Effect of aerobic fitness on capillary blood volume and diffusing membrane capacity responses to exercise

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Key points

- Endurance trained athletes exhibit enhanced cardiovascular function compared to non-athletes, although it is considered that exercise training does not enhance lung structure and function.
- An increased pulmonary capillary blood volume at rest is associated with a higher $\dot{V}_{O_2}\text{max}$.
- In the present study, we compared the diffusion capacity, pulmonary capillary blood volume and diffusing membrane capacity responses to exercise in endurance-trained males compared to non-trained males.
- Exercise diffusion capacity was greater in athletes, secondary to an increased membrane diffusing capacity, and not pulmonary capillary blood volume.
- Endurance-trained athletes appear to have differences within the pulmonary membrane that facilitate the increased O$_2$ demand needed for high-level exercise.

Abstract

Endurance-trained athletes exhibit enhanced cardiovascular function compared to non-athletes, although it is generally accepted that exercise training does not enhance lung structure and function. Recent work has shown that an increased resting pulmonary capillary blood volume ($V_C$) is associated with a higher maximum oxygen consumption ($\dot{V}_{O_2}\text{max}$), although there have been no studies to date examining how aerobic fitness affects the $V_C$ response to exercise. Based on previous work, we hypothesized that endurance-trained athletes will have greater $V_C$ compared to non-athletes during cycling exercise. Fifteen endurance-trained athletes (HI: $\dot{V}_{O_2}\text{max}$ 64.6 $\pm$ 1.8 ml kg$^{-1}$ min$^{-1}$) and 14 non-endurance trained males (LO: $\dot{V}_{O_2}\text{max}$ 45.0 $\pm$ 1.2 ml kg$^{-1}$ min$^{-1}$) were matched for age and height. Haemoglobin-corrected diffusion capacity (DLCO), $V_C$ and diffusing membrane capacity ($D_M$) were determined using the Roughton and Forster (1957) multiple fraction of inspired O$_2$ (F$I_{O_2}$)-DLCO method at baseline and during incremental cycle exercise up to 90% of peak O$_2$ consumption. During exercise, both groups exhibited increases in DLCO, $D_M$ and $V_C$ with exercise intensity. Athletes had a greater DLCO and greater $D_M$ at 80 and 90% of $\dot{V}_{O_2}\text{max}$ compared to non-athletes. However, $V_C$ was not different between groups during exercise. In contrast to our hypothesis, exercise $V_C$ was not greater in endurance-trained subjects compared to controls; rather, the increased DLCO in athletes at peak exercise was secondary to an enhanced $D_M$. These findings suggest that endurance-trained athletes appear to have differences within the pulmonary membrane that facilitate the increased O$_2$ demand needed for high-level exercise.

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Abbreviations  
DLCO, pulmonary diffusion capacity for carbon monoxide; DLO₂, O₂ diffusion in the lung; Dₘ, pulmonary membrane diffusing capacity; F/plain;O₂, fraction of inspired O₂; HI-Fit, endurance-trained athlete subjects; LO-Fit, non-endurance trained subjects; PAP, pulmonary arterial pressure; PAWP, pulmonary arterial wedge pressure; P(ao), partial pressure of alveolar O₂; PVR, pulmonary vascular resistance; PWP, pulmonary wedge pressure; Q, cardiac output; RER, respiratory exchange ratio; TLC, total lung capacity; V(A), alveolar volume; V(C), pulmonary capillary blood volume; V(CO₂), carbon dioxide production; V(O₂), oxygen consumption; V(O₂,max), maximum oxygen consumption.

Introduction

With incremental exercise, pulmonary diffusion capacity (DLCO) must increase with exercise to meet the increased O₂ demand; otherwise, a diffusion limitation may occur, which may lead to an increased alveolar–arterial oxygen difference, and exercise-induced arterial hypoxemia (Stickland et al. 2013). From rest to peak exercise, diffusion capacity, as evaluated by the diffusing capacity for carbon monoxide (DLCO), increases up to 150% (Mostyn et al. 1963; Turino 1963; Warren & Jennings, 1984; Tamhane et al. 2001; Taylor et al. 2014) as a result of increases in pulmonary capillary blood volume (V(C)) and diffusing membrane capacity (Dₘ) (Hsia, 2002). As cardiac output (Q) increases to meet O₂ delivery requirements during incremental exercise, the increase in right ventricular pressure results in an increase in pulmonary arterial pressure (PAP) (Reeves et al. 2005), which in turn increases V(C) through recruitment and distension of the pulmonary capillaries (Reeves & Taylor, 2011). Capillary recruitment results in a concurrent increase in Dₘ because previously unperfused alveoli now receive capillary blood, thereby increasing total surface area for gas exchange. Recruitment and distension of pulmonary capillaries also serve to reduce pulmonary vascular resistance (PVR), thereby attenuating the increase in pulmonary artery pressure with exercise (Stickland et al. 2013; Laughlin et al. 2013).

Endurance-trained athletes exhibit enhanced cardiovascular function compared to non-athletes (Stickland et al. 2006); however, it is generally accepted that exercise training does not affect lung structure and function (Hagberg et al. 1988; Womack et al. 2000; Green et al. 2008). Recent work has shown that individuals with a higher maximum oxygen consumption (V(O₂,max)) have greater resting pulmonary V(C) (Lalande et al. 2012). Combined with data demonstrating that resting pulmonary artery and pulmonary wedge pressures (PAWP) are probably lower in athletes (Levine et al. 1991; Stickland et al. 2006), this suggests that more fit individuals may have a more distensible pulmonary circulation (Laughlin et al. 2013). These findings would suggest that there might be differences in the pulmonary vasculature between endurance trained vs. untrained subjects that may be related to V(O₂,max). Because endurance-trained athletes have a greater V(O₂,max), they would require a greater ability to increase diffusion capacity with exercise to prevent or limit a diffusion limitation and gas exchange impairment. Although it has traditionally been assumed that the pulmonary vasculature is insensitive to exercise training (Johnson et al. 1960; Stickland et al. 2006; Green et al. 2008), and that pulmonary capillary recruitment plateaus at a critical exercise intensity and/or recruitment is not affect by training (Warren et al. 1991), recent work conducted at rest suggests that the exercise response may be different between trained and untrained individuals (Lalonde et al. 2012).

There are a number of studies examining diffusion capacity during exercise (Mostyn et al. 1963; Warren & Jennings, 1984; Tamhane et al. 2001; Taylor et al. 2014). However, none of these studies have looked at exercise above 80% of V(O₂,max), and thus it is not fully understood exactly how V(C) and Dₘ contribute to the increased DLCO during exercise, or how aerobic fitness may modulate this response. We hypothesized that endurance-trained athletes would have an increased DLCO, V(C), and Dₘ compared to non-athletes during exercise. To test this, we adapted the Roughton and Forster (1957) multiple fraction of inspired O₂ (F(İ)O₂)–DLCO technique (Roughton & Forster, 1957) to determine the effect of aerobic fitness on DLCO, V(C), and Dₘ during incremental exercise. If DLCO, and thus V(C) and/or Dₘ, were found to be higher during exercise in highly fit athletes, this would suggest that an element of pulmonary diffusion is enhanced in these individuals, facilitating the increased O₂ demand needed for high-level exercise.

Methods

Ethical approval

All subjects provided their written, informed consent to the study, which was approved by the Human Research Ethics Board of the University of Alberta. The study conformed with the standards set by latest revision of the Declaration of Helsinki.

Fourteen non-endurance-trained males (LO: V̇O₂,max mean ± SD: 45.0 ± 4.4 ml kg⁻¹ min⁻¹) and 15 endurance-trained males (HI: V̇O₂,max: 64.6 ± 6.9 ml kg⁻¹ min⁻¹) volunteered for the present study. All subjects were non-smokers, had normal pulmonary function and had no history of pulmonary or cardiovascular disease.
Subject characteristics and pulmonary function data are presented in Table 1.

**Study design overview**

At a preliminary testing session, subjects underwent an incremental test of $V_{O_{2}}\max$ on a cycle ergometer to determine their aerobic fitness. At least 48 h later, subjects returned to the laboratory for the exercise DLCO sessions. Subjects performed multiple-FI$_2$O$_2$ DLCO manoeuvres when exercising at power outputs corresponding to 30%, 50%, 70%, 80% and 90% of their previously determined $V_{O_{2}}\max$. With three FI$_2$O$_2$ and five exercise intensities, each subject performed a minimum of 15 DLCO manoeuvres. The DLCO sessions were spread out over 3 days, with the order of workloads and the FI$_2$O$_2$ randomized.

**Preliminary testing**

Subjects reported to the laboratory and their completed physical activity readiness questionnaires were screened for any cardiopulmonary disorders and/or medications, and then the subjects completed resting pulmonary function testing. Subjects then performed a test for $V_{O_{2}}\max$ (Encore229 Vmax; SensorMedics, Yorba Linda, CA, USA) using an incremental test to volitional fatigue on a cycle ergometer (Ergoselect II 1200; Ergoline, Blitz, Germany). Initial power output was set to 50 W and the power output was increased by 25 W every 2 min until ventilatory threshold, and each stage above ventilatory threshold was characterized by increments of 25 W every 1 min. Confirmation of $V_{O_{2}}\max$ required that three of four conditions were satisfied: volitional exhaustion; a respiratory exchange ratio (RER) greater than 1.1; increases in oxygen consumption <100 ml min$^{-1}$ with further increase in power output; and reaching age-predicted maximum heart rate. Additionally, $Q_{A}$ was evaluated using impedance cardiography during the incremental exercise test (PhysioFlow; Manatec Biomedical LLC, Ebersviller, France).

**Exercise DLCO sessions**

No less than 48 h after preliminary testing, subjects returned to the laboratory for further testing. Lung diffusing capacity for carbon monoxide (DLCO) was determined using the single-breath breath-hold technique (MacIntyre, 2005) at baseline and during exercise (Encore V62J Autobox; SensorMedics, Yorba Linda, CA, USA). Haemoglobin concentration ([Hb]) was measured at the beginning of each session (HemoCue AB, Angelholm, Sweden) to correct DLCO for [Hb] using the equation (Marrades et al. 2011): DLCO$_{adj}$ = DLCO $\times \frac{10.22 + [Hb]}{1.7 \times [Hb]}$. To calculate $V_{C}$ and $D_M$, DLCO breath holds at three different FI$_2$O$_2$ values (0.21, 0.40, 0.60) were performed during steady-state at each exercise intensity, with 2 min of washout time between trials. Methane (0.3%) was used in each gas mixture to determine alveolar volume ($V_A$) and gas equilibration.

The order of the FI$_2$O$_2$ and exercise intensity was randomized, and completed over three different days, separated by at least 48 h, to minimize COHb build up and fatigue. Prior to data collection, subjects were coached in the proper breath hold manoeuvre. Immediately prior to each DLCO manoeuvre, each subject pre-breathed five breaths of gas from a Douglas bag at the respective FI$_2$O$_2$ to the specific DLCO gas to be used to ensure alveolar PO$_2$ was stable. After pre-breathing, subjects were instructed to inhale to total lung capacity (TLC), and to perform a breath hold for 6 s, avoiding Valsalva or Mullerian manoeuvres. 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 $V_A$ for a trial and/or breath

| Table 1. Subject characteristics and pulmonary function | LO-Fit | HI-Fit |
|--------------------------------------------------------|--------|--------|
| N                                                       | 14     | 15     |
| Mean                                                                 |
| Age (years)                                             | 26.1 ± 1.8 |
| Height (m)                                              | 1.77 ± 0.02 |
| Mass (kg)                                               | 85.5 ± 4.9 |
| $V_{O_{2}}\max$ (l min$^{-1}$)                          | 3.81 ± 0.18 |
| $V_{O_{2}}\max$ (ml kg$^{-1}$ min$^{-1}$)               | 45.0 ± 1.2 |
| TLC (litres)                                            | 6.99 ± 0.24 |
| FEV$_1$ (litres)                                        | 4.38 ± 0.14 |
| FEV$_1$/FVC (%)                                         | 5.56 ± 0.17 |
| FEF$_{25-75}$ (%)                                       | 78.9 ± 1.4 |
| FEV$_{1}$/FVC (%) for 1 s                               | 113.4 ± 5.6 |
| FEV$_{1}$/FVC (%) for 2 s                               | 99.0 ± 3.1 |
| FEV$_{1}$/FVC (%) for 3 s                               | 105.3 ± 2.6 |
| FEV$_{1}$/FVC (%) for 4 s                               | 96.6 ± 2.8 |
| FEV$_{1}$/FVC (%) for 5 s                               | 102.0 ± 2.7 |
| FEV$_{1}$/FVC (%) for 6 s                               | 95.4 ± 1.6 |
| FEV$_{1}$/FVC (%) for 7 s                               | 113.4 ± 5.6 |
| FEV$_{1}$/FVC (%) for 8 s                               | 113.4 ± 5.6 |
| FEV$_{1}$/FVC (%) for 9 s                               | 113.4 ± 5.6 |
| FEV$_{1}$/FVC (%) for 10 s                              | 113.4 ± 5.6 |
| FEV$_{1}$/FVC (%) for 11 s                              | 113.4 ± 5.6 |
| FEV$_{1}$/FVC (%) for 12 s                              | 113.4 ± 5.6 |
| FEV$_{1}$/FVC (%) for 13 s                              | 113.4 ± 5.6 |
| FEV$_{1}$/FVC (%) for 14 s                              | 113.4 ± 5.6 |
| FEV$_{1}$/FVC (%) for 15 s                              | 113.4 ± 5.6 |
| Mean % Predicted                                        | 78.9 ± 1.4 |
| % Predicted                                             | 113.4 ± 5.6 |

Values are expressed as the mean ± SE. FEV$_{1}$, forced expired volume in 1 s; FVC, forced vital capacity. *Significantly greater than the LO-Fit group ($P < 0.05$).
hold time was not within 5% of previous trials. The $V_A$ for each individual trial was similar at baseline, submaximal exercise and peak exercise (Table 2).

Pulmonary $V_C$ and $D_M$ were determined using the Roughton and Forster (1957) multiple-$F$LO DLCO breath-hold technique with the equation: $1/DLCO = 1/D_M + 1/\theta_CO \times V_C$ and $\theta_CO$ (i.e. the reaction rate of CO with haemoglobin) was calculated using the equation: $1/\theta_CO = 0.0058 \times P_{aCO} + 0.73$ (Roughton & Forster, 1957). $P_{aCO}$ was calculated from the equation: $P_{aCO} = F_{O_2}(P_{bar} - P_{H_2O}) - P_{aCO} \times (1 - F_{O_2})/RER$.

As with previous studies (Sansores et al. 1995; Smith et al. 2015), we did not correct for carboxyhaemoglobin because subjects were non-smokers (Sansores et al. 1995; West, 2012) and 2 min between DLCO breath-hold tests was shown to be sufficient to clear CO from the lungs (Blakemore et al. 1957). Also, there is evidence that multiple short breath hold manoeuvres (~5 s) do not appreciably decrease DLCO until COHb is greater than 6% (Zavorsky, 2013). Finally, exercise promotes clearance of CO from the lungs and blood (Zavorsky et al. 2012).

Partial pressure of arterial CO$_2$ ($P_{aCO_2}$) was estimated from end-tidal CO$_2$ values. For each workload, the relationship between $1/DLCO$ and $1/\theta_CO$ for the three $F_{O_2}$ values were plotted, and a regression equation was calculated. The minimum acceptable $r^2$ value was set to 0.95, and DLCO manoeuvres were repeated when $r^2$ values were outside of this range. Values for $1/V_C$ (slope) and $1/D_M$ (y-intercept) were then determined (Roughton & Forster, 1957).

### Statistical analysis

For all inferential analyses, the probability of a type I error was set at 0.05. Group data for each variable are expressed as the mean ± SE unless otherwise indicated. Statistical analysis was performed using two-way repeated measures ANOVA (SigmaPlot, version 11.1; Systat Software, Inc., San Jose, CA, USA) to evaluate the effect of aerobic fitness (HI-Fit vs. LO-Fit), on the diffusion capacity response (dependent variables: DLCO, $D_M$, $V_C$) to exercise (six levels of exercise: baseline, 30%, 50%, 70%, 80% and 90% of $V_{O_2,max}$). Where there were main effects of exercise intensity, Fisher’s least significant difference was performed to determine whether there was a plateau effect. Finally, three pre-planned comparison $t$ tests were performed as post hoc tests to determine differences between HI- and LO-Fit subjects in DLCO, $D_M$ and $V_C$ at baseline, 70% and 90% of $V_{O_2,max}$.

### Results

All subjects tolerated the study phases and procedures well. Descriptive characteristics of all participants are provided in Table 1.

### Effect of fitness on exercise DLCO

At baseline, DLCO was higher in the HI-Fit group compared to the LO-Fit group ($P = 0.047$). With incremental exercise, both LO- and HI-Fit subjects exhibited an increased DLCO with increasing oxygen
Pulmonary diffusion capacity is enhanced during exercise in athletes

Pulmonary VC

At baseline, VC was higher in the HI-Fit group compared to the LO-Fit group (P = 0.005). With incremental exercise (Fig. 2), both LO- and HI-Fit subjects increased VC with increasing oxygen consumption (P < 0.001), although there was no statistical difference between groups during exercise (P = 0.498). When VC was expressed relative to Q, VC/Q was greater in HI-Fit at baseline (P = 0.002) but not at 70% (P = 0.403) or 90% (P = 0.229) of VO2max (Table 2).

There was a significant, positive correlation between baseline pulmonary VC and individual VO2max (r = 0.509, P = 0.004) (Fig. 3A), although pulmonary VC at the 90% workload was not correlated with individual VO2max (r = 0.012, P = 0.949) (Fig. 3B). When plotted against peak Q, both baseline VC (r = 0.573, P = 0.001) (Fig. 4A) and peak VC were significantly correlated with peak Q (r = 0.368, P = 0.049) (Fig. 4B).

Membrane DM

At baseline, DM was not different between HI-Fit and LO-Fit groups (P = 0.83). With incremental exercise, both LO- and HI-Fit subjects increased DM with increasing oxygen consumption (P < 0.001). During exercise at 70% and 90% of VO2max, HI-Fit subjects had a greater DM compared to LO-Fit subjects (Fig. 5). Likewise, DM/Q was greater at peak exercise in HI-Fit as compared to LO-Fit subjects. Both groups exhibited a plateau in their respective DM responses to exercise, with HI-Fit showing no significant increase in DM above 50% of VO2max (P = 0.582) and, in LO-Fit, after 30% of VO2max (P = 0.655). When DM was expressed relative to Q, DM/Q was not different at baseline (P = 0.320) or 70% (P = 0.401), although DM/Q was greater in the HI-Fit group at 90% (P = 0.048) of VO2max (Table 2).

Unlike VC, baseline DM was not correlated with VO2max (r = 0.011, P = 0.951) (Fig. 3C); however, DM at the 90% workload was correlated with VO2max (r = 0.425, P = 0.021) (Fig. 3D). Baseline DM was not correlated with peak Q (r = 0.193, P = 0.315) (Fig. 4C); however, peak DM was significantly correlated with peak Q (r = 0.378, P = 0.043) (Fig. 4D).

Discussion

The present study examined the effect of aerobic fitness on DLCO, pulmonary VC, and DM response during

Figure 1. Diffusing capacity response to exercise

*DLCO was greater in HI-Fit subjects at baseline (P = 0.047), at 70% (P = 0.028) and at 90% of VO2max (P = 0.013) compared to LO-Fit subjects.
exercise. Consistent with previous work, the pulmonary $V_c$ in athletes was higher than in non-athletes at baseline, and $V_c$ was related to $\dot{V}_{O_2,\text{max}}$ (Lalande et al. 2012). However, in contrast to our hypothesis, we found that the greater exercise DLCO in endurance-trained athletes is not secondary to an increased pulmonary $V_c$ but rather to an increased $D_M$. These data suggest that endurance-trained athletes appear to have differences within the alveolar-capillary membrane that facilitate the increased $O_2$ delivery needed at peak exercise.

**Diffusion capacity during exercise**

In the present study, we found that athletes have a greater DLCO at baseline and during exercise compared to non-athletes. Aside from pulmonary $V_c$ and $D_M$, diffusion capacity is affected by [Hb] and $V_A$ (Roughton & Forster, 1957; Hlastala et al. 1976; Rose et al. 1979). To determine the difference in $V_c$ and $D_M$ between athletes and non-athletes, we aimed to minimize these confounding factors by correcting DLCO for [Hb] (Marrades et al. 2016).
2011) and $V_A$. Interestingly, despite being matched for height, the HI-Fit group had greater $V_A$ and the between-group differences in DLCO disappeared when expressed relative to $V_A$, suggesting that the enhanced DLCO in endurance-trained athletes stems from a larger alveolar volume (Table 1). This is consistent with work in high-level swimmers showing an increased $V_A$ compared to control subjects (Armour et al. 1993). Importantly, however, peak $D_M$ remains greater in the HI-Fit subjects when correcting for $V_A$ (Table 2), indicating that the differences in membrane diffusion are independent of alveolar volume.

**Pulmonary $V_C$**

The increase in diffusion capacity with exercise results from increases in gas exchange surface area, with increasing $D_M$ and pulmonary $V_C$, secondary to recruitment and distension of capillaries (Johnson et al. 1960). It was previously theorized that pulmonary $V_C$
may plateau despite an increasing $Q$ because of a morphological limitation of pulmonary $V_c$ expansion, resulting in a decreased pulmonary capillary transit time with increasing exercise intensity, which may predispose athletes to a diffusion limitation (Warren et al. 1991). In the present study, $V_c$ was not significantly different between HI- or LO-Fit subjects with incremental exercise, and there was no apparent plateau in $V_c$ in either group. The lack of plateau in $V_c$ is consistent with previous work in humans (Mostyn et al. 1963; Warren et al. 1991; Johnson & Hsia, 1994), and animals (Wagner & Latham, 1975; Hsia et al. 1993, 1994) showing a lack of plateau in DLCO up to 80% of $V_{O_{2,\text{max}}}$, and suggests that reserves for diffusion and $V_c$ are substantial and not completely exhausted with maximal exercise (Johnson et al. 2010).

**$D_M$**

Fick’s first Law of Diffusion (1855) states that diffusion of gas across a membrane is determined by: (1) surface area; (2) thickness of the membrane; (3) difference in partial pressure across the membrane; and (4) the diffusing property of the gas (Fick, 1855). In the present study, we observed an increase in $D_M$ in both HI- and LO-Fit groups during incremental exercise. $D_M$ reflects the available alveolar-capillary surface area for gas exchange (Hsia, 2002; Laughlin et al. 2013) and is increased by unfolding and distension of alveolar septa during lung inflation, and recruitment of capillaries associated with previously un-perfused alveoli (Hsia, 2002). $D_M$ was greater during exercise in HI-Fit participants compared to LO-Fit participants, with the greatest difference seen at maximal exercise. There are a number of possible reasons that may explain why HI-Fit participants are able to increase their $D_M$ more than LO-Fit participants at high levels of exercise. Alveolar volume can affect $D_M$ (Gehr et al. 1978; Colebatch & Ng, 1992); however, $D_M/V_A$ at peak exercise was still greater in HI-Fit participants (Table 2), suggesting that there may be a morphological difference in the lungs of HI-Fit athletes beyond having larger lungs that contributes to the increased $D_M$. Because $D_M$ cannot be measured in un-perfused alveoli, this may suggest that $D_M$ represents pulmonary capillary recruitment (Lewis et al. 1958; Johnson & Hsia, 1994; Lalande et al. 2012; Bartesaghi et al. 2014) and that capillary recruitment may be greater in endurance-trained athletes.

As previously mentioned, the increase in pulmonary artery pressure and $Q$ during exercise recruits previously unperfused capillaries, thereby increasing membrane conductance (i.e. $D_M$). Computational modelling would suggest that the increase in $Q$ with exercise also improves regional erythrocyte spacing (haematocrit), which may account for 30–50% of the increase in $D_M$ from rest to peak exercise (Hsia et al. 1999; Hsia, 2002). Therefore, $Q$ may play a role in the increased $D_M$ in HI-Fit subjects. As conceptualized by Hsia et al. (1992), the effectiveness of pulmonary vascular recruitment and diffusion during exercise can be evaluated by the ratio of DLCO/$Q$. The DLCO/$Q$ ratio progressively declines with incremental exercise, and would contribute to gas exchange impairment once DLCO/$Q$ drops below a critical value (Hsia, 2002). By extension, evaluating $D_M$ and $V_c$ relative to $Q$ would normalize these variables for the greater $Q$ in HI-Fit subjects. The current data show that DLCO/$Q$ and $V_c/Q$ are similar between HI- vs. LO-Fit subjects during exercise; however, $D_M/Q$ was greater in HI-Fit subjects at peak exercise (Table 2). These results demonstrate that the increased $D_M$ observed in HI-Fit at peak exercise is not explained by a greater $Q$.

It is accepted that $V_{O_{2,\text{max}}}$ in healthy subjects is limited by $O_2$ availability (Wagner, 1996a). Although $Q$ is predominant in $O_2$ delivery, a theoretical analysis of the factors that determine $V_{O_{2,\text{max}}}$ found that $O_2$ diffusion in the lung (DLCO/$Q$) was as influential as $Q$ in determining $V_{O_{2,\text{max}}}$ (Wagner, 1996a, 1996b). Based on the principle of mass-balance for $O_2$ exchange, highly fit individuals with a high $V_{O_{2,\text{max}}}$ require a greater DLCO compared to less fit individuals (Wagner, 1996a). A failure to increase DLCO relative to the increased $O_2$ demand during exercise would result in a gas exchange impairment (Stickland et al. 2013). In this context, the finding that highly fit athletes have an increased pulmonary membrane diffusing capacity compared to less fit subjects (Fig. 5), and that $D_M$ at peak exercise is correlated with $V_{O_{2,\text{max}}}$ (Fig. 3D), is entirely consistent with the importance of $O_2$ supply relative to $O_2$ delivery in the determination of $V_{O_{2,\text{max}}}$ (Wagner, 1996a).

**Pulmonary vascular adaptation in athletes**

The initial increase in pulmonary artery pressure with exercise is considered to recruit and distend pulmonary capillaries previously under-perfused at rest (Johnson & Hsia, 1994; Hsia, 2002; Reeves & Taylor, 2011), increasing the cross-sectional area of the pulmonary capillary network, reducing pulmonary vascular resistance and increasing $V_c$ (Short et al. 1996; La Gerche et al. 2010; Reeves & Taylor, 2011; Lalande et al. 2012). Although the individual contribution of capillary recruitment or distension to the overall increase in $V_c$ has yet to be determined (Staub et al. 1962; Reeves et al. 2005), a greater resting $V_c$ has been attributed to an enhanced pulmonary vascular distensibility and, subsequently, a higher $V_{O_{2,\text{max}}}$ (Lalande et al. 2012). There is evidence that athletes have the same or lower left arterial pressure (pulmonary wedge pressure; PWP) compared to non-athletes at rest and during exercise, as well as similar or lower PAP (Levine et al. 1991; Stickland et al. 2006). Pressure within the pulmonary
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V at similar...et al. 2010; Smith et al. because exposure to high O2 were greater in HI-Fit athletes during exercise. Regardless of the assumptions...event, the shortened breath-hold time would probably not have affected between-group differences. The American Thoracic Society (1995) guidelines for DLCO measurements recommend a 10 s single breath-hold technique. The present study employed a 6 s single breath hold because our subjects had difficulty with relatively long breath holds during high intensity exercise, and thus it is possible that our measurement of DLCO may be underestimated. However, a 6 s breath hold has not been shown to affect single breath DLCO measurement in healthy individuals (Graham et al. 1985). Importantly, all participants in the present study followed the same technique, and thus the shortened breath-hold time would probably not have affected between-group differences.

Every 1% increase in CO backpressure (evaluated by % COHb) would diminish DLCO by 1% (Cotes et al. 1972; Graham et al. 2002). However, we did not adjust for CO backpressure, consistent with other reports in the literature (Sansores et al. 1995; Smith et al. 2015). We conducted additional pilot work and determined that COHb increases less than 3% over the course of a typical study day, whereby subjects perform six DLCO manoeuvres during exercise. Based on these results, as well as previous studies (Graham et al. 2002; Ceridon et al. 2010), the resultant effect on VC and DM calculation is less than 3% and probably inconsequential.
Although we corrected DLCO for baseline (resting) [Hb] (Marrades et al. 2011), there are reports suggesting that exercise causes an increase in [Hb], with the increase similar in both sedentary and endurance trained subjects (Martin et al. 1985). In pilot work, we found a 1.6% increase in [Hb] with exercise, which, if unaccounted for, would underestimate $D_V$ and $V_C$ by 3% and 5%, respectively. Importantly, there is no evidence that the [Hb] response to exercise is different in trained individuals (Martin et al. 1985).

The present study was designed to minimize the potential effect of increasing COHb and exercise [Hb] on DLCO, $V_C$, and $D_M$ measurements: (1) the order of $F_{1\text{O}_2}$-DLCO manoeuvres was randomized; (2) the order of exercise intensity was also randomized; (3) at least 2 min separated DLCO manoeuvres; (4) subjects did not perform more than six breath holds in a single testing session; (5) data collection was spread over 3 days (with at least 1 week between tests); and (6) importantly, there is no evidence that the rise in COHb following multiple DLCO manoeuvres is different in highly fit athletes compared to sedentary subjects, and thus the between-group differences observed in the present study would not be obscured by an increasing COHb. Therefore, although the CO back-pressure and [Hb] changes with exercise may have had a minor effect on the data, these potential changes would not have been sufficient to explain the differences observed between our LO and HI-Fit groups.

The baseline measurements of DLCO, $V_C$ and $D_M$ were taken during the preliminary day, before the incremental exercise test. However, $Q$ and ventilation during baseline in both groups were greater than would be expected for true resting values. Although we made considerations in providing a quiet, resting environment for all of our study participants, it is not certain whether the pre-exercise levels of arousal are similar between fitness groups and, thus, the differences in DLCO and $V_C$ at baseline may not reflect true resting values. However, the pre-exercise absolute values for DLCO and $V_C$ are consistent with previous data showing that aerobic fitness is positively correlated with a greater resting $V_C$ (Lalande et al. 2012).

Finally, the cross-sectional design of the present study only allows for speculation of the effect of endurance training on diffusion capacity and the pulmonary $V_C$ response during exercise. To our knowledge, only one study has examined longitudinal changes in lung function and diffusion at rest and during exercise, finding that prepubescent swimmers had greater exercise DLCO, secondary to greater total lung volume, after 3 years of training compared to non-trained peers (Andrew et al. 1972). Future studies should investigate changes in the pulmonary vascular with chronic exercise training of previously un-trained adult humans, as well as the associated changes in pulmonary $V_C$ and $D_M$.

Conclusions

We examined the effect of aerobic fitness on diffusion capacity, pulmonary $V_C$ and $D_M$ responses to exercise. We found that athletes with a high $V_{O_{max}}$ have an increased diffusion capacity during high intensity exercise, secondary to a greater $D_M$, and do not have a greater pulmonary $V_C$ compared to non-athletes. These findings suggest that endurance-trained athletes appear to have differences within the alveolar-capillary membrane that facilitate the increased $O_2$ demand required during high-intensity exercise.

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Additional information

Competing interests

The authors declare that they have no competing interests.

Author contributions

VT, MMB and MKS contributed to the conception and design of the experiments; collection, analysis and interpretation of data; and drafting of the article or its critical revision for important intellectual content. The study was performed in the Clinical Physiology Laboratory at the University of Alberta. All authors approved the final version of the manuscript. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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