RESEARCH ARTICLE

Are there sex differences in the capillary blood volume and diffusing capacity response to exercise?

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Bouwsema MM, Tedjasaputra V, Stickland MK. Are there sex differences in the capillary blood volume and diffusing capacity response to exercise? J Appl Physiol 122: 460–469, 2017. First published December 8, 2016; doi:10.1152/japplphysiol.00389.2016.—Previous work suggests that women may exhibit a greater respiratory limitation in exercise compared with height-matched men. Diffusion capacity (DLCO) increases with incremental exercise, and the smaller lungs of women may limit membrane diffusing capacity (Dm) and pulmonary capillary blood volume (Vc) in response to the increased oxygen demand. We hypothesized that women would have lower DLCO, Dm relative to cardiac output (DLCO/QO2), Vc, and pulmonary transit time, secondary to lower Vc at peak exercise. Sixteen women (112 ± 12% predicted relative VO2peak) and sixteen men (118 ± 22% predicted relative VO2peak) were matched for height and weight. Hemoglobin-corrected diffusion capacity (DLCO), Vc, and Dm were determined via the multiple-FIO2 DLCO technique at rest and during incremental exercise up to 90% of VO2peak. Both groups increased DLCO, Vc, and Dm with exercise intensity, but women had 20% lower DLCO (P < 0.001), 18% lower Vc (P = 0.002), and 22% lower Dm (P < 0.001) compared with men across all workloads, and neither group exhibited a plateau in Vc. When expressed relative to alveolar volume (Va), the between-sex difference was eliminated. The drop in DLCO/QO2 was proportionally less in women than men, and mean pulmonary transit time did not drop below 0.3 s in either group. Women demonstrate consistently lower DLCO, Vc, and Dm compared with height-matched men during exercise; however, these differences disappear with correction for lung size. These results suggest that after differences in lung volume are accounted for there is no intrinsic sex difference in DLCO, Vc, or Dm response to exercise.

NEW & NOTEWORTHY Women demonstrate lower diffusion capacity-to-cardiac output ratio (DLCO/QO2), pulmonary capillary blood volume (Vc), and membrane diffusing capacity (Dm) compared with height-matched men during exercise. However, these differences disappear after correction for lung size. These results suggest that after differences in lung volume are accounted for, there is no intrinsic sex difference in DLCO, Vc, or Dm response to exercise.

OXYGEN DIFFUSING CAPACITY (DLCO) increases during exercise to meet the rising oxygen demand (23). Diffusing capacity is influenced by pulmonary capillary blood volume (Vc) and membrane diffusing capacity (Dm); as pulmonary capillaries are recruited and distended during exercise, more alveoli are perfused and thus the overall surface area available for gas exchange increases. A failure to increase surface area in response to the increase in metabolic demand with exercise would result in a diffusion limitation and impairment in gas exchange (23).

There are a number of studies examining sex differences in pulmonary physiology during exercise (4, 5, 7, 9, 22). Previous work suggests that women may develop greater gas exchange impairment during exercise compared with their age- and height-matched male counterparts and thus may be at a higher risk of exercise-induced arterial hypoxemia (EIAH) (7, 9). Women have smaller lungs compared with age- and height-matched men (17) and exhibit lower pulmonary diffusing capacity (DLCO) at rest (20) and during exercise (22) compared with men (24), which might predispose women to gas exchange impairment. Our recent work has shown that men do not exhibit an upper limit of diffusion capacity with incremental exercise (24), but it has been speculated that women may reach an upper limit in diffusion capacity during exercise because of their smaller lungs (17).

The effectiveness of pulmonary vascular recruitment and diffusion during exercise can be evaluated by the ratio of DLCO to cardiac output (DLCO/QO2) (10). As the proportional increase in Q is greater than the increase in DLCO with exercise, DLCO/QO2 declines progressively with exercise intensity (10), and when DLCO/QO2 is reduced below a critical level, diffusion limitation and a gas exchange impairment could develop (11). Should women fail to increase DLCO proportionally to Q, this may explain the greater prevalence of EIAH previously reported in women (9).

Accordingly, the purpose of the present study was to examine the diffusion capacity response to incremental exercise in height-matched men and women. To examine this, this study adapted the Roughton and Forster (1957) multiple-FIO2 DLCO method to determine Vc and Dm at rest and during incremental exercise in men and women (19). We hypothesized that because of the smaller size of their lungs women would have lower Vc and Dm during incremental exercise compared with men and, as a result, have lower DLCO/QO2 and pulmonary transit time (PTT) at peak exercise, secondary to a reduced Vc.

METHODS

Subjects. Sixteen women and sixteen height-matched men were recruited for this study. All subjects gave written informed consent to
participate in the study, which was approved by the Human Research Ethics Board of the University of Alberta. All subjects were nonsmokers, had normal pulmonary function, and were physically active. Subject characteristics and pulmonary function data are presented in Table 1. From the total sample of subjects, two additional subgroups were selected on the criteria of fitness, with one group matched according to absolute VO2peak (l/min) and a second highly fit subgroup matched by percentage of predicted relative VO2peak. Of note, seven of the male subjects studied also participated in a recent investigation by our laboratory examining the effect of aerobic fitness on DLCO, Dm, and Vc (24).

During the midluteal phase, progesterone and estrogen are elevated and thus represent the greatest hormonal difference between men and women (20, 21). Previous work suggests that exercise DLCO is greatest during the midluteal phase compared with the early follicular phase (22). We aimed to maximize the potential hormonal differences between sexes, and thus testing took place during the midluteal phase of the menstrual cycle. Additionally, all female participants were on hormonal contraceptives at the time of data collection, in the form of oral contraceptives or an intrauterine device. As DLCO and Vc fluctuate across the menstrual cycle because of changing hormone levels (22), this criterion reduced the hormone variance between testing days.

Study design overview. Consistent with our previous work (24), subjects initially underwent a full pulmonary function test, followed by an incremental VO2peak test on a cycle ergometer. At least 48 h later, subjects returned to the laboratory for the exercise DLCO sessions. Subjects performed multiple-FIO2 DLCO maneuvers while exercising at power outputs corresponding to 30%, 50%, 70%, and 90% of their previously determined VO2peak, ventilatory threshold (VT), and 25 W above that threshold (VT25). The DLCO sessions were spread out over 2–3 days, and the order of the workloads was randomized.

Preliminary screening. Subjects reported to the laboratory, were cleared for exercise according to the physical activity readiness questionnaire (PAR-Q), and were screened for any cardiopulmonary contraindications or an intrauterine device. As DLCO and Vc fluctuate across the menstrual cycle because of changing hormone levels (22), this criterion reduced the hormone variance between testing days.

Exercise DLCO sessions. No less than 48 h after preliminary testing, subjects returned to the laboratory for further testing. Hemoglobin-corrected lung diffusing capacity for carbon monoxide (DLCO) was determined with the single-breath breath-hold technique (15) at rest and during exercise (Encore V62J Autobox, SensorMedics). Hemoglobin concentration ([Hb]) was measured at the beginning of each session (HemoCue 201+, HemoCue, Angelholm, Sweden), and DLCO was adjusted for [Hb] with the following equation (16):

$$DLCO_{adj} = DLCO \times \frac{10.22 + [Hb]}{1.7 \times [Hb]}$$

DLCO breath holds at three different FIO2 values (0.21, 0.40, 0.60) were performed at each exercise workload, with at least 2 min of washout time between trials. Methane (0.3%) was used in each gas mixture to measure alveolar volume (VA) and determine adequate gas equilibration.

During low-intensity workloads, subjects exercised continuously for a minimum of 2 min to ensure that a steady state was achieved. Steady state was confirmed by a consistent heart rate (<3 beats/min (bpm) change over 1 min). For the high-intensity workloads (i.e., VT25 and 90% of VO2peak), a discontinuous protocol was used such that there was active recovery (2 min) between each DLCO maneuver to limit fatigue. During these exercise trials, DLCO maneuvers were performed once heart rate reached the value consistent with that obtained at the equivalent workload during the graded exercise test. It typically took 2 min to reach the target heart rate.

The orders of the FIO2 and workloads were randomized and completed over 2 or 3 different days to minimize carboxyhemoglobin (HbCO) buildup and subject fatigue. Before data collection, subjects were coached in the proper breath-hold maneuver.

Before each DLCO maneuver, each subject rebreathed five breaths of gas from a Douglas bag at the respective FIO2 to the specific DLCO gas to be used. Subjects were then instructed to inhale to total lung capacity (TLC) and to perform a breath hold for 6 s, avoiding Valsalva or Müllerian maneuvers. During the exhalation, the methane tracing was monitored to ensure that the slope was horizontal, indicating that the test gas was well equilibrated in the lung. The trial was repeated if VA for a trial and/or breath-hold time was not within 5% of previous trials (<±0.3 s). The VA for each individual trial was similar at rest, submaximal exercise, and peak exercise.

Vc and Dm were determined with the equation

$$\frac{1}{DLCO} = \frac{1}{Dm} + \frac{1}{\theta CO \times Vc}$$

Theta (θCO) was calculated from the equation $$1/\theta CO = 0.0058 \times PAO2 + 0.73$$ (19). Alveolar PO2 (PAO2) was calculated with the standard alveolar air equation (26), with measured barometric pressure, water vapor pressure predicted based on body temperature during exercise, and respiratory exchange ratio at each exercise workload. PAO2 was estimated from end-tidal CO2 values (14).

For each workload, the relationships between 1/DLCO and 1/θCO for the three FIO2 values were plotted and a regression equation calculated. The minimum acceptable r2 value was set to 0.95, and DLCO maneuvers were

Table 1. Subject demographics of all men and women

<table>
<thead>
<tr>
<th>All Men</th>
<th>All Women</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Age, yr</td>
<td>26 ± 6.4</td>
<td>22 ± 2.7</td>
</tr>
<tr>
<td>Height, cm</td>
<td>173 ± 3.4</td>
<td>172.5 ± 6.9</td>
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<tr>
<td>Weight, kg</td>
<td>75.3 ± 11.8</td>
<td>67.9 ± 8.7</td>
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<tr>
<td>Resting Dm, ml·min⁻¹·mmHg⁻¹</td>
<td>33.5 ± 1.7</td>
<td>25.1 ± 1.1</td>
</tr>
<tr>
<td>Resting Vc, ml</td>
<td>89.3 ± 17.7</td>
<td>76.0 ± 21.2</td>
</tr>
<tr>
<td>Resting Dm, ml·min⁻¹·mmHg⁻¹</td>
<td>71.4 ± 6.0</td>
<td>49.3 ± 4.8</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>15.2 ± 1.0</td>
<td>12.8 ± 0.6</td>
</tr>
<tr>
<td>TLC, liters</td>
<td>6.94 ± 0.97</td>
<td>6.58 ± 0.56</td>
</tr>
<tr>
<td>% Predicted TLC, liters</td>
<td>100 ± 13</td>
<td>93 ± 9</td>
</tr>
<tr>
<td>FEV1 liters</td>
<td>4.46 ± 0.58</td>
<td>3.54 ± 0.39</td>
</tr>
<tr>
<td>% Predicted FEV1, liters</td>
<td>102 ± 13</td>
<td>93 ± 11</td>
</tr>
<tr>
<td>FVC, liters</td>
<td>5.62 ± 0.71</td>
<td>4.45 ± 0.46</td>
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<tr>
<td>% Predicted FVC, liters</td>
<td>107 ± 12</td>
<td>98 ± 11</td>
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<tr>
<td>FEV1/FVC</td>
<td>0.80 ± 0.06</td>
<td>0.80 ± 0.07</td>
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<tr>
<td>RV, liters</td>
<td>1.22 ± 0.44</td>
<td>1.18 ± 0.33</td>
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<tr>
<td>% Predicted RV, liters</td>
<td>78 ± 26</td>
<td>80 ± 22</td>
</tr>
<tr>
<td>% Predicted V02peak, l/min</td>
<td>119 ± 23</td>
<td>131 ± 18</td>
</tr>
<tr>
<td>% Predicted V02peak, ml·kg⁻¹·min⁻¹</td>
<td>118 ± 22</td>
<td>112 ± 12</td>
</tr>
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</table>

Values are means ± SD. DLCO, pulmonary diffusion capacity for carbon monoxide; Vc, pulmonary capillary blood volume; Dm, pulmonary membrane diffusing capacity; TLC, total lung capacity; FEV1, forced expired volume in 1 s; FVC, forced vital capacity; RV, residual volume; V02peak, peak O2 consumption; SpO2, arterial oxygen saturation. *Significantly different vs. female group (P < 0.05).

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repeated when \( r^2 \) values were outside this range. Values for \( 1/V_c \) (slope) and \( 1/D_m \) (y-intercept) were then determined (19).

As with previous reports (20, 22, 24), we did not correct for \( HbCO \) because subjects were nonsmokers (20), 2 min between \( DLCO \) breath-hold tests has been shown to sufficiently clear CO from the lungs (18a), and a <3% increase in \( HbCO \) does not appreciably affect \( V_c \) and \( D_m \) measurements with this technique (24). There is also evidence that multiple short breath-hold maneuvers (6 s) do not appreciably decrease \( DLCO \) until \( HbCO \) is \( \geq 6\% \) (29), and exercise promotes clearance of CO from the lungs and blood (27).

**Statistical analysis.** For all inferential analyses, the probability of type I error was set at 0.05. Statistical analysis was performed with a two-way repeated-measures ANOVA (SPSS Statistics for Windows, version 22.0, IBM, Armonk, NY) to evaluate between-sex differences (two groups: men, women) in the diffusing capacity response (dependent variables: \( DLCO, V_c, D_m \)) to exercise (7 levels of exercise: rest, 30\%, 50\%, 70\%, VT, VT + 25, 90\% of \( V_O2peak \)). Two preplanned comparison t-tests were performed to determine differences between male and female subjects in \( DLCO, V_c, D_m, \) and \( PTT \) at 70\% and 90\% of \( V_O2peak \), and a Bonferroni correction was used for multiple comparisons. Post hoc power calculations determined that with a sample size of \( n = 16 \) in each group it is possible to detect a 15-ml difference in \( V_c \). To further examine sex differences while accounting for the potential confounding effect of fitness, subgroup analysis compared our most fit men and women (matched according to \% of predicted relative \( V_O2peak \)) (13) as well as women vs. men matched for absolute \( V_O2peak \) (l/min).

**RESULTS**

**All men and women.** All subjects tolerated the study procedures well. Descriptive characteristics of all men and women are reported in Table 1.

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**Fig. 1.** Diffusing capacity, pulmonary capillary blood volume, and membrane diffusing capacity responses to exercise in all male (\( n = 16 \)) and female (\( n = 16 \)) subjects (means ± SE). **Left:** response in relation to absolute \( V_O2 \) (l/min). **Right:** response in relation to \% of \( V_O2peak \). Note that preplanned comparisons were performed only at 70\% and 90\% of \( V_O2peak \). *Significantly different from female group (\( P < 0.025 \)) \( DLCO \), pulmonary diffusion capacity for carbon monoxide; \( V_c \), pulmonary capillary blood volume; \( D_m \), pulmonary membrane diffusing capacity.
Both men and women increased \( \text{DLCO} \), \( \text{Vc} \), and \( \text{Dm} \) with incremental exercise (\( P < 0.001 \)). Compared with women, men had greater \( \text{DLCO} \) (\( P < 0.001 \)), \( \text{Vc} \) (\( P = 0.002 \)), and \( \text{Dm} \) (\( P < 0.001 \)) at rest and during every exercise intensity. As a preplanned comparison, unpaired \( t \)-tests indicated that compared with women, men had significantly greater \( \text{DLCO} \) and \( \text{Vc} \) at 70% of exercise (\( P = <0.001, 0.006 \), respectively) and at 90% of exercise (\( P = 0.006, 0.020 \), respectively). Men had greater \( \text{Dm} \) at 70% of exercise (\( P = 0.005 \)) but not at 90% of exercise (\( P = 0.043 \)). Group values are illustrated in Fig. 1.

When values were normalized by the measured \( \text{VA} \), there was no effect of sex for \( \text{DLCO/VA}, \text{Vc/VA}, \text{and Dm/VA} \) (\( P = 0.928, 0.916, 0.694 \), respectively) between men and women at any workload (Fig. 2). When \( \text{DLCO} \) was expressed relative to \( \dot{Q} \), \( \text{DLCO/}\dot{Q} \) was greater in men at 70% (\( P < 0.001 \)) but not 90% (\( P = 0.073 \)) of \( \dot{V}_{\text{O2peak}} \) (Table 2).

**Highly fit men and women.** The subgroup of highly fit men and women were matched according to their percentage of predicted relative \( \dot{V}_{\text{O2peak}} \) (ml·kg\(^{-1}\)·min\(^{-1}\)) (men 137 \pm 11%; women 138 \pm 25%) and similar percentage of predicted pulmonary function [TLC, forced expired volume in 1 s (FEV1), forced vital capacity (FVC), residual volume (RV)].

Figure 3 illustrates a main effect for sex on \( \text{DLCO}, \text{Vc}, \text{and Dm} \), where highly fit men had higher values in relation to
highly fit women ($P = 0.001, 0.018, 0.002$, respectively). Preplanned comparisons at 70% and 90% of VO$_{2peak}$ showed greater DL$_{CO}$ in highly fit men compared with women ($P = 0.018, 0.025$). There are no significant differences in these workloads with regard to Vc at 70% and 90% of VO$_{2peak}$ ($P = 0.049, 0.222$) or Dm at 70% and 90% of VO$_{2peak}$ ($P = 0.158, 0.072$).

When values were expressed relative to lung size (i.e., V$_A$), there was no effect of sex for DL$_{CO}$/V$A$, Vc/V$A$, and Dm/V$A$ ($P = 0.185, 0.085, 0.979$, respectively) between highly fit men and women at any exercise intensity (Fig. 3). When DL$_{CO}$ was expressed relative to Q, DL$_{CO}$/Q was greater in highly fit men only at rest ($P = 0.011$) (Table 2).

**Men and women matched for absolute VO$_{2peak}$ (l/min).** The subgroup of men and women matched according to their absolute VO$_{2peak}$ were also matched for height (men 172.1 ± 3.4 cm, women 174.5 ± 7.1 cm) and had similar percentages of predicted pulmonary function (TLC, FEV$_1$, FVC, RV).

There was no main effect of sex on exercise DL$_{CO}$, Vc, and Dm ($P = 0.252, 0.460, 0.116$, respectively) (Fig. 4).

When values were expressed relative to V$_A$, there was no difference between the sexes in DL$_{CO}$/V$A$, Vc/V$A$, and Dm/V$A$ ($P = 0.728, 0.871, 0.862$, respectively) (Fig. 4). When DL$_{CO}$ was expressed relative to Q, there was no difference in DL$_{CO}$/Q between men and women at rest or during exercise (Table 2).

**Pulmonary capillary transit time.** Group values are found in Fig. 5. PTT decreased with exercise in all men and women ($P < 0.001$). Women consistently had lower PTT than men ($P = 0.001$); however, the mean PTT did not drop below 0.3 s in either group. PTT in highly fit men and women decreased with exercise ($P < 0.001$), but there was no difference between highly fit men and highly fit women ($P = 0.476$). PTT in men and women matched for absolute VO$_{2peak}$ (l/min) decreased with exercise intensity ($P < 0.001$), but no main effect of sex was observed ($P = 0.151$).

**DISCUSSION**

This study aimed to compare sex differences in the pulmonary capillary blood volume response to incremental exercise.
As expected, women demonstrated consistently lower DLCO, Vc, and Dm during incremental exercise compared with height-matched men. Sex differences were eliminated when the smaller VA in women was accounted for, suggesting that the greater DLCO, Vc, and Dm during exercise in men is explained by greater lung size. Together, these data suggest that the pulmonary capillary blood volume response is proportional to lung size and is adequate to meet individual oxygen demand during exercise.

Pulmonary capillary blood volume. Warren et al. (1991) theorized that Vc might reach an upper limit at high exercise intensities in athletes because of a structural limitation in capillary size and number, despite a continual increase in Q (25). However, as women have smaller lungs, their lungs likely have fewer and smaller capillaries (17), and thus women would have less capacity to increase Vc and DLCO with exercise. However, when men and women are compared, a similar response in absolute Vc with increasing exercise intensity is observed, indicating that pulmonary capillary recruitment and distension in women occurs similarly to men. Of note, the subanalysis of fit men and women (Fig. 3, middle) would suggest that both groups may develop a plateau in Vc with incremental exercise; however, no upper limit on Vc response at peak exercise was previously observed in men regardless of
As the women sampled in the present study would not be considered elite endurance athletes and did not develop EIAH, it would be of interest to examine the Vc and Dm responses to exercise in elite endurance-trained women who develop an impairment in gas exchange with exercise.

Size differences. The relationship between pulmonary vascular recruitment and diffusion during exercise is represented by DLCO/Q (10). It has been suggested that diffusion limitation and gas exchange impairment could develop when DLCO/Q is reduced below a critical level during exercise (11). In the present study, the DLCO-to-Q ratio was less in women compared with men at rest and at moderate exercise but not at peak. Moreover, from rest to peak exercise, women demonstrated a 36% reduction in DLCO/Q compared with a 65% drop in men, whereas the highly fit women and men had similar DLCO/Q responses at rest and throughout exercise. While very few studies have identified a critical DLCO/Q contributing to gas exchange impairment during high-level exercise, there is evidence that DLCO/Q can drop by up to 34% in healthy subjects during submaximal exercise (60 W) without any associated diffusion limitation (12). However, it is acknowledged that subjects in the present study had greater peak oxygen consumption than in previous work (12). While we did not examine women previously known to develop a significant impairment in gas exchange with exercise, these data suggest that the decrease in DLCO/Q in women was unlikely to contribute to gas exchange impairment, and that the drop in DLCO/Q was pro-

Fig. 4. Diffusing capacity, pulmonary capillary blood volume, and membrane diffusing capacity response to exercise, and values corrected for alveolar volume, in male (n = 7) and female (n = 7) subjects matched for absolute VO2 (means ± SE). Left: DLCO, Vc, and Dm in relation to % of VO2peak. Right: DLCO, Vc, and Dm corrected for V̇A in relation to % of VO2peak. Note that preplanned comparisons were performed only at 70% and 90% of VO2peak. *Significantly different from female group (P < 0.025). DLCO, pulmonary diffusion capacity for carbon monoxide; Vc, pulmonary capillary blood volume; Dm, pulmonary membrane diffusing capacity; DLCO/VA, pulmonary diffusion capacity for carbon monoxide divided by alveolar volume; Vc/VA, pulmonary capillary blood volume divided by alveolar volume; Dm/VA, pulmonary membrane diffusing capacity divided by alveolar volume.

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portionally less than that observed in men. This is consistent with previous work by Olfert et al. (2004) demonstrating that the ratio $\text{DLO}_2/\dot{V}Q$ (which estimates the pulmonary end-capillary diffusion equilibrium), as well as estimates of diffusion limitation during normoxic exercise, were not different between men and women matched for height and aerobic fitness (18). Combined with previous work, these data suggest that there is no apparent intrinsic sex difference in the $\text{DLCO}$, $\dot{V}c$, $\dot{D}m$, or $\text{DLCO}/\dot{V}Q$ response to exercise that would contribute to greater gas exchange impairment in these women.

**Pulmonary transit time.** Mean PTT is calculated by expressing capillary blood volume relative to cardiac output ($\dot{V}c/\dot{Q}$). As $\dot{Q}$ increases with exercise more than capillary blood volume recruitment, mean pulmonary capillary transit time declines progressively with exercise. It has been suggested that pulmonary capillary transit time must be at least 0.3–0.4 s to allow for adequate red blood cell oxygenation (2, 26). If $\dot{V}c$ reaches an upper limit during exercise, this would result in a reduction in mean PTT, which may cause gas exchange impairment secondary to diffusion limitation (25). Mean PTT was indeed

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**Fig. 5.** Mean pulmonary transit time responses to exercise (means ± SE). A: all male ($n=16$) and female ($n=16$) subjects. B: highly fit male ($n=7$) and female ($n=7$) subjects. C: male ($n=7$) and female ($n=7$) subjects matched for absolute $\dot{V}O_2\text{peak}$. Left: response in relation to absolute $\dot{V}O_2$ (l/min). Right: response in relation to % of $\dot{V}O_2\text{peak}$. Note that preplanned comparisons were performed only at 70% and 90% of $\dot{V}O_2\text{peak}$. *Significantly different from female group ($P < 0.025$). PTT: pulmonary transit time. Dotted line denotes critical time point for red blood cell oxygenation at 0.3 s.
higher in men compared with women at peak exercise, and a similar response was seen in men and women matched for absolute $\dot{V}O_{2\text{peak}}$. Previous work has shown that highly fit women appear to be at the greatest risk of gas exchange impairment (9). Subgroup analysis of our fit men and women found no sex difference in mean PTT. Furthermore, mean PTT did not drop below 0.3 s (even at peak exercise) in either the highly fit men or the highly fit women. Of note, as the fit men and women sampled would not be considered elite endurance athletes based on $\dot{V}O_{2\text{peak}}$ alternate responses might have been observed in more fit subjects. Zavorsky et al. (2003) have suggested that the pulmonary vasculature adapts to increases in blood volume to preserve PTT (28), while others have suggested that it may not be mean PTT but rather segmental PTT that contributes to pulmonary gas exchange impairment (8). Our results indicate that mean PTT does not drop critically low in either men or women, suggesting that PTT is unlikely to contribute to any between-sex differences in gas exchange.

**Hormonal differences.** Both $Dl_{\text{CO}}$ (20) and $Vc$ (21) are greatest in women at rest during the mid to late luteal phase of the menstrual cycle, when circulating progesterone and estrogen are greatest, suggesting that one or both of these hormones may influence diffusion capacity. Smith et al. (2015) found that $Dl_{\text{CO}}$ during heavy exercise was 10–15% greater during the midluteal phase compared with the early follicular phase, secondary to a 25% increase in $Vc$, without a concurrent change in $Dm$ (22). Importantly, in the studies by Sansores et al. (20) and Smith et al. (22), women had lower $Dl_{\text{CO}}$, $Vc$, and $Dm$ compared with male control subjects regardless of menstrual cycle phase, indicating that variables other than hormones contribute to the sex difference in diffusion capacity. In the present study, female subjects were tested during the midluteal phase of their menstrual cycle, the time point at which $Dl_{\text{CO}}$ and $Vc$ would be highest (20–22). Furthermore, all women recruited were on hormonal contraceptives to increase hormonal stability across testing days. We attempted to maximize $Dl_{\text{CO}}$ and $Vc$ values in women relative to men by testing women on hormonal contraceptives during their luteal phase, when estrogen and progesterone are greatest (and thus when the greatest hormonal difference between men and women is present).

**Considerations.** Methodological limitations relevant to the multiple-breath $Dl_{\text{CO}}$ technique have been discussed in our recent publication (24). Briefly, there is no consensus as to the correct value for $\theta$ in calculating $Vc$ and $Dm$ from $Dl_{\text{CO}}$, as the original method did not provide specific values (19). The present study used $\alpha = 0.0058$ and $\beta = 0.73$ as recommended by Ceridon et al. (2010), where a pH of 8.0 and moderate red cell permeability are assumed (1), which we believe is best for $Vc$ and $Dm$ calculations during exercise and was used in our previous investigation (24).

A 6-s breath-hold time was used in the present study as opposed to the standard 10-s $Dl_{\text{CO}}$ breath hold (15). Previous work has demonstrated identical $Dl_{\text{CO}}$ values from 6-s and 10-s breath holds in healthy subjects (3), and in the present study any changes in $Dl_{\text{CO}}$ would affect men and women similarly. To further improve our $Dl_{\text{CO}}$ methodology, all breath holds were within 0.3 s, serial $VA$ values were reproducible (i.e., <5%), and $Dl_{\text{CO}}$ maneuvers were repeated in the rare event that the $r^2$ value for the $1/Dl_{\text{CO}}$ regression was <0.95. By using this strict methodology, we were able to demonstrate coefficients of variation for $Dl_{\text{CO}}$ (21% $FIO_2$) at rest and exercise of 5.23% and 6.53%, respectively, which is below the ATS reference standard for repeated $Dl_{\text{CO}}$ measurements (15), and low coefficients of variation for $Vc$ and $Dm$ at rest (8.90% and 10.59%, respectively) and during exercise (4.54% and 14.86%, respectively).

As noted above, the female subjects sampled did not develop significant EIAH, as evaluated by $SpO_2$ values (Table 2). It is possible that women who develop gas exchange impairment and subsequently EIAH may have a divergent $Dl_{\text{CO}}$, $Vc$, and $Dm$ response to exercise. Additionally, elite endurance athletes were not specifically recruited; our highly fit group was created from within our existing cohort and should not be considered highly trained endurance athletes. Thus our results might have been different had we recruited women with demonstrated gas exchange impairment with exercise and/or elite endurance athletes.

**Conclusions.** This study examined the effect of sex on the capillary blood volume and diffusion response to exercise. Women demonstrated consistently lower $Dl_{\text{CO}}$, $Vc$, and $Dm$ during incremental exercise compared with height-matched men. However, the sex differences, including those observed in the highly fit subgroup and the subgroup of men and women matched for absolute $\dot{V}O_{2\text{peak}}$, were eliminated when $VA$ was accounted for. Additionally, the PTT and $Dl_{\text{CO}}/Q$ responses to exercise in men and women were similar and unlikely to contribute to any between-sex differences in gas exchange. These findings suggest that $Vc$ and $Dm$ responses in healthy men and women are proportional to lung size and are adequate to meet individual oxygen demand during exercise.

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**

M.M.B., V.T., and M.K.S. conceived and designed research; M.M.B., V.T., and M.K.S. interpreted results of experiments; M.M.B., V.T., and M.K.S. performed experiments; M.M.B., V.T., and M.K.S. analyzed data; M.M.B., V.T., and M.K.S. drafted manuscript; M.M.B., V.T., and M.K.S. prepared figures; M.M.B., V.T., and M.K.S. edited and revised manuscript; M.M.B., V.T., and M.K.S. approved final version of manuscript.

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