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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 $\dot{V}_{O_2max}$.
- 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, allthough 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 ($\dot{V}_{O_2max}$), 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: $\dot{V}_{O_2max}$ 64.6 ± 1.8 ml kg^{-1} min^{-1}) and 14 non-endurance trained males (LO: $\dot{V}_{O_2max}$ 45.0 ± 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_IO_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 $\dot{V}_{O_2max}$ 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; DLO_2, O_2 diffusion in the lung; D_M, pulmonary membrane diffusing capacity; F_IO_2, fraction of inspired O_2; HI-Fit, endurance-trained athlete subjects; LO-Fit, non-endurance trained subjects; PAP, pulmonary arterial pressure; PAWP, pulmonary arterial wedge pressure; P_{aO_2}, partial pressure of alveolar O_2; 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; $\dot{V}_{CO_2}$, carbon dioxide production; $\dot{V}_{O_2}$, oxygen consumption; $\dot{V}_{O_2max}$, maximum oxygen consumption.

Introduction

With incremental exercise, pulmonary diffusion capacity (DLCO) must increase with exercise to meet the increased O_2 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 (D_M) (Hsia, 2002). As cardiac output (Q) increases to meet O_2 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 D_M because previously unperfused alveoli now receive capillary blood, thereby increasing total surface area for gas exchange. Recruitment and distension of pulmonary capillaries also serves 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 ($\dot{V}_{O_2max}$) 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 $\dot{V}_{O_2max}$. Because endurance-trained athletes have a greater $\dot{V}_{O_2max}$, 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 (Lalande *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 $\dot{V}_{O_2max}$, and thus it is not fully understood exactly how V_C and D_M 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 D_M compared to non-athletes during exercise. To test this, we adapted the Roughton and Forster (1957) multiple fraction of inspired O_2 (F_IO_2)–DLCO technique (Roughton & Forster, 1957) to determine the effect of aerobic fitness on DLCO, V_C, and D_M during incremental exercise. If DLCO, and thus V_C and/or D_M, 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 O_2 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: $\dot{V}_{O_2max}$ mean ± SD: 45.0 ± 4.4 ml kg^{-1} min^{-1}) and 15 endurance-trained males (HI: $\dot{V}_{O_2max}$: 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.

Table 1. Subject characteristics and pulmonary function

	LO-Fit		HI-Fit	
	Mean	% Predicted	Mean	% Predicted
N	14		15	
Age (years)	26.1 ± 1.8		26.7 ± 6.8	
Height (m)	1.77 ± 0.02		1.77 ± 0.02	
Mass (kg)	85.5 ± 4.9		77.9 ± 2.0	
$\dot{V}_{O_2max}$ (l min^{-1})	3.81 ± 0.18	113.4 ± 5.6	5.04 ± 0.20*	152.4 ± 7.4*
$\dot{V}_{O_2max}$ (ml kg^{-1} min^{-1})	45.0 ± 1.2	99.0 ± 3.1	64.6 ± 1.8*	144.5 ± 5.4*
TLC (litres)	6.99 ± 0.24	105.3 ± 2.6	7.69 ± 0.25	113.4 ± 2.8
FEV_1 (litres)	4.38 ± 0.14	96.6 ± 2.8	5.04 ± 0.18*	109.6 ± 3.3*
FVC (litres)	5.56 ± 0.17	102.0 ± 2.7	6.28 ± 0.20*	112.4 ± 2.5*
FEV_1/FVC (%)	78.9 ± 1.4	95.4 ± 1.6	80.1 ± 1.4	97.2 ± 1.8

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 ($P < 0.05$).

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 $\dot{V}_{O_2max}$ 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-F_IO_2 DLCO manoeuvres when exercising at power outputs corresponding to 30%, 50%, 70%, 80% and 90% of their previously determined $\dot{V}_{O_2max}$. With three F_IO_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 F_IO_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 $\dot{V}_{O_2max}$ (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 $\dot{V}_{O_2max}$ 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 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 201+; 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 F_IO_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 F_IO_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 F_IO_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 Müllerian 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 2. Physiological responses at baseline and during exercise at 70% and 90% of $\dot{V}_{O_2max}$

	Baseline		70%		90%	
	LO	HI	LO	HI	LO	HI
PO (W)			185 ± 10	263 ± 13*	254 ± 12	347 ± 17*
$\dot{V}_{O_2}$ (l min^{-1})	0.49 ± 0.04	0.55 ± 0.05	2.62 ± 0.16	3.51 ± 0.19*	3.43 ± 0.20	4.51 ± 0.23*
$\dot{V}_{CO_2}$ (l min^{-1})	0.42 ± 0.04	0.46 ± 0.04	2.63 ± 0.17	3.37 ± 0.15*	3.80 ± 0.21	4.80 ± 0.23*
RER	0.88 ± 0.08	0.88 ± 0.08	1.00 ± 0.02	0.96 ± 0.03	1.11 ± 0.02	1.07 ± 0.03
V_E (l min^{-1})	14.8 ± 1.2	15.2 ± 1.2	67.7 ± 4.8	91.1 ± 4.8*	109.0 ± 8.0	146.1 ± 9.1*
V_T (litres)	0.82 ± 0.04	0.95 ± 0.06*	2.24 ± 0.15	3.03 ± 0.21*	2.82 ± 0.18	3.40 ± 0.17*
RR (breaths min^{-1})	17.2 ± 2.2	16.4 ± 1.6	28.8 ± 3.1	30.8 ± 1.8	36.8 ± 4.3	43.3 ± 2.3
V_A (litres)	6.68 ± 0.26	7.55 ± 0.31*	6.67 ± 0.20	7.49 ± 0.26*	6.87 ± 0.22	7.64 ± 0.29*
Q (l min^{-1})	7.6 ± 0.4	7.2 ± 0.4	17.5 ± 1.2	20.0 ± 1.3*	19.9 ± 1.2	22.2 ± 1.7*
SV (ml)	99 ± 5	108 ± 8	119 ± 7	126 ± 9	117 ± 8	123 ± 9
HR (breaths min^{-1})	77 ± 3	68 ± 4*	147 ± 5	159 ± 4*	173 ± 6	179 ± 3
DLCO/V_A	5.1 ± 0.3	5.0 ± 0.2	7.4 ± 0.4	7.2 ± 0.3	7.4 ± 0.3	7.7 ± 0.3
V_C/V_A	12.9 ± 0.8	13.5 ± 0.6	18.8 ± 1.5	18.9 ± 0.9	20.8 ± 1.6	20.3 ± 1.2
D_M/V_A	11.4 ± 1.0	10.3 ± 0.9	16.6 ± 1.3	16.7 ± 1.8	14.8 ± 1.4	18.2 ± 1.8*
DLCO/Q	4.6 ± 0.3	5.4 ± 0.3*	2.9 ± 0.2	2.9 ± 0.1	2.6 ± 0.2	2.7 ± 0.2
V_C/Q	11.4 ± 0.9	14.4 ± 0.9*	7.21 ± 0.5	7.36 ± 0.6	7.3 ± 0.6	6.9 ± 0.4
D_M/Q	10.3 ± 1.2	10.9 ± 0.9	6.8 ± 0.5	6.6 ± 0.6	5.1 ± 0.4	6.6 ± 0.9*

Values are the mean ± SE. PO, power output; V_E, minute ventilation; V_T, tidal volume; RR, respiratory rate; SV, stroke volume; HR, heart rate. *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_IO_2 DLCO breath-hold technique with the equation: $1/\text{DLCO} = 1/D_M + 1/\theta_{CO} \times V_C$ and θ_{CO} (i.e. the reaction rate of CO with haemoglobin) was calculated using the equation: $1/\theta_{CO} = 0.0058 \times P_{aO_2} + 0.73$ (Roughton & Forster, 1957). P_{aO_2} was calculated from the equation: $P_{aO_2} = F_IO_2(P_{Bar} - P_{H_2O}) - P_{aCO_2} \times (1 - F_IO_2)/\text{RER}$.

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 $1/\theta$ for the three F_IO_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 $\dot{V}_{O_2max}$). 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 $\dot{V}_{O_2max}$.

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

consumption ($P < 0.001$). DLCO was higher in HI-Fit subjects compared to LO-Fit subjects at 70% ($P = 0.028$) and 90% ($P = 0.013$) of $\dot{V}_{O_2max}$ (Fig. 1).

Although TLC was not statistically different between groups ($P = 0.052$), V_A was significantly larger in HI-Fit (mean ± SE: HI-Fit 7.55 ± 0.31; LO-Fit 6.68 ± 0.26 l, $P = 0.013$). When DLCO was corrected for V_A, both HI- and LO-Fit groups increased DLCO/V_A with exercise intensity, although there were no differences between HI- and LO-Fit groups (Table 2). When DLCO was expressed relative to Q, DLCO/Q was greater in HI-Fit at baseline ($P = 0.019$) but not at 70% ($P = 0.335$) or 90% ($P = 0.459$) of $\dot{V}_{O_2max}$ (Table 2).

Pulmonary V_C

At baseline, V_C 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 V_C with increasing oxygen consumption ($P < 0.001$), although there was no statistical difference between groups during exercise ($P = 0.498$). When V_C was expressed relative to Q, V_C/Q was greater in HI-Fit at baseline ($P = 0.002$) but not at 70% ($P = 0.403$) or 90% ($P = 0.229$) of $\dot{V}_{O_2max}$ (Table 2).

There was a significant, positive correlation between baseline pulmonary V_C and individual $\dot{V}_{O_2max}$ ($r = 0.509$, $P = 0.004$) (Fig. 3*A*), although pulmonary V_C at the 90% workload was not correlated with individual $\dot{V}_{O_2max}$ ($r = 0.012$, $P = 0.949$) (Fig. 3*B*). When plotted against peak Q, both baseline V_C ($r = 0.573$, $P = 0.001$) (Fig. 4*A*) and peak V_C were significantly correlated with peak Q ($r = 0.368$, $P = 0.049$) (Fig. 4*B*).

Membrane D_M

At baseline, D_M was not different between HI-Fit and LO-Fit groups ($P = 0.83$). With incremental exercise, both LO- and HI-Fit subjects increased D_M with increasing oxygen consumption ($P < 0.001$). During exercise at 70% and 90% of $\dot{V}_{O_2max}$, HI-Fit subjects had a greater D_M compared to LO-Fit subjects (Fig. 5). Likewise, D_M/V_A was greater at peak exercise in HI-Fit as compared to LO-Fit subjects. Both groups exhibited a plateau in their respective D_M responses to exercise, with HI-Fit showing no significant increase in D_M above 50% of $\dot{V}_{O_2max}$ ($P = 0.582$) and, in LO-Fit, after 30% of $\dot{V}_{O_2max}$ ($P = 0.655$). When D_M was expressed relative to Q, D_M/Q was not different at baseline ($P = 0.320$) or 70% ($P = 0.401$), although D_M/Q was greater in the HI-Fit group at 90% ($P = 0.048$) of $\dot{V}_{O_2max}$ (Table 2).

Unlike V_C, baseline D_M was not correlated with $\dot{V}_{O_2max}$ ($r = 0.011$, $P = 0.951$) (Fig. 3*C*); however, D_M at the 90% workload was correlated with $\dot{V}_{O_2max}$ ($r = 0.425$, $P = 0.021$) (Fig. 3*D*). Baseline D_M was not correlated with peak Q ($r = 0.193$, $P = 0.315$) (Fig. 4*C*); however, peak D_M was significantly correlated with peak Q ($r = 0.378$, $P = 0.043$) (Fig. 4*D*).

Discussion

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

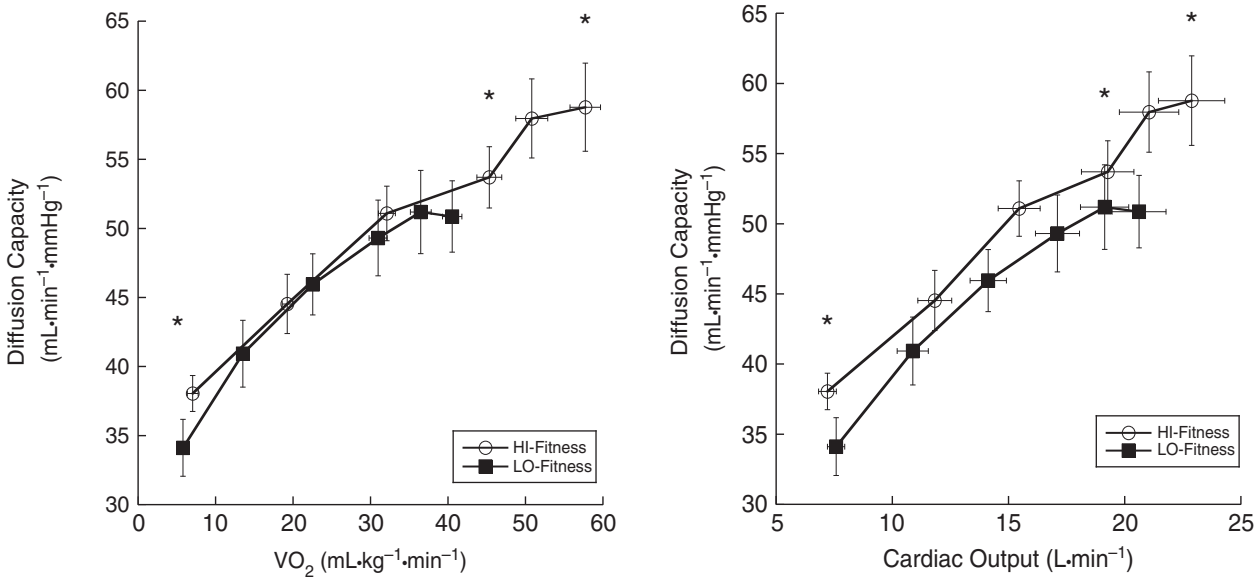

Figure 1. Diffusing capacity response to exercise
*DLCO was greater in HI-Fit subjects at baseline ($P = 0.047$), at 70% ($P = 0.028$) and at 90% of $\dot{V}_{O_2max}$ ($P = 0.013$) compared to LO-Fit subjects.

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 $\dot{V}_{O_2max}$ (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.*

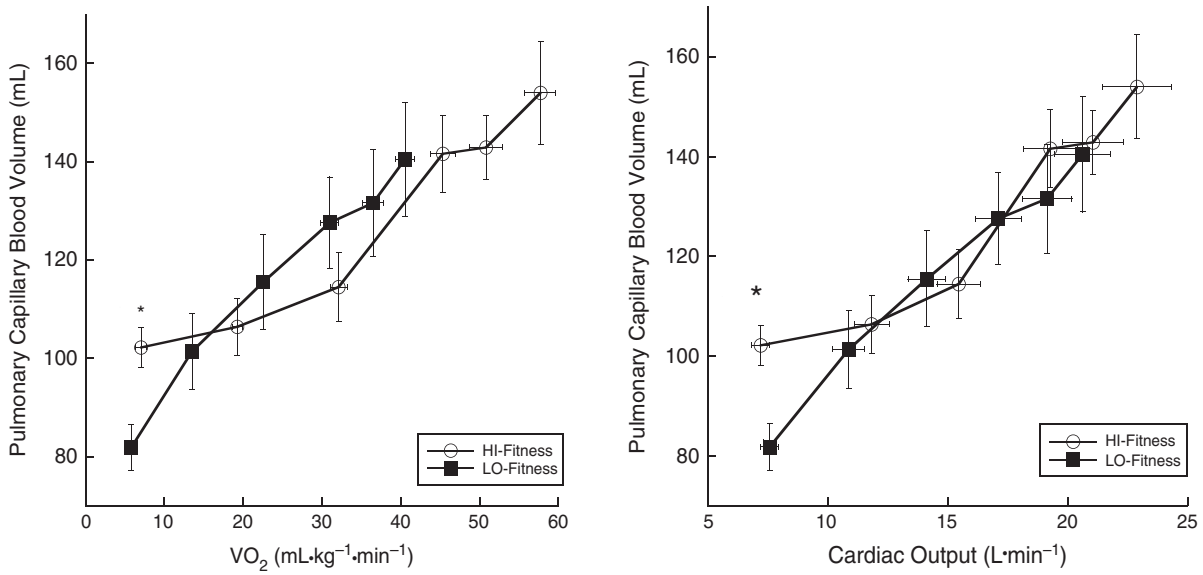

Figure 2. Pulmonary V_C response to exercise
*V_C was significantly higher in HI-Fit subjects compared to LO-Fit subjects only at baseline ($P = 0.005$).

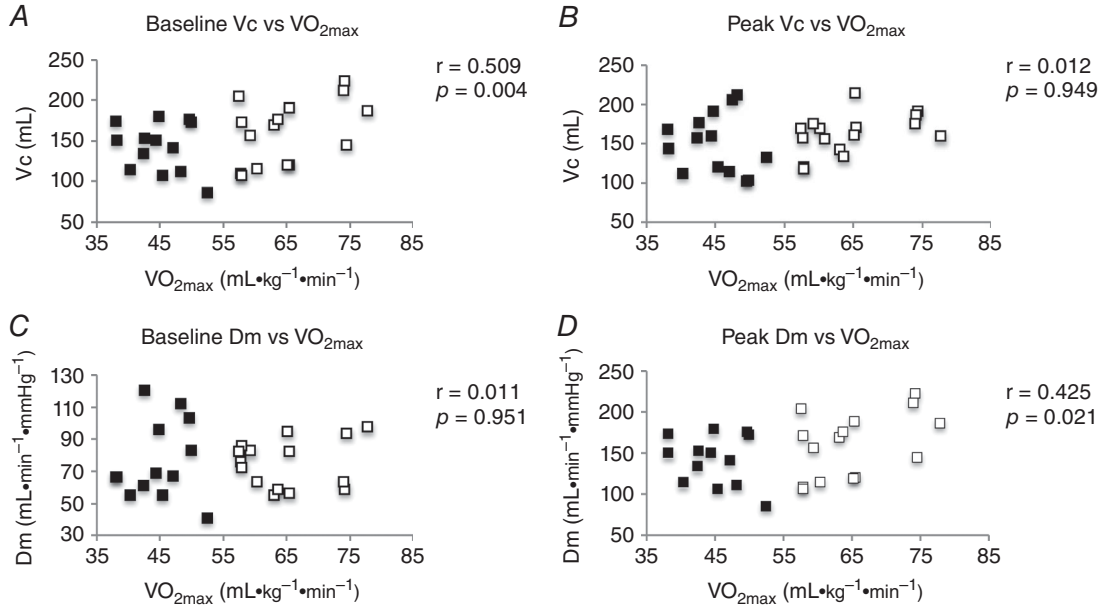

Figure 3. Correlations of individual V_C and D_M to $\dot{V}_{O_2max}$
A, baseline V_C. *B*, V_C at 90% $\dot{V}_{O_2max}$. *C*, Baseline D_M. *D*, D_M at 90% $\dot{V}_{O_2max}$. Black squares, LO-Fit group; white squares, HI-Fit group.

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

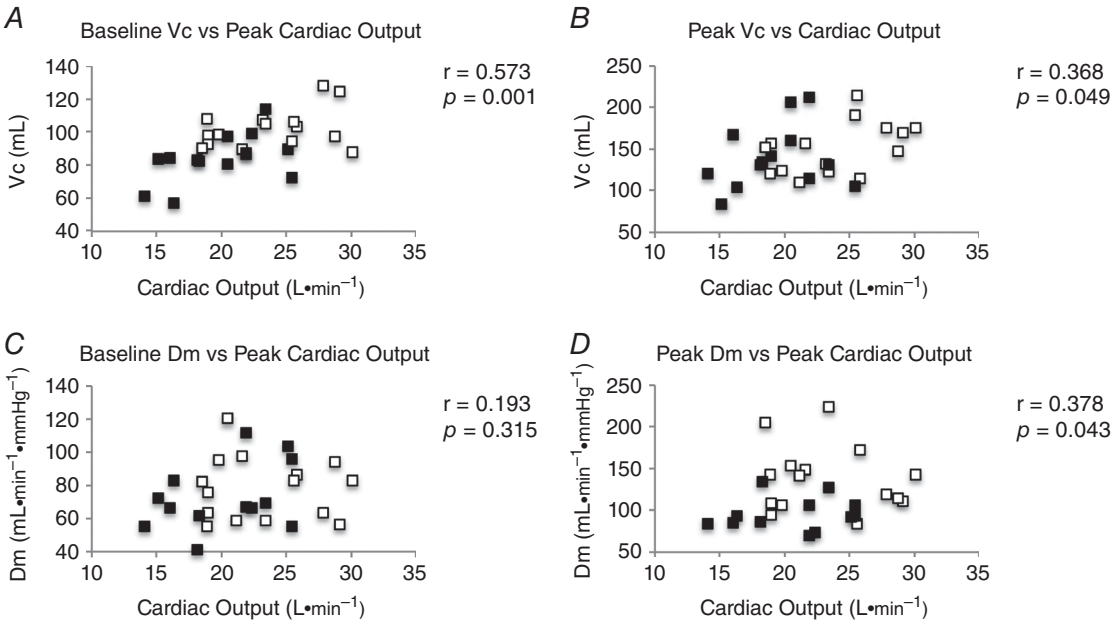

Figure 4. Correlations of individual D_M and V_C to peak Q
A, baseline V_C. *B*, V_C at 90% $\dot{V}_{O_2max}$. *C*, Baseline D_M. *D*, D_M at 90% $\dot{V}_{O_2max}$. Black squares, LO-Fit group; white squares, HI-Fit group.

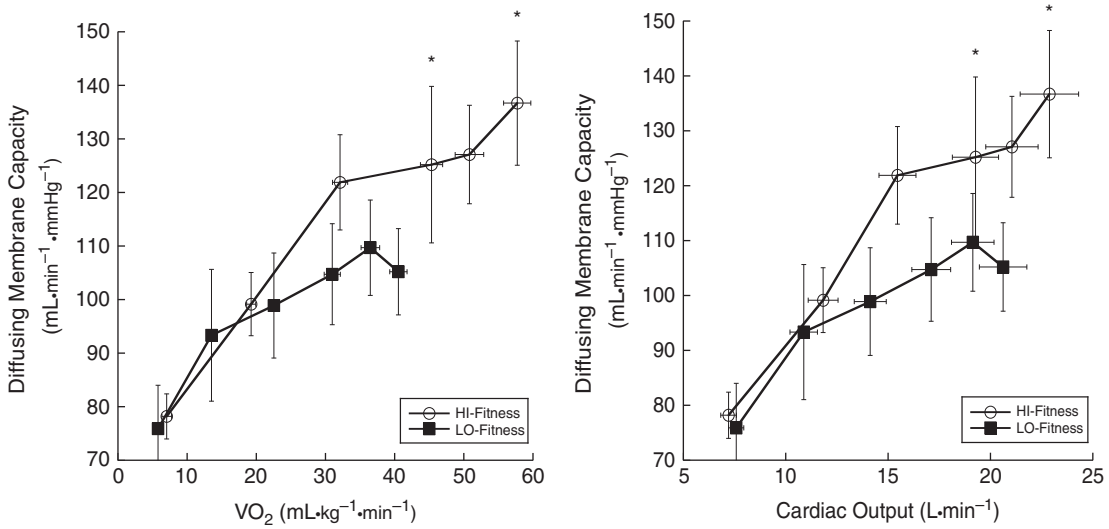

Figure 5. D_M during exercise
*D_M was significantly greater in HI-Fit subjects compared to LO-Fit subjects during exercise at 70% ($P = 0.035$) and at 90% of $\dot{V}_{O_2max}$ ($P = 0.006$).

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 $\dot{V}_{O_2max}$, 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 $\dot{V}_{O_2max}$ in healthy subjects is limited by O_2 availability (Wagner, 1996*a*). Although Q is predominant in O_2 delivery, a theoretical analysis of the factors that determine $\dot{V}_{O_2max}$ found that O_2 diffusion in the lung (DLO_2) was as influential as Q in determining $\dot{V}_{O_2max}$ (Wagner, 1996*a*, 1996*b*). Based on the principle of mass-balance for O_2 exchange, highly fit individuals with a high $\dot{V}_{O_2max}$require a greater DLO_2 compared to less fit individuals (Wagner, 1996*a*). A failure to increase DLO_2 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 $\dot{V}_{O_2max}$ (Fig. 3*D*), is entirely consistent with the importance of O_2 supply relative to O_2 delivery in the determination of $\dot{V}_{O_2max}$ (Wagner, 1996*a*).

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 $\dot{V}_{O_2max}$ (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

capillaries is uncertain, although the best estimation is approximately half the difference between PAP and PWP (West, 2012). Thus, an elevated resting V_C at similar Q, but lower pressure within the pulmonary capillaries would suggest that athletes have a more compliant pulmonary vasculature compared to non-athletes, and that this enhanced pulmonary compliance may be related to increased $\dot{V}_{O_2max}$ (Fig. 3*A*). Work by La Gerche *et al.* (2010) demonstrated that pulmonary vascular compliance is important in decreasing PVR, preventing excessive right ventricular afterload, and thereby enhancing right ventricular function and Q during exercise. Thus, the response of the pulmonary vasculature to increasing pulmonary artery pressures during exercise will directly affect Q and, ultimately, $\dot{V}_{O_2max}$. Taken together, these studies suggest that endurance-trained athletes may have beneficial adaptations within the pulmonary vasculature that enable greater Q and $\dot{V}_{O_2max}$.

Recent work by Brown *et al.* (2015) shows that lung density is positively correlated to diffusion capacity, and lung size is negatively correlated to lung density. This suggests that the number of alveolar units are very similar between large and small lungs in healthy humans (Brown *et al.* 2015) and, thus, when normalized for the larger alveolar size (and presumably surface area), it would be expected that the athlete lung would have a decreased diffusion capacity, and thus decreased D_M. However, in the present study, DLCO and D_M were greater in HI-Fit athletes during exercise. It is possible that endurance-trained athletes may have thinner alveolar-capillary membranes to compensate for a decreased lung density; however, this remains speculative without histological evaluation of membrane thickness.

The results of the present study are consistent with other evidence that endurance-trained athletes have greater diffusion capacity at rest (Armour *et al.* 1993; Degens *et al.* 2013) and during exercise (Mostyn *et al.* 1963; Armour *et al.* 1993; Degens *et al.* 2013). However, none of these studies have examined DLCO responses to exercise above 80% of $\dot{V}_{O_2max}$, and only one has measured V_C and D_M during exercise (Mostyn *et al.* 1963). Mostyn *et al.* (1963) studied DLCO, V_C, and D_M in athletes at rest and during steady-state treadmill exercise at a mean $\dot{V}_{O_2}$ of 2.0 l min^{-1}, finding that athletes have an increased DLCO during submaximal exercise secondary to an increased D_M, and not an increased V_C (Mostyn *et al.* 1963). However, this previous investigation pre-breathed gas containing F_IO_2 of 0.6 and 1.0 for 5 min before performing the respective DLCO breath hold (Mostyn *et al.* 1963), which may affect V_C because exposure to high O_2 has been shown to alter the distribution of pulmonary blood (Ley *et al.* 2007). Although the results of the present study are consistent with the findings of this landmark study (Mostyn *et al.* 1963), our methodology provides greater detail of the DLCO, D_M and V_C responses from baseline to near-maximal exercise.

Study limitations

It is assumed that θ_{co} does not change with exercise (Johnson *et al.* 1960) because there is evidence showing that θ_{co} is relatively insensitive to changes in pH (Roughton & Forster, 1957). When Roughton and Forster (1957) first introduced their method of estimating V_C and D_M, the recommended values for α (temperature- and pH-dependent coefficient linked to kinetic reactions of CO with Hb) and β (i.e. ratio of red-cell membrane to red cell interior permeability) were not explicitly given for the calculation of θ_{co}. As a result, several studies have used different variants of these constants (Hsia, 2002; Ceridon *et al.* 2010; Smith *et al.* 2015), ultimately affecting their respective V_C and D_M calculations. The assumption of a pH of 8.0 was addressed by Forster (1987) in a subsequent study, providing a correction for α that accounts for a pH of 7.4. More recently, Ceridon *et al.* (2010) employed Forster's corrected α, and found highly improbable values for D_M in all subjects. For this reason, the present study used $\alpha = 0.0058$ and $\beta = 0.73$ as recommended by Ceridon *et al.* (2010), which probably provides the best estimation of V_C and D_M during exercise. Regardless of the assumptions for θ_{co} determination, the same value was used for both trained and untrained groups, and therefore the calculated differences in V_C and D_M are probably not a result of the value given for θ_{co}.

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 V_C and D_M 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_M 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_IO_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 $\dot{V}_{O_2max}$ 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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