Pulmonary gas exchange efficiency during exercise breathing normoxic and hypoxic gas in adults born very preterm with low diffusion capacity

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Submitted 4 April 2014; accepted in final form 23 June 2014

Duke JW, Elliott JE, Laurie SS, Beasley KM, Mangum TS, Hawn JA, Gladstone IM, Lovering AT. Pulmonary gas exchange efficiency during exercise breathing normoxic and hypoxic gas in adults born very preterm with low diffusion capacity. J Appl Physiol 117: 473–481, 2014. First published June 26, 2014; doi:10.1152/japplphysiol.00307.2014.—Adults with a history of very preterm birth (<32 wk gestational age; PRET) have reduced lung function and significantly lower lung diffusion capacity for carbon monoxide (DLCO) relative to individuals born at term (CONT). Low DLCO may predispose PRET to diffusion limitation during exercise, particularly while breathing hypoxic gas because of a reduced O2 driving gradient and pulmonary capillary transit time. We hypothesized that PRET would have significantly worse pulmonary gas exchange efficiency [i.e., increased alveolar-to-arterial Po2 difference (AaDO2)] during exercise breathing room air or hypoxic gas (FIO2 = 0.12) compared with CONT. To test this hypothesis, we compared the AaDO2 in PRET (n = 13) with a clinically mild reduction in DLCO (72 ± 7% of predicted) and CONT (n = 14) with normal DLCO (105 ± 10% of predicted) pre- and during exercise breathing room air and hypoxic gas. Measurements of temperature-corrected arterial blood gases, and direct measure of O2 saturation (SaO2), were made prior to and during exercise at 25, 50, and 75% of peak oxygen consumption (VO2peak) while breathing room air and hypoxic gas. In addition to DLCO, pulmonary function and exercise capacity were significantly less in PRET. Despite PRET having low DLCO, no differences were observed in the AaDO2 or SaO2 pre- or during exercise breathing room air or hypoxic gas compared with CONT. Although our findings were unexpected, we conclude that reduced pulmonary function and low DLCO resulting from very preterm birth does not cause a measureable reduction in pulmonary gas exchange efficiency.

diffusion limitation; ex-preterms; exercise; hypoxia; pulmonary gas exchange efficiency

very preterm birth (<32 wk gestational age) results in arrested development of the lungs causing abnormal alveologenesis and pulmonary vasculogenesis/angiogenesis (20, 43, 49, 52). The functional consequences of this remain poorly defined, although previous studies have measured a significantly reduced aerobic exercise capacity, lung function, and lung diffusion capacity in ex-preterms compared with those born at full term (2, 16, 26, 31, 39, 40, 54, 62). A mild impairment of pulmonary diffusing capacity for carbon monoxide (DLCO), defined as lower than the lower limit of normal but greater than 60% of predicted (44), is observed in ex-preterms and is suggestive of a reduced alveolar and pulmonary capillary surface area relative to those born at full term, which could theoretically lead to less surface area for gas exchange. For this reason, it has previously been suggested that low DLCO in ex-preterms may predispose these individuals to an impairment in pulmonary gas exchange efficiency during exercise, specifically by increasing the likelihood for incomplete alveolar-end capillary O2 diffusion equilibration, compared with those who were born at full term (12, 16, 39–41).

Pulmonary gas exchange efficiency is defined and quantified by the alveolar-to-arterial Po2 difference (AaDO2) and, in addition to diffusion limitation, can be negatively affected by ventilation to perfusion heterogeneity (VA/Q) and right-to-left shunt (7). VA/Q is considered to be the dominant contributor to the AaDO2 in normoxia, while the contribution from diffusion limitation is likely minimal except during very high workloads (VO2 > 3 l/min) in highly trained athletes (17). Conversely, during exercise breathing hypoxic gas mixtures when alveolar Po2 is significantly reduced compared with room air, the effect of VA/Q and shunt are minimized and as a result, diffusion limitation is suggested to be the primary contributor to the AaDO2 (45, 46, 53, 58, 61).

Lovering et al. (31) directly measured the AaDO2 and demonstrated that pulmonary gas exchange efficiency does not differ between adults with a history of very preterm birth (PRET) and matched controls born at full term (CONT) during exercise breathing room air (31). Although DLCO in that previous study was significantly lower in PRET compared with CONT, the PRET group mean DLCO was still 105 ± 18% of predicted. Therefore, it remains possible that PRET with a clinically mild reduction in DLCO may be predisposed to developing a diffusion limitation during exercise breathing room air compared with CONT with relatively high DLCO. Breathing hypoxic gas (FiO2 = 0.12) at rest and during exercise is known to cause a diffusion limitation and increase the AaDO2 in healthy humans with normal, healthy lungs (55). Accordingly, the combination of exercise in hypoxia in PRET subjects with a low DLCO may be required to sufficiently challenge the pulmonary gas exchange capacity of the lungs of these individuals.

The purpose of this study was to determine if PRET, with low DLCO (~70% of predicted), would have worse pulmonary gas exchange efficiency during exercise breathing room air (normoxia; Eugene, OR: elevation 130 m) and hypoxic gas (FiO2 = 0.12; hypoxia) compared with CONT, with normal DLCO (~105% of predicted). We hypothesized that the AaDO2 would be significantly larger during exercise breathing room air and hypoxic gas in PRET with low DLCO compared with CONT with normal DLCO.
METHODS

The study was approved by the University of Oregon Office of Responsible Conduct of Research. All subjects were informed orally and in writing of the nature of and risks associated with the study and provided written, informed consent before participation. The current study includes some subjects whose data have previously been published by our group (31). Nevertheless, the present study utilized different PRET and CONT group inclusion criteria (specifically low/normal DLCO) to allow us to address questions about DLCO and its impact on pulmonary gas exchange efficiency during exercise breathing room air and hypoxic gas. A total of 47 nonsmoking individuals aged 18–31 yr volunteered for the study. From the initial n = 47 individuals who volunteered to participate in the study, n = 31 were PRET and n = 16 were CONT. We wished to compare PRET individuals with a clinically mild reduction in diffusion capacity to CONT individuals with normal diffusion capacity. A clinically mild diffusion impairment is defined by the American Thoracic/European Respiratory Societies (ATS/ERS) to be a DLCO that is less than the lower limit of normal (LLN) and greater than 60% of predicted, and a moderate reduction in diffusion capacity are individuals with a %predicted DLCO of 40–60% (44). Using two different reference equations (14, 63) and the age and height and the appropriate equation for males and females we calculated the predicted DLCO and LLN. The LLN is defined as the standard error of the estimate for the regression equation used multiplied by the z-score of the 5th percentile (i.e., 3.469 × 1.645 = 5.7 ml·min⁻¹·mmHg⁻¹) (44). This quantity is then subtracted from the calculated DLCO from the prediction equations (14, 63) for each individual and it is this value that is the LLN expected for each individual. For the PRET subjects we included all individuals that had a measured DLCO that was less than the LLN (n = 13). Twelve of the 13 included had mild reduction in diffusion capacity and one had a moderate reduction in diffusion capacity. The remaining PRET subjects (n = 18) had DLCO that was >LLN and were not considered for further analysis. For the CONT subjects we used the same prediction equations discussed above and calculated the %predicted DLCO and included all individuals who attained ≥95% (n = 12). The remaining CONT subjects (n = 4) had a %predicted DLCO that was <95% and were not considered for further analysis. Diagnosis and classification of very preterm birth with and without bronchopulmonary dysplasia (BPD) was determined by a board-certified neonatologist (I. M. Gladstone) using medical records and the criteria outlined in the NICHD/NHLBI/ORD Workshop Summary (27), which has been validated (9). Using these criteria all subjects with BPD were classified as having “New BPD.” Individuals in the PRET group with BPD had either mild (n = 4) or moderate (n = 3) severity BPD based on these criteria (27).

Visit 1

Echocardiographic screening. Subjects underwent a comprehensive echocardiographic screening (Philips Sonos 5500) to rule out cardiac abnormalities. A saline-contrast echocardiography screening was done to detect the presence of a patent foramen ovale (PFO) as we have previously published (11, 31). Briefly, a 20- to 22-gauge intravenous catheter was placed in a superficial vein in the arm for the injection of agitated saline-contrast microbubbles. Microbubbles were created by 10–15 s of agitation of 3 ml of sterile saline and 1 ml of room air. It was determined that subjects had a PFO if microbubbles appeared in the left heart in <3 heartbeats following right heart opacification with or without the release of a Valsalva maneuver (11). Only CONT subjects with a PFO were excluded because we have previously found that a PFO is associated with an increased AaDO2 at rest only (33). Of the n = 13 included in the PRET group, n = 7 had a PFO.

Resting pulmonary function and lung diffusive capacity for CO. Baseline pulmonary function was determined using computerized spirometry (MedGraphics, Ultima PFX, St. Paul, MN) according to standards set forth by the ATS/ERS (38). CONT subjects were required to attain a forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) ≥90% predicted. Lung volumes and capacities were determined using whole body plethysmography (MedGraphics, Elite Series Plethysmograph) according to ATS/ERS standards (34, 59). Lung diffusive capacity for carbon monoxide (DLCO) was determined using the single-breath, breath-hold technique (34). Predicted values for pulmonary function and capacities were obtained using the appropriate predictive equations (14, 18, 63).

Visit 2

V̇O2peak testing. Metabolic rate was determined at rest and during exercise in the forward leaning aerobar position as previously published from our laboratory (31) using a metabolic cart (MedGraphics, Ultima PFX). Subjects performed a progressive cycle ergometer (Excalibur Sport, Lode) exercise test to volitional exhaustion while breathing room air to determine maximal oxygen consumption (V̇O2peak) and maximum power output (W). Subjects breathed room air through a low-resistance two-way nonrebreathing valve (2400 series, Hans Rudolph) for all exercise in Visits 2–3.

Visit 3

Instrumentation. A 20-gauge × 1.75-in. radial artery catheter (Arrow International, Reading, PA) was placed under local anesthesia [1% lidocaine, 2% by volume nitroglycerine (5 mg/ml) to minimize vasospasm] by a cardiologist (J. A. Hawn). A core temperature pill (CorTemp HQ, Palmetto, FL) was ingested for the measurement of core body temperature. Intestinal temperature measured during exercise with a telemetric ingestible pill has been shown to be in good agreement with esophageal temperature and to differ by ~0.5°C with a tendency for temperature measured with the ingestible pill to be higher than esophageal temperature (4). A 20- to 22-gauge intravenous catheter was placed in the opposite arm for the injection of agitated saline contrast for the detection of blood flow through intrapulmonary arteriovenous anastomoses (IPAVA) at rest and during exercise at each intensity breathing room air and hypoxic gas.

Exercise protocol breathing normoxia and hypoxia. Subjects rested for 10–15 min on the bike and then exercised for 3 min each at 25, 50, and 75% of the power output attained at V̇O2peak in normoxia with a 10-min break, breathing room air, between each of the three exercise bouts. Subjects performed exercise while breathing room air (normoxia) first and then repeated the exercise protocol at the same absolute power output as in normoxia, but while breathing a hypoxic gas mixture (FiO2 = 0.12) after resting off the bike for ~45 min while breathing room air. Pulmonary gas exchange efficiency was quantified by the AaDO2, as before (31–33), using temperature-corrected arterial blood gases (Siemens, RAPIDLab 248, Erlangen, Germany). Barometric pressure was measured daily using a solid-state transducer barometer (7400 series Perception II, Davis Instruments). Arterial oxygen saturation (SaO2) was measured with a CO-oximeter (Radiometer, OSM-3, Copenhagen, Denmark). Exercise-induced arterial hypoxemia and poor pulmonary gas exchange efficiency were defined as SaO2 < 95% and/or AaDO2 ≥ 30 Torr during exercise in normoxia, respectively (7). Metabolic and ventilatory data were measured continuously throughout exercise using a metabolic system as has been previously done in our laboratory (10, 31).

Data Analysis

Blood flow through IPAVA was qualitatively scored from 0 to 5 based on the number and spatial distribution of saline bubbles in the left ventricle (i.e., bubble scores), as before (10, 11, 31). A bubble score of “0” indicates no blood flow through IPAVA and a bubble score of “5” indicates a significant volume of blood flow through IPAVA. All statistical analyses were performed using GraphPad Prism statistical software (v5.0d) and alpha was set to equal P = 0.05 a priori. Two-way (2 × 2) ANOVAs were computed to test for...
Table 1. Gestational age and birth weight

<table>
<thead>
<tr>
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<th>PRET, n = 13</th>
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<tbody>
<tr>
<td>Average age, wk</td>
<td>27.8 ± 2.2</td>
</tr>
<tr>
<td>Maximum age, wk</td>
<td>32</td>
</tr>
<tr>
<td>Minimum age, wk</td>
<td>25</td>
</tr>
<tr>
<td>Median age, wk</td>
<td>27</td>
</tr>
<tr>
<td>Average birth weight, kg</td>
<td>1.08 ± 0.43</td>
</tr>
<tr>
<td>Maximum birth weight, kg</td>
<td>2.17</td>
</tr>
<tr>
<td>Minimum birth weight, kg</td>
<td>0.68</td>
</tr>
<tr>
<td>Median birth weight, kg</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Values are means ± SD. Very preterm birth (PRET) group includes n = 7 adults with a history of bronchopulmonary dysplasia (BPD) and n = 6 adults without a history of BPD.

differences between groups on anthropometric, aerobic capacity/peak workload, and pulmonary function variables. This type of analysis was chosen to test for a potential sex effect, in addition to the hypothesized birth status effect, because aerobic capacity and absolute pulmonary function values are known to differ between men and women (18). Separately for normoxia and hypoxia, multiple two-way (2 × 4) mixed-model ANOVAs were computed to determine where and if differences existed between groups during exercise. When a significant omnibus test was observed, Bonferroni adjusted post hoc (t-tests) tests were computed to elucidate significant pairwise differences of interest. Specifically, we tested for differences with respect to preexercise (e.g., 25% vs. preexercise, 50% vs. preexercise, etc.) and between groups at each time point (e.g., CONT vs. PRET preexercise, CONT vs. PRET at 25%, etc.). We did not include both normoxia and hypoxia in the statistical model because we were not interested in testing hypotheses between normoxia and hypoxia; rather we were interested in how groups differed within normoxia or hypoxia. Because bubble scores do not follow a normal distribution, a Mann-Whitney U-test was computed to compare the median bubble scores between PRET and CONT during exercise breathing room air and hypoxic gas at 75% VO$_2$ (i.e., separately for room air and hypoxia).

RESULTS

Birth and Prematurity, Anthropometric, Pulmonary Function, Lung Diffusive Capacity, and VO$_2$peak

Values for gestational age and birth weight for PRET are presented in Table 1. Anthropometric, pulmonary function, and exercise capacity data are presented in Table 2. The PRET group was made up of six women and seven men, and the CONT group was made up of six women and eight men. The PRET group was significantly shorter by 10 cm than the CONT. VO$_2$peak, %predicted VO$_2$peak achieved, peak workload, and %predicted peak workload achieved were all significantly lower in the PRET group. PRET had reduced pulmonary function compared with CONT. Specifically, and by design, DLCO was significantly lower in the PRET group. DLCO %predicted was also significantly lower in PRET compared with CONT (range = 55–81% for PRET and 95–129% for CONT). There was a significant effect of sex on height, mass, peak workload, and absolute values for FVC, FEV$_1$, forced mid-expiratory flow (FEF25–75%), slow vital capacity (SVC), inspiratory capacity (IC), expiratory reserve volume (ERV), FRC, residual volume (RV), total lung capacity (TLC), and DLCO, which was expected (18). However, the effect of sex was not statistically significant when the % predicted values were compared, which is not surprising as the prediction equations take sex into account. Independent of sex, there was a significant effect of birth status on height, and absolute values for VO$_2$peak, peak workload, FVC, FEV$_1$, FEV$_1$/FVC, FEF25–75%, SVC, IC, ERV, FRC, TLC, and DLCO and % predicted values for VO$_2$peak and peak workload. FVC, FEV$_1$, FEV$_1$/FVC, FEF25–75%, SVC, IC, ERV, FRC, TLC, and DLCO. Because of these findings we proceeded with our analyses between PRET and CONT with men and women combined in their respective groups.

Pulmonary Gas Exchange Efficiency During Exercise

Because VO$_2$peak and peak power output were significantly different between CONT and PRET we made comparisons at relative intensities (%VO$_2$peak). Power output was significantly different between groups at all exercise intensities (Table 3). Arterial blood gas and pulmonary gas exchange data are presented in Figs. 1, 2, and 3 and Table 3. During exercise breathing room air the AaDO$_2$ increased significantly during exercise at 50% of VO$_2$peak in PRET and at 75% of VO$_2$peak in both groups, yet there were no differences between PRET and CONT preexercise or during any intensity of exercise. During

Table 2. Anthropometric, VO$_2$peak, resting pulmonary function, and diffusion capacity data

<table>
<thead>
<tr>
<th></th>
<th>CONT, n = 14 (6 women)</th>
<th>PRET, n = 13 (6 women)</th>
</tr>
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<tbody>
<tr>
<td>Age, yr</td>
<td>22 ± 3</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>Height, cm</td>
<td>177 ± 10</td>
<td>168 ± 8*</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>72 ± 12</td>
<td>65 ± 10</td>
</tr>
<tr>
<td>VO$_2$peak, ml·kg$^{-1}$·min$^{-1}$</td>
<td>48 ± 9</td>
<td>35 ± 9*</td>
</tr>
<tr>
<td>(PRET)</td>
<td>(126 ± 18)</td>
<td>(92 ± 14)*</td>
</tr>
<tr>
<td>Peak power output, W</td>
<td>318 ± 81</td>
<td>185 ± 59*</td>
</tr>
<tr>
<td>(CONT)</td>
<td>(149 ± 21)</td>
<td>(96 ± 20)*</td>
</tr>
<tr>
<td>FVC, liters</td>
<td>5.4 ± 1.3</td>
<td>4.3 ± 0.8*</td>
</tr>
<tr>
<td>(PRET)</td>
<td>(106 ± 8)</td>
<td>(96 ± 6)*</td>
</tr>
<tr>
<td>SVC, liters</td>
<td>5.6 ± 1.3</td>
<td>4.2 ± 0.9*</td>
</tr>
<tr>
<td>(CONT)</td>
<td>(111 ± 11)</td>
<td>(94 ± 10)*</td>
</tr>
<tr>
<td>FEV$_1$, liters</td>
<td>4.5 ± 1.0</td>
<td>3.2 ± 0.7*</td>
</tr>
<tr>
<td>(PRET)</td>
<td>(105 ± 9)</td>
<td>(84 ± 14)*</td>
</tr>
<tr>
<td>FEV$_1$/FVC, %</td>
<td>83 ± 6</td>
<td>75.2 ± 9.6*</td>
</tr>
<tr>
<td>(CONT)</td>
<td>(98 ± 6)</td>
<td>(88 ± 11)*</td>
</tr>
<tr>
<td>FEF25–75%, l/s</td>
<td>4.5 ± 1.1</td>
<td>2.8 ± 1.0*</td>
</tr>
<tr>
<td>(PRET)</td>
<td>(100 ± 19)</td>
<td>(67 ± 24)*</td>
</tr>
<tr>
<td>FRC pleth, liters</td>
<td>3.0 ± 0.9</td>
<td>3.0 ± 0.7*</td>
</tr>
<tr>
<td>(CONT)</td>
<td>(115 ± 14)</td>
<td>(102 ± 20)</td>
</tr>
<tr>
<td>IC, liters</td>
<td>3.4 ± 0.9</td>
<td>2.8 ± 0.7*</td>
</tr>
<tr>
<td>(PRET)</td>
<td>(106 ± 13)</td>
<td>(97 ± 16)</td>
</tr>
<tr>
<td>ERV, liters</td>
<td>2.2 ± 0.6</td>
<td>1.5 ± 0.8*</td>
</tr>
<tr>
<td>(CONT)</td>
<td>(119 ± 21)</td>
<td>(90 ± 6)*</td>
</tr>
<tr>
<td>TLC, liters</td>
<td>7.3 ± 1.7</td>
<td>5.7 ± 1.0*</td>
</tr>
<tr>
<td>(PRET)</td>
<td>(110 ± 10)</td>
<td>(99 ± 8)*</td>
</tr>
<tr>
<td>RV, liters</td>
<td>1.6 ± 0.6</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td>(CONT)</td>
<td>(108 ± 26)</td>
<td>(118 ± 45)</td>
</tr>
<tr>
<td>DLCO, ml·min$^{-1}$·Torr$^{-1}$</td>
<td>38.2 ± 8.6</td>
<td>24.5 ± 5.1*</td>
</tr>
<tr>
<td>(PRET)</td>
<td>(105 ± 10)</td>
<td>(72 ± 7)*</td>
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</table>

All values are means ± SD. (Values in parentheses are means ± SD % of predicted.) VO$_2$peak, peak oxygen consumption; FVC, forced vital capacity; SVC, slow vital capacity; FEV$_1$, forced expiratory volume in 1 s; FEF25–75%, forced mid-expiratory flow; FRC pleth, functional residual capacity determined by whole body plethysmography; IC, inspiratory capacity; ERV, expiratory reserve volume; RV, residual volume; TLC, total lung capacity; DLCO, diffusion capacity for carbon monoxide. *P < 0.05, Welch’s t-test compared with full term (CONT).
exercise breathing hypoxic gas the AaDO\textsubscript{2} significantly increased with respect to preexercise in both PRET and CONT at all intensities of exercise. As one would expect, AaDO\textsubscript{2} was significantly related to \( V\text{O}_{2}\text{peak} \) (ml-kg\(^{-1}\)-min\(^{-1}\)) pre- and during exercise breathing room air and hypoxic gas (Fig. 3). The lines represent the least-squares regression line for each group in each condition. The slopes did not differ statistically between PRET and CONT in either condition (\( P > 0.05 \)), but the intercept was significantly greater in PRET in hypoxia (\( P < 0.05 \)). Exercise-induced arterial hypoxemia did not develop at any exercise intensity for either group. SaO\textsubscript{2} during exercise breathing room air significantly decreased relative to preexercise during exercise at 75% of \( V\text{O}_{2}\text{peak} \) in CONT only (99% to 97% SaO\textsubscript{2}). During exercise breathing hypoxic gas, SaO\textsubscript{2} significantly decreased relative to preexercise during exercise at all intensities in both groups, but did not differ between groups preexercise or any exercise intensity.

Arterial PO\textsubscript{2}, PCO\textsubscript{2}, and pH data are presented in Table 3. No differences in arterial O\textsubscript{2} content (CaO\textsubscript{2}), total hemoglobin (Hb), and hematocrit (Hct) existed between groups at any time point while breathing room air or hypoxic gas. Median bubble score did not differ between PRET and CONT during exercise while breathing room air or hypoxic gas.

**Cardiorespiratory Data**

Power output was significantly greater in CONT compared with PRET at all intensities. As expected all cardiopulmonary variables (Table 3) increased relative to preexercise during exercise breathing room air and hypoxic gas in both groups.
DISCUSSION

The purpose of this study was to determine if pulmonary gas exchange efficiency was significantly worse at rest and during exercise breathing room air and hypoxic gas in adults with a history of very preterm birth and a clinically mild impairment in DLCO compared with adults born at full term with normal DLCO. The main finding of the present study was that there was no difference in pulmonary gas exchange efficiency between groups at rest or during exercise while breathing either room air or hypoxic gas.

We developed this study because prior work has suggested that PRET would have a reduced pulmonary gas exchange efficiency because of the reduced lung diffusion capacity observed in this population (12, 16, 39–41). Additionally, our prior work demonstrated no difference in the AaDO2 between PRET and CONT during exercise breathing room air, but this sample included individuals with “normal” (i.e., >100%) DLCO and, therefore, it is possible there was not sufficient group separation to elucidate a difference in AaDO2 between PRET and CONT (31). Therefore, in the present study we used three strategies to create unfavorable conditions for complete O2 diffusion and equilibration. First, individuals performed exercise at 25–75% VO2peak to significantly increase cardiac output to reduce mean red blood cell pulmonary capillary transit time through the lung. This small change in mean red blood cell transit time would have a minimal impact in normoxic conditions, but could have a significant impact in hypoxic conditions when a 0.1–0.3 s reduction could be the difference between complete and incomplete equilibration. Second, individuals breathed hypoxic gas at rest and during exercise to decrease the driving gradient for O2 diffusion. We chose a FiO2 = 0.12 because this reduction in alveolar PO2 to ~50–60 Torr is theoretically sufficient to cause a diffusion limitation at rest and during exercise (55) although still allowing subjects to exercise at the same absolute workload as while breathing room air. Third, we preferentially selected PRET subjects with a clinically mild impairment in DLCO and CONT subjects with normal DLCO. During exercise at altitude or while breathing hypoxic gas, it has been demonstrated, using the multiple inert gas elimination technique in healthy individuals, that diffusion limitation is the main contributor to the AaDO2 (53, 57, 58). Therefore, we expected that PRET subjects in our study would be more susceptible to diffusion impairments. Despite selecting all of these conditions, there was no significant difference in AaDO2 between PRET and CONT. Thus the combination of obstructive lung disease and low DLCO had no functional consequence on pulmonary gas exchange efficiency at rest or during exercise when breathing either room air or hypoxic gas.

![Fig. 1. Values are means ± SD. Alveolar-to-arterial PO2 difference (AaDO2) breathing room air (A) and hypoxic gas (B) pre- and during exercise at 25, 50, and 75% of peak oxygen consumption (VO2peak) in full-term controls (CONT) and adults with a history of very preterm birth (PRET). †P < 0.05 vs. preexercise CONT and PRET.](image1)

![Fig. 2. Values are means ± SD. Oxygen saturation (SaO2) breathing room air (A) and hypoxic gas (B) pre- and during exercise at 25, 50, and 75% of VO2peak in CONT and PRET. †P < 0.05 vs. preexercise CONT only, † †P < 0.05 vs. preexercise CONT and PRET.](image2)
Because there were both men and women in each group, one potential limitation of this study is the sex differences between groups. There were a slightly greater proportion of women in the PRET group compared with the CONT group (46% vs. 43%). Potentially, this could have biased our data in favor of the PRET having a significantly greater AaDO2 for three reasons. First, it has previously been suggested that women have worse pulmonary gas exchange than men (19, 37) and it has been demonstrated that women have a greater incidence of exercise-induced arterial hypoxemia than men (8). However, we did not observe a difference in AaDO2 between the PRET and CONT group and neither developed exercise-induced arterial hypoxemia so the slight difference in the proportion of women in each group had little impact on our findings. Second, we had no difference between groups on tHb, Hct, and CaO2. This is important because the diffusion of CO from alveolar air to blood is dependent upon tHb (47) and with no difference between groups on tHb this cannot be an explanation for the observed difference in DLCO. To ensure that even the minor observed difference in tHb between groups (<1 g/dl) did not impact our group differences we can correct the DLCO % predicted using our measured arterial tHb data and published formulas (34, 36). Making the correction for tHb had no impact on the % predicted in either group and did not narrow the absolute difference of 32% between groups. Third, women are generally shorter than men and have smaller lungs and a smaller alveolar volume; this could have predisposed the PRET group to having a lower DLCO and subsequently a smaller area across which gas exchange can occur. When DLCO is corrected for alveolar volume the CONT still have a significantly greater lung diffusion capacity than the PRET, a finding that is consistent with the previous literature (3). This is an important correction in the current study given the CONT were significantly taller than the PRET, irrespective of sex. One final concern with respect to potential sex differences is that we did not control for ovarian hormone status and this could impact ventilatory chemosensitivity and therefore some of our measures. MacNutt et al. (35) demonstrated that the hypoxic and hypercapnic ventilatory responses did not differ between menstrual cycle phases in women studied throughout a single menstrual cycle. This was true for exercise as well. Therefore, we do not think that including females in the study and not controlling for menstrual cycle phase (i.e., ovarian hormone status) had a significant impact on our results.

To determine why the AaDO2 did not differ between groups we can explore the causes of pulmonary gas exchange inefficiency. The three causes of pulmonary gas exchange inefficiency are 1) right-to-left shunt, 2) V\(\dot{A}/Q\) mismatch, and 3) diffusion limitation (7). During exercise in normoxia the existing literature suggests that the increased AaDO2 is due almost entirely to either V\(\dot{A}/Q\) mismatch or diffusion limitation, depending on the exercise intensity (13, 17, 48, 53). Right-to-left shunt (e.g., intracardiac, intrapulmonary, or postpulmonary) could also contribute to the AaDO2 during exercise in normoxia particularly because of the large difference between mixed venous and arterial Po2. In this scenario, a shunt as small as 1–3% of Q\(T\) could account for up to 20–25 Torr of the AaDO2, as calculated using published equations (30, 60). During exercise at altitude or while breathing hypoxic gas at sea level, the difference between mixed venous and arterial Po2 becomes smaller such that the same magnitude of shunt (1–3% of Q\(T\)) would have a much smaller impact on the AaDO2. Additionally, the contribution of V\(\dot{A}/Q\) mismatch to the AaDO2 during exercise in hypoxia is also less (45, 46, 61). Therefore, diffusion limitation is likely the primary contributor to the AaDO2 at altitude or while breathing hypoxic gas at sea level, especially during exercise. Although at rest and all relative exercise intensities the AaDO2 did not differ between groups in room air or hypoxia, one could examine the rate of the increase in AaDO2 with the increase in V\(\dot{O}_2\) as we have done in Fig. 3. The lines represent the least-squares regression lines for each group in each condition. There was no difference in the slopes between PRET and CONT in either condition. *Significantly greater intercept in PRET.

![Graph](http://jap.physiology.org/)

**Fig. 3.** AaDO2 vs. \(\dot{V}_O_2\) pre- and during exercise breathing room air (A) and hypoxic gas (B). The lines (solid = PRET and dashed = CONT) are least-squares line of best fit in each condition. There was no difference in the slopes between PRET and CONT in either condition. *Significantly greater intercept in PRET.
irrespective of condition. The intercept was significantly greater by ~1 Torr in the PRET in hypoxia which would suggest that at a given \( V_O2 \) the PRET had a significantly greater AA\( O2 \) than the CONT. However, because of the inherent variability in the AA\( O2 \) this ~1 Torr difference in intercepts may not be physiologically or clinically meaningful.

Our study was designed to create unfavorable conditions for complete end-capillary \( O2 \) diffusion equilibration. Despite an experimental paradigm designed to maximize the likelihood of detecting differences in pulmonary gas exchange efficiency resulting from diffusion limitation, we were surprised that PRET and CONT subjects continued to demonstrate no difference in the AA\( O2 \). \( V\alpha/Q\) mismatch and diffusion limitation have long been considered the primary causes for the increased AA\( O2 \) during exercise while breathing normoxic and hypoxic gas. Because we made no direct measures of \( V\alpha/Q \) mismatch or diffusion limitation during exercise in this study, we cannot comment directly on their contribution to the AA\( O2 \) in these study groups. However, with little differences in the AA\( O2 \) between groups we can assume that \( V\alpha/Q \) matching and diffusion limitation were contributing equally to the AA\( O2 \) between PRET and CONT individuals. Nevertheless, we would expect PRET to be more susceptible to \( V\alpha/Q \) mismatch resulting in a larger AA\( O2 \) because of their underdeveloped lungs (50) relative to CONT.

Blood flow through IPAVA did not differ between PRET and CONT during exercise at 75% of \( V_{O2peak} \) while breathing room air and hypoxic gas. Whether or not blood flow through IPAVA acts as a source of shunt is controversial (21), but even if blood flow through these pathways did act as a shunt it would have contributed to the AA\( O2 \) equally in both groups because bubble scores did not significantly differ. This is despite there being \( n = 7 \) PRET with an intracardiac shunt (PFO) that would be expected to contribute to the AA\( O2 \) at rest only (33).

It is conceptually difficult to envisage that a diffusion limitation would exist or develop during exercise in normoxia, especially in individuals who are not elite endurance athletes with excessively large maximal cardiac outputs (i.e., >30 l/min) mainly due to the ~3-fold increase in pulmonary capillary blood volume that occurs during maximal exercise to keep transit time sufficient for complete \( O2 \) equilibration (6, 65). Furthermore, there are data in animals (1, 5, 23, 24) and humans (64) that have suggested that pulmonary gas exchange inefficiency may not be related to pulmonary capillary red blood cell transit times. However, with exercise in hypoxia it is possible that a diffusion limitation could occur because of the reduced \( O2 \) driving gradient and increased cardiac output (i.e., potentially reduced pulmonary capillary red blood cell transit time). This assumption is supported by data obtained using the multiple inert gas elimination technique (13, 53, 57, 58).

PRET subjects in our study had reduced lung function, suggestive of obstructive lung disease and significantly reduced DL\( CO \) relative to CONT that was considered a clinically mild impairment in diffusion capacity, but had normal pulmonary gas exchange efficiency. It may be possible that a ~35% difference in % predicted DL\( CO \) (i.e., diffusion capacity) is not sufficient to predispose someone for developing a diffusion limitation during exercise. Data in >8,000 individuals with various obstructive or restrictive lung diseases support this and demonstrate that the prevalence of significant desaturation during exercise at sea level (decrease of Sp\( O2 \) ~4%) was not significant until DL\( CO \) fell below 62% of predicted (15). However, these data were obtained from a 3-min submaximal stepping protocol and fingertip pulse oximetry so these data would be comparable only to our mild intensity (25% of \( V_{O2peak} \)) data. Using our data, there was no relationship between the AA\( O2 \) during exercise breathing hypoxic gas at 75% of \( V_{O2peak} \) and DL\( CO \) at rest (Fig. 4). Thus it is possible that a mean DL\( CO \) of ~70% of predicted is not sufficient to result in a functional decrease in pulmonary gas exchange efficiency, such that a significant relationship may develop for DL\( CO \) % of predicted less than 70%, but this remains speculative.

There were some small measurement errors that resulted in a few negative AA\( O2 \) values at rest and during low-intensity exercise (7 of 216 total AA\( O2 \)s; 5 at rest, 2 during exercise at 25% \( V_{O2peak} \)). Of the seven negative AA\( O2 \) values we had in our study, all were between 0 and ~3 Torr. Others have previously reported negative AA\( O2 \) values preexercise and during low-intensity exercise (22, 28, 42, 51, 56). There are two possible reasons for the negative AA\( O2 \) values reported in these previous studies and our present study: 1) that resting measurements were, perhaps, not made under true resting conditions because subjects were anticipating exercise, and 2) very small (~1%) measurement errors of \( P_{aCO2} \), \( V_{O2} \), and \( V_{CO2} \) could have a large impact on the calculation of alveolar \( P_{O2} \). Our preexercise measurements were not made under true resting conditions because they were resting on the cycle ergometer anticipating exercise. The HR data in breathing room air and hypoxic gas in Table 2 clearly demonstrate this. Because we cannot be certain exactly where the measurement error occurred and because a negative AA\( O2 \) is physiologically impossible, we removed the negative values from the AA\( O2 \) analyses. There was one negative \( AaDO2 \) at rest in the PRET group in both normoxia and hypoxia and one during exercise at 25% \( V_{O2peak} \) in normoxia, one in the CONT group at rest and one during exercise at 25% of \( V_{O2peak} \) in normoxia, and two in CONT at rest in hypoxia. We used “available case analysis” to handle missing data from a statistical standpoint (29). This method is preferred because it keeps as many samples in the analysis as possible and does not involve

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**Fig. 4.** AA\( O2 \) vs. lung diffusion capacity for carbon monoxide (DL\( CO \))/ventilation (VA). Solid line is the least-squares line of best fit for all data (PRET and CONT combined). The relationship between the AA\( O2 \) during exercise at 75% of \( V_{O2peak} \) while breathing hypoxic gas and DL\( CO \)/VA at rest is not statistically significant \((R^2 = 0.09; P > 0.05)\). Standard error of the estimate (SEE) is 3.3.
missing data imputation, which would alter the true variance of the sample and could result in a type I error.

**Summary and Conclusions**

The purpose of this study was to determine if pulmonary gas exchange efficiency was significantly worse at rest and during exercise breathing room air and hypoxic gas in adults with a history of very preterm birth and a reduced lung diffusion capacity compared with adults born at full term with normal to high lung diffusion capacity for CO. Despite selecting an FiO₂ suggested to cause a diffusion limitation at rest and during exercise (55) and preferentially selecting PRET individuals with a reduced DLCO, we did not measure a significant difference in AaDO₂ between PRET and CONT. There are several conclusions that can be drawn from these data. First, very preterm birth, and presumably arrested lung development, caused a significant reduction in pulmonary function and DLCO, but had no functional effect on pulmonary gas exchange efficiency during exercise breathing room air or hypoxic gas. Second, the significant absolute difference in % predicted DLCO between PRET and CONT was not sufficient to cause a functional decrease in pulmonary gas exchange efficiency during exercise breathing room air or hypoxic gas. Third, although DLCO at rest does not represent lung diffusion capacity for O₂ during exercise, these parameters would increase proportionally during exercise and this has shown to occur in exercising dogs with and without lung resection (25). Nevertheless, a 30% reduction in DLCO had no measureable impact on pulmonary gas exchange efficiency, which supports data in exercising dogs that suggest the lungs have a tremendous ability to increase diffusion capacity during exercise to protect against developing a diffusion limitation (5, 23, 24). How impaired lung function, poor exercise capacity, and reduced lung diffusion in young adults born very preterm will change with increasing age, and the subsequent impact this may have on their pulmonary gas exchange efficiency, remains completely unexplored, yet will be important to understand as this population continues to increase in number and age.

**GRANTS**

This study was supported by American Heart Association Scientist Development Grant 2280238 (A. T. lovering), the American Physiological Society’s Giles F. Filley Memorial Award for Excellence in Respiratory Physiology and Medicine (A. T. Lovering), and the Medical Research Foundation of Oregon Early Clinical Investigator Award (J. W. Duke).

**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


