Hypoxia-induced intrapulmonary arteriovenous shunting at rest in healthy humans

Steven S. Laurie, Ximeng Yang, Jonathan E. Elliott, Kara M. Beasley, and Andrew T. Lovering

Department of Human Physiology, University of Oregon, Eugene, Oregon

Submitted 11 February 2010; accepted in final form 4 August 2010

Laurie SS, Yang X, Elliott JE, Beasley KM, Lovering AT. Hypoxia-induced intrapulmonary arteriovenous shunting at rest in healthy humans. J Appl Physiol 109: 1072–1079, 2010. First published August 5, 2010; doi:10.1152/japplphysiol.00150.2010.—Intrapulmonary arteriovenous (IPAV) shunting has been shown to occur at rest in some subjects breathing a hypoxic gas mixture [fraction of inspired oxygen (FIO2) = 0.12] for brief periods of time. In the present study we set out to determine if IPAV shunting could be induced at rest in all subjects exposed to hypoxia for 30 min. Twelve subjects (6 women) breathed four levels of hypoxia (FIO2 = 0.16, 0.14, 0.12, and 0.10) for 30 min each in either an ascending or descending order with a 15-min normoxic break between bouts. Saline contrast echocardiography was used to detect IPAV shunt and a shunt score (0–5) was assigned based on contrast in the left ventricle with a shunt score ≥ 2 considered significant. Pulmonary artery systolic pressure (PASP) was determined using Doppler ultrasound. The total number of subjects demonstrating shunt scores ≥ 2 for FIO2 = 0.16, 0.14, 0.12, and 0.10 was 1/12, 7/12, 9/12, and 12/12, respectively. Shunt scores were variable between subjects but significantly greater than normoxia for FIO2 = 0.12 and 0.10. Shunt scores correlated with peripheral measurements of arterial oxygen saturation (SpO2) (r = −0.67) and PASP (r = 0.44), despite an increased shunt score but no increase in PASP while breathing an FIO2 = 0.12. It is unknown how hypoxia induces the opening of IPAV shunts, but these vessels may be controlled via similar mechanisms as systemic vessels that vasodilate in response to hypoxia. Despite intersubject variability our results indicate significant IPAV shunting occurs at rest in all subjects breathing an FIO2 = 0.10 for 30 min.

Hypoxia; altitude; saline contrast echocardiography; pulmonary artery pressure

LARGE-DIAMETER intrapulmonary arteriovenous (IPAV) shunt pathways are recruited during submaximal through maximal exercise as detected by saline contrast echocardiography (12, 21, 35–37). While it was originally suggested that the mechanism opening IPAV shunts may be due to increased pulmonary vascular pressures or flows (12), more recent work using both hypoxia (21) and hyperoxia (23) suggests a role for oxygen tension in the regulation of these inducible IPAV shunt pathways as well.

For example, breathing 100% O2 prevented IPAV shunting during submaximal exercise and prevented or reduced shunt magnitude at maximal exercise in healthy humans (23). Conversely, while IPAV shunting is not detected at rest in normoxia, breathing a hypoxic gas mixture (12% O2) induced shunting in 3/9 subjects at rest (21). Limitations in that study’s design do not allow for generalizable conclusions to be made regarding the effect of hypoxia at rest because only a single level of hypoxia was used and the time subjects breathed hypoxia was not reported. Consequently, it remains unclear if breathing some level of hypoxia could open IPAV shunt pathways in all healthy human subjects and if the stimulus to open these pathways is directly related to the fraction of inspired oxygen (FIO2), the duration of hypoxic exposure, some secondary physiological effect in response to the hypoxic exposure, or some combination of the above.

The purpose of this study was to determine if we could open IPAV shunts in all subjects at rest. We tested the hypothesis that 30 min of breathing an FIO2 = 0.10 at rest would result in shunting in all subjects.

METHODS

The University of Oregon Office for Protection of Human Subjects approved this study, and each subject gave written informed consent before participation. All studies were performed according to the Declaration of Helsinki.

Subjects. Sixteen healthy nonsmoking subjects (8 women) aged 20–40 yr with no history of cardiopulmonary disease volunteered to participate in the study.

Cardiac screening. A registered diagnostic cardiac sonographer with over 20 years experience conducted all ultrasound imaging using a Philips Sonos 7500. An echocardiographic screening was performed to rule out any cardiac abnormalities involving the ventricular outflow tract, valvular function, ventricular function, the great arteries, and the pericardium. There were no signs of ischemia or heart disease in any subjects. Right atrial pressure was estimated to be normal and assigned a value of 5 mmHg in all subjects because a quick inspiration resulted in greater than 50% collapse of the inferior vena cava (20).

A Valsalva-induced patent foramen ovale was revealed during a bubble study by the appearance of saline contrast bubbles in the left ventricle <3 heart beats after the initial appearance of bubbles in the right ventricle in two male subjects during screening, and they were excluded from participation. Additionally, two female subjects had 1–3 bubbles appear in the left ventricle >5 heart beats after the appearance of contrast in the right ventricle suggestive of a possible arteriovenous malformation (AVM) and were therefore excluded from participation. Thus the remaining 12 subjects (6 women) completed the remainder of the study.

Pulmonary function testing. Comprehensive spirometry, including forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), forced midexpiratory flows (FEF25–75%), and peak expiratory flows, was determined using a MedGraphics Ultima PFX computerized spirometry system (St. Paul, MN) according to American Thoracic Society/European Respiratory Society (ATS/ERS) standards (28). Total lung capacity (TLC) was determined using whole body plethysmography with a MedGraphics Plethysmograph 1085D according to ATS/ERS standards (45). Following this test, lung diffusion capacity for carbon monoxide (DLco) was determined using the single-breath, breath-hold technique according to ATS/ERS standards for breath-hold timing (Jones and Meade) and alveolar sample collection (24) (MedGraphics Ultima PFX, Breeze v.6.3.006).

Saline contrast echocardiography. A 22-gauge intravenous catheter with saline lock was placed into a peripheral vein in the arm with...
a three-way stopcock attached to the end. Two additional four-way stopcocks were attached in series with a 10-ml syringe attached to each with 1 ml of air in one and 3 ml of sterile saline in the other. Subjects reclined and rolled slightly onto their left side so that a clear four-chamber apical echocardiogram could be obtained. Saline contrast bubbles were created by manually pushing the two syringe plungers back and forth for 15 s before a forced hand injection of the agitated saline (12, 21).

These peripherally injected saline bubbles are echogenic and appear as a “cloud of echoes” in the right atria and ventricle. Every injection resulted in complete opacification of the right ventricle. With no right-to-left shunting, the pulmonary microcirculation clears these bubbles and no contrast bubbles are visualized in the left atrium or ventricle during the subsequent 20 cardiac cycles (7, 27). Because these subjects were previously screened and it was determined that no bubbles appeared in the left heart at rest in noroxia, IPAV shunting was defined as the appearance of contrast bubbles in the left ventricle after a minimum of three cardiac cycles from the initial appearance of contrast in the right side of the heart (46).

Using a previously published scoring system (23), a 0–5 score was assigned after each injection based on the greatest density and spatial distribution of bubbles in the left ventricle of a single cardiac cycle during the subsequent 20 heart beats. Accordingly, 0 = zero bubbles; 1 = 1–3 bubbles; 2 = 4–12 bubbles; 3 = >12 bubble bolus; 4 = >12 bubbles heterogeneously distributed; and 5 = >12 bubbles homogeneously distributed (23). A shunt score ≥2 was considered significant. To validate the reproducibility of this scoring system in our lab, two independent observers who were blinded to the conditions scored 119 images with shunt scores spanning the 0–5 scale. Every image was assigned the same score by both observers, and therefore we are confident that a single observer could accurately score the remaining images.

Peripheral estimate of oxygen saturation (SpO2) was continuously measured using a Nellcor Oximax N-600 pulse oximeter (Tyco) with forehead sensor (Oximax Max-Fast). These data were continuously fed into the same data acquisition software (Breeze v.6.3.006) that collected breath-by-breath metabolic data from the subject. No direct measurements of arterial oxygen saturation (SaO2) were taken in this study, but we have found a strong correlation between SaO2 (Radiometer, OSM-3, Copenhagen, Denmark) and SpO2 measurements (n = 213) conducted in our own lab (unpublished) across a range of values (SaO2 = 63.5–99.5) observed in this study (y = 1.04x – 5.83, where y = %SaO2, and x = %SpO2, r² = 0.978). A Bland-Altman plot of these data revealed a mean difference of SpO2 – SaO2 of 2.15 with a standard deviation of 1.31; this indicates that our SpO2 measurements slightly overestimate the SaO2 measurements. The slope of the difference between SpO2 and SaO2 vs. the mean of SpO2 and SaO2 was −0.0506 ± 0.01 and was statistically significantly different from zero for the 213 pairs of measurements.

Resting hypoxia: FIO2 = 0.16, 0.14, 0.12, 0.10. Medical grade (USP) nitrogen and air were mixed through a gas blender to create the four levels of hypoxia (FIO2 = 0.16, 0.14, 0.12, 0.10). These hypoxic gas mixtures were administered to subjects in either an ascending or descending order, and a 15-min room air (normoxic) break was provided between each bout. Four subjects completed both ascending and descending orders (separate days), and there was no difference in shunt scores for a given FIO2 between these orders (Friedman’s test, Dunn’s multiple comparison posttest).

Breath-by-breath metabolic data (MedGraphics Ultima PFX, Breeze v.6.3.006), including oxygen consumption (VO2), carbon dioxide production (VCO2), respiratory exchange ratio (RER), minute ventilation (Ve), and end-tidal gas sampling, were collected for 5 min of normoxia and throughout each 30-min bout of hypoxic exposure. Mean alveolar PO2 (PAO2) was calculated from the alveolar gas equation \[ PAO2 = (P_A - 47) \times FIO2 - PACO2 \times (FIO2 - 1 - FIO2)^{RER} \], where barometric pressure (P_A) = 750 Torr and end-tidal CO2 (PETCO2) was substituted for alveolar PCO2 (PACO2) (2). At minute 4 of normoxia, agitated saline contrast was injected and a shunt score assigned. Continuous-wave Doppler ultrasound using color flow imaging was used to measure tricuspid regurgitation peak velocity (v) and this was used to estimate pulmonary artery systolic pressure (PASP) using the simplified Bernoulli equation and an assumed right atrial pressure of 5 mmHg (4v² + 5 mmHg). This technique has been shown to correlate well with direct measurements of PASP (r = 0.89–0.97) (1, 4, 8, 20, 47), with one of these groups (1) showing the mean difference between direct invasive measurements and estimates using Doppler ultrasound to be −0.5 ± 8 mmHg. Heart rate (HR) was continuously monitored from lead II of the ECG. From minutes 5 to 35 subjects breathed the hypoxic gas mixture and had agitated saline contrast injections and cardiac measurements repeated at 5-min intervals for a total of six injections while breathing each level of hypoxia. Thus, for each of the four hypoxic bouts, shunt scores and cardiac measurements were obtained at a total of seven time points and metabolic measurements were averaged over ~20 s from the time of each saline contrast injection.

Statistics. All statistical calculations were made using GraphPad Prism statistical software (v5.0b), except for statistical correlations, which were run using SAS (version 9.2, SAS Institute, 2004). All metabolic, SpO2, and HR measurements represent 20-s averages from the time of saline contrast injection. Mean PASP for all subjects was analyzed using a one-way ANOVA with Bonferroni posttest. Shunt scores were analyzed using a Friedman’s test with Dunn’s multiple comparison posttest. Within-subject, nested correlations (r_w) between shunt score and SpO2 and PASP were analyzed using a mixed-model regression with the data nested within subjects using the initial normoxic time point and all six hypoxic time points for all four levels of hypoxia (n = 300).

RESULTS

Lung function and maximal oxygen uptake. Anthropometric, pulmonary function, maximal oxygen consumption, and diffusion capacity data were within normal limits and are presented in Table 1.

Resting cardiopulmonary measurements. Individual subject shunt scores during initial room air exposure and following 30-min exposure to an FIO2 = 0.16, 0.14, 0.12, and 0.10 are shown in Table 2, with the mean metabolic data of all subjects presented in Table 3.

Contrast echocardiography while breathing hypoxic gas mixture. Representative echocardiograms of all six (0–5) shunt scores are shown in Fig. 1. A shunt score ≥2 was considered significant to indicate the presence of IPAV shunting. Accordingly, the total number of subjects demonstrating shunt scores ≥2 for FIO2 = 0.16, 0.14, 0.12, and 0.10 were 1/12, 7/12, 9/12, and 12/12, respectively. Figure 2 shows the shunt scores for each subject in normoxia and after breathing each level of hypoxia for 30 min. For FIO2 = 0.12 and 0.10, shunt scores were significantly greater than shunt scores in normoxia (Fig. 2). Additionally, for an FIO2 = 0.12 and 0.10, shunt scores were greater after 30-min exposure than after 5-min exposure (Fig. 3).

Oxygen saturation and PASP. SpO2 was plotted against corresponding shunt scores for all subjects during the initial normoxic exposure and all time points during the four hypoxic exposures (n = 300) (Fig. 4). There was no difference in mean PASP between any of the normoxic PASP measurements before each level of hypoxia. SpO2 and shunt score were significantly correlated (r_w = −0.673, P < .001), as were PASP and shunt score (r_w = 0.436, P < .001).
DISCUSSION

The purpose of this study was to determine if breathing hypoxia could open IPAV shunt vessels in all subjects at rest. Four levels of hypoxia (FiO2 = 0.16, 0.14, 0.12, 0.10) were chosen that spanned the level of hypoxia (FiO2 = 0.12) used by Lovering et al. (21) in which 3/9 subjects demonstrated shunt at rest. While that study used a single FiO2 and did not standardize the duration of hypoxic exposure at rest, subjects in the present study breathed each level of hypoxia for 30 min.

We found that at rest after 30 min breathing an FiO2 = 0.10, 100% of subjects displayed IPAV shunting with shunt score ≥ 2. Furthermore, shunt scores were significantly greater for an FiO2 = 0.12 and 0.10 than shunt scores in normoxia.

Methodological considerations. The use of saline contrast echocardiography to detect IPAV shunt has been used during normoxic exercise (12), hypoxic exercise (21), and hyperoxic exercise (23) to differentiate the observed range of bubbles seen from a trivial amount to the near complete opacification of the left ventricle. While this scoring system should not be assumed to represent a certain shunt fraction, it is intended to highlight the variability in bubble density in the left ventricle under different conditions. A variety of qualitative scoring systems have been developed and modified for use with saline contrast echocardiography (3, 32, 39). Thus our use of the present scoring system is not without precedent.

The present study suggests that hypoxia indeed regulates the inducible pulmonary vasculature in a manner opposite to that of the conventional pulmonary circulation. However, because hypoxia has effects at multiple levels systemically, we discuss below some potential mechanisms regulating these inducible shunt pathways.

1) Hypoxic pulmonary vasoconstriction and PASP. It has been known for some time that hypoxia causes pulmonary vasoconstriction (HPV) and that this leads to an increase in pulmonary artery pressure (6, 29, 42). Because the maximum HPV response can take up to 2 h (11), we limited the hypoxic exposures to 30 min and provided 15-min normoxic breaks between hypoxic bouts to limit complete HPV and thus limit the rise of PASP. We are confident that the normoxic breaks sufficiently eliminated any increased pressure due to HPV from the previous hypoxic exposures as there was no difference in PASP between any of the normoxic time points before each hypoxic exposure. Furthermore, there was no difference in PASP between normoxia and 30-min exposure to FiO2 = 0.16, 0.14, or 0.12 despite that 7/12 and 9/12 subjects demonstrated shunt at FiO2 = 0.14 and 0.12, respectively. Thus, even though there was a statistically significant correlation between PASP and shunt score, the effect of hypoxia on IPAV shunting does not appear to be a result of an increased PASP.

2) Hypoxia-induced IPAV shunting: stimulus PO2. The hypoxic environment in the pulmonary system that induces HPV of the arteriole smooth muscle is described by a stimulus oxygen tension that reflects the combination of PAO2 and mixed

Table 1. Anthropometrics, pulmonary function, and \( \dot{V}O_2_{\text{max}} \) data

<table>
<thead>
<tr>
<th>Ages, yr</th>
<th>Men (n = 6)</th>
<th>Women (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.5 ± 8.5</td>
<td>22.3 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>178.9 ± 6.7</td>
<td>167.6 ± 6.2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>75.0 ± 8.3</td>
<td>58.7 ± 4.7</td>
</tr>
<tr>
<td>( \dot{V}O_2_{\text{max}}, \text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1} )</td>
<td>52.7 ± 4.2</td>
<td>50.1 ± 8.2</td>
</tr>
<tr>
<td>FVC, liters</td>
<td>5.5 ± 1.0 (99.2 ± 13.1%)</td>
<td>4.3 ± 0.5 (108.7 ± 11.8%)</td>
</tr>
<tr>
<td>FEV₁, liters</td>
<td>4.3 ± 0.7 (95.2 ± 12.0%)</td>
<td>3.7 ± 0.5 (106.7 ± 16.1%)</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>0.78 ± 0.05 (95.7 ± 12.0%)</td>
<td>0.85 ± 0.07 (98.7 ± 7.3%)</td>
</tr>
<tr>
<td>FEF25–50, l/s</td>
<td>3.6 ± 0.8 (83.0 ± 22.5%)</td>
<td>4.0 ± 1.1 (105.2 ± 28.9%)</td>
</tr>
<tr>
<td>DLCO, ml/min·torr⁻¹</td>
<td>37.8 ± 3.5 (116 ± 2.1%)</td>
<td>27.8 ± 3.7 (104.7 ± 9.9%)</td>
</tr>
</tbody>
</table>

All values are means ± SD. Values in parenthesis are percent predicted. \( \dot{V}O_2_{\text{max}} \), maximal oxygen consumption; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; FEF25–50, forced midexpiratory flow; DLCO, diffusion capacity for carbon monoxide.

Table 2. Shunt scores in each subject during initial room air exposure and following 30-min exposure to each level of hypoxia

<table>
<thead>
<tr>
<th>FiO₂</th>
<th>Subject</th>
<th>0.21</th>
<th>0.16</th>
<th>0.14</th>
<th>0.12*</th>
<th>0.10*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

FiO₂, fraction of inspired oxygen. *Significantly different compared with shunt score breathing FiO₂ = 0.21, \( P < 0.05 \) (Friedman’s test, Dunn’s posttest).
venous PO2 (PvO2) (5, 25). Breathing a hypoxic gas mixture at rest results in a decrease in both the PAO2 as well as PvO2 compared with normoxia (43). If the regulation of the smooth muscle controlling IPAV shunting is regulated by the same stimulus oxygen tension as that inducing HPV, a change in either the PAO2 or PvO2 throughout the 30-min hypoxic exposures could augment the degree of IPAV shunting.

Between 5 min and 30 min exposure to an FIO2 = 0.16 and 0.14, the mean PAO2 fell 2.5 Torr and shunt scores remained unchanged. But for an FIO2 = 0.12 and 0.10, shunt score increased significantly between 5 min and 30 min (P < 0.001) (Fig. 3) and PAO2 fell by 4.7 and 6.2 Torr, respectively. This reduction in PAO2 could stimulate an oxygen sensor located in either the airways or alveoli but will also lead to a reduction in arterial PO2 (PaO2) and PvO2, the latter of which contributes to the stimulus PO2 of the pulmonary vasculature for HPV. While the PAO2 has a greater effect on HPV than the PvO2, the contribution of PvO2 to HPV increases compared with its contribution during normoxia as PAO2 is reduced (25), with individual smooth muscle cells responding to the local PO2 gradient of their environment. Thus, while the PvO2 contributes minimally during rest in normoxia, the FIO2s used in this study span a range that would indicate an increasing contribution by the PvO2 to the vasoreactivity of the pulmonary vasculature as FIO2 decreases. Therefore, it is possible that the hypoxic exposures eliciting IPAV shunting during an FIO2 = 0.12 and 0.10 may span a PO2-dependent threshold located in the pulmonary arterial vasculature that induces IPAV shunting as PvO2 decreases below a critical value.

While we did not measure the PvO2 in this study, Wagner et al. (43) did measure the PvO2 at rest and during exercise at sea level and simulated altitude. At rest in normoxia, the PvO2 is 36 ± 2 Torr and drops during both submaximal normoxic exercise (~120 W) and hypoxic exposure at rest to 19 ± 2

Table 3. Cardiopulmonary measurements during initial room air exposure and following 30-min exposure to each level of hypoxia

<table>
<thead>
<tr>
<th>FIO2</th>
<th>VO2, l/min</th>
<th>VCO2, l/min</th>
<th>RER</th>
<th>VE, l/min</th>
<th>PAO2, mmHg</th>
<th>PtcO2, mmHg</th>
<th>SpO2, %</th>
<th>HR, beats/min</th>
<th>PASP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.21</td>
<td>0.28 ± 0.06</td>
<td>0.28 ± 0.07</td>
<td>0.89 ± 0.12</td>
<td>10.5 ± 1.1</td>
<td>104.7 ± 6.4</td>
<td>38.4 ± 3.0</td>
<td>99.6 ± 1.0</td>
<td>59 ± 13</td>
<td>28.4 ± 4.9</td>
</tr>
<tr>
<td>0.16</td>
<td>0.28 ± 0.07</td>
<td>0.25 ± 0.06</td>
<td>0.89 ± 0.07</td>
<td>11.3 ± 1.5</td>
<td>71.1 ± 5.1</td>
<td>37.5 ± 2.7</td>
<td>95.4 ± 1.9</td>
<td>63 ± 14</td>
<td>31.0 ± 3.6</td>
</tr>
<tr>
<td>0.14</td>
<td>0.29 ± 0.07</td>
<td>0.26 ± 0.06</td>
<td>0.91 ± 0.06</td>
<td>11.7 ± 1.7</td>
<td>58.5 ± 3.9</td>
<td>36.7 ± 2.4</td>
<td>90.9 ± 2.7</td>
<td>64 ± 15</td>
<td>30.0 ± 3.6</td>
</tr>
<tr>
<td>0.12</td>
<td>0.30 ± 0.06</td>
<td>0.27 ± 0.05</td>
<td>0.91 ± 0.05</td>
<td>12.3 ± 1.3</td>
<td>46.5 ± 2.9</td>
<td>34.9 ± 2.4</td>
<td>79.8 ± 4.7</td>
<td>72 ± 17</td>
<td>30.4 ± 5.3</td>
</tr>
<tr>
<td>0.10</td>
<td>0.30 ± 0.05</td>
<td>0.28 ± 0.06</td>
<td>0.93 ± 0.06</td>
<td>13.3 ± 1.2</td>
<td>36.1 ± 2.6</td>
<td>32.2 ± 2.9</td>
<td>65.6 ± 6.8</td>
<td>80 ± 17</td>
<td>33.3 ± 5.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. VO2, oxygen consumption; VCO2, carbon dioxide production; RER, respiratory exchange ratio; VE, minute ventilation; PAO2, alveolar PO2; PtcO2, end-tidal PCO2; SpO2, peripheral estimate of oxygen saturation; HR, heart rate; PASP, pulmonary artery systolic pressure.
Accordingly, previous work that has demonstrated exercise-induced IPAV shunting (12) may reflect changes in the PvP2 and be the result of a similar mechanism as that which occurs during hypoxia-induced IPAV shunting at rest. However, we do not have any data to directly support or refute this idea.

3) Hypoxia-induced IPAV shunting: arterial PO2. It is also possible that despite changes in oxygen tension in the pulmonary vasculature, an oxygen-sensing mechanism outside the pulmonary circulation could play a role in the regulation of IPAV shunt vessels. The stimulation of the peripheral chemoreceptor by PaO2 could somehow be influencing the patency of IPAV shunt vessels via an unknown mechanism. While we did not make direct measurements of blood gases in this study, each subject demonstrated an increase in shunt score as their SpO2 dropped. This led to a significant correlation between shunt score and SpO2, despite variability in SpO2 at shunt onset and variability in SpO2 for any given shunt score (Fig. 4). Just as there exists considerable variability in sensitivity of oxygen sensing by the peripheral chemoreceptor between individuals (18, 40), a similar variability may exist between individuals in the responsiveness of IPAV shunts to hypoxia. Future work should be conducted to determine if the variability in peripheral chemoreceptor sensitivity to hypoxia between individuals explains the variability we see in the regulation of IPAV shunt vessels in response to hypoxia.

4) Possible mechanism for hypoxic pulmonary vasodilation. Interestingly, the opening of IPAV shunt vessels in response to hypoxia represents a vascular response that is opposite to that of the conventional pulmonary vasculature. However, there is precedent for such vascular responses in the pulmonary circulation. Blood flows through a patent ductus arteriosus during fetal development with a relatively hypoxic PO2 of approximately 16–19 Torr (34). Postnatal increases in PO2 lead to inhibition of a delayed rectifier K+ channel inducing membrane depolarization, a subsequent increase in intracellular Ca2+ concentration (38), and thus vasoconstriction of the ductus arteriosus through direct effects on the vascular smooth muscle (14). Once closed, however, the ductus arteriosus becomes the ductus ligamentum and is no longer an inducible vessel.

While this mechanism has been identified in the ductus arteriosus, it is plausible that this type of vascular channel also exists throughout other vascular beds within the pulmonary circulation that induce vasodilation in response to hypoxia, as they exist in other vascular beds throughout the body (9, 15, 30). In our study, the hypoxic exposures may be reducing the PAO2 and PvP2 to a stimulus oxygen tension that directly stimulates the dilation of IPAV shunt vessels via a similar mechanism as that regulating the smooth muscle in the ductus arteriosus or other vasculature such as the coronary or cerebral arteries that dilate in response to hypoxia (9, 15, 30).
Furthermore, the opening of a \(K_{\text{ATP}}\) channel in the isolated ferret lung has been identified to allow \(K^+\) efflux, hyperpolarization, and vasodilation (14). While the levels of hypoxia in this study may not have been low enough to sufficiently lower intracellular ATP concentrations and open these channels in the normal pulmonary vasculature, it has been shown that \(K_{\text{ATP}}\) channels in the myocardium can be activated in hypoxia even when intracellular ATP concentrations do not drop below normoxic controls (10). Thus it is possible that hypoxia opens \(K_{\text{ATP}}\) channels on IPAV shunt vessels resulting in their vasodilation. Because it appears that only a small percentage of the entire cardiac output is diverted through IPAV shunt vessels (22), opening of these vessels may not be sufficient to lower resistance throughout the entire pulmonary circulation due to HPV of the conventional pulmonary vasculature.

Alternatively, it may be that these shunt pathways help to minimize further increases in pulmonary artery pressure that would otherwise occur due to HPV in the absence of these shunt vessels. However, while increases in left atrial pressure recruit the normal pulmonary vasculature for this very reason of limiting increases in pressure, breathing hypoxia at rest does not appear to increase left atrial pressure (17) and is therefore probably not the mechanism recruiting IPAV shunt vessels.

5) Reconciling gas exchange-dependent and anatomic-based techniques. The results of this study clearly indicate a role of oxygen tension in the regulation of IPAV shunts. Recent work by Vogiatzis et al. (41) using the 100% oxygen technique reported that intrapulmonary shunts represent a very small contribution to the worsening of gas exchange during exercise, especially while breathing hypoxia. However, their rationale for such a conclusion did not take into consideration the possibility that breathing 100% oxygen may in fact close inducible pathways that were open during normoxic or hypoxic exercise. Work by Lovering and colleagues recently demonstrated that breathing 100% oxygen does in fact prevent IPAV shunting in all subjects at submaximal exercise (23). Consequently, the assumption by Vogiatzis et al. that IPAV shunting persists equally during exercise while breathing 100% oxygen, normoxic, and hypoxic may not be a valid assumption. Rather, the only right-to-left shunt detected by the 100% oxygen technique would be from noninducible sources such as bronchial and Thebesian venous drainage into the left heart or the presence of a patent foramen ovale. As such, their assumption that the degree of shunt detected using 100% oxygen (0.5%) can be applied to conditions while breathing either normoxic or hypoxic would result in an underestimation of shunt. Therefore, their assertion that there would need to be a “major change in functional anatomy of the lungs” (41) caused by changing the \(FIO_2\) is in fact entirely possible and supported by previous work (23, 26, 31) and our present study.

The transpulmonary passage of saline contrast has not been directly demonstrated to represent a true right-to-left shunt. As such, future research needs to be done to quantify the shunt fraction that occurs from the opening of inducible IPAV pathways, if any, and to determine if the quantified shunt fraction could account for any pulmonary gas exchange impairment by directly measuring the \(P_O_2\) in the arterial and mixed venous blood in subjects breathing hypoxia at rest. While this level of invasiveness was not conducted in the present study, we can calculate estimates of gas exchange efficiency [alveolar-arterial oxygen difference (A-aDO2)] and the venous admixture required to account for the changes in A-aDO2 based on mean \(SpO_2\) values from Table 2. Assuming cardiac output increased relative to heart rate (with a fixed stroke volume) and using our linear transformation to adjust our \(SpO_2\) measurements to probable \(SaO_2\) values, we subsequently used equations by Severinghaus (33) to correct for pH and were therefore able to estimate \(Pao_2\), A-aDO2 and the required venous admixture at each \(FIO_2\). We calculated A-aDO2 values of 5.3, 5.0, 4.3, 6.4, and 6.4 mmHg for the five levels of oxygen (\(FIO_2 = 0.21, 0.16, 0.14, 0.12,\) and 0.10), respectively.

In order for our calculated A-aDO2 values to remain relatively constant with progressively lower \(FIO_2\), venous admixture would have to increase. For the five levels of inspired oxygen (\(FIO_2 = 0.21, 0.16, 0.14, 0.12,\) and 0.10) the respective total venous admixture [including low ventilation/perfusion (V/Q), bronchial and Thebesian drainage, intracardiac, and intrapulmonary shunt] would have needed to be 1.6%, 4.7%, 8.0%, 26.0%, and 40.7% of cardiac output to account for the entire calculated A-aDO2. Interestingly, this study demonstrated that shunt scores increased in parallel with the increases in calculated venous admixture that occurred with decreasing \(FIO_2\). Specifically, at an \(FIO_2 = 0.21, 0.16,\) and 0.14, the calculated venous admixture was relatively small. This is not surprising because diffusion limitation and ventilation/perfusion inequality play a minimal, if any, role in pulmonary gas exchange inefficiency at rest at these levels of hypoxia (16, 43, 44) and we also demonstrated minimal intrapulmonary shunting. However, when subjects breathed an \(FIO_2 = 0.12\), the calculated venous admixture increased to 26.0%. At rest, with this level of hypoxia, ventilation/perfusion inequality remains similar to that in normoxia (16, 43, 44), diffusion limitation can account for half of the A-aDO2 (43) but intrapulmonary shunt scores became significantly greater than in normoxia, paralleling the significant increase in calculated venous admixture. Furthermore, for an \(FIO_2 = 0.10\), the calculated venous admixture necessary to account for a 4.4 mmHg A-aDO2 increased to 40.7%. At this \(FIO_2\), all subjects demonstrated transpulmonary passage of saline contrast bubbles and contributions from ventilation/perfusion inequality and diffusion limitation would be expected to have increasing contributions to pulmonary gas exchange inefficiency at rest (43, 44). It is important to note that we are not suggesting that intrapulmonary shunt represents the entire required venous admixture for the A-aDO2 at each \(FIO_2\). As pointed out above, V/Q heterogeneity and diffusion limitation are involved to some degree. However, the increase in the degree of transpulmonary passage of saline contrast bubbles seen with a decreasing \(FIO_2\) supports the idea that hypoxia-induced intrapulmonary shunt could contribute significantly to the pulmonary gas exchange efficiency that occurs in hypoxia at rest.

Summary. We have shown that exposure to a gas mixture with an \(FIO_2 = 0.10\) at rest opens IPAV shunt pathways in all healthy adult subjects. A rise in PASP does not appear to be recruiting these pathways; rather, the stimulus oxygen tension resulting from both the \(Pao_2\) and \(PvO_2\) may be regulating IPAV shunt vessels via a similar mechanism as that inherent to fetal vessels such as the ductus arteriosus or other systemic vascular beds. Despite the individual variability in the level of hypoxia...
necessary to induce IPAV shunting, it appears oxygen plays an obligatory role in the regulation of IPAV shunt vessels.

ACKNOWLEDGMENTS

We thank Randy Goodman, RDSC, for invaluable expertise in obtaining all echocardiographic images.

GRANTS

This study was funded by Oregon Health and Sciences University Medical Research Foundation Grant 0820 and from the American Physiological Society’s Giles F. Filley Memorial Award for Excellence in Respiratory Physiology and Medicine (A. T. Lovering). Funds for statistical support provided by the Univ. of Oregon office of the vice president for Research.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


