/min increments until cadence could no longer be maintained at >50 rpm (i.e., \( V_{\text{O}2\max} \) was reached). Exercise data are presented, unless otherwise specified, as the highest iso-workload achieved within an individual subject across SL, ALT1, and ALT16. For example, subjects may have achieved ≥160 W at SL and ALT16, but only completed 130 W at ALT1; in this case, the iso-workload reported would be data at 130 W, from each SL, ALT1, and ALT16.

Pulmonary Artery Systolic Pressure and Cardiac Output

Pulmonary artery systolic pressure (PASP) was assessed from the peak velocity of the tricuspid regurgitation using saline contrast enhanced Doppler ultrasound (Sonosite Micromamm, Bothell, WA). This was applied to the modified Bernoulli equation (4\( v^2 + P_{\text{RA}} \)), where \( v \) is the tricuspid regurgitation velocity envelope and \( P_{\text{RA}} \) is right atrial pressure, according to the guidelines of the American Society for Echocardiography (35, 60, 83). A small volume (~0.5 ml) of air agitated with 3 ml of sterile saline was injected and used to help delineate the tricuspid regurgitation velocity envelope. Cardiac output (\( Q_T \)) was calculated as before (67) with heart rate obtained from the ECG and stroke volume estimates derived from intra-arterial blood pressure tracings obtained via a saline-filled pressure transducer (Utah Medical, Salt Lake City, UT) positioned at heart level and attached to the radial artery catheter (6, 68). Neither \( Q_T \) nor PASP were obtained at maximal exercise.

Acute Mountain Sickness

Acute mountain sickness was assessed using the criteria outlined in the AltitudeOmics overview paper (66), and peripheral \( O_2 \) saturation (SpO\(_2\); Respironics GO finger oximeter) was assessed at the same time point AMS was assessed.

Hemoglobin Mass

Hemoglobin mass was measured using the optimized carbon monoxide rebreathing method (55, 64) with minor modifications. For a detailed description of the methodology we refer the reader to the primary report of these data in the AltitudeOmics series (61).

Arterial Blood Gases, Body Temperature, and Blood Lactate

At rest and at the end of each 3-min submaximal workload, a 3 ml radial artery blood sample was drawn anaerobically over 10–15 s into a heparinized syringe and rapidly analyzed in duplicate (or triplicate if time permitted) for arterial \( P_{\text{O}_2} \) (PaO\(_2\)), arterial \( P_{\text{CO}_2} \) (PaCO\(_2\)), and arterial pH with a blood-gas analyzer calibrated daily with tonometered whole blood (Siemens RAPIDLab 248, Erlangen, Germany). Arterial blood gases were corrected for body temperature (13, 33, 65) based on readings from the ingested core temperature pill. Arterial \( O_2 \) saturation (SaO\(_2\)) and total hemoglobin (Hb) were measured with CO-oximetry (Radiometer OSM3, Copenhagen, Denmark). Hematocrit was analyzed in triplicate at rest and in single measurements for each workload using the microcapillary tube centrifugation method (M24 Centrifuge, LW Scientific, Lawrenceville, GA). Blood lactate was analyzed in duplicate using the Lactate Plus hand held meter and lactate test strips (Nova Biomedical, Waltham, MA).

Calculations

Alveolar \( P_{\text{O}_2} \) (\( P_{\text{A}O_2} \)) was calculated using the ideal gas equation, as before (15, 17, 43), and temperature-corrected \( P_{\text{aCO}_2} \), and a respiratory quotient (RER) from a 15-s average of metabolic data corresponding to the time and duration of the arterial blood draw:

\[
P_{\text{A}O_2} = \left[ \frac{\left( P_B - e^{-0.0589489 \times T_B + 1.689589} \times F_{\text{I}O_2}\right)}{1 - F_{\text{I}O_2} + \frac{F_{\text{I}O_2}}{P_{\text{aCO}_2} \times \text{RER}}} \right]
\]

where \( T_B \) is core body temperature for temperature correction of water vapor pressure, \( F_{\text{I}O_2} \) is fraction of inspired \( O_2 \), \( \text{RER} \) is the respiratory exchange ratio (\( V_{\text{CO}_2}/V_{\text{O}_2} \)), and \( P_{\text{aCO}_2} \) is barometric pressure that was measured daily (Greisinger electronic, GBP 3300). The \( \text{a-DO}_2 \) was determined at rest and during exercise as the difference between the temperature-corrected \( P_{\text{aO}_2} \) and corresponding \( P_{\text{A}O_2} \).

Measures of \( O_2 \) content were calculated from the standard content equation:

\[
O_2\text{ content} = \left[ 1.39 \times tHb \left( \frac{SO_2}{100} \right) \right] + (0.003 \times P_{\text{O}_2})
\]

using an \( O_2 \) carrying capacity of 1.39 ml \( O_2/g \) Hb and directly measured \( tHb \) (g Hb/dl). For arterial \( O_2 \) content (CaO\(_2\)) \( O_2 \) represents arterial \( O_2 \) saturation (SaO\(_2\)) and temperature-corrected \( P_{\text{O}_2} \). The SaO\(_2\) used to calculate CaO\(_2\) in Tables 2–4 is CO-oximetry measured Hb O2% (i.e., functional saturation; \( \text{HbO}_2\% \)) from the Kelman equation (34) assuming complete alveolar-capillary \( O_2 \) equilibration such that end-capillary \( P_{\text{O}_2} \) (\( P_{\text{c}O_2} \)) was equal to \( P_{\text{A}O_2} \). Mixed venous \( O_2 \) content (CvO\(_2\)) \( O_2 \) was calculated using the Fick principle of mass balance \( [V_{\text{O}_2} = Q_T \times (\text{CaO}_2 - \text{CvO}_2)] \) using measured CaO\(_2\), \( V_{\text{O}_2} \), and an estimate of total \( Q_T \) as described above.

The fraction of venous admixture (\( Q_{VA}/Q_T \)) accounting for the entirety of the A–a\( O_2 \) was calculated from the shunt equation (5) using the previously calculated \( O_2 \) content values:

\[
\frac{Q_{VA}}{Q_T} = \frac{Cc'O_2 - \text{CaO}_2}{Cc'O_2 - \text{CvO}_2}
\]

Alveolar ventilation (\( V_A \)) was calculated using the measured \( V_{\text{CO}_2} \) and temperature-corrected \( P_{\text{aCO}_2} \):

\[
V_A = \left( \frac{V_{CO_2} \times 863}{P_{\text{aCO}_2}} \right)
\]

Statistical Analyses

All statistical calculations were made using GraphPad Prism statistical software (v. 5.0d), and significance was set to \( P < 0.05 \). In both the PFO– and PFO+ groups, measured and calculated physiological variables were compared across time points (e.g., SL, ALT1, and ALT16) using a one-way ANOVA. A Newman-Keuls multiple comparison post hoc test was used to determine specific pairwise differences between groups and time points. Comparisons were determined a priori and performed two times (at rest and during exercise) in the PFO– and PFO+ groups. Differences in measured and calculated variables, between the PFO– and PFO+ groups at rest
Baseline values for anthropometric, exercise, hematological, and pulmonary function data at SL for the PFO− (n = 10) and PFO+ (n = 11) groups are presented in Table 1. Cardiopulmonary data at rest and during iso-workload exercise for SL, ALT1, and ALT16 for the PFO− and PFO+ groups are presented in Tables 2 and 3, respectively. Cardiopulmonary and arterial blood gas data obtained at V̇O_{2max} for SL, ALT1, and ALT16 for the PFO− and PFO+ groups are presented in Table 4. The mean iso-workload in the PFO− and PFO+ groups was 150 ± 24 and 136 ± 28 W, respectively, which was not different between groups. The presentation of results will sequentially describe data collected at rest and during iso-workload exercise at SL, ALT1, and ALT16 in the PFO− and PFO+ groups, and conclude with data obtained at V̇O_{2max}.

### Table 1. Anthropometric, exercise, hematological, and pulmonary function data

<table>
<thead>
<tr>
<th></th>
<th>PFO− (n = 10)</th>
<th>PFO+ (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>21.1 ± 1.4</td>
<td>20.6 ± 1.6</td>
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<tr>
<td>Sex, F</td>
<td></td>
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<tr>
<td>Height, cm</td>
<td>178.4 ± 6.1</td>
<td>173.4 ± 8.8</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>71.5 ± 8.4</td>
<td>68.1 ± 9.7</td>
</tr>
<tr>
<td>V̇O_{2max}, ml·kg⁻¹·min⁻¹</td>
<td>46.8 ± 7.1</td>
<td>44.0 ± 6.2</td>
</tr>
<tr>
<td>Peak power output, W</td>
<td>311 ± 67</td>
<td>268 ± 64</td>
</tr>
<tr>
<td>Ferritin, ng/ml</td>
<td>61.3 ± 30.6</td>
<td>44.0 ± 32.7</td>
</tr>
<tr>
<td>Iron, μg/dl</td>
<td>129.5 ± 47.9</td>
<td>134.1 ± 61.4</td>
</tr>
<tr>
<td>FVC, liters</td>
<td>5.48 ± 0.92</td>
<td>4.73 ± 1.04</td>
</tr>
<tr>
<td>SVC, liters</td>
<td>5.61 ± 1.08</td>
<td>5.01 ± 1.18</td>
</tr>
<tr>
<td>FVC, %predicted</td>
<td>100.0 ± 11.0</td>
<td>100.4 ± 11.3</td>
</tr>
<tr>
<td>TLC, liters</td>
<td>7.16 ± 1.07</td>
<td>6.28 ± 1.33</td>
</tr>
<tr>
<td>FeLV, %predicted</td>
<td>101.3 ± 9.0</td>
<td>101.9 ± 7.9</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>85.4 ± 9.8</td>
<td>86.4 ± 5.3</td>
</tr>
<tr>
<td>FEV1/FVC, %predicted</td>
<td>100.9 ± 11.6</td>
<td>100.6 ± 6.1</td>
</tr>
<tr>
<td>FEV1/VA</td>
<td>6.00 ± 0.71</td>
<td>5.61 ± 1.03</td>
</tr>
<tr>
<td>DLco/VA, %predicted</td>
<td>124.1 ± 17.5</td>
<td>113.2 ± 21.4</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of subjects without (PFO−) or with patent foramen ovale (PFO+). FVC, forced vital capacity; SVC, slow vital capacity; FEV1, forced expiratory volume in 1 s; FEF25–75, forced expiratory flow from 25 to 75% of FVC; TLC, total lung capacity; DLco, diffusion capacity of carbon monoxide; DLco/VA, DLco/alveolar volume. †P < 0.05 compared with PFO−.

### Table 2. Cardiopulmonary and arterial blood gas data at rest in the PFO− and PFO+ groups at sea level, acute hypoxia, and after acclimatization to high altitude

<table>
<thead>
<tr>
<th></th>
<th>SL</th>
<th>PFO−</th>
<th>PFO+</th>
<th>ALTI</th>
<th>PFO−</th>
<th>PFO+</th>
<th>ALTI6</th>
<th>PFO−</th>
<th>PFO+</th>
</tr>
</thead>
<tbody>
<tr>
<td>V̇E, l/min</td>
<td>14.5 ± 4.7</td>
<td>19.4 ± 4.4</td>
<td>16.7 ± 4.9</td>
<td>26.2 ± 8.9</td>
<td>18.7 ± 6.5</td>
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<td>V̇A, l/min</td>
<td>8.2 ± 3.8</td>
<td>12.3 ± 2.7</td>
<td>10.6 ± 4.8</td>
<td>19.4 ± 7.8</td>
<td>12.4 ± 5.1</td>
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<tr>
<td>V̇O_{2}/V̇E</td>
<td>0.42 ± 0.06</td>
<td>0.37 ± 0.11</td>
<td>0.42 ± 0.06</td>
<td>0.38 ± 0.16</td>
<td>0.41 ± 0.05</td>
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<tr>
<td>V̇O₂, l/min</td>
<td>0.34 ± 0.10</td>
<td>0.43 ± 0.14</td>
<td>0.34 ± 0.12</td>
<td>0.43 ± 0.06</td>
<td>0.35 ± 0.08</td>
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<tr>
<td>RC, l/min</td>
<td>0.32 ± 0.12</td>
<td>0.39 ± 0.10</td>
<td>0.32 ± 0.11</td>
<td>0.40 ± 0.11</td>
<td>0.29 ± 0.09</td>
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<tr>
<td>RER</td>
<td>0.92 ± 0.08</td>
<td>0.93 ± 0.09</td>
<td>0.97 ± 0.09</td>
<td>0.90 ± 0.07</td>
<td>0.83 ± 0.09</td>
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<tr>
<td>V̇E/V̇O₂</td>
<td>43.0 ± 5.7</td>
<td>47.0 ± 7.1</td>
<td>51.4 ± 8.4</td>
<td>61.4 ± 15.2</td>
<td>53.2 ± 9.6</td>
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<tr>
<td>HR, beats/min</td>
<td>77 ± 15</td>
<td>87 ± 16</td>
<td>89 ± 16*</td>
<td>93 ± 16</td>
<td>92 ± 14*</td>
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<tr>
<td>SL</td>
<td>86 ± 18</td>
<td>90 ± 23</td>
<td>83 ± 13</td>
<td>73 ± 16</td>
<td>75 ± 16</td>
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<tr>
<td>Qt, l/min</td>
<td>6.5 ± 1.6</td>
<td>7.7 ± 1.5</td>
<td>7.3 ± 1.4</td>
<td>6.6 ± 1.2</td>
<td>6.9 ± 1.2</td>
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<tr>
<td>PASP, mmHg</td>
<td>30.4 ± 6.4</td>
<td>36.6 ± 6.6</td>
<td>31.8 ± 5.1</td>
<td>34.1 ± 4.0</td>
<td>31.9 ± 5.6</td>
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<tr>
<td>CO₂, ml O₂/dl</td>
<td>20.1 ± 1.5</td>
<td>16.4 ± 1.3*</td>
<td>15.3 ± 1.5*</td>
<td>20.8 ± 1.9</td>
<td>17.7 ± 3.2*</td>
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<tr>
<td>pH</td>
<td>7.43 ± 0.03</td>
<td>7.51 ± 0.02</td>
<td>7.51 ± 0.03*</td>
<td>7.51 ± 0.03*</td>
<td>7.50 ± 0.03*</td>
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<tr>
<td>HCO₃⁻, mmol/l</td>
<td>21.98 ± 9.2</td>
<td>20.70 ± 1.31</td>
<td>20.82 ± 2.05</td>
<td>14.36 ± 1.83</td>
<td>15.75 ± 1.13*</td>
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<tr>
<td>BE, mmol/l</td>
<td>-1.87 ± 1.73</td>
<td>-1.97 ± 1.14</td>
<td>-1.87 ± 1.56</td>
<td>-7.83 ± 1.70</td>
<td>-6.71 ± 0.81*</td>
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<tr>
<td>Hct, %</td>
<td>44.5 ± 3.0</td>
<td>44.3 ± 3.3</td>
<td>42.4 ± 3.5</td>
<td>51.7 ± 3.2*</td>
<td>47.2 ± 6.1*</td>
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<tr>
<td>Hb, g/dl</td>
<td>14.8 ± 1.1</td>
<td>15.1 ± 1.0</td>
<td>13.9 ± 1.5</td>
<td>17.2 ± 1.4*</td>
<td>15.0 ± 2.4</td>
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<tr>
<td>Core temp, °C</td>
<td>37.1 ± 0.4</td>
<td>36.9 ± 0.5</td>
<td>37.1 ± 0.4</td>
<td>37.0 ± 0.9</td>
<td>37.0 ± 0.4</td>
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<tr>
<td>Lactate, mmol/l</td>
<td>0.98 ± 0.42</td>
<td>1.16 ± 0.29</td>
<td>1.26 ± 0.59</td>
<td>1.03 ± 0.31</td>
<td>1.04 ± 0.31</td>
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<tr>
<td>n, max</td>
<td>10</td>
<td>6</td>
<td>9</td>
<td>11</td>
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Values are means ± SD; SL, sea level; ALTI, acute hypoxia; ALTI6, after acclimatization to high altitude; V̇E, minute ventilation; V̇A, alveolar ventilation; V̇O_{2}/V̇E, dead space-to-tidal volume ratio; V̇O₂, oxygen consumption; V̇CO₂, carbon dioxide production; RER, respiratory exchange ratio; HR, heart rate; SV, stroke volume; Qt, cardiac output; PASP, pulmonary artery systemic pressure; CO₂, arterial oxygen content; pH, arterial pH; HCO₃⁻, arterial bicarbonate; BE, arterial base excess; Hct, arterial hematocrit; Hb, arterial total hemoglobin; Core temp, core body temperature. *P < 0.05 compared with SL; †P < 0.05 compared with ALTI1; ‡P < 0.05 compared with PFO−.

### Results

#### Overview

No differences were observed between PFO− and PFO+ groups in baseline anthropometric, exercise, or hematological variables at SL (Table 1). The greater number of females in the PFO+ group (n = 7) explains the observed differences in absolute pulmonary function values and results from the known differences in absolute lung volumes between males and females (26). However, when the pulmonary function data were expressed as percent predicted, or when DLco was corrected for alveolar volume, there were no statistical differences. Of note, preliminary analyses between male and female subjects revealed no differences other than a larger CaO₂ in males as a result of increased Hb across SL, ALT1, and ALT16 (66).
Table 3. Cardiopulmonary and arterial blood gas data during iso-workload exercise in the PFO− and PFO+ groups at sea level, acute hypoxia, and after acclimatization to high altitude

<table>
<thead>
<tr>
<th></th>
<th>PFO−</th>
<th>PFO+</th>
<th>PFO−</th>
<th>PFO+</th>
<th>PFO−</th>
<th>PFO+</th>
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<td>SL</td>
<td>ALT1</td>
<td>ALT16</td>
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<td></td>
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<tr>
<td>VE, l/min</td>
<td>143.0</td>
<td>124.1</td>
<td>140.1</td>
<td>148.6</td>
<td>146.1</td>
<td>131.3</td>
</tr>
<tr>
<td>V̇A, l/min</td>
<td>98.3</td>
<td>93.2</td>
<td>98.0</td>
<td>91.9</td>
<td>91.9</td>
<td>91.9</td>
</tr>
<tr>
<td>VE/V̇A</td>
<td>3.27</td>
<td>3.09</td>
<td>3.27</td>
<td>3.27</td>
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<td>3.27</td>
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<tr>
<td>V̇O₂, l/min</td>
<td>2.62</td>
<td>2.41</td>
<td>2.62</td>
<td>2.62</td>
<td>2.62</td>
<td>2.62</td>
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<tr>
<td>V̇CO₂, l/min</td>
<td>3.70</td>
<td>3.45</td>
<td>3.70</td>
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<tr>
<td>RER</td>
<td>0.06</td>
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<tr>
<td>HR, beats/min</td>
<td>189.0</td>
<td>189.0</td>
<td>175.0</td>
<td>175.0</td>
<td>175.0</td>
<td>175.0</td>
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<tr>
<td>CaO₂, ml O₂/dl</td>
<td>39.5</td>
<td>39.5</td>
<td>39.5</td>
<td>39.5</td>
<td>39.5</td>
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<tr>
<td>PaO₂, mmHg</td>
<td>175.0</td>
<td>175.0</td>
<td>175.0</td>
<td>175.0</td>
<td>175.0</td>
<td>175.0</td>
</tr>
<tr>
<td>PaCO₂, mmHg</td>
<td>4.78</td>
<td>4.78</td>
<td>4.78</td>
<td>4.78</td>
<td>4.78</td>
<td>4.78</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>3.70</td>
<td>3.70</td>
<td>3.70</td>
<td>3.70</td>
<td>3.70</td>
<td>3.70</td>
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<tr>
<td>n, max</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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</table>

Values are means ± SD. See Table 2 for definitions. *P < 0.05 compared with SL; †P < 0.05 compared with ALT1; ‡P < 0.05 compared with PFO−.
Effect of PFO on Acclimatization to High Altitude • Elliott JE et al.

Fig. 1. Pulmonary gas exchange efficiency (A and B), alveolar PO2 (PAO2; C and D), arterial PO2 (PaO2; E and F), arterial PCO2 (PaCO2; G and H), and arterial O2 saturation (SaO2; I and J) for the groups without patent foramen ovale (PFO− group) and with PFO (PFO+ group) at rest and during iso-workload exercise at sea level (SL), acute hypoxia (ALT1), and after acclimatization to high altitude (ALT16). A-aDO2, alveolar-to-arterial P O2 difference. Data presented are means ± SE (see Tables 2 and 3 for respective n values), *P < 0.05 compared with SL, †P < 0.05 compared with ALT1, ‡P < 0.05 compared with the PFO− group.
subjects developed AMS, while only 60% of PFO− subjects did \( (P < 0.001) \). Likewise, SpO2 was significantly lower in PFO+ subjects \( (73 \pm 8\% \) than in PFO− subjects \( (81 \pm 7\% \) \( (P < 0.04) \).

**Day 5 at 5,260 m (ALT5)**

The incidence of AMS decreased to 40% in the PFO+ group and to 10% in the PFO− group \( (P < 0.08) \) while SpO2 was not significantly different between groups, PFO+ subjects having 78 \± 4% and PFO− subjects 79 \± 7%.

**Acclimatization to 5,260 m (ALT16)**

Pulmonary gas exchange efficiency. Unlike the PFO− group, the A-aDO2 at rest \( (\text{Fig. 1A}) \) and during exercise \( (\text{Fig. 1B}) \) in the PFO+ group did not decrease compared with ALT1. Furthermore, although there was only a trend for the A-aDO2 to be greater in the PFO+ group compared with the PFO− group \( (P = 0.063) \) at rest \( (\text{Fig. 1A}) \), the A-aDO2 was significantly greater in the PFO+ group compared with the PFO− group during exercise \( (\text{Fig. 1B}) \). This difference in pulmonary gas exchange efficiency between the PFO− and PFO+ groups at ALT16 can also be illustrated by calculating the difference in the total \( Q_{VA}/Q_T \) required to account for the entire A-aDO2 for each group between ALT1 and ALT16 \( (\text{Fig. 3}) \). From ALT1 to ALT16 at rest, the PFO− group showed a \(~21\%\) reduction in calculated \( Q_{VA}/Q_T \), which was greater than the \(~13\%\) reduction in the PFO+ group \( (\text{Fig. 3}) \). Similarly, from ALT1 to ALT16 during exercise, calculated \( Q_{VA}/Q_T \) in the PFO− group decreased by \(~18\%\), which was also greater than the \(~11\%\) decrease in the PFO+ group \( (\text{Fig. 3}) \).

Ventilatory acclimatization and arterial blood gases. Importantly, both the PFO− and PFO+ groups demonstrated a reduction in \( P_{ACO2} \) and an increase in \( P_{AO2} \) compared with ALT1, consistent with acclimatization to high altitude \( (\text{Fig. 1, E and G}) \). That said, the PFO+ group had no decrease in A-aDO2 from ALT1 to ALT16, and they demonstrated a less pronounced degree of ventilatory acclimatization compared with the PFO− group as evidenced by a significantly greater \( P_{ACO2} \) and lower \( P_{AO2} \), and therefore a lower calculated \( V_A \). Only the PFO− group increased \( V_A \) at rest at ALT16 compared with ALT1, and \( V_A \) was less in the PFO+ group compared with the PFO− group at rest at ALT16 \( (\text{Table 2}) \). Consequently, the PFO+ group had reduced resting \( P_{AO2} \), \( P_{ACO2} \), and \( SaO2 \) and increased \( P_{ACO2} \) compared with the PFO− group \( (\text{Fig. 1, C, E, G, I}) \) at rest at ALT16, and both \( P_{AO2} \) and \( SaO2 \) had not improved after acclimatization compared with ALT1 \( (\text{Fig. 1, C and I}) \). This reduced degree of ventilatory acclimatization can also be illustrated by examining the change in resting \( VE \) from SL to ALT16 relative to the change in \( SaO2 \) not increasing at ALT16 compared with ALT1 in the PFO+ group \( (\text{Fig. 2}) \). This contrasts with the PFO− group who showed an increase in the change in resting \( VE \) from SL to ALT16 relative to the change in \( SaO2 \) at ALT16 compared with ALT1 \( (\text{Fig. 2}) \).

The difference in \( VA \) between the PFO− and PFO+ groups at ALT16 was present only at rest and not during exercise at ALT16. Nevertheless, only the PFO− group increased \( P_{AO2} \) \( (\text{Fig. 1D}) \) and decreased \( P_{ACO2} \) \( (\text{Fig. 1H}) \) during exercise at ALT16 compared with ALT1. The PFO− and PFO+ groups increased \( P_{AO2} \) and \( SaO2 \) during exercise at ALT16 compared with exercise at ALT1; however, during exercise at ALT16, \( P_{AO2} \) and \( SaO2 \) in the PFO+ group were lower compared with the PFO− group \( (\text{Fig. 1, F and J}) \).

Acute mountain sickness. At ALT16 all subjects were free of AMS symptoms as would be expected after 16 days of high-altitude acclimatization.

Hemoglobin and hematocrit. \( tHb \) and \( Hct \) were less in the PFO+ group compared with the PFO− group and only the PFO− group increased \( tHb \) and \( Hct \) compared with ALT1. However, \( Hb \) mass increased compared with SL in 19/21 subjects and the 2 subjects who lacked an increase in \( Hb \) mass were PFO+ females \( (61) \). Statistical analysis of the PFO+ group without these two subjects shows no differences between the PFO− and PFO+ groups in \( tHb \) \( (P = 0.20) \) or \( Hct \) \( (P = 0.21) \) at ALT16; therefore, in both the PFO− and PFO+ groups \( tHb \) and \( Hct \) increased compared with ALT1.


Maximal Exercise

Data presented at maximal exercise differ from that of iso-workload exercise in two ways: 1) the workload achieved by each subject was not consistent across SL, ALT1, and ALT16 (i.e., data were collected at $V_{O2max}$ not iso-workload); and 2) all available data are presented including subjects with incomplete data across study days. These points should be taken into consideration, particularly when interpreting the comparison of data across SL, ALT1, and ALT16. That said, the overall physiological responses observed at $V_{O2max}$ at SL and ALT1 were similar between the PFO− and PFO+ groups (Table 4). However, at ALT16, the PFO+ group had a lower $V_{e}$, $P_{AO2}$, and $S_{A}O2$ compared with the PFO− group, despite reaching similar peak workloads. Additionally, $V_{O2max}$ or peak workload did not increase at ALT16 compared with ALT1 in either the PFO− and PFO+ groups, despite $O2$ carrying capacity being returned to SL values.

DISCUSSION

The primary purposes of this study were to investigate the effect of a PFO on pulmonary gas exchange efficiency, represented by the A-aDO2, at rest and during exercise at sea level (SL), after rapid transport to 5,260 m (ALT1), and again at 5,260 m after 16 days of high-altitude acclimatization (ALT16) in healthy, PFO−/H11001 and PFO−/H11002 sea level natives as well as to investigate the presence of a PFO on AMS susceptibility. The novel findings in this study are, in the PFO+ group: 1) there was a significantly increased incidence of AMS at ALT1; 2) pulmonary gas exchange efficiency did not improve (i.e., the A-aDO2 did not decrease) at rest or during exercise following acclimatization to 5,260 m; and 3) there was blunt ventilatory acclimatization to 5,260 m.

Terminology

In this manuscript we use the term “pulmonary gas exchange efficiency” as being synonymous with the A-aDO2 (13). Determination of the A-aDO2 is accomplished through directly sampling arterial blood for $P_{AO2}$, and comparing this to the calculated $P_{AO2}$. Factors that can potentially affect the resulting A-aDO2 include: 1) alveolar ventilation-to-perfusion inequality ($V_{A}/Q_{O2}$), 2) incomplete end-capillary $P_{O2}$ equilibration, and 3) right-to-left shunt, which includes intrapulmonary shunt (i.e., an area of the lung with a $V_{A}/Q_{O2} = 0$), and extrapulmonary shunt (i.e., the venous blood from the bronchial and Thebesian circulations). Additionally, subjects with a PFO would also have a potential source of intracardiac right-to-left shunt. These factors can thus be divided into pulmonary (i.e., $V_{A}/Q_{O2}$ inequality, diffusion limitation, and intrapulmonary shunt) and nonpulmonary factors (i.e., extrapulmonary and intracardiac shunt). Taken together, because determination of the A-aDO2 via arterial blood gas analysis reflects contributions from all factors (pulmonary and nonpulmonary), we use the terminology “pulmonary gas exchange efficiency” to describe the A-aDO2 for both PFO+ and PFO− subjects, despite the nonpulmonary factor, intracardiac shunt, present in PFO+ subjects. Historically every study investigating the A-aDO2 at altitude has used this conventional terminology despite not distinguishing between subjects with or without a PFO.

Sea Level (SL)

Although this study did not directly assess contributions from $V_{A}/Q_{O2}$ inequality or diffusion limitation, previous work suggests that diffusion limitation represents a minimal contribution to the A-aDO2 during submaximal exercise ($V_{O2} < 2.0$ l/min) in healthy humans at SL (25, 31, 57, 70, 72) such that the majority of the A-aDO2 is explained by $V_{A}/Q_{O2}$ inequality and right-to-left shunt. Accordingly, during iso-workload exercise ($V_{O2} \sim 2.0$ l/min) in our healthy subject population, at SL $V_{A}/Q_{O2}$ inequality and right-to-left shunt were likely the predominant contributors to the measured A-aDO2. In both PFO− and PFO+ subjects this would include extrapulmonary shunt, and intrapulmonary shunt, if any. However, subjects in the PFO+ group also have an additional source of shunt, which is intracardiac shunt via the PFO.

Right-to-left blood flow through the PFO is dependent upon right atrial pressure exceeding left atrial pressure, which can occur transiently during the normal respiratory cycle, likely at end inspiration when systemic venous return is augmented by reduced intrathoracic pressure (21, 82). Therefore, during normal respiration, right-to-left blood flow through the PFO would be expected to be intermittent and variable in volume. In the current study the A-aDO2 was not different between the PFO− and PFO+ groups at rest or during exercise at SL, suggesting that the degree of blood flow through the PFO was not great enough to impact the A-aDO2 at SL.

Acute Ascent to 5,260 m (ALT1)

At 5,260 m (PB ~406 mmHg) inspired $P_{O2}$ is lowered to ~75 mmHg, significantly reducing $P_{AO2}$, and reducing the contribution from $V_{A}/Q_{O2}$ inequality on the A-aDO2 while the contribution of diffusion limitation on the A-aDO2 increases (53, 54, 78). The effect that a given volume of right-to-left shunt has on the A-aDO2 is also lessened in hypoxia due to the difference between mixed venous $P_{O2}$ ($P_{Vo2}$) and $P_{AO2}$ becoming less. For this reason, if the shunt fraction via the PFO was constant from SL to ALT1, this additional 0.5–2.0% shunt (as it was calculated to be at SL) would account for between 1 and 3 mmHg of the measured A-aDO2 at ALT1. Therefore, it should not be surprising that at ALT1 the PFO− and PFO+ groups had a similar A-aDO2 both at rest and during exercise (Fig. 1B).

Previous work suggested that the presence of a PFO may facilitate an exaggerated pulmonary hypertensive response to high altitude, thereby predisposing these subjects to the development of HAPE (6). In that work, of the 16 HAPE-susceptible subjects studied, 11 were PFO+, and PASP increased by 57 ± 12 mmHg at 4,550 m. In the current work, at a similar altitude, PASP in the PFO+ group was ~32 ± 6 mmHg. Although not conclusive, our data suggest that HAPE susceptibility may depend more on an exaggerated pulmonary vascular response to hypoxia rather than on the presence of PFO. However, we do not know if the blood flow through the PFO in our subjects was similar or different to that in the PFO subjects in the HAPE study, so direct comparisons should be made with caution. Furthermore, previous work has speculated that a potential reason some subjects experience a greater degree of arterial $O2$ desaturation at altitude may be the presence of a PFO (41).
**Acclimatization to 5,260 m (ALT16)**

Acclimatization to hypobaric hypoxia is characterized by a multitude of physiological adaptations, notably a time-dependent increase in ventilation (77). Compared with acute hypoxia, ventilatory acclimatization helps to increase CaO2 by increasing PaO2 and the driving gradient for O2 diffusion at the alveolar-capillary interface, increasing PaO2 and, therefore, SaO2. Consequently, compared with acute hypoxia the further increase inVA, and therefore PaO2, with acclimatization would theoretically reduce the relative contributions from VA/Q inequality and diffusion limitation while increasing the relative contribution of right-to-left shunt on the A-aDO2. Indeed, an increase in VA should equate to improved VA/Q matching by way of lessening potential disparities in the Po2 between alveoli (20, 56, 63). Diffusion limitation would theoretically also be reduced compared with ALT1 by way of an increased driving gradient for O2 diffusion (73), increased PvO2, and a potential improvement in diffusing capacity for O2 (1). Last, a given volume of right-to-left shunt would have a greater effect on the A-aDO2 due to the difference between PvO2 and PaO2 increasing at ALT16 compared with ALT1.

**Rest.** Absence of an increased VA, calculated in part from a significantly increased PaCO2 at rest at ALT16 in the PFO+ group may partially explain the absence of a reduction in A-aDO2 compared with ALT1. The less pronounced degree of ventilatory acclimatization in the PFO+ group corresponded to a lower PaO2, which, compared with the PFO− group, may increase the relative contribution and potential for VA/Q inequality and particularly diffusion limitation to contribute to the A-aDO2. Additionally, continued right-to-left shunt via the PFO could have also contributed to the lack of improvement in A-aDO2 at rest at ALT16. However, the effect of this right-to-left shunt via the PFO, although increased compared with ALT1, would still likely be minimal considering the magnitude of the increase in PaO2 from ALT1 to ALT16 (40 ± 5 mmHg at ALT1 vs. 46 ± 3 mmHg at ALT16). Conversely, in the PFO− group PaO2 increased from 40 ± 4 mmHg at ALT1 to 53 ± 4 mmHg at ALT16, approximately twice as much as of the PFO+ group (P = 0.0003). Importantly, small changes in PaO2 in this range on the oxygen-hemoglobin dissociation curve correspond to large changes in SaO2, and thus CaO2. Indeed, had PaO2 increased in the PFO+ group to the same degree as it did in the PFO− group, SaO2 in the PFO+ group would have increased from ~83% to ~88%, corresponding to CaO2 increasing from ~18 ml O2/dl blood to ~19 ml O2/dl blood.

The PFO+ subjects had a lower VA despite the presence of a potentially increased drive to breathe from a combination of low O2 and higher CO2, which would be potentiated after acclimatization (11). Why the PFO+ group had a lesser degree of ventilatory acclimatization (higher PaCO2) compared with the PFO− group remains unknown, although we speculate that this may actually represent a beneficial response to hypoxia in subjects with an intracardiac right-to-left shunt (i.e., the PFO+ group). Increasing ventilation and therefore raising PaO2 would increase PaO2 in PFO− subjects and reduce PaCO2 resulting in a left-shifted oxygen-hemoglobin dissociation curve that would facilitate oxygen loading at the lung. In PFO+ subjects, due to the continued presence of right-to-left shunt via the PFO, an increase in ventilation in PFO− subjects would not be as effective in increasing PaO2 and decreasing PaCO2. Therefore, the metabolic demand associated with increasing ventilation would potentially benefit PFO+ subjects to a lesser degree in terms of raising PaO2, compared with PFO− subjects. Furthermore, considering pH and temperature were not different between the PFO− and PFO+ groups, the lower VA in the PFO+ group resulted in a higher PaCO2 and thus a right-shifted oxygen-hemoglobin dissociation curve. Estimating this shift based on the calculated standard p50 values using the Hill equation (29) (Hill coefficient = 2.7), the PFO+ group had a higher standard p50 (29 ± 1 mmHg) compared with the PFO− group (28 ± 1 mmHg) (P = 0.036) at ALT16. This would theoretically facilitate the unloading of O2 at the tissue, which would be beneficial for PFO+ subjects considering their impaired ability to raise PaO2 and thus SaO2, due to right-to-left shunt via the PFO. Nevertheless, this theoretical interpretation should be taken into context with work showing that small changes in the p50 do not influence O2 extraction in skeletal muscle due to the strong influence of the Bohr effect and local temperature (74). Interestingly, in animals with an intracardiac right-to-left shunt and in children with cyanotic congenital heart disease, the presence of a right-shifted oxygen-hemoglobin dissociation curve has also been hypothesized to be a possible compensatory mechanism for facilitating O2 unloading and therefore reducing tissue hypoxia in conditions when increasing ventilation would be ineffective in increasing the PaO2 of the shunted blood (51, 80). Although exercising muscle may not require this right shift to enhance unloading of O2, such a shift may still be beneficial for O2 unloading in the brain and other tissues, which are not as acidic as exercising skeletal muscle, and therefore have less of a Bohr effect on O2 unloading.

**Iso-workload exercise.** During exercise the A-aDO2 is greater in acute hypoxia compared with SL and decreases following acclimatization to high altitude compared with acute hypoxia (4, 9, 12, 45), yet the cause of this subsequent improvement in A-aDO2 remains speculative. Considering the sample sizes of these prior studies (n = 6–10), statistically we would expect each study to have 2–4 PFO+ subjects, and it is unknown to what extent such subjects could potentially have influenced the findings in these previous studies. In the current...
study when the A-aDO2 data from the PFO− and PFO+ groups is pooled, as in these previous studies, pulmonary gas exchange efficiency improves following acclimatization to hypobaric hypoxia (Fig. 4). However, by prospectively identifying PFO− and PFO+ subjects, the current work suggests that this reduction in the A-aDO2 after acclimatization was not present in the PFO+ subjects in our study. As previously discussed, right-to-left blood flow through the PFO would be expected to be intermittent and variable in volume and dependent on a sufficient pressure gradient between the right and left atria. Using PASP as an estimate for this potential pressure gradient, PASP was higher at rest and during exercise at ALT16 in PFO+ and PFO− subjects. Thus there was a potential for greater blood flow across a PFO at ALT16.

In contrast to rest, V\textsubscript{A} and PAO\textsubscript{2} were not different between the PFO− and PFO+ groups during iso-workload exercise at ALT16. It remains unknown why differences in ventilation between the PFO− and PFO+ groups did not persist during exercise, but could be due to differences in the control of ventilation during exercise compared with rest (19, 76). This suggests that contributions from V\textsubscript{A}/Q\textsubscript{˙} inequality and diffusion limitation to the A-aDO2 during exercise may be relatively equal between the PFO− and PFO+ groups. However, while not directly measured, we cannot rule out the possibility that differences between the PFO− and PFO+ groups in terms of V\textsubscript{A}/Q\textsubscript{˙} inequality and diffusion limitation existed. Nevertheless, the intracardiac right-to-left shunt via the PFO in the PFO+ group could have also contributed to the lower PaO\textsubscript{2} in the PFO+ group (Fig. 1E) and therefore contributed to the lack of improvement in A-aDO2 compared with ALT1 and significantly greater A-aDO2 compared with the PFO− group at ALT16 (Fig. 1B). The calculated volume of venous admixture required to account for the difference in A-aDO2 during exercise at ALT16 between the PFO− and PFO+ groups was ~7%. This ~7% difference between the PFO− and PFO+ groups includes all sources of venous admixture, yet this does not preclude the possibility that the intracardiac right-to-left shunt in the PFO+ group was contributing to the lack of improvement in pulmonary gas exchange efficiency expected to occur with acclimatization to high altitude.

Although our exercise data at ALT16 indicate worse pulmonary gas exchange efficiency in the PFO+ group, this did not translate into differences in functional exercise capacity that have been described previously (66). However, neither group had an A-aDO2 > 25 mmHg at this submaximal workload, and therefore it is unlikely that exercise capacity would be limited due to pulmonary gas exchange inefficiency. Alternatively, the lack of functional difference between groups may also be the result of the right-shifted oxygen-hemoglobin dissociation curve that facilitated the unloading of oxygen despite the fact that pulmonary gas exchange efficiency did not improve with acclimatization and ventilatory acclimatization was less than PFO− subjects.

Maximal Exercise

As previously reported (66) \( \tilde{V}O_{2\text{max}} \) or peak workload did not increase at ALT16 compared with ALT1, in both the PFO− and PFO+ groups, despite CaO\textsubscript{2} increasing at ALT16 compared with ALT1. The reason(s) why \( \tilde{V}O_{2\text{max}} \) did not increase with acclimatization remain unknown, but support prior work that also showed no improvement in \( \tilde{V}O_{2\text{max}} \) with acclimatization to high altitude (9, 45, 62, 69, 79). Work by Calbet et al. (9) suggests that through a combination of reduced maximal \( Q_T \) and redistribution of blood flow to noncontracting tissue, the cardiovascular system does not increase \( O_2 \) delivery to the exercising muscles after acclimatization to high altitude, despite an apparent “reserve capacity” for \( O_2 \) delivery. Work in this area is ongoing; e.g., the level of hemoconcentration present after acclimatization has little to no impact on \( Q_T \) or \( \tilde{V}O_{2\text{max}} \) (8, 10). Our study was not designed to specifically investigate this question, yet does provide insight into the area, and we refer the reader to the overview paper from the AltitudeOmics series for further discussion (66).

Limitations

Subjects. Important limitations to the overall study have been previously outlined in detail (66). Several limitations specific to the current work should be highlighted. First, we have previously reported that there was no significant sex vs. time interaction (66). However, in the present study, 2/10 and 7/11 subjects were female in the PFO− and PFO+ groups, respectively. As previously mentioned, no differences were observed between the PFO− and PFO+ groups in SL baseline anthropometric, exercise, or hematological variables. Differences in absolute pulmonary function values appear to be due to the known differences in absolute lung volumes between males and females (26) as pulmonary function data, expressed as percent predicted, were not different between sexes, with the exception of uncorrected DLCO. But, after correcting DLCO for individual alveolar volumes (DLCO/VA), there were no differences in absolute or percent-predicted DLCO/VA. Furthermore, recent work by Duke et al. (15) has shown that in subjects with chronic lung disease, a mildly reduced DLCO does not impact the A-aDO2 at rest or during exercise up to 75% \( \tilde{V}O_{2\text{peak}} \)-breathing room air or 12% \( O_2 \). Additionally, although it has been reported that women may have more severe pulmonary gas exchange inefficiency during exercise (14, 28), these studies have been done in women where the presence or absence of a PFO was unknown. Clearly, studies in women with and without PFO should be completed to determine if sex is a more important determinant affecting the A-aDO2 than the presence of a PFO.

Additional physiological variables that could be influenced by the imbalance in sex include measures of Hb and Hct. Compared with SL, Hb mass increased at ALT16 in 19/21 subjects (61). The two subjects who did not have an increase in Hb mass were PFO+ females (61). Removal of these two PFO+ subjects from the data set results in no differences between the PFO− and PFO+ groups in Hb (P = 0.20) or Hct (P = 0.21) at ALT16. Nevertheless, neither of these measures were primary outcome variables for the current study, and it remains unknown why these two PFO+ female subjects displayed no increase in Hb mass from SL to ALT16.

Methodology. Another limitation to the current work is the methodology used to determine \( Q_T \). Specifically, \( Q_T \) was calculated, as before (67), with heart rate obtained from the ECG and stroke volume estimates derived from intra-arterial blood pressure tracings obtained via a saline-filled pressure transducer positioned at heart level and attached to the radial artery catheter (6, 68). Importantly, although \( Q_T \) is involved in...
calculation of $\dot{Q}_{VA}/\dot{Q}_T$, it is not involved in the quantification of the A-aDO$_2$ or any of the other primary outcome variables, so this potential limitation is restricted to the calculated $\dot{Q}_{VA}/\dot{Q}_T$, which, nevertheless, corresponds well with the measured impact on pulmonary gas exchange.

**Timeline for acclimatization.** Our study design did not permit performing investigations in subjects for longer acclimatization periods and therefore it is unknown if our findings would be attenuated or augmented had the subjects been allowed to live at high altitude for a longer duration. To speculate on this topic is difficult considering the plethora of physiological factors that would need to be taken into consideration. Nevertheless, how pulmonary artery pressure changes with acclimatization in PFO$-$ and PFO$+$ subjects is one particularly important factor. Subjects in the current study did not have an exaggerated pulmonary hypertensive response to acute or chronic hypoxia. However, if pulmonary artery pressure continued to increase during longer periods of acclimatization, blood flow through the right-to-left shunt in PFO$+$ subjects could increase. As previously mentioned, this interesting idea was proposed as an explanation for why subjects with and without PFO are susceptible or resistant to high-altitude pulmonary edema (2). Future work in this area would benefit from longer durations at high altitude and direct measures of pulmonary arterial and left-atrial pressure that were unavailable in the current study.

**Summary**

The current study aimed to assess the impact of a PFO on pulmonary gas exchange efficiency at rest and during exercise at SL, with acute transport to 5,260 m, and after living at 5,260 m for 16 days. We identified an improvement in pulmonary gas exchange efficiency with acclimatization to high-altitude similar to previous investigations; however, this finding was not present in PFO$+$ subjects. The contribution of this right-to-left shunt through the PFO to pulmonary gas exchange efficiency is reduced at altitude and may only partially explain the lack of improvement in pulmonary gas exchange efficiency at ALT16. PFO$+$ subjects demonstrated a less pronounced degree of ventilatory acclimatization to 5,260 m as determined by a significantly greater PaCO$_2$, concomitant with a greater A-aDO$_2$ and lower PaO$_2$ and SaO$_2$. Furthermore, our data at ALT1 suggest the incidence of AMS may be greater in PFO$+$ subjects providing precedence for future work in this area. Although future work needs to corroborate these findings, we speculate that this reduction in ventilatory acclimatization may be beneficial in PFO$+$ subjects by limiting the metabolic cost of hyperventilation, which would not effectively increase PaO$_2$ in the presence of a right-to-left shunt. Ultimately, when O$_2$ loading is hindered by the presence of an intracardiac right-to-left shunt, a more effective strategy may be to ventilate less, resulting in a right-shifted oxygen-hemoglobin dissociation curve that facilitates O$_2$ unloading at the brain and other tissues, which are not as acidic as exercising skeletal muscle, and therefore have less of a Bohr effect on O$_2$ unloading.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


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