The pulmonary vasculature during exercise in health and mild chronic obstructive pulmonary disease

by

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Abstract

The purpose of this dissertation was to characterize the response of the pulmonary vasculature to exercise in health (including a pharmacological intervention) and in individuals with mild chronic obstructive lung disease. The first project studied the effect of a dopamine receptor blockade on the gas exchange response during exercise in 12 healthy males (age: 25 ± 6 years, $\dot{V}O_{2max}$ = 58.6 ± 6.5 $mL \cdot kg^{-1} \cdot min^{-1}$). Previous research has demonstrated recruitment of Intrapulmonary (IP) shunt during exercise, which may contribute to gas exchange impairment. IP shunts are recruited with dopamine infusion and endogenous dopamine increases with exercise intensity. It was hypothesized that dopamine receptor blockade would reduce the recruitment of IP shunt during exercise and reduce gas exchange impairment. Administration of 20 mg metoclopramide did not reduce IP shunt recruitment, as assessed by agitated saline contrast echocardiography. However, the alveolar-arterial oxygen difference was reduced by 23%, and increased arterial oxygen saturation by 0.8% during exercise at 85% of $\dot{V}O_{2max}$, indicating an improvement in gas exchange impairment. Also, dopamine receptor blockade reduced peak oxygen consumption by 5.5% and time-to-exhaustion while exercising at 90% of $\dot{V}O_{2max}$ by 38.6%. As dopamine is a known pulmonary vasodilator, this study suggests that endogenous dopamine is important to the normal cardiopulmonary response to exercise, and is necessary for optimal high-intensity exercise performance.

Two subsequent projects examined the relative contributions of pulmonary capillary blood volume (Vc) and membrane diffusing capacity (Dm) to the overall increase in diffusing capacity (DLCO) during exercise. Endurance-trained athletes exhibit enhanced cardiovascular function compared to sedentary non-athletes, but it is accepted that exercise training does not enhance lung structure and function. The second study investigated differences between 15 endurance-

trained athletes ($\dot{V}O_{2max}$ = 64.6 ± 6.9 $mL \cdot kg^{-1} \cdot min^{-1}$) and 14 age- and height-matched sedentary controls ($\dot{V}O_{2max}$ = 45.0 ± 4.4 $mL \cdot kg^{-1} \cdot min^{-1}$). We found that athletes had 15% greater DLCO during exercise at 90% of $\dot{V}O_{2max}$, secondary to 30% greater Dm compared to sedentary controls. No difference in pulmonary capillary blood volume (Vc) was observed. These data suggest that athletes appear to have an enhanced pulmonary membrane that facilitates the increased oxygen uptake during high-level exercise.

The third project examined the DLCO response to exercise in mild chronic obstructive pulmonary disease (COPD). Previous research has shown that mild COPD patients exhibit pulmonary vascular impairment, indicating that the impact of the disease is worse than spirometry testing would suggest. We recruited 15 mild COPD (FEV_1/FVC = 64 ± 3, FEV_1 = 93 ± 13% of predicted) and 15 height-, sex-, and age-matched controls, and measured DLCO, Vc, and Dm at rest and during exercise. We observed 22% lower DLCO at rest and during exercise in mild COPD, which is primarily caused by 24% lower capillary blood volume. No difference in membrane diffusing capacity was observed between groups. These results suggest that, despite the relatively minor airway obstruction, individuals with mild COPD exhibit some pulmonary vascular impairment that precedes changes to the alveolar interface, such as emphysema. Together, this dissertation contributes to the understanding of the importance of the pulmonary vasculature to cardiovascular function and pulmonary gas exchange during exercise in health and mild chronic obstructive lung disease.

Preface

All of the work presented henceforth was conducted in the Clinical Physiology Laboratory at the University of Alberta. All projects received ethical approval from the University of Alberta Human Ethics Research Board.

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Dedication

All that I am, or hope to be, I owe to my angel mother.

-Abraham Lincoln

This dissertation is dedicated to the memory of my late mother Kellyna Rusli Tedjasaputra, and my late grandmother Kartika Salim, who passed away in the final days of my writing this dissertation. Your eternal legacy lives in these pages.

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To my fellow colleagues, the brightest and most young curious minds I have had the pleasure of working with: Linn Moore, Wade Michaelchuk, Sophie Collins, Andrew Brotto, Bradley Byers, Karishma Kapoor, Desi Fuhr, and Salmina Ahmed, I will forever cherish pick up hockey at Lake Louise, potato Mondays, and chocolate Fridays. Thank you to my clinical collaborators Drs. Sean Van Diepen, Tracey Bryan, Mohit Bhutani, Eric Wong, Marc Bibeau, and Ron Damant for assisting in recruitment and data collection. I would like to especially thank my best friend and colleague Devin Phillips, who was always a willing source of scientific and philosophical

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As they say, life is a highway. On to the next adventure!

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Definition
A-aDO_2	Alveolar-arterial PO_2 difference
BP	Blood pressure
CC	Carotid chemoreceptors
CO	Carbon monoxide
COHb	Carboxyhemoglobin
COPD	Chronic obstructive pulmonary disease
DA	Dopamine
DLCO	Pulmonary diffusing capacity for CO
DLNO	Pulmonary diffusing capacity for nitric oxide
DLO_2	Pulmonary diffusing capacity for O_2
Dm	Membrane diffusing capacity
EELV	End expiratory lung volume
EIAH	Exercise induced arterial hypoxemia
FEV_1	Forced expired volume in 1 second
F_IO_2	Fraction of inspired oxygen
FRC	Functional residual capacity
FVC	Forced vital capacity
GOLD	Global initiative for chronic lung disease
Hb	Hemoglobin
HR	Heart rate

IC	Inspiratory capacity
IP shunt	Intrapulmonary shunt (alternate- IPAVA)
IPAVA	Intrapulmonary arteriovenous anastomosis
IRV	Inspiratory reserve volume
IV	Intravenous
MIGET	Multiple inert gas elimination technique
$PaCO_2$	Arterial PCO_2
PaO_2	Arterial PO_2
PAO_2	Alveolar PO_2
PAP	Pulmonary artery pressure
PASP	Pulmonary artery systolic pressure, from echocardiography
PAWP	Pulmonary artery wedge pressure
$P_{ET}CO_2$	End tidal CO_2
$P_{ET}O_2$	End tidal O_2
PFO	Patent formen ovale
PVR	Pulmonary vascular resistance
$\dot{Q}$	Cardiac output
$\dot{Q}s/\dot{Q}t$	Shunt fraction
RER	Respiratory equivalent ratio
RV	Residual volume
SaO_2	Oxyhemoglobin saturation
SV	Stroke volume
TLC	Total lung capacity

TTE	Time-to-exhaustion
V_A	Alveolar volume
$\dot{V}_A/\dot{Q}$	Ventilation to perfusion ratio
Vc	Pulmonary capillary blood volume
$\dot{V}_E$	Minute ventilation
$\dot{V}_E/\dot{V}CO_2$	Ventilatory equivalent for CO_2
$\dot{V}_E/\dot{V}O_2$	Ventilatory equivalent for O_2
$\dot{V}O_{2max}$	Maximal oxygen consumption
$\dot{V}O_{2peak}$	Peak oxygen consumption

CHAPTER 1

Introduction

1.1 General Introduction

During exercise, cardiac output must increase to meet the greater metabolic demand. As right ventricle contractility increases, pulmonary arterial blood pressure increases, resulting in the recruitment and distension of pulmonary capillaries[1]. This increase in the cross sectional area of the pulmonary capillaries serves two purposes: to decrease pulmonary vascular resistance to prevent excessive right ventricular afterload, optimizing cardiac output[6]; and, to increase total surface area for gas exchange. Thus, the response of the pulmonary vasculature (pulmonary arteries, arterioles, capillaries, and intrapulmonary arteriovenous anastomoses - IPAVA) to increasing pulmonary artery pressure directly affects both cardiac output and the gas exchange properties of the lung. An IPAVA is a large arterio-venous connection in the lung that bypasses the pulmonary capillaries. It is thought that IPAVA recruitment serves as a 'pop-off' valve to protect the lung from large increases in pulmonary artery pressure during exercise[2-5]. Notably, a lack of IPAVA recruitment during exercise results in high pulmonary artery pressure and low cardiac output[2], suggesting that IPAVA recruitment is integral to normal cardiopulmonary function.

Measurement of the blood volume in the pulmonary capillaries provides insight into the function of the pulmonary vasculature. Previous research has shown that individuals with the highest resting pulmonary capillary blood volume had the greatest $\dot{V}O_{2max}$ and greater pulmonary vascular distensibility, suggesting that the pulmonary vasculature (and especially capillary blood volume) is an important contributor to exercise performance and aerobic capacity[3].

Conversely, failure of the pulmonary vasculature to respond properly to exercise would thus impair cardiac output and pulmonary gas exchange. There is evidence suggesting that patients with chronic obstructive pulmonary disease (COPD) may exhibit pulmonary vascular

dysfunction[6,7]. COPD is a respiratory disease largely caused by smoking, and is characterized by partially reversible airway obstruction and pulmonary capillary destruction[8]. Patients with COPD experience exaggerated breathlessness (dyspnea) during activity, which greatly impairs their ability to perform daily tasks and consequently reduces quality of life[9].

Exertional dyspnea has been studied extensively in COPD, and results from a phenomenon described as neuromechanical dissociation, which is the uncoupling of the central drive to breathe from the mechanical ventilation of the lung[10]. The control of breathing requires a synchronized effort between the central nervous system, lungs, and cardiovascular system. The signal to breathe originates in the respiratory centers in the brain stem, initiating ventilation of fresh air into the alveoli, where gas is exchanged with the blood in the pulmonary capillaries (Figure 1-1). This process is mediated by afferent mechanical feedback from the lung and chemoreceptors located in the cardiovascular and neurovascular systems which respond to changes in arterial blood gases[11]. Together, afferent signaling modifies the central drive to breathe. If the lung cannot adequately respond to the increased central drive due to mechanical limitations such as dynamic hyperinflation, the sensation of dyspnea arises[10]. Figure 1-1 provides a simplified summary of the major afferent signals associated with exertional dyspnea in COPD.

The pulmonary vasculature plays an important role in afferent feedback. Increases in pulmonary artery pressure stretches the lung vessels, activating juxta-capillary receptors[12] and increases ventilation[13,14]. Patients with advanced COPD have been shown to have increased pulmonary artery pressure[6] due to inflammation and remodeling of the smooth muscle in the pulmonary arteries[15]. Pulmonary vascular dysfunction has also been reported in mild COPD[16-18], which would increase pulmonary vascular resistance and therefore require an elevated pulmonary artery pressure to maintain flow. As pulmonary capillary recruitment is vital to blunt the rise in

pulmonary artery pressure during exercise, reduced pulmonary capillary recruitment or distention could impact cardiac output, dyspnea, and ultimately exercise tolerance. From the gas exchange perspective, reduced blood flow to the pulmonary capillaries reduces surface area for diffusion, which may lead to hypoxemia. Thus, it is important to characterize pulmonary capillary blood volume in COPD, as it may indicate impairment in the vessels of the lung. However, to date there have been no studies of the pulmonary capillary blood volume response to exercise in COPD.

Taken together, this dissertation focuses on the working relationship between the heart and pulmonary blood vessels to maintain adequate blood flow and gas exchange to meet the increased metabolic demand of exercise. Examining this response in mild COPD would provide insight into underlying vascular pathology and would help to better understand the progression of this early lung disease.

1.2 Purpose of Dissertation

This document consists of three experiments designed to investigate the complex responses of the pulmonary vasculature during exercise in selected states of health and lung disease. The purpose of this dissertation was to examine the spectrum of responses of the pulmonary vasculature to exercise from endurance-trained athletes to chronic obstructive lung disease patients. The physiological scope of this dissertation concerned the determinants of pulmonary gas exchange, namely the alveolar-capillary interface and intrapulmonary arteriovenous anastomoses, represented in the bottom-left box in Figure 1-1.

The first experiment was an interventional study to determine the effect of a dopamine-2 receptor blockade on exercise pulmonary gas exchange, intrapulmonary arteriovenous anastomosis

recruitment, and exercise tolerance in healthy, relatively fit young men[5]. We hypothesized that dopamine receptor blockade would reduce intrapulmonary shunt recruitment and improve pulmonary gas exchange. The results indicated that dopamine receptor blockade improved pulmonary gas exchange, as measured by the Alveolar-arterial O_2 difference ($A\text{-}aDO_2$), but decreased exercise tolerance. Intrapulmonary shunt recruitment, as measured by agitated saline contrast echocardiography, was not affected by dopamine blockade. These data suggest that endogenous dopamine is important for the normal healthy response to exercise, and that intrapulmonary shunt recruitment was not related to the change in $A\text{-}aDO_2$. This study was the first to demonstrate that dopamine blockade can be used to experimentally modulate the normal pulmonary vascular response to exercise. This pharmacological intervention could be used in future studies to model pulmonary vascular disease acutely.

The second study was a cross-sectional, descriptive experiment determining the pulmonary diffusion capacity (DLCO) response to exercise in endurance-trained males compared to sedentary controls[19]. It was hypothesized that trained subjects would have increased DLCO secondary to greater pulmonary capillary blood volume (Vc) due to enhanced pulmonary vascular compliance. We found that endurance-trained athletes had greater diffusion capacity during exercise, secondary to increased diffusing membrane capacity (Dm), and not Vc. These data suggest that athletes may have morphological differences in the alveolar-capillary membrane that facilitate greater diffusion of oxygen at higher exercise intensities. While other studies have examined diffusion capacity in athletes at rest[20] and during exercise[21,22], none have examined DLCO responses to exercise above 80% of $\dot{V}O_{2max}$, and only one had measured Vc and Dm during exercise[22]. Together, the first two experiments of this dissertation provide insight into the healthy normal response of the pulmonary vasculature to exercise.

The third experiment was designed to examine the pulmonary vascular response to exercise in patients with mild COPD. It was hypothesized that the DLCO, Vc and Dm response to exercise would be lower in COPD compared to age-, sex-, and height-matched healthy control subjects. The results of this study indicated that DLCO was lower in COPD both at rest, and during exercise compared to controls, and this decrease in DLCO was secondary to lower Vc and not Dm. The results suggest that despite the relatively minor airflow obstruction in mild COPD, the response of the pulmonary microvasculature to exercise is blunted, which may impact pulmonary gas exchange. This work provides additional insight into the possible mechanisms of exertional dyspnea in mild COPD, and is an important contribution to the understanding of the progression of pulmonary vascular dysfunction in this disease.

1.3 Summary of Dissertation

Collectively, this dissertation contributes to the understanding of the pulmonary circulation under the stress of exercise in healthy individuals and in mild chronic obstructive lung disease. Study 1 highlights the importance of dopamine to the normal response of the pulmonary vasculature to exercise. Study 2 provides some of the first evidence that some aspect of gas exchange is enhanced in aerobic trained athletes. Finally, Study 3 indicates that despite their relatively mild airway obstruction, mild COPD patients exhibit a deficiency in their pulmonary vascular response to exercise.

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1.5 Figures

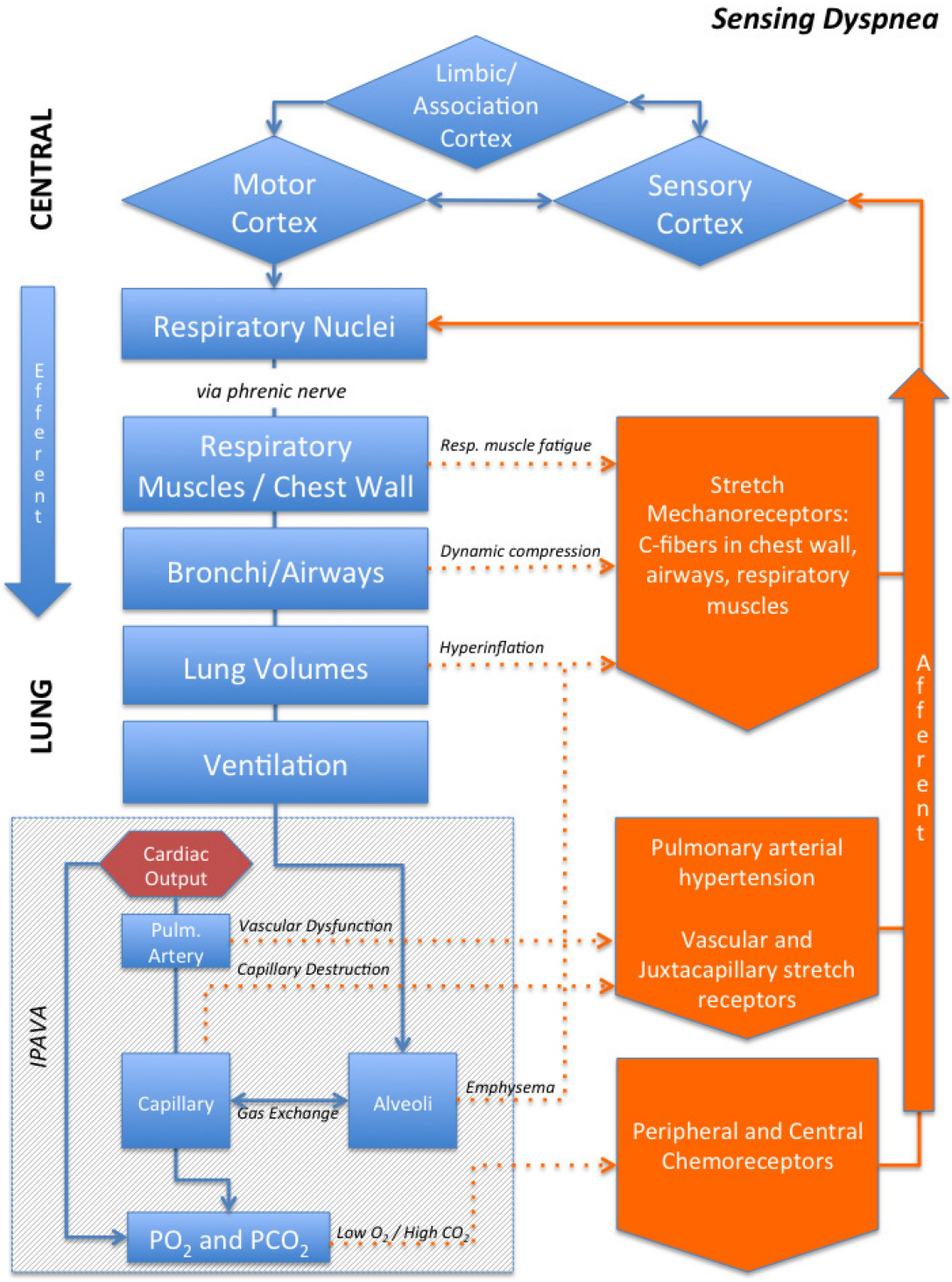

Figure 1-1: Summary of the control of breathing. Efferent signals (blue) initiate ventilation in the lung, and the drive to breathe is modified by afferent signaling (orange). The bottom-left box represents the physiological scope of this dissertation. IPAVA, Intrapulmonary Arteriovenous Anastomosis. This figure adapted from Burki (2010) and O'Donnell *et al*. (2007)[23,24].

CHAPTER 2

Review of Literature

2.1 Pulmonary Gas Exchange

The primary function of the lungs is gas exchange: the process by which oxygen is brought in from the environment into the alveoli and diffused into the blood, and carbon dioxide is removed. This process requires several steps: alveolar ventilation, the delivery of fresh gas from the environment into the alveoli, gas diffusion across the alveolar wall into the pulmonary capillary blood, and gas diffusion into plasma and red blood cells. The efficiency of pulmonary gas exchange is determined by the difference in partial pressure of O_2 in the alveoli (P_AO_2) and the artery (P_aO_2), termed the alveolar-arterial oxygen difference, or A-aDO_2[1].

At rest, pulmonary gas exchange is quite efficient, as resting A-aDO_2 is approximately 5 mmHg[1]. However, during exercise the A-aDO_2 progressively increases with increasing work rates[2,3]. A large increase in A-aDO_2 can result in a drop in the P_aO_2 < 92 mmHg (exercise-induced arterial hypoxemia) and arterial desaturation, particularly in aerobically-trained athletes[4]. An increased A-aDO_2 is a result of intrapulmonary shunt[5,6], ventilation-perfusion ($\dot{V}_A/\dot{Q}$) inequality[7-9], and diffusion limitation[10]. The majority of studies examining gas exchange impairment during exercise have utilized the multiple inert gas elimination technique (MIGET)[2,3,7,9]. This technique measures the pulmonary exchange of six different inert gases dissolved together in saline or dextrose, infused intravenously[11]. Those measurements are then used to compute the distribution of ventilation/perfusion ratios that best explain the simultaneous exchange of the six gases in the lungs. In addition to determining $\dot{V}_A/\dot{Q}$ mismatch, data derived from MIGET can identify and quantify diffusion limitation, and intrapulmonary shunt[11]. The mechanisms and contribution of these factors to the exercise-induced increased A-aDO_2 will be discussed briefly.

2.2 Intrapulmonary Shunt

Studies using MIGET have found no evidence that intrapulmonary shunt contributes to the A-aDO_2 during exercise[8,11,12]. However, several studies have demonstrated that anatomical shunts are recruited and appear to be related to the gas exchange impairment during exercise using other methods[5,6,13,14]. There is evidence that intrapulmonary shunts can be recruited pharmacologically by increasing cardiac output with exogenous dopamine[15], dobutamine[15], and epinephrine[16]. With the exception of dobutamine, these inotropes are of interest as they increase endogenously during exercise. The mechanism in which intrapulmonary shunts are recruited during exercise is not yet completely understood. Taken together, these studies suggest that the regulation of intrapulmonary shunt recruitment is multi-factorial, but oppose several MIGET gas exchange studies showing a lack of evidence for right-to-left shunt during exercise[8,11,12]. Thus, the existence of intrapulmonary shunt recruitment during exercise remains controversial, and has been the subject of debate over its contribution to the A-aDO_2 during exercise[17-19].

2.3 Ventilation-Perfusion Inequality

Ventilation-perfusion ($\dot{V}_A/\dot{Q}$) inequality is improved with modest exercise[20]. However, with increasing exercise intensity and duration[2,12,21], $\dot{V}_A/\dot{Q}$ inequality worsens in most individuals, resulting in an increase in A-aDO_2[8,10,22,23]. During exercise intensity below a $\dot{V}O_2$ of 4.0 $L \cdot min^{-1}$, $\dot{V}_A/\dot{Q}$ inequality constitutes the majority of the measured A-aDO_2[21]. Although controversial, exercise-induced $\dot{V}_A/\dot{Q}$ inequality is theorized to be secondary to the development of transient sub-clinical pulmonary edema[24,25]. It is hypothesized that exposure of the pulmonary capillaries to sustained high perfusion pressure results in accumulation of fluid in the interstitial space[7]. This would result in the mechanical compression of small airways and blood vessels, thereby impairing $\dot{V}_A/\dot{Q}$ matching[24]. Furthermore, $\dot{V}_A/\dot{Q}$ inequality persists well after exercise is

terminated, despite cardiac output and ventilation returning to baseline values, suggesting that the development of edema is not transient[7]. In the animal model, exercise increases $\dot{V}_A/\dot{Q}$ inequality in swine[9] and histological examination of lung tissues following exercise demonstrates accumulation of fluid surrounding the lung vessels[24]. Together, these studies support the idea that exercise-induced pulmonary edema contributes to ventilation perfusion inequality.

2.4 Diffusion Limitation

The diffusing capacity of the lung is determined by several factors, including the diffusing capacity of the alveolar-capillary membrane (Dm), pulmonary capillary blood volume (Vc), hemoglobin concentration [Hb], the shape of the oxyhemoglobin dissociation curve, and interactions between PO_2 and PCO_2[1,26]. At sea level, complete equilibrium of partial pressures of O_2 and CO_2 in the alveoli and pulmonary capillary blood occurs in approximately 0.25 s at rest[1,26,27]. Thus, if the transit time of red blood cells through the pulmonary capillaries is reduced below 0.25 s, a diffusion limitation could occur, preventing the equilibration of gases in alveoli and pulmonary capillary blood[28].

Diffusion limitation may also occur if the distance across the membrane is increased. It might be expected that the alveolar-capillary membrane may thicken with the development of interstitial edema during exercise. However, previous research suggests that fluid accumulation is unlikely to affect the alveolar membrane until the edema is advanced[29], and thus no diffusion limitation would be expected with the relatively mild exercise-induced pulmonary edema[30]. This lack of diffusion limitation with mild edema is consistent with a previous study showing no diffusion limitation after rapid saline infusion administration, which caused a volume overload-induced interstitial edema[31]. Further, research by Stickland *et al*. (2006) shows evidence that at a given cardiac output, endurance trained athletes have lower pulmonary arterial wedge pressure

(PAWP), and therefore lower pulmonary capillary pressure[32], suggesting that the development of exercise-induced pulmonary edema is actually *less* likely in endurance trained subjects compared to non-athletes.

During high intensity exercise, cardiac output can increase up to five-fold[33]. Thus it is possible that red blood cell transit time in some pulmonary capillaries could decrease and subsequently contribute to gas exchange impairment[10]. Studies using MIGET have demonstrated that during high intensity exercise, the A-aDO_2 observed is greater than the predicted A-aDO_2 from $\dot{V}_A/\dot{Q}$ mismatch and intrapulmonary shunt[2,21]. This difference between the predicted and observed A-aDO_2 is said to represent a diffusion limitation[12,21]. In normal healthy subjects, a diffusion limitation is observed during exercise above a $\dot{V}O_2$ of 3.5 $L \cdot min^{-1}$ [34], and this diffusion limitation increases with exercise intensity[8,22]. As endurance-trained individuals can easily sustain exercise above 3.5 $L \cdot min^{-1}$, diffusion limitation is often observed in these individuals[10].

2.5 Pulmonary Diffusion

It is important to appreciate that pulmonary diffusing capacity must meet the increased oxygen demand to minimize any diffusion limitation. Pulmonary diffusion (DL) is the conductance of oxygen (DLO_2) or carbon monoxide (DLCO) from the alveolar space to hemoglobin in the capillary space. In a lung with perfect ventilation-perfusion matching, DLO_2 is defined as:

$$DLO_2 = \frac{\dot{V}O_2}{P_AO_2 - P_cO_2}$$

Where $\dot{V}O_2$ is defined as the oxygen consumption, P_AO_2 is the pressure of oxygen in the alveoli, and the P_cO_2 is the mean PO_2 passing through pulmonary capillaries. Measurement of P_cO_2 is assumed to be equal to the P_aO_2, and the denominator term of the above equation is equivalent to

the A-aDO_2[1]. Thus, as oxygen consumption increases during exercise, the diffusing capacity must rise to meet the increased oxygen demand to avoid an increased A-aDO_2.

The uptake of most soluble gases such as nitric oxide or acetylene is limited by, and varies with pulmonary blood flow[35]. In contrast, carbon monoxide has a strong affinity for hemoglobin[36], and the red cell mass acts as an infinite sink to absorb CO. Thus, carbon monoxide uptake is not dependent on flow (i.e. cardiac output), but rather diffusion[37]. For practical reasons, DLO_2 is difficult to calculate. Factors that would affect the diffusion of O_2 would also affect diffusion of CO, and thus carbon monoxide is commonly used to assess the diffusing capacity of the lungs[38].

2.6 Membrane Diffusing Capacity and Pulmonary Capillary Blood Volume

The transfer of carbon monoxide from the alveolar space to the pulmonary capillary blood is influenced by several factors[39,40], including alveolar volume, pulmonary capillary blood volume (Vc), alveolar-capillary membrane thickness, pH, and hemoglobin concentration[41]. Roughton and Forster (1957) conceived a simplified model to quantify lung diffusing capacity for carbon monoxide, and thus oxygen[40]. This simplified model partitions the overall conductance of CO in the lung into two components, the diffusing capacity across the alveolar-capillary barrier (Dm), and the reaction of carbon monoxide to hemoglobin in the pulmonary capillary blood. These are expressed as a series of resistances in the following equation.

$$\frac{1}{DL} = \frac{1}{Dm} + \frac{1}{\theta_{co} \times Vc}$$

Where θ_{CO} is the reaction rate of CO with hemoglobin (mL of gas uptake per mmHg pressure gradient per mL of whole blood in vitro), and Vc is the pulmonary capillary blood volume in mL. The above equation for 1/DL assumes the following[39,40,42]:

1) Gas phase resistance in the alveoli is inconsequential

2) Septal tissue and erythrocyte membranes do not interact with each other

3) Alveolar PO_2 does not affect Dm or Vc in a significant way

The third assumption is vital to the Roughton and Forster (1957) method of Dm and Vc measurement, as it involves the measurement of DLCO at varying alveolar oxygen tensions (P_AO_2)[40]. Binding of carbon monoxide to hemoglobin in blood is dependent on oxygen tension in the lung, such that increasing P_AO_2 will cause competitive inhibition for CO for heme sites on hemoglobin[40,43,44]. Therefore, DLCO is inversely related to the F_IO_2.

The Roughton and Forster (1957) multiple-F_IO_2 DLCO method utilizes this relationship to determine membrane diffusing capacity and capillary blood volume[40]. A number of studies have calculated Vc and Dm using these standard DLCO measurements at varying oxygen tensions[39,45,46]. DLCO is measured at three levels of F_IO_2, and the relationship of $1/\theta_{co}$ vs. 1/DLCO is plotted.

The equation for 1/θco as a function of the P_AO_2 is[40]:

$$\frac{1}{\theta_{co}} = \alpha \times P_AO_2 + \beta$$

Alpha (α) is a temperature- and pH-dependent coefficient linked to kinetic reactions of CO with Hb, and β is the ratio of red-cell membrane to red cell interior permeability. In their original publication, Roughton and Forster (1957) do not explicitly recommend specific values for α and β. Because of this, several studies have used variants of the constants in the above equation[39,40,42]. It is assumed that θco does not change with exercise, as θco is relatively insensitive to changes in pH[43]. In a recent study examining the effect of changing θco related constants on the calculation of pulmonary capillary blood volume and membrane diffusing capacity, Ceridon *et al.* (2012) found substantial variability in calculated values relating to the θ_{co}

equation[39]. However, they concluded that the equation assuming pH = 8.0, and λ = 2.5 for moderate red cell permeability, α = 0.0058 and β = 0.73 is the recommended equation for studies of Vc and Dm during exercise[39].

The assumption of a pH of 8.0 was addressed by Forster (1987) in a subsequent publication, providing a correction for α, taking into account a pH of 7.4[47]. However, when Ceridon *et al.* (2010) employed this correction, they found highly unlikely values for Dm across all subjects, thus confirming their recommended equation[39]. As pH becomes more acidotic during exercise, the use of this particular equation presents a limitation in the determination of Dm and Vc.

The final term in the calculation of θco is the alveolar PO_2 calculated from the following equation.

$$P_AO_2 = FIO_2 \left(P_{Bar} - P_{H_2O}\right) P_aCO_2 \times \frac{1 - FIO_2}{RER}$$

Where FIO_2 = fraction of O_2 in inhaled gas, P_{Bar} = atmospheric pressure, P_{H2O} = saturation vapor pressure of water, usually 47 mmHg at 37°C at rest, and RER = respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$).

2.7 Diffusing Capacity Response to Exercise

Diffusing capacity increases during exercise up to 150% of resting values, without reaching an upper limit with respect to cardiac output[48-52]. The increase in diffusing capacity occurs from increases in gas exchange surface area, as a result of increases in membrane diffusing capacity (Dm) and capillary blood volume (Vc) secondary to recruitment and distension of capillaries[41]. Diffusing capacity is also increased due to an increased capillary hematocrit, homogeneous distribution of red blood cells within and among capillaries[42], and increasing alveolar volume (V_A)[53]. Thus, it is important to consider differences in [Hb] and V_A in the measurement of DLCO

during exercise. The membrane diffusing capacity reflects the available alveolar-capillary surface area, and is inversely related to the barrier thickness, summarized in the following equation:

$$Dm = \mathrm{k} \times \frac{\text{surface area}}{\text{barrier thickness}}$$

Where k is the diffusion constant, representing gas permeability in lung tissue and plasma[26]. During exercise, membrane diffusing capacity can increase up to 50% above resting values[41,54]. An increase in Dm is a result of increasing surface area by way of unfolding and distension of alveolar septa associated with lung inflation[26,42], and recruitment of capillaries associated with previously un-perfused alveoli. As the Dm in an un-perfused alveolus is 0, this would suggest that Dm may be a surrogate index of pulmonary capillary recruitment[41,54,55].

The increase in pulmonary capillary blood volume during exercise reflects the recruitment and distention of pulmonary capillaries. Capillaries may be recruited and distended by an increase in plasma volume or red cell mass[56], as demonstrated by animal perfusion studies[57]. However, only an increase in red cell mass can increase pulmonary gas exchange. As cardiac output increases during exercise, thoracic blood volume increases[58], and the number of perfused capillaries (and thus Vc) directly increases concurrently with the rise in pulmonary arterial pressure[56,57]. This increases the surface area for pulmonary gas exchange. In contrast to Dm, Vc can increase up to 100% above resting values during maximal exercise[41,54]. This is consistent with data suggesting that the Vc response to exercise is dominated by pulmonary capillary distention and not recruitment[41,55]. However, the separate contributions of recruitment and distention to the increase in pulmonary capillary blood volume cannot directly be extrapolated from a Vc measurement alone[59].

It was previously theorized that pulmonary capillary blood volume would plateau during incremental exercise because of a morphological limitation of pulmonary capillary blood volume expansion[60]. A plateau in pulmonary capillary blood volume would result in a decreased pulmonary capillary transit time with further exercise intensity[60]. However, there is evidence that Vc does not plateau during exercise in healthy individuals[60-63]. This suggests that the capillary reserves for diffusion are not completely exhausted with maximal exercise[26].

2.8 Pulmonary Circulation

A pressure gradient must exist in order for blood to flow across the lung. The total pulmonary blood flow across the lung is considered the cardiac output. To increase cardiac output during exercise, pulmonary driving pressure (inflow – outflow pressure) must increase, and pulmonary vascular resistance must decrease[59]. As the right ventricle contracts, pressure in the pulmonary artery (PAP) increases more so than the left atrial pressure, which is the outflow pressure of the pulmonary circulation[59].

Pulmonary hemodynamic measurements in humans are routinely made using right heart catherization. Pulmonary artery and left atrial pressures are measured invasively using a balloon-tipped pulmonary artery catheter known as a Swan-Ganz catheter[64]. Briefly, a Swan-Ganz catheter is placed into the right ventricle under guidance of pressure monitoring. The balloon is then inflated and is carried by blood flow through the right heart into the pulmonary artery. When the catheter is confirmed to be in the pulmonary artery by the pressure tracing, the pressure measured distal to the balloon tip is said to be pulmonary arterial pressure. To determine the left atrial pressure, the catheter is advanced further until it is wedged in a smaller branch of the pulmonary artery. The pressure measured distal to the balloon is referred to as wedge pressure (Pw), equivalent to mean left atrial pressure[59].

Pulmonary vascular resistance (PVR) is calculated as the difference between mean pulmonary artery pressure (PAP) and wedge pressure (Pw) divided by the cardiac output:

$$PVR = \frac{PAP - Pw}{Q}$$

Direct measurement of pulmonary capillary pressure is not possible. However, the best estimation of pulmonary capillary pressure is halfway between PAP and Pw[65,66].

2.9 Pulmonary Circulation During Exercise

During exercise, cardiac output increases to meet the increasing oxygen demand. In order to increase cardiac output, pulmonary arterial pressure may rise up to 40 mmHg in humans[67]. The increased PAP presents a significant potential stress to the pulmonary circulation[68], as the pulmonary capillaries are susceptible to stress failure[69]. To blunt the rise in PAP, pulmonary vascular resistance must decrease during exercise[67].

At rest, pulmonary vascular resistance is quite low, but with the onset of exercise, PVR decreases a further 40-60%[59]. The mechanism for the decreased PVR during exercise is multifactorial[56]. The initial increase in PAP with exercise is believed to recruit and distend pulmonary capillaries that were previously hypo-perfused at rest[42,59,63]. The net result is an increase in the cross-sectional area of the pulmonary capillary network and a reduction in vascular resistance[59]. There is also evidence that pulmonary vascular tone is actively reduced by endothelial and neurohumoral factors during exercise[67,70]. Finally, it is possible that recruitment of anatomical intrapulmonary shunts may decrease pulmonary vascular resistance[6,55,71]. Working together, these factors decrease PVR in order to prevent substantial increases in PAP during exercise.

The relative contributions of capillary recruitment and distension to the overall increase in pulmonary capillary blood volume during exercise is still unresolved[59]. Research in an animal

model has demonstrated an increase in pulmonary capillary recruitment with a moderate increase in blood flow in isolated canine lungs, resulting in a more homogenous distribution of capillary transit times and capillary perfusion[72]. However, no additional recruitment was observed when blood flow was increased further, suggesting that full recruitment of capillaries occurs with modest increments of flow[59]. Extrapolating this research to the human, this would suggest that mild exercise fully recruits the pulmonary microcirculation in the upright lung[59]. Further increases in exercise intensity subsequently increase pulmonary capillary pressure, resulting in distension of pulmonary capillaries. Together, recruitment and distension of pulmonary capillaries increase pulmonary capillary blood volume, which increases surface area for gas exchange and maximize transit time of red blood cells.

Measures of resting capillary blood volume provide insight into the distensibility of the pulmonary vasculature, and the relationship of the pulmonary vasculature to exercise tolerance. As discussed below, and later in Chapter 4, a high maximal oxygen consumption rate ($\dot{V}O_{2max}$) is correlated with an increased resting pulmonary capillary blood volume[55]. Lalande *et al.* (2012) examined the relationship between Vc and pulmonary vascular distensibility during exercise in subjects of varying fitness[55]. Pulmonary vascular distensibility was assessed using agitated saline contrast echocardiography, as well as echo-derived estimation of mean pulmonary artery pressure and cardiac output. While many studies utilize agitated saline contrast as an indicator of intrapulmonary shunt recruitment[6,13,15], Lalande *et al.* (2012) interpreted the passage of agitated saline as increased pulmonary capillary distensibility, secondary to alveolar corner capillary distension up to 20 μm[55]. This suggestion of large-diameter pulmonary capillaries conflicts with other research showing that fully distended pulmonary capillaries are 15 μm or less in diameter[73].

The findings of Lalande *et al.* (2012) imply that Vc increases more due to capillary distension than recruitment at high levels of exercise[41,55], consistent with increased diameter of pulmonary capillaries during exercise. Accordingly, the increase in capillary diameter decreases pulmonary vascular resistance. As individuals with the highest resting Vc had the greatest $\dot{V}O_{2max}$, this suggests that pulmonary vascular distensibility may be related to aerobic exercise capacity in healthy humans[55]. Together, these data suggest that Vc, the pulmonary vasculature, and pulmonary vascular distensibility are important contributors to $\dot{V}O_{2max}$[74].

Of note, an increase in resting Vc is also well documented in heart failure patients[75-79], which is associated with chronic elevations in left atrial pressure[80]. Pulmonary arterial pressure is subsequently increased in order to maintain the pressure gradient required for pulmonary blood flow. Accordingly, pulmonary capillary pressure increases as well, recruiting and distending pulmonary capillaries and increasing Vc[79]. Thus, the finding of increased resting Vc in athletes is paradoxical in the context of similar findings in this diseased population.

Failure to decrease pulmonary vascular resistance with exercise is associated with pulmonary vascular dysfunction, like that observed in conditions such as pulmonary arterial hypertension or COPD[81,82]. An exaggerated increase in PAP during exercise is associated with an increase in minute ventilation, secondary to juxtacapillary receptor (J-receptor) activation within the pulmonary vasculature[83]. This may explain the excessive ventilation, dyspnea, and breathing discomfort observed in pulmonary arterial hypertension or COPD during exercise.

Work by Paintal (1969) detailed the stimulation of these J-receptors, which are sensory nerve endings located in the alveolar walls in juxtaposition to pulmonary capillaries which are innervated by the vagus nerve[83]. The rise in pulmonary arterial and capillary pressure resulted in

the excitation of these J-receptors, thereby increasing ventilation through afferent feedback[83]. More recent research has associated lung hyperinflation[84] and sensations of mouth, throat, and chest discomfort with J-receptor activation[85]. Further studies acutely increasing PAP by way of lower body positive pressure[86] and rapid saline infusion[87] during exercise have also observed exaggerated ventilation. Taken together, these studies suggest that elevations in PAP can cause a reflex-mediated increase in minute ventilation.

In summary, an increase in pulmonary capillary recruitment and distension during exercise serve to increase gas exchange surface area, slowing red cell passage through the lung to preserve transit time, and to decrease pulmonary vascular resistance to prevent large increases in pulmonary arterial pressure.

2.10 Dopamine Receptor Blockade

In addition to recruitment of pulmonary capillary recruitment and distension, there is evidence that pulmonary vascular resistance is reduced by endogenous dopamine during exercise[88]. The first study of this dissertation explored the effect of a dopamine blockade on exercise pulmonary gas exchange and exercise performance. The complete manuscript for this study can be found in Chapter 3, but will be discussed here briefly.

In low doses, dopamine decreases pulmonary vascular resistance, secondary to vasodilation in the pulmonary vasculature in humans[15,89,90] and in animals[91-93]. At rest, exogenous dopamine recruits anatomical intrapulmonary shunts as evaluated by agitated saline contrast echocardiography[15]. There is speculation that anatomical intrapulmonary shunts are recruited during exercise to help improve cardiac output by offloading the right ventricle. It was hypothesized that the exercise-induced increase in endogenous dopamine during exercise is

partly responsible for the recruitment of intrapulmonary shunts, and would correspondingly impair pulmonary gas exchange.

To test this, we antagonized dopamine receptors with a dopamine receptor blockade (metoclopramide) with the intention of reducing intrapulmonary shunt recruitment during exercise. As predicted, dopamine blockade reduced the pulmonary gas exchange impairment at peak exercise, but did not significantly affect intrapulmonary shunt recruitment as assessed by agitated saline contrast echocardiography. While pulmonary gas exchange was improved, dopamine blockade decreased cardiac output at peak exercise, resulting in a markedly decreased exercise performance.

While pulmonary arterial pressure was not measured, the increase in minute ventilation and decreased stroke volume are consistent with an increased PAP, which would stimulate J-receptors in the lung and exaggerate ventilation[83]. These results suggest that despite contributing to pulmonary gas exchange impairment during exercise, increasing endogenous dopamine is important for the control of pulmonary vascular pressure, and is critical for a normal cardiopulmonary response to exercise.

2.11 Pulmonary Capillary Blood Volume Response in Endurance-Trained Males

The second study of this dissertation examined the possible effect of aerobic fitness on pulmonary capillary blood volume and membrane diffusing capacity response to exercise in males. The manuscript in whole can be found in Chapter 4 of this dissertation but will be discussed briefly here.

Endurance-trained athletes exhibit enhanced cardiovascular function compared to non-athletes[32]. However, it is generally accepted that exercise training does not enhance lung structure and

function[94-96]. The evidence that a higher pulmonary capillary blood volume at rest is related to a higher $\dot{V}O_{2max}$[55] suggests that fitter individuals have a more distensible pulmonary circulation[56]. In keeping with this idea, it would be expected that a more distensible pulmonary circulation would result in a greater reduction in pulmonary vascular resistance with exercise[97]. To date, there has been little research on the effect of fitness on the Vc response to exercise[61]. We hypothesized that endurance-trained athletes would have a greater Vc response to exercise compared to non-athletes.

To test this, we recruited 15 athletes ($\dot{V}O_{2max}$ mean ± SD = 64.6 ± 6.9 $mL \cdot kg^{-1} \cdot min^{-1}$) and 14 non-athletes ($\dot{V}O_{2max}$ = 45.0 ± 4.4 $mL \cdot kg^{-1} \cdot min^{-1}$), and measured DLCO, Vc, and Dm at various exercise intensities. Consistent with a previous study[55], a higher resting Vc was significantly correlated with a higher $\dot{V}O_{2max}$. Diffusion capacity in athletes was greater at 85% and 90% of their $\dot{V}O_{2max}$ compared to non-athletes. Contrary to our hypothesis, Vc was not significantly greater in athletes compared to non-athletes. Instead, the greater DLCO at peak exercise in athletes was secondary to an enhanced diffusing membrane capacity.

These data suggest that endurance-trained athletes appear to have differences within the pulmonary membrane that facilitate increased oxygen delivery at peak exercise. It is possible that the increased Dm indicates an increased gas exchange surface area in athletes. Alternately, the increased lung volume and ventilation during high levels of exercise may thin the alveolar-capillary interface, decreasing resistance for diffusion across the membrane.

2.12 Chronic Obstructive Pulmonary Disease (COPD)

Chronic obstructive pulmonary disease (COPD) is a respiratory disorder largely caused by smoking, and is characterized by progressive, partially reversible airway obstruction and

pulmonary capillary destruction[98]. The severity of COPD is determined by the extent of airway obstruction as measured by pulmonary function testing. In patients with an FEV_1/FVC below the lower limit of normal (set at the level of the 5th percentile of the population)[99], disease severity is categorized primarily by FEV_1 % predicted, as outlined by the Global Initiative for Chronic Lung Disease (GOLD)[100,101]:

GOLD 1: Mild COPD $FEV_1 \geq 80\%$ predicted

GOLD 2: Moderate COPD FEV_1 between 50% and 80% predicted

GOLD 3: Severe COPD FEV_1 between 30% and 50% predicted

GOLD 4: Very severe COPD $FEV_1 < 30\%$ predicted

2.13 Dyspnea in COPD

COPD patients experience significant dyspnea, or breathlessness[102,103] on exertion, which profoundly affects health-related quality of life[98,104-106]. Importantly, increased exertional dyspnea is related to a reduction in daily physical activity[103,107], which is an important risk factor for COPD disease progression, mortality, and hospitalizations[102,108]. While the exact cause of exertional dyspnea is not completely understood, progress has been made in understanding some of the mechanisms of this complex system.

Control of respiration originates in the central nervous system, generating a motor pattern transmitted via the phrenic nerve to the respiratory muscles to initiate ventilation[102,109]. Variations in the central motor output depend on afferent feedback from various mechano- and chemoreceptors in lung, cardiovascular, and brain tissue[109,110]. The respiratory afferents resulting from an increase in breathing and/or the perception of dyspnea in COPD are triggered by dysfunction in both static and dynamic respiratory mechanics[110,111]. The changes in ventilatory mechanics in COPD are caused in part by the development of expiratory flow limitation. This is

due to permanent parenchymal destruction (emphysema) and airway dysfunction in the form of small airway inflammation, mucosal edema, airway remodeling/fibrosis, and mucous impaction[112].

Emphysema reduces lung elastic recoil pressure, further reducing driving pressure for expiratory flow through narrowed and poorly tethered airways[112]. The inspiratory capacity (IC) is the maximal volume of air that can be inhaled after a spontaneous expiration to end expiratory lung volume (EELV). The increased compliance of a lung with COPD increases the resting end expiratory lung volume, shifting inspiratory volume upwards causing a "static" lung hyperinflation. During exercise, this hyperinflation becomes worse as the dynamic EELV increases, and because total lung capacity does not change with exercise, the IC is decreased[113,114]. This is termed dynamic hyperinflation, which worsens with increasing dead space. Breathing frequency increases to compensate for an inability to expand tidal volume, which leads to greater dead space ventilation and eventual respiratory muscle fatigue[115].

These mechanical limitations to ventilation in COPD are relayed in afferent signals to the respiratory centers. Dyspnea occurs when the central neural output does not produce the expected result of adequate ventilation, and this is termed neuromechanical dissociation[116]. While decreasing IC is strongly associated with dyspnea, recent research in more advanced COPD suggests that it is the critical inspiratory reserve volume (IRV) and not IC that dictates the true mechanical limit at which further increases in ventilation are not possible[113]. Dyspnea rises as tidal volume expands to reach a minimal IRV regardless if dynamic hyperinflation is present. This suggests that the intensity of dyspnea is related to the reduction in IRV (and thus constraining tidal volume expansion) and not necessarily the extent of acute (dynamic) hyperinflation during exercise[113].

To summarize, exertional dyspnea in COPD results from the imbalance between the central demand for breathing and the ability of the lung to adequately ventilate during exercise. With an increase in disease severity, there is a progressive decline in inspiratory reserve volume, resulting in an inability to increase tidal volume and ventilation in response to the increased metabolic demand of exercise[114].

2.14 Mild COPD

There have been many studies exploring the mechanisms of dyspnea in severe COPD, including increased chemoreceptor sensitivity, dynamic hyperinflation, muscle weakness, and decreased chest wall compliance[106,115,117,118]. However, little is known regarding the underlying mechanisms for both the greater exertional dyspnea and potentiated ventilatory response to exercise in mild COPD.

More recently, evolving work in patients with mild COPD has demonstrated a greater impairment than previously appreciated[108,119,120]. Despite exhibiting a relatively preserved lung function ($FEV_1 \geq 80\%$ of predicted) and minimal dyspnea at rest, patients with mild COPD experience substantially greater dyspnea during exertion and reduced exercise tolerance (reduced peak oxygen consumption) compared to age-matched controls[119]. This indicates that the impact of the disease is worse than would be suggested by lung function testing alone. Furthermore, mild COPD patients have an exaggerated ventilatory response to exercise as evaluated by an increased $\dot{V}_E/\dot{V}CO_2$ response, and combined with airflow obstruction, this would potentiate exertional dyspnea[107,119,121].

Recent research has shown that mild COPD patients have a resting diffusing capacity (DLCO) that is 20% below healthy age-matched controls[119]. As reviewed earlier, both components of

DLCO, membrane diffusing capacity and pulmonary capillary blood volume, must increase in order to maintain adequate gas exchange and arterial blood gases during exercise[40]. However, there is very little work examining which component of DLCO explains the low diffusion capacity in mild COPD at rest[122]. Furthermore, there is no information regarding any impairment to the Vc or Dm response to exercise in COPD.

2.15 Pulmonary Vascular Dysfunction in Mild COPD

As outlined previously, an increasing Vc reflects the recruitment and distention of pulmonary capillaries. In addition to improving gas exchange, capillary recruitment and distension reduces pulmonary vascular resistance and prevents large increases in pulmonary arterial pressure (PAP) with increasing cardiac output (i.e. pulmonary blood flow)[56]. Much like changes seen in the systemic vasculature in COPD[123-126], there are also unfavorable changes to the pulmonary vascular bed in COPD[81]. Changes in pulmonary capillary blood volume response to exercise may indicate unfavorable changes to the pulmonary vasculature.

If Vc fails to sufficiently increase during exercise due to capillary destruction and/or upstream vascular impairment, a greater PAP would be expected for a given cardiac output. As discussed earlier, an abnormally increased PAP has been shown to consequently increase ventilation and greater dyspnea[86,87], consistent with J-receptor stimulation in the pulmonary vasculature[83,85]. Patients with COPD share some similarities to pulmonary arterial hypertension patients (PAH)[81], as excessive exercise ventilation is generally a poor prognosis in both diseases[127]. Further, PAH patients exhibit ventilatory inefficiency and dyspnea during exercise, consequently decreasing exercise tolerance and increasing mortality[128-130]. Finally, there is evidence that aging affects the Vc response to exercise[131,132]. Thus, any study involving mild COPD patients must be compared to age-matched control subjects.

2.16 Dopamine Blockade in COPD

As reviewed earlier in section 2.10, dopamine receptors in the lung vasculature[133,134] appear to be important in the regulation of pulmonary vascular pressure[15]. At low doses, dopamine is a pulmonary vasodilator and decreases pulmonary vascular resistance, thereby increasing in pulmonary blood flow without a concurrent increase in pulmonary artery pressure[92]. The first study in this dissertation (Chapter 3) provides evidence that blocking dopamine receptors negatively affects exercise performance, possibly due to an increased pulmonary vascular resistance. However, it is not known how dopamine blockade affects the pulmonary capillary response to exercise in COPD. If dopamine blockade does not affect the Vc response to exercise in COPD, this would indicate that the Vc response in COPD is independent of circulating dopamine and thus a blunted Vc response to exercise may be due to capillary destruction and not vascular impairment. Conversely, if dopamine blockade further reduces Vc during exercise in mild COPD, greater ventilation, dyspnea and further impaired exercise tolerance would be expected. If Vc response to exercise is worsened with dopamine blockade in mild COPD, this may suggest that their capillary vascular bed is not completely dysfunctional, and thus may be a target for therapeutic intervention. Furthermore, if based on these findings, dopamine blockade decreases Vc and increases dyspnea, metoclopramide could be used to experimentally simulate pulmonary vascular dysfunction in healthy subjects.

2.17 Dissertation Purpose

This dissertation is comprised of three research projects investigating the response of the pulmonary vasculature to exercise in health and chronic obstructive lung disease. With the increased metabolic demand during exercise, the pulmonary vasculature must respond to increase total surface area for gas exchange and to reduce right ventricular afterload and maintain

adequate cardiac output. Thus, any changes to the pulmonary vasculature, secondary to pharmacological intervention[135], exercise training status[136], or disease state would have a significant outcome on exercise pulmonary gas exchange, cardiac output, and overall exercise tolerance.

2.18 References

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CHAPTER 3

Dopamine Receptor Blockade Improves Pulmonary Gas Exchange but Decreases Exercise Performance in Healthy Humans

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3.1 Introduction

Intense aerobic exercise has been shown to decrease the efficiency of gas exchange in highly trained humans, as demonstrated by an increase in the alveolar-arterial oxygen difference (A-aDO_2)[1,2]. Increased A-aDO_2 during exercise was classically thought to be a result of diffusion O_2 limitation[1,3] or $\dot{V}_A/\dot{Q}$ inequality[1,4,5] secondary to transient interstitial pulmonary edema or reduced pulmonary transit time. More recent research suggests that intrapulmonary (IP) shunt vessels are recruited during exercise[6,7], and appear related to gas exchange impairment[7]. However, this anatomical evidence is in contrast to the considerable inert gas data which has not shown evidence of right-to-left shunt during exercise[8-10]. Thus, the mechanism of IP shunt recruitment and its association with A-aDO_2 is the subject of an unresolved debate[11-13]

During exercise, endogenous dopamine (DA) concentrations increase curvilinearly with intensity[14]. Importantly, there is evidence that dopamine causes gas exchange impairment, as dopamine infusion increases shunt fraction ($\dot{Q}s/\dot{Q}t$) in resting supine humans[15] and right-to-left shunt ($\dot{V}_A/\dot{Q} = 0$) in critically ill patients[16]. Dopamine also decreases pulmonary vascular resistance secondary to vasodilation in the pulmonary vasculature in humans[15,17,18] and animal models[19-21]. Additionally, our previous investigation reported that dopamine increased recruitment of IP shunt vessels with the concurrent increase in shunt fraction[15].

We hypothesized that increases in dopamine concentration[3] are responsible for the recruitment of IP shunt[15] and correspondingly impair gas exchange with exercise. Thus, dopamine blockade during exercise would improve gas exchange and reduce IP shunt. Additionally, in contrast to their detrimental effect on gas exchange, it has been speculated that these shunts may be important to help improve cardiac output[7,22-24], $\dot{V}O_{2max}$, and exercise tolerance by reducing pulmonary vascular resistance, and thereby offloading the right ventricle. Therefore, we also

hypothesized that despite improving gas exchange, administration of a dopamine blockade would be detrimental to exercise performance.

3.2 Methods

3.2.1 Ethical Approval

This study was approved by the Human Research Ethics Board (Biomedical Panel) at the University of Alberta. All procedures conformed to the Declaration of Helsinki. All participants gave written, informed consent to participate.

3.2.2 Study Design Overview

This study consisted of four phases that were separated by at least a week. *Phase 1:* Preliminary testing including pre-screening and progressive incremental exercise test (n = 12). *Phase 2:* Evaluation of pulmonary gas exchange and intrapulmonary shunt recruitment during exercise with or without dopamine blockade. To validate this protocol, a separate sample of participants completed an identical protocol, but with placebo at both time points (n = 15). *Phase 3*: Time-to-exhaustion (TTE) trials at 85% of $\dot{V}O_{2max}$, with or without dopamine blockade (order randomized and separated by at least 48 hours). *Phase 4*: Incremental exercise test to $\dot{V}O_{2max}$ on a cycle ergometer with or without dopamine blockade, with order randomized and separated by at least 48 hours (n = 15).

3.2.3 Phase 1: Preliminary Testing

Twelve healthy, non-smoking male participants (mean age ± SD: 25 ± 6 yr, $\dot{V}O_{2max}$ = 4.39 ± 0.59 $L \cdot min^{-1}$; 56.6 $mL \cdot kg^{-1} \cdot min^{-1}$) participated in phases 1-3. Participants completed physical activity readiness questionnaires (PAR-Q), were screened for any cardiopulmonary disorders and/or medications, and were screened for presence of a patent foramen ovale (PFO) with Doppler

echocardiography and agitated saline contrast procedures if no intra-atrial flow was demonstrated with Doppler imaging[25]. No PFOs were observed in any study participant. Additionally, participants were screened for risks associated with ingestion of a telemetry pill. Participants then performed an incremental cycle (Ergoselect II 1200 Ergoline, Blitz, Germany) test to volitional fatigue to determine $\dot{V}O_{2max}$ (Encore229 Vmax, SensorMedics, Yorba Linda, CA). The initial power output was set to 50 W and the power output was increased by 25 W every 2 min until ventilatory threshold was reached, identified by a systematic increase in both the slope of the $\dot{V}_E/\dot{V}O_2$ and RER-power output curve[26]. Each stage above ventilatory threshold was characterized by increments of 25 W each minute. The criterion for confirmation of $\dot{V}O_{2max}$ required that at least 3 of the 4 following conditions were met: volitional exhaustion; a respiratory exchange ratio (RER) greater than 1.1; increases in oxygen consumption less than 100 $mL \cdot min^{-1}$ with further increases in power output; and, reaching age-predicted maximum heart rate.

3.2.4 Phase 2: Evaluation of Gas Exchange and Intrapulmonary Shunt Recruitment

No less than 48 h after preliminary testing, participants returned to complete the gas exchange/intrapulmonary shunt trial. Participants ingested a placebo pill and were then instrumented with an antecubital intravenous catheter, and a radial artery catheter for blood sampling (detailed in *instrumentation and measurements* below). Echocardiograms were performed at rest and during exercise at 30%, 50%, 70%, and 85% of the power output at previously determined $\dot{V}O_{2max}$. Measurements were taken approximately 3 minutes into each 6-minute stage during steady state exercise. The relative power outputs were chosen in order to characterize the effect of the drug across varying intensities. After the first exercise period, participants ingested dopamine blockade, (metoclopramide, 20 mg orally), and recovered for 60

minutes before repeating an identical exercise protocol. Participants were blinded to the order of placebo and metoclopramide ingestion.

Metoclopramide. Metoclopramide is a dopamine-2 receptor antagonist, and time to peak systemic bioavailabilty of oral metoclopramide is < 60 minutes[27]. Previous studies using metoclopramide have administered doses up to 40 mg[28,29]. However, through pilot work, we determined that 20 mg was an effective dose to elicit an increase in exercise ventilation, with no apparent side effects.

Instrumentation and measurements. A 22-gauge intravenous catheter (Insyte-W, BD, Franklin Lakes, NJ, USA) was inserted into an antecubital vein, and attached via 6-inch extension tubing to a 3-way stopcock for agitation and injection of agitated saline contrast for contrast echocardiography[15]. A 20-gauge angiocatheter (Insyte-W, BD, Franklin Lakes, NJ, USA) was inserted into the radial artery using local anesthesia (1% lidocaine). Both catheters were kept patent using a pressurized flush system of normal saline, and samples were immediately analyzed with an ABL800 FLEX blood gas analyzer for PaO_2, $PaCO_2$, and SaO_2 (Radiometer Medical Aps, Brønshøj, Denmark). Core temperature was measured via telemetry capsule concurrent to arterial blood gas sampling in order to correct for increasing temperature (VitalSense, Mini Mitter Co. Inc., Bend, OR, USA). Respiratory gas-exchange data were collected using a metabolic measurement system (Encore229 Vmax, SensorMedics, Yorba Linda, CA). Pulse oxygen saturation (SpO_2) was measured using finger pulse oximetry (N-595; Nellcor Oximax, Boulder, CO, USA). Heart rate was measured by electrocardiography (CardioSoft, GG Medical Systems, Milwaukee, WI, USA). Cardiac output was determined beat-by-beat with impedance cardiography (PhysioFlow, Manatec, Paris, France) which has shown good agreement with the direct Fick method in previous studies[30,31]. All instruments were

calibrated before each exercise session, and calibrations were verified at the conclusion of each session.

Contrast Echocardiography. Agitated saline contrast echocardiography was used to quantify intracardiac and intrapulmonary shunt, according to previously published methodologies[7,15,24,32]. Briefly, 10 mL of saline was combined with 0.5 mL of air, and the solution was forcefully agitated through a three-way stopcock between two syringes to form fine suspended bubbles, then injected into the antecubital IV catheter. A four-chamber view of the heart was recorded prior to, and during contrast injection with a minimum of 20 cardiac cycles recorded. All echocardiograms were performed by one experienced sonographer (ECHOPac, Vivid Q, GE), and were later analyzed by a cardiologist who was blinded to experimental conditions (exercise intensity and drug condition). Intra-cardiac shunt was graded by the appearance of contrast in the left ventricle in less than 5 cardiac cycles. The appearance of contrast in the left ventricle after 5 cardiac cycles suggests an IP shunt. No participant had evidence of an intra-cardiac shunt during the study. Shunt was scored based on a previously described scoring system used in our laboratory[7,15] and others[32,33]. We attempted to obtain Doppler derived pulmonary arterial systolic pressure data at each workload; however, the lack of sufficient tricuspid regurgitant jet in most participants prevented these calculations.

Protocol Validation. To address any potential order effect, a separate sample of participants (n = 15) completed an identical study protocol to the gas exchange experiment, but were given placebo pills at both Time 1 (T_1) and Time 2 (T_2). Importantly, core temperature returned to baseline resting levels before the second bout of exercise began (rest: T_1 36.82 ± 1.24 ^{o}C and T_2 37.18 ± 0.20 ^{o}C, P = 0.67). Oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio (RER), minute ventilation ($\dot{V}_E$), heart rate (HR), and SpO_2 were not

different between T_1 and T_2 at rest or at any exercise intensity, and the change in core temperature from rest was not significantly different between T_1 and T_2 at any exercise intensity. The lack of an observed difference between the two incremental exercise sessions with placebo alone suggests no order effect from the protocol.

3.2.5 Phase 3: Time to Fatigue

Participants completed two time-to-exhaustion (TTE) trials (placebo vs. blockade, order randomized) on two separate days, at the same time of day. One hour before arrival to the laboratory, participants ingested placebo or dopamine blockade pills. Participants warmed-up at a self-selected intensity and duration, and then began a TTE trial at a power output corresponding to 85% of $\dot{V}O_{2max}$. Time was recorded from the onset of exercise to volitional exhaustion, defined as the inability of the participant to maintain a minimum cadence of 60 rpm. At least 48 hours, but not more than a week later, participants completed the remaining trial in the alternate condition.

3.2.6 Phase 4: Incremental Exercise Testing

A separate sample of fifteen healthy, non-smoking male participants (mean age ± SE: 24 ± 1 yr; height: 1.77 ± 0.02 m; mass: 80.3 ± 2.3 kg; $\dot{V}O_{2max}$: 4.02 ± 0.19 $L \cdot min^{-1}$; 52.1 ± 2.4 $mL \cdot kg^{-1} \cdot min^{-1}$) participated in phase 4. This sample included 5 participants from phases 1-3.

Participants reported to the laboratory and ingested either a placebo pill or dopamine blockade. After 1 hour, participants completed an incremental exercise test to volitional exhaustion (described above in preliminary testing). At least 48 hours, but not more than a week later, participants completed the remaining trial in the alternate condition.

3.2.7 Statistical Analysis

Statistical analysis was performed using two-way repeated-measures ANOVA (SigmaPlot, v.11.1, Systat Software, Inc., San Jose, CA, USA). For the evaluation of gas exchange and shunt, physiological responses ($\dot{V}O_2$, $\dot{V}CO_2$, RER, $\dot{V}_E$, HR, core temperature), gas exchange variables (PAO_2, PaO_2, $PaCO_2$, A-aDO_2), and cardiovascular responses ($\dot{Q}$, HR, SV, systolic and diastolic BP, and mean arterial pressure), there were two repeated measures: exercise intensity and drug condition, with five levels of exercise intensity (rest, 30%, 50%, 70%, and 85% of $\dot{V}O_{2max}$) and two levels of drug condition (placebo, blockade). For the evaluation of IP shunt recruitment (shunt score), there were 3 levels of exercise intensity (rest, 70%, and 85% of $\dot{V}O_{2max}$) and 2 levels of drug condition (placebo and blockade). When main effects were found, Fisher's least significant difference post hoc tests were used. Linear regression was performed to determine relationships at peak exercise intensity between 1) Change in cardiac output and A-aDO_2 2) Change in A-aDO_2 and change in IP shunt score and 3) Change in shunt score and change in cardiac output. Note, some data were missing from 2 participants at peak exercise due to some technical difficulties, and therefore, correlations were conducted with only 10 participants. Paired t-tests were used to evaluate the effect on dopamine blockade on exercise performance: Time-to-Exhaustion, $\dot{V}O_{2max}$, RER_{max}, $\dot{Q}_{max}$, HR_{max}, SV_{max}, peak power, and SpO_2. The order of these tests (placebo, blockade) was randomized. We accepted a type I error of 0.05 to determine a significant difference between variables.

3.3 Results

All participants tolerated the study phases and procedures well.

3.3.1 Effect of Dopamine Blockade on Pulmonary Gas Exchange

Descriptive characteristics of participants in the gas exchange experiment are reported in Table 3-1. A summary of physiological response to exercise is given in Table 3-2. There were no significant differences in O_2 consumption or CO_2 production between the placebo and blockade conditions. In general, any differences in cardiovascular or gas exchange responses between conditions occurred at the highest stages of the protocol 70% and 85% of $\dot{V}O_{2max}$.

Ventilation. Minute ventilation was increased 3.5% with the blockade at 70% of $\dot{V}O_{2max}$, and 13.8% at 85% of $\dot{V}O_{2max}$, which was driven by significant increases in respiratory rate at 70% and at 85% of $\dot{V}O_{2max}$. Tidal volume did not change significantly with blockade at any time point. Correspondingly, $PaCO_2$ was decreased at 70% and 85% of $\dot{V}O_{2max}$, consistent with increased alveolar ventilation.

Cardiovascular response. A summary of cardiovascular response is given in Table 3-3. Heart rate was significantly increased by blockade at rest, 30%, and 50% of $\dot{V}O_{2max}$, but not at 70% or 85%. Systolic blood pressure (SBP) was decreased at rest and 85% of $\dot{V}O_{2max}$. Diastolic blood pressure (DBP) was not different at any time point during exercise with blockade. Mean arterial pressure (MAP) was significantly decreased by blockade at 85% $\dot{V}O_{2max}$ (P=0.019). Cardiac output was significantly lower by 9.3% in the blockade condition at 85% of $\dot{V}O_{2max}$ but not at any other workloads.

Pulmonary Gas Exchange. Alveolar PO_2 was not changed with dopamine blockade at any level of exercise, but arterial PO_2 was significantly higher at 70% (P = 0.016) and at 85% of $\dot{V}O_{2max}$ (P = 0.049). Accordingly, there was a significant decrease in A-aDO_2 at 85% of $\dot{V}O_{2max}$ (P = 0.038)

and arterial O_2 saturation was increased at 70% (P = 0.024) and 85% of $\dot{V}O_{2max}$ (P = 0.001, Figure 3-1).

There was a significant, positive correlation between the change in cardiac output and the change in A-aDO_2 with dopamine blockade at the 85% workload (r = 0.694, P = 0.021, Figure 3-2 TOP).

3.3.2 Intrapulmonary Shunt Recruitment

Intrapulmonary shunt score increased with exercise, but no difference was observed between dopamine blockade and placebo at any intensity. Of 12 participants, 5 showed a mean decrease in shunt score of -1.4 ± 0.9 with dopamine blockade, but there was no correlation between decreased shunt score and decreased A-aDO_2 (Figure 3-2 MIDDLE) or change in shunt score and cardiac output at 85% of $\dot{V}O_{2max}$ (Figure 3-2 BOTTOM).

3.3.3 Functional Significance

Time to exhaustion. During the time-to-exhaustion (TTE) trials, dopamine blockade decreased exercise performance at 85% of $\dot{V}O_{2max}$ by 38.6% (P = 0.032). At exercise termination, cardiac output was decreased in the blockade condition by 9.3% compared to placebo (P = 0.042). The decrease in cardiac output with dopamine blockade was driven primarily by the 12.4% decrease in stroke volume (P = 0.044), as no significant change in heart rate was observed (Table 3-4). Dopamine blockade increased O_2 saturation by 0.8% at the end of the TTE (P < 0.001).

Incremental exercise response. A summary of the effects of dopamine blockade on graded exercise performance is presented in Table 3-4. Dopamine blockade decreased maximal exercise performance as $\dot{V}O_{2max}$ was decreased by 5.5% (P = 0.013), peak power output was decreased by 7.1% (P < 0.001), and maximum HR decreased 2.2% (P = 0.011). Ventilation was increased at

90% of $\dot{V}O_{2max}$ with blockade, but peak ventilation was decreased likely because of a reduction in $\dot{V}O_{2max}$ (Figure 3-3).

3.4 Discussion

This study examined the effects of a dopamine receptor blockade on pulmonary gas exchange, intrapulmonary shunt recruitment, and exercise performance. Consistent with previous studies, A-aDO_2 was increased during intense aerobic exercise[1,2,8]. Dopamine blockade decreased peak exercise A-aDO_2 by 22.3%, indicating an improvement in gas exchange. Within the limitations of utilizing agitated saline contrast echocardiography, dopamine receptor blockade did not appear to affect IP shunt, and the individual improvement in A-aDO_2 was unrelated to the individual change in IP shunt score. Despite improving gas exchange, dopamine blockade decreased exercise performance, as measured by $\dot{V}O_{2max}$ and time-to-exhaustion at 85% of $\dot{V}O_{2max}$. The results of this study suggest that endogenous dopamine is important to the normal cardiopulmonary response to exercise.

3.4.1 Pulmonary Gas Exchange During Exercise

We have previously shown that exercise-induced IP shunt recruitment is related to the decreases in pulmonary gas exchange[7]. The mechanisms by which these shunts are recruited, and their contribution to gas exchange remain the subject of an unresolved debate[12,13]. Dopamine increases with exercise intensity[3], exogenous dopamine impairs gas exchange in healthy[15] and ill participants[16]. Contrast echocardiography indicates that IP shunts are recruited with exogenous dopamine[15,32]. Therefore, we hypothesized that blocking dopamine receptors with metoclopramide would decrease IP shunt and improve pulmonary gas exchange during exercise. The results show that blocking dopamine receptors reduces the pulmonary gas exchange impairment at peak exercise, but the improvement in gas exchange appears to be unrelated to a

decrease in IP shunt recruitment as evaluated by saline contrast echocardiography. Further, the individual change in A-aDO_2 at peak exercise was not related to the individual change in IP shunt score following dopamine blockade (Figure 3-2 MIDDLE), suggesting that anatomical IP shunt, as evaluated by agitated saline contrast transmission, is not related to gas exchange.

While the improvement in gas exchange with dopamine blockade may be unrelated to IP shunt, there is evidence that the improvement in gas exchange was secondary to a reduction in cardiac output. In addition to the 23% improvement in A-aDO_2 at the highest level of exercise, dopamine blockade resulted in a 10% reduction in cardiac output, secondary to a 7.8% reduction in stroke volume. Further, the reduction in cardiac output was correlated with the improvement in A-aDO_2 (see Figure 3-2 TOP). While pulmonary transit time was not determined, these findings highlight the importance of cardiac output as a determinant of gas exchange impairment and are consistent with early suggestions of a diffusion limitation secondary to reduced pulmonary transit time during heavy exercise[1,2,34].

In a previous investigation Dopamine infusion at rest recruited intrapulmonary shunt and increased $\dot{Q}s/\dot{Q}t$[15]. However, there was no change in the A-aDO_2 due to the concurrent increase in mixed venous oxygen content, resulting from the increased cardiac output. In the current study, oxygen consumption during the highest level of exercise was unchanged by dopamine blockade, while cardiac output was reduced. Therefore, mixed venous oxygen content would have been reduced as a result of increased peripheral O_2 extraction. Based on principles of gas exchange, a reduction in mixed venous PO_2 by itself would cause an *increase* in A-aDO_2[35]. However, the *improvement* in A-aDO_2 with dopamine blockade, despite a reduction in mixed venous PO_2, indicates that some component of gas exchange (likely ventilation/perfusion matching) was improved following dopamine blockade.

The increased ventilation with dopamine blockade caused a significant reduction in $PaCO_2$ and subsequent increase in pH (Table 3-2), which would have resulted in a leftward shift of the oxygen-hemoglobin dissociation curve[36]. Based on previous calculations[37], the 0.4 unit increase in pH following dopamine blockade would have increased SaO_2 by 0.4%. These calculations suggest that approximately half of the improvement in SaO_2 with dopamine blockade can be explained by a shift in the oxygen-hemoglobin dissociation curve, with the remaining improvement the result of enhanced gas exchange.

3.4.2 Cardiovascular Effects of Dopamine Blockade

While dopamine blockade seems to improve pulmonary gas exchange, we observed a contrasting effect on exercise tolerance. Decreases were seen in measures of peak exercise performance ($\dot{V}O_{2max}$ and peak power) and time to exhaustion cycling at a power output corresponding to 85% of $\dot{V}O_{2max}$. While $\dot{V}O_2$ was not measured during the time-to-exhaustion trial, the decrease in TTE was consistent with decreased cardiac output, secondary to a reduction in stroke volume. Based on the gas exchange data, the increase in PO_2 and SaO_2 observed with dopamine blockade would have increased arterial O_2 content by only 1%, which is in contrast to the 10% decrease in cardiac output. The resultant 9% decrease in convective O_2 delivery would explain the diminished exercise performance with dopamine blockade despite improved pulmonary gas exchange.

While low dose dopamine acts as a pulmonary vasodilator in humans[20] and animals[19,21], blocking dopamine receptors increases PAP secondary to increased pulmonary vascular resistance[20]. Further, dopamine blockade has been shown to decrease exercise tolerance[38]. Acutely increasing PAP via lower body positive pressure[39] and saline infusion[40], increases the ventilatory response to exercise. While we were unsuccessful in determining PAP in the present study, as none of our participants had sufficient tricuspid regurgitation to estimate pulmonary artery systolic

pressure[41], we observed an increase in ventilation and a decrease in stroke volume during exercise with dopamine blockade, which could be due to an potentiated PAP response to exercise, secondary to J-receptor stimulation in the lung[42]. Although speculative, it is plausible that dopamine blockade increased PAP, limiting cardiac output, and by extension, exercise performance due to increased right ventricular afterload[7,22]. The results of the present study highlight the importance of endogenous dopamine in the normal cardiopulmonary response to exercise.

Previous work has demonstrated that dopamine receptor agonists inhibit carotid chemoreceptors (CC) in humans[43] and animals[44,45], decreasing sympathetic vasoconstrictor outflow[43,44,46], and ventilatory drive during exercise[47]. Conversely, dopamine blockade may sensitize the CC, increasing sympathetic outflow, decreasing skeletal muscle blood flow[43] and increasing the ventilatory drive during heavy exercise[47]. In the current study, peak cardiac output was reduced by dopamine blockade. Yet, systolic and diastolic blood pressures were not different, suggesting that sympathetic outflow may have been increased secondary to heightened CC activity. Additional work is needed to evaluate the effect of dopamine blockade on CC activity/sensitivity and sympathetic control of cardiovascular function.

3.4.3 Study Limitations

Agitated saline contrast echocardiography has been utilized in several studies to assess intrapulmonary shunt recruitment during exercise, but the practice has been debated in a recent Point:Counterpoint discussion[12,13]. Several contributors pointed out shortcomings of this technique, primarily the qualitative nature of the interpretation of images, and the inability to determine the size of micro-bubbles in contrast solution[11]. Thus, in the current study it is possible that the method of detection was not sufficiently sensitive to detect such subtle changes between

drug and placebo conditions. Acknowledging this limitation, the improvement in pulmonary gas exchange observed with dopamine blockade does not appear to be related to IP shunt, as assessed by agitated saline contrast.

We attempted to determine PASP via tricuspid regurgitant velocity. However, due to our young and healthy sample, we were not able to obtain a suitable signal. As a supplement to our non-invasive cardiac output estimation, we attempted to measure aortic velocity-time integral. However, due to movement artifact during exercise, we were not able to maintain an acceptable window to obtain a high quality Doppler-derived cardiac output measurement. Future experiments should employ the multiple inert gas elimination technique to confirm if dopamine blockade decreases diffusion limitation during exercise, or attenuates ventilation-perfusion inequality during exercise.

It is possible that not all dopamine-2 receptors may have been blocked in our experiment. To the best of our knowledge, there is no method to evaluate if dopamine receptors are fully blocked in humans, and we acknowledge this limitation. The 38.6% reduction in time to exhaustion with dopamine-2 blockade was larger than what would be expected by the 9.3% reduction in cardiac output. As metoclopramide was given systemically, it may have influenced central (i.e. brain) dopamine receptors. Long term and/or high dose metoclopramide increases the risk of the development of neurological and muscle control disorders such as tardive dyskinesia[48,49], suggesting that central dopamine-2 receptors may be important for motor control and/or motor output and exercise. Thus, it is possible that the decreased exercise performance observed with dopamine blockade may be partly explained by central dopamine-2 receptor antagonism.

Finally, because dopamine blockade reduced $\dot{V}O_{2max}$, all workloads in the blockade condition represent a greater relative percentage of maximal effort. This presents a potential limitation in drawing comparison between the blockade and placebo condition. However, we aimed to examine the cardiopulmonary response to dopamine blockade at the same rate of oxygen demand, and therefore compare the two conditions across the same absolute workloads.

3.5 Conclusion

We examined the effect of a dopamine-2 receptor blockade on the cardiopulmonary response to exercise. We found that blocking dopamine-2 receptors with 20 mg metoclopramide improved gas exchange during near-peak exercise; however, cardiac output and exercise tolerance were decreased. IP shunt recruitment, as evaluated by saline contrast echocardiography, was unaffected with dopamine blockade, suggesting that the improvement in gas exchange was unrelated to a decrease in IP shunt. These findings suggest that endogenous dopamine contributes to exercise-induced gas exchange impairment; however, dopamine is an important mediator of the normal convective oxygen delivery during exercise by optimizing blood flow, cardiac output and exercise performance.

3.6 References

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3.7 Figures and Tables

Table 3-1. Participant characteristics (n = 12)

	Means ± SD	Range
Age, yr	25 ± 6	20-39
Height, m	1.78 ± 0.05	1.68-1.87
Mass, kg	75.8 ± 7.8	63.6-91.0
$\dot{V}O_{2max}$, $L \cdot min^{-1}$	4.39 ± 0.59	2.96-5.33
$\dot{V}O_{2max}$, $mL \cdot kg^{-1} \cdot min^{-1}$	58.6 ± 6.5	46.6-67.3
$\dot{V}_{Emax}$, $L \cdot min^{-1}$	152.5 ± 32.4	98.9-217.2
Peak power output, w	352 ± 63	250-425
HR_{max}, $b \cdot min^{-1}$	184 ± 9	171-201

$\dot{V}O_{2max}$, maximal O_2 consumption; $\dot{V}_{Emax}$, maximal ventilation; HR_{max}, maximal heart rate.

Table 3-2. Physiological responses to graded exercise with dopamine blockade (n=12)

		Rest	30%	50%	70%	85%
PO (W)			67 ± 16	142 ± 24	234 ± 38	290 ± 47
$\dot{V}O_2$, $L \cdot min^{-1}$	Placebo	0.41 ± 0.01	1.35 ± 0.06	2.22 ± 0.12	2.97 ± 0.19	3.65 ± 0.26
	Blockade	0.40 ± 0.02	1.43 ± 0.06	2.24 ± 0.10	3.14 ± 0.18	3.69 ± 0.26
$\dot{V}CO_2$, $L \cdot min^{-1}$	Placebo	0.33 ± 0 .11	1.15 ± 0.05	2.17±0.07	3.34 ± 0.19	4.20 ± 0.32
	Blockade	0.35 ± 0.02	1.23 ± 0.07	2.19 ± 0.09	3.54± 0.16	4.25 ± 0.29
RER	Placebo	0.80 ± 0.02	0.85 ± 0.02	0.97 ± 0.02	1.02 ± 0.01	1.06 ± 0.01
	Blockade	0.84 ± 0.03	0.86 ± 0.02	0.96 ± 0.02	1.03 ±0.02	1.05 ± 0.01
$\dot{V}_E$, $L \cdot min^{-1}$	Placebo	10.8 ± 0.5	32.1 ± 1.6	51.2 ± 2.4	95.6 ± 5.6	123.8 ± 8.7
	Blockade	12.5 ± 0.5	33.9 ± 1.9	55.2 ± 2.5	99.0 ± 5.1*	140.9 ± 11.0*
$PaCO_2$, mmHg	Placebo	36.7 ± 0.7	37.8 ± 0.7	38.3 ± 0.9	36.2 ± 0.3	32.4 ± 0.6
	Blockade	35.7 ± 1.3	37.1 ± 0.7	37.7 ± 0.7	34.6 ± 0.7*	30.4 ± 0.7*
pH	Placebo	7.42 ± 0.002	7.41 ± 0.002	7.39 ± 0.002	7.33 ± 0.004	7.31 ± 0.005
	Blockade	7.41 ± 0.002	7.40 ± 0.001	7.39 ± 0.003	7.36 ± 0.004*	7.35 ± 0.004*
ΔT, ^oC	Placebo		0.06 ± 0.14	0.20 ± 0.16	0.52 ± 0.16	0.67 ± 0.16
	Blockade		0.05 ± 0.02	0.21 ± 0.04	0.62 ± 0.07	0.73 ± 0.07
Dyspnea	Placebo	0.0 ± 0.0	1.2 ± 0.4	3.1 ± 1.2	5.0 ± 1.3	8.1 ± 1.1
	Blockade	0.0 ± 0.0	1.3 ± 0.5	3.1 ± 1.1	5.7 ± 0.9	8.6 ± 1.0

Values are means ± SE. PO, Power output; $PaCO_2$, partial pressure of arterial CO_2; ΔT, change in core temperature from rest; Dyspnea, breathlessness score (1-10); Leg fatigue, fatigue score (1-10). Significantly different from placebo *P<0.05.

Table 3-3. Cardiovascular responses to graded exercise with dopamine blockade (n=12)

		Rest	30%	50%	70%	85%
$\dot{Q}$, $L \cdot min^{-1}$	Placebo	7.4 ± 0.3	10.3 ± 0.7	14.6± 1.0	19.4 ± 1.3	22.2 ± 1.6
	Blockade	7.8 ± 0.3	11.4 ± 0.6	14.7± 0.9	19.1 ±1.0	20.0 ± 1.1*
HR, $b \cdot min^{-1}$	Placebo	58 ± 3	95 ± 2	131± 3	159 ± 4	178 ± 4
	Blockade	75 ± 3	12 ± 2	139 ± 4	163 ± 4	178 ± 3
SV, mL	Placebo	90 ± 5	103 ± 8	125 ± 7	127 ± 9	129 ± 4
	Blockade	97 ± 4	113 ± 6	128 ± 7	129 ± 7	119 ± 10
SBP, mmHg	Placebo	111 ± 3	133 ± 4	161 ± 5	179 ± 3	195 ± 4
	Blockade	103 ± 2*	129 ± 3	153 ± 5	175 ± 7	188 ± 5*
DBP, mmHg	Placebo	66 ± 2	59 ± 1	61 ± 1	62 ± 1	61 ± 1
	Blockade	65 ± 2	63 ± 2	62 ± 2	63 ± 2	65 ± 2
MAP, mmHg	Placebo	81 ± 2	83 ± 2	93 ± 3	99 ± 5	105 ± 1
	Blockade	76 ± 2	83 ± 1	88 ± 1	96 ± 2	103 ± 1*

Values are means ± SE. $\dot{Q}$, cardiac output; SV, stroke volume; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure = (1/3(SBP) + 2/3(DBP). Significantly different from placebo *$P<0.05$

Table 3-4. Exercise performance response to dopamine blockade

	Placebo	Blockade	% Change
Time to Exhaustion Test at 90% of $\dot{V}O_{2max}$ (n = 12)			
TTE, s	500 ± 53	307 ± 27	-38.6*
$\dot{Q}_{max,}$ $L \cdot min^{-1}$	21.4 ± 1.4	19.4 ± 1.0	-9.3*
HR_{max}, $b \cdot min^{-1}$	181 ± 2	188 ± 2	+3.9
SV_{max}, mL	118 ± 9	103 ± 8	-12.6*
SpO_2, %	95.3 ± 0.2	96.1 ± 0.2	+1.0$^{\sharp}$
Graded Exercise Test (n = 15)			
$\dot{V}O_{2max}$, $L \cdot min^{-1}$	4.02 ± 0.19	3.80 ± 0.17	-5.5*
RER_{max}	1.15 ± 0.01	1.12 ± 0.01	-3.0
HR_{max}, $b \cdot min^{-1}$	184 ± 2	180 ± 4	-2.2*
Peak power, W	311 ± 14	289 ± 13	-7.1*
$\dot{V}_{Emax,}$ $L \cdot min^{-1}$	141.1 ± 8.8	127.6 ± 8.9	-9.6*

Values are means ± SE. % Change is the percent difference in blockade condition from placebo at maximal exercise. $\dot{Q}_{max}$, maximal cardiac output during the TTE test; SV_{max} maximal stroke volume during TTE, time to exhaustion cycling at 90% of $\dot{V}O_{2max}$; SpO_2, O_2 saturation at end of TTE. $\dot{V}_{Emax}$, minute ventilation at $\dot{V}O_{2max}$. *P< 0.05, $^{\sharp}$P <0.01

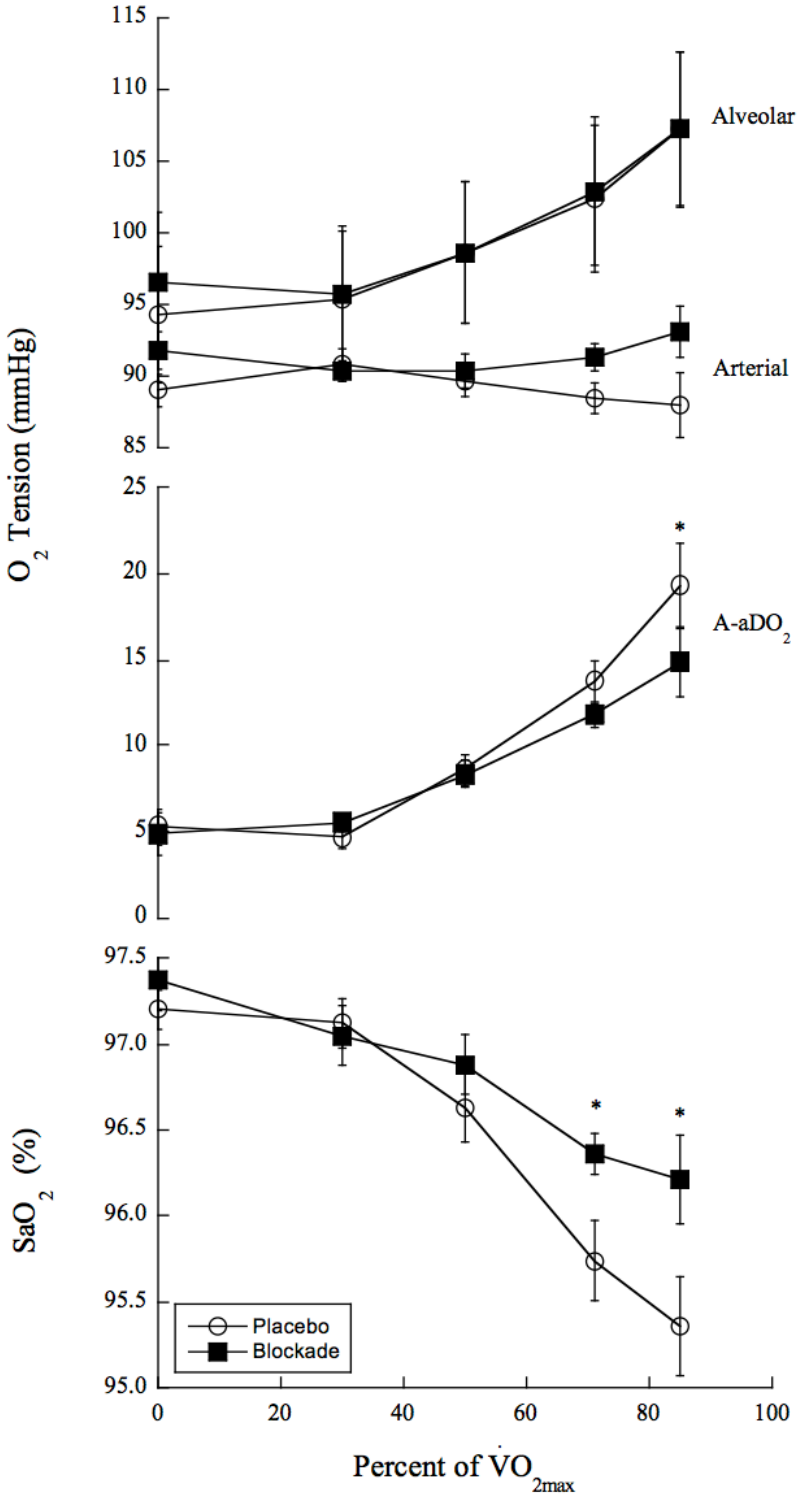
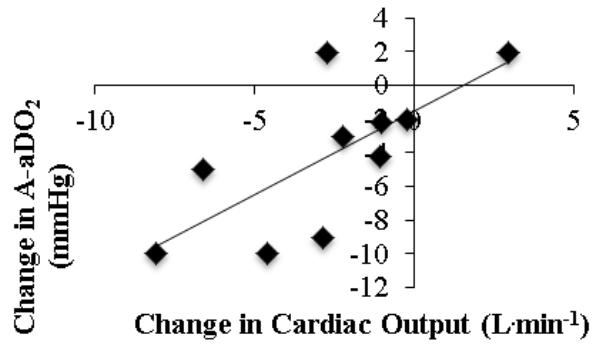
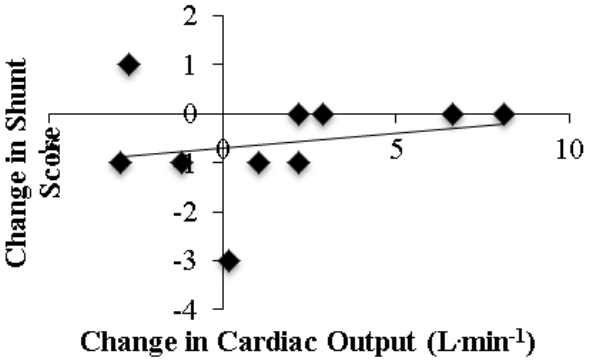


Figure 3-1. Mean ± SE pulmonary gas exchange response to dopamine blockade during exercise**.** A-aDO_2 (middle) was significantly decreased with dopamine blockade at 85% of $\dot{V}O_{2max}$ ($^{*}P< 0.05$). SaO_2 was significantly increased with dopamine blockade at 70% and 85% of $\dot{V}O_{2max}$. (n=12, n=10 at 85% of $\dot{V}O_{2max}$).





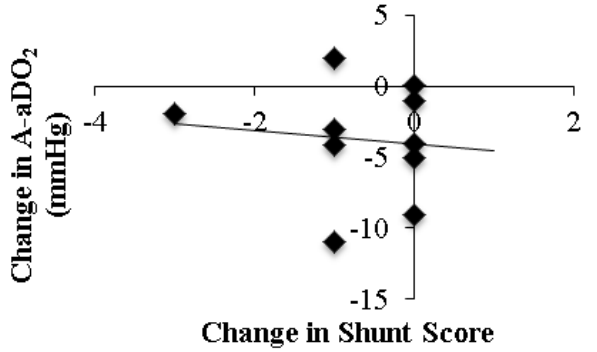


Figure 3-2. Correlations. TOP-Relationship between individual changes in cardiac output and A-aDO_2 with blockade at 85% of $\dot{V}O_{2max}$ (R^2 = 0.50, *P* = 0.02), n=10.
MIDDLE-Relationship between individual changes in A-aDO_2 and shunt score with blockade at 85% of $\dot{V}O_{2max}$ (R^2 = 0.04, *P* = 0.30), n = 10.
BOTTOM-Individual changes in Cardiac Output and Shunt Score with blockade at 85% of $\dot{V}O_{2max}$ There was no statistically significant positive correlation (R^2 = 0.01, *p* = 0.58), n = 10.

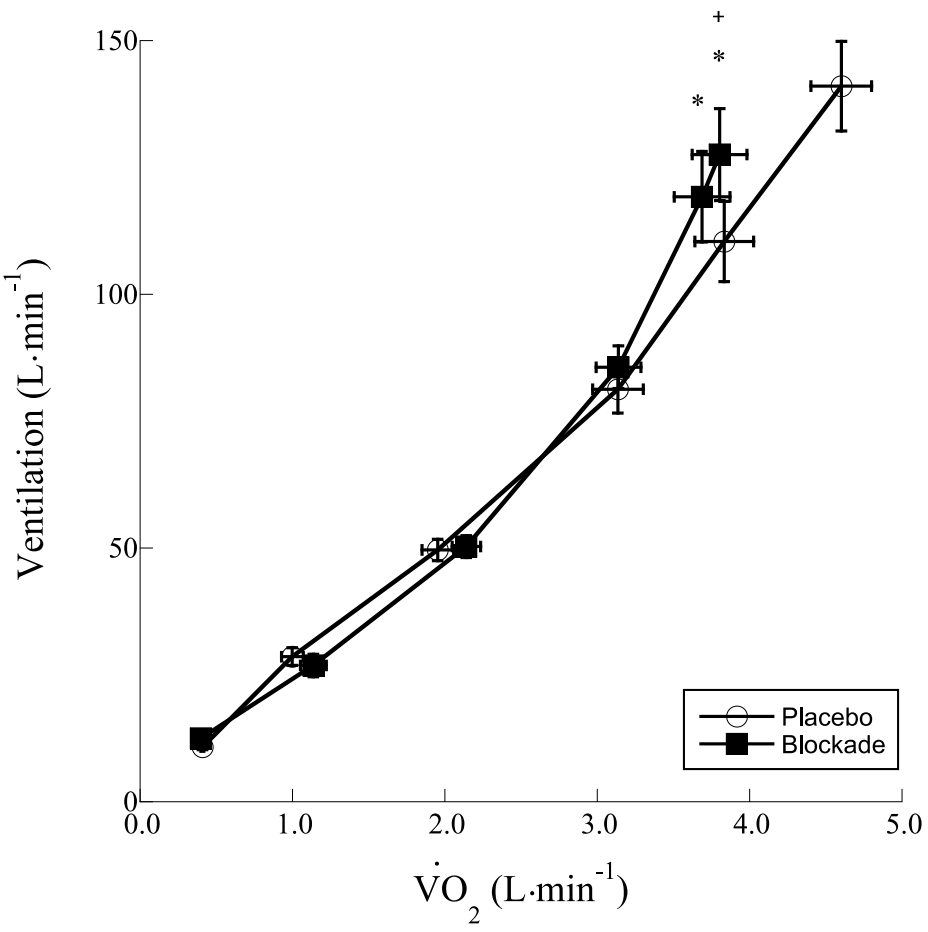


Figure 3-3: Mean (± SE) ventilatory response to graded exercise with dopamine blockade. Ventilation was increased in blockade condition at 85% but decreased at 100% of $\dot{V}O_{2max}$ $^{*}P < 0.05$. Blockade decreased $\dot{V}O_{2max}$ $+P < 0.05$ (n = 15).

CHAPTER 4

Effect of Aerobic Fitness on Capillary Blood Volume and Diffusing Membrane Capacity Response to Exercise

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4.1 Introduction

With incremental exercise, pulmonary diffusion capacity (DLCO) must increase with exercise to meet the greater oxygen demand, otherwise, a diffusion limitation might occur, leading to a greater alveolar-arterial oxygen difference and ultimately, exercise-induced arterial hypoxemia[1]. From rest to peak exercise, diffusion capacity, as evaluated by the diffusing capacity for carbon monoxide (DLCO), increases up to 150%[2-5] due to expansions in pulmonary capillary blood volume (Vc) and membrane diffusing capacity (Dm)[6]. As cardiac output increases to meet oxygen delivery requirements during incremental exercise, the increase in right ventricular pressure results in an augmented pulmonary arterial pressure (PAP)[7], which in turn expands Vc through recruitment and distension of the pulmonary capillaries[8]. Capillary recruitment results in a concurrent increase in Dm, as previously un-perfused 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[1,9].

Highly fit individuals exhibit enhanced cardiovascular function compared to low fit indivduals[10]. However, it is generally accepted that exercise training does not affect lung structure and function[11-13]. Recent research has shown that individuals with a higher $\dot{V}O_{2max}$ have greater resting pulmonary capillary blood volume[14]. Furthermore, there is evidence that that resting pulmonary artery and pulmonary wedge pressures (PAWP) are likely lower in highly fit individuals[10,15]. Taken together, these data suggest that the pulmonary circulation in highly fit individuals is more distensible[9], and thus there might be differences in the pulmonary vasculature between highly fit and low fit subjects that relate to $\dot{V}O_{2max}$. As highly fit individuals have a greater $\dot{V}O_{2max}$, they would require a greater ability to increase diffusion capacity with exercise,

in order to prevent diffusion limitation and gas exchange impairment. While it has traditionally been assumed that the pulmonary vasculature is insensitive to exercise training[10,13,16], and that pulmonary capillary recruitment plateaus at a critical exercise intensity and/or recruitment is not affected by training[3], a recent study conducted at rest suggests that the exercise response may be different between highly-fit and low-fit individuals[14].

There are a number of studies examining diffusion capacity during exercise[2-5,17], however, none of these have looked at exercise above 80% of $\dot{V}O_{2max}$. Thus, it is not fully understood exactly how Vc and Dm contribute to the increased DLCO during exercise, or how aerobic fitness may modulate this response. We hypothesized that the highly fit group would have a greater DLCO, Vc, and Dm, compared to the low fit group during exercise. To test this, we adapted the Roughton and Forster (1957) multiple F_IO_2–DLCO technique[18] to determine the effect of aerobic fitness on DLCO, Vc, and Dm during incremental exercise. If DLCO, and thus Vc and/or Dm were found to be higher during exercise in the highly fit, 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.

4.2 Methods

4.2.1 Ethical Approval

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

Fourteen LO-fit males (LO: $\dot{V}O_{2max}$ mean ± SD: 45.0 ± 4.4 $mL \cdot kg^{-1} \cdot min^{-1}$) and 15 HI-fit males (HI: $\dot{V}O_{2max}$: 64.6 ± 6.9 $mL \cdot kg^{-1} \cdot min^{-1}$) volunteered for this study. All subjects were nonsmokers, had normal pulmonary function, and had no history of pulmonary or cardiovascular disease. Subjects in the LO-Fit group self-reported to be recreationally active, exercising at moderate intensity for at least one hour, three times a week. All individuals in the HI-Fit group self-reported exercise at high intensity for at least one hour, at a minimum of five times a week. Subject characteristics and pulmonary function data are presented in Table 4-1.

4.2.2 Study Design Overview

At a preliminary testing session, subjects completed 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 maneuvers while 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 maneuvers. The DLCO sessions were spread out over three days, with the order of workloads and the F_IO_2 randomized.

4.2.3 Preliminary Testing

Subjects reported to the laboratory, completed Physical Activity Readiness Questionnaires (PAR-Q), were screened for cardiopulmonary disorders and/or medications, and completed resting pulmonary function testing. Subjects then performed a test for $\dot{V}O_{2max}$ (Encore229 Vmax, SensorMedics, Yorba Linda, CA) 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 power output was increased by 25 W every two minutes until ventilatory threshold, and every minute beyond ventilatory threshold. Confirmation of $\dot{V}O_{2max}$ required that three of the four following conditions were satisfied: volitional exhaustion; a respiratory exchange ratio (RER) greater than 1.1; increases in oxygen consumption < 100 $mL \cdot min^{-1}$ with further increase in power output; and reaching age-predicted maximum heart rate. Percent predicted $\dot{V}O_{2max}$ was determined using the following equations[19]:

$$\text{Absolute } \dot{V}O_{2max\ predicted} = 4.2 - (0.033 \times \text{Age})$$

$$\text{Relative } \dot{V}O_{2max\ predicted} = 60 - (0.55 \times \text{Age})$$

Additionally, cardiac output was evaluated using impedance cardiography during the incremental exercise test (PhysioFlow, Manatec Biomedical LLC, Ebersviller, France).

4.2.4 Exercise DLCO Sessions

No less than 48 h after preliminary testing, subjects returned to the laboratory for further testing. Lung diffusion capacity for carbon monoxide (DLCO) was determined using the single-breath breath-hold technique[20] at baseline and during exercise (Encore V62J Autobox, SensorMedics, Yorba Linda, CA). Hemoglobin concentration [Hb] was measured at the beginning of each

session (HemoCue 201+, HemoCue AB, Angelholm, Sweden) to correct DLCO for [Hb] using the equation[21]:

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

To calculate Vc and Dm, 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 two minutes of wash out 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 maneuver. Immediately prior to each DLCO maneuver, each subject pre-breathed five breaths of gas from a Douglas bag at the respective FIO_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 TLC, and to perform a breath hold for six seconds, 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 V_A was not within 5% of previous trials, or if breath hold time was not 6.0 ± 0.3 s. The V_A for each individual trial was similar at baseline, submaximal exercise, and peak exercise (Table 4-2).

Pulmonary capillary blood volume (Vc) and membrane diffusing capacity (Dm) were determined using the Roughton and Forster (1957) multiple-F_IO_2 DLCO breath-hold technique with the equation:

$$\frac{1}{DL} = \frac{1}{Dm} + \frac{1}{\theta_{co} \times Vc}$$

θ_{CO} was calculated using the equation[18]:

$$\frac{1}{\theta_{CO}} = 0.0058 \times P_A O_2 + 0.73$$

P_AO_2 was calculated from the equation:

$$P_AO_2 = FIO_2 \left(P_{Bar} - P_{H_2O}\right) PaCO_2 \times \frac{1 - FIO_2}{RER}$$

As with previous reports[22,23], we did not correct for carboxyhemoglobin as subjects were non-smokers[24], and two minutes between DLCO breath-hold tests has been shown to sufficiently clear CO from the lungs[25]. Also, there is evidence that multiple short breath hold maneuvers (~5 s) do not appreciably decrease DLCO until COHb is greater than 6%[26]. Finally, exercise promotes clearance of CO from the lungs and blood due to increased cardiac output and ventilation[27].

$PaCO_2$ was estimated from end tidal CO_2 values. For each workload, the relationship between 1/DLCO and 1/θ 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 maneuvers were repeated when r^2 values were outside of this range. Values for 1/Vc (slope) and 1/Dm (y-intercept) were then determined[18].

4.2.5 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 mean ± standard error unless otherwise indicated. Two-way repeated

measures ANOVA (SigmaPlot, v.11.1, Systat Software, Inc., San Jose, CA, USA) was used to evaluate the effect of aerobic fitness (HI-fit vs. LO-fit), on the diffusion capacity response (dependent variables: DLCO, Dm, Vc) 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 (LSD) was used to determine if 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, Dm, and Vc at baseline, 70%, and 90% of $\dot{V}O_{2max}$.

4.3 Results

All subjects tolerated the study phases and procedures well. Descriptive characteristics of all participants are found in Table 4-1.

4.3.1 Effect of Fitness on Exercise Diffusion Capacity (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 greater in HI-fit subjects compared to LO-fit subjects at 70% ($P = 0.028$) and 90% ($P = 0.013$) of $\dot{V}O_{2max}$ (Figure 4-1).

While total lung capacity (TLC) was not statistically different between groups ($P = 0.052$), alveolar volume (V_A) was significantly larger in HI-fit (mean $V_A \pm$ SE = HI: 7.55 ± 0.31; LO: 6.68 ± 0.26 L, $P = 0.013$). When DLCO was corrected for alveolar volume, both HI-and LO-fit groups increased DLCO/V_A with exercise intensity, but there were no differences between HI- and LO-fit groups (Table 4-2). When DLCO was expressed relative to cardiac output ($\dot{Q}$), DLCO/$\dot{Q}$ was greater in HI-fit at baseline ($P = 0.019$), but not at 70% ($P = 0.335$) or 90% of $\dot{V}O_{2max}$ ($P = 0.459$, Table 4-2).

4.3.2 Pulmonary Capillary Blood Volume (Vc)

At baseline, Vc was higher in the HI-fit group compared to the LO-fit group ($P = 0.005$). With incremental exercise both LO- and HI-fit subjects increased Vc with increasing oxygen consumption ($P < 0.001$), but there was no statistical difference between groups during exercise (Figure 4-2, $P = 0.498$). When Vc was expressed relative to cardiac output ($\dot{Q}$), Vc/$\dot{Q}$ 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 4-2).

4.3.3 Membrane Diffusing Capacity (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 $\dot{V}O_{2max}$, HI-fit subjects had greater Dm than LO-fit subjects (Figure 4-3). Likewise, Dm/V_A was greater at peak exercise in HI-fit as compared to LO-fit. Both groups exhibited a plateau in their respective Dm response to exercise, with HI-fit showing no significant increase in Dm 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 Dm was expressed relative to cardiac output ($\dot{Q}$), Dm/$\dot{Q}$ was not different at baseline ($P = 0.320$), or 70% ($P = 0.401$), but Dm/$\dot{Q}$ was greater in HI-fit group at 90% ($P = 0.048$) of $\dot{V}O_{2max}$ (Table 4-2).

4.3.4. Correlations

There was a significant, positive correlation between baseline pulmonary capillary blood volume and individual $\dot{V}O_{2max}$ ($r = 0.57$, $P = 0.001$, Figure 4-4A). Unlike Vc, baseline Dm was not correlated to $\dot{V}O_{2max}$ ($r = 0.19$, $P = 0.315$, Figure 4-4B). Pulmonary capillary blood volume at the 90% workload was not correlated to individual $\dot{V}O_{2max}$ ($r = 0.01$, $P = 0.949$, Figure 4-4D).

However, diffusing membrane capacity at the 90% workload was correlated with $\dot{V}O_{2max}$ (r = 0.38, P = 0.049, Figure 4-4D).

4.4 Discussion

The current study examined the effect of aerobic fitness on diffusion capacity, pulmonary capillary blood volume, and diffusing membrane capacity response during exercise. Consistent with previous work, the pulmonary capillary blood volume in the highly fit group was higher compared to low fit at baseline, and Vc was related to $\dot{V}O_{2max}$[14]. However, contrary to our hypothesis, we found that the greater exercise DLCO in the highly fit group is secondary to increased membrane diffusing capacity, and not pulmonary capillary blood volume. These data suggest that highly fit individuals appear to have differences within the alveolar-capillary membrane that facilitate the increased O_2 delivery needed at peak exercise.

4.4.1 Diffusion Capacity During Exercise

In the current study, we found that the HI-fit group has greater DLCO at baseline and during exercise compared to the LO-fit group. Aside from pulmonary capillary blood volume (Vc) and membrane diffusing capacity (Dm), diffusion capacity is affected by hemoglobin concentration [Hb], and alveolar volume (V_A)[18,28,29]. In order to determine the difference in Vc and Dm between HI- and LO-fit individuals,, we aimed to minimize these confounding factors by correcting DLCO for [Hb][21], and V_A. Interestingly, despite no significant difference in 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 HI-fit individuals stems from a larger alveolar volume (Table 4-1). This is consistent with research in high-level swimmers showing an increased V_A compared to control subjects[30]. However, peak Dm remains greater in

the HI-fit subjects when correcting for V_A (Table 4-2), indicating that the differences in membrane diffusing capacity are independent of alveolar volume.

4.4.2 Pulmonary Capillary Blood Volume

The increase in diffusion capacity with exercise results from increases in gas exchange surface area, with increasing membrane diffusing capacity and pulmonary capillary blood volume, secondary to recruitment and distension of capillaries[16]. It was previously theorized that pulmonary capillary blood volume may plateau despite an increasing cardiac output because of a morphological limitation of pulmonary capillary blood volume expansion, resulting in a decreased pulmonary capillary transit time with increasing exercise intensity, which may predispose HI-fit individuals to a diffusion limitation[3]. In the current study, Vc was not significantly different between HI- or LO-fit subjects with incremental exercise, and there was no apparent plateau in Vc in either group. The lack of plateau in Vc is consistent with previous research in humans[2,31] and animals[32-34], showing no upper limit to DLCO up to 80% of $\dot{V}O_{2max}$, which suggests that reserves for diffusion and capillary blood volume are substantial and not completely exhausted with maximal exercise[35].

4.4.3 Diffusing Membrane Capacity

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[36]. In the current study, we observed an increase in membrane diffusing capacity (Dm) in both HI- and LO-Fit groups during incremental exercise. Membrane diffusing capacity reflects the available alveolar-capillary surface area for gas exchange[6,9], and is increased by unfolding and distension of alveolar septa during lung inflation, and recruitment of capillaries associated with previously un-perfused alveoli[6]. Dm 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 membrane diffusing capacity more than LO-fit participants at high levels of exercise. Alveolar volume can affect Dm[37,38]; however, Dm/V_A at peak exercise was still greater in HI-fit participants (Table 4-2), suggesting that there may be a morphological difference in the lungs of HI-fit subjects beyond having larger lungs that contributes to the increased Dm. As Dm cannot be measured in un-perfused alveoli, this suggests that Dm is an index of pulmonary capillary recruitment[14,31,39], and that capillary recruitment may be greater in HI-fit individuals.

As previously mentioned, the increase in pulmonary artery pressure and cardiac output during exercise recruits previously unperfused capillaries, thereby increasing membrane conductance (i.e. Dm). Computational modelling would suggest that the increase in cardiac output with exercise also improves regional erythrocyte spacing (hematocrit), which may account for 30 to 50% of the increase in Dm from rest to peak exercise[6,40]. Therefore, cardiac output may play a role in the increased Dm 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 to cardiac output ($DLCO/\dot{Q}$). The $DLCO/\dot{Q}$ ratio progressively declines with incremental exercise, and would contribute to gas exchange impairment once $DLCO/\dot{Q}$ drops below a critical value[6]. By extension, evaluating Dm and Vc relative to $\dot{Q}$ would normalize these variables for the greater cardiac output in HI-fit subjects. The current data found that $DLCO/\dot{Q}$ and $Vc/\dot{Q}$ are similar between HI- vs. LO-fit subjects during exercise; however, $Dm/\dot{Q}$ was greater in HI-fit subjects at peak exercise (Table 4-2). These results suggest that the increased Dm observed in HI-fit at peak exercise is not explained by greater cardiac output.

It is accepted that $\dot{V}O_{2max}$ in healthy subjects is limited by O_2 availability[40]. While cardiac output is predominant in O_2 delivery, a theoretical analysis of factors that determine $\dot{V}O_{2max}$ found that O_2 diffusion in the lung (DLO_2) was as influential as cardiac output in determining $\dot{V}O_{2max}$[41,42]. 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 as compared to less fit individuals[41]. A failure to increase DLO_2 relative to the increased O_2 demand during exercise would result in a gas exchange impairment[1]. In this context, the finding that highly fit individuals have an increased pulmonary membrane diffusing capacity compared to less fit subjects (Figure 4-3), and that Dm at peak exercise is correlated with $\dot{V}O_{2max}$ (Figure 4-4D) is entirely consistent with the importance of O_2 supply relative to O_2 delivery in the determination of $\dot{V}O_{2max}$[41].

4.4.4 Pulmonary Vascular Adaptation in the Highly Fit

The initial increase in pulmonary artery pressure with exercise is believed to recruit and distend pulmonary capillaries previously under-perfused at rest[6,8,31], increasing cross-sectional area of the pulmonary capillary network, reducing pulmonary vascular resistance, and increasing Vc[8,14,43,44]. While the individual contribution of capillary recruitment or distension to the overall increase in Vc has yet to be determined[7,45], a greater resting Vc has been attributed to an enhanced pulmonary vascular distensibility, and subsequently a higher $\dot{V}O_{2max}$ [14]. There is evidence that HI-fit individuals have the same or lower left arterial pressure (pulmonary wedge pressure, PWP) compared to LO-fit individuals at rest and during exercise as well as similar or lower PAP[10,15]. Pressure within the pulmonary capillaries is uncertain, but the best estimation is approximately half the difference between PAP and PWP[24]. Thus, an elevated resting Vc at similar cardiac output, but lower pulmonary capillary pressure would suggest that highly fit individuals have a more compliant pulmonary vasculature compared to low fit counterparts, and

that this enhanced pulmonary compliance may be related to increased $\dot{V}O_{2max}$ (Figure 4-4A). Work by La Gerche *et al.* (2010) demonstrated that pulmonary vascular compliance is important in decreasing PVR, preventing excessive right ventricular (RV) afterload, and thereby enhancing RV function and cardiac output during exercise[44]. Thus, the response of the pulmonary vasculature to increasing pulmonary artery pressures during exercise will directly affect cardiac output and ultimately, $\dot{V}O_{2max}$. Taken together, these studies suggest that highly fit individuals may have beneficial adaptations within the pulmonary vasculature that enable greater cardiac output and $\dot{V}O_{2max}$.

Recent research 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[46], 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 Dm. However, in the current study, DLCO and Dm were *greater* in HI-fit subjects during exercise. It is possible that highly fit individuals 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 our study are consistent with other evidence that highly fit individuals have greater diffusion capacity at rest[30,47] and during exercise[2,30,47]. However, none of these studies have examined DLCO response to exercise above 80% of $\dot{V}O_{2max}$, and only one has measured Vc and Dm during exercise. Mostyn *et al.* (1963) studied DLCO, Vc, and Dm in highly fit subjects at rest and during steady state treadmill exercise at a mean $\dot{V}O_2$ of 2.0 $L \cdot min^{-1}$, finding that highly fit individuals have an increased DLCO during submaximal exercise secondary to increased Dm,

and not greater Vc[2]. However, this previous investigation used pre-breathing gas containing F_IO_2 of 0.6 and 1.0 for 5 minutes before performing the respective DLCO breath hold which may affect Vc, as exposure to high O_2 has been shown to alter distribution of pulmonary blood[48]. While the results of our study are consistent with the findings of this landmark study[2], our methodology provides greater detail of the DLCO, Dm, and Vc responses from baseline to near-maximal exercise.

4.4.5 Study Limitations

It is assumed that theta-CO (θ_{co}) does not change with exercise[16], as there is evidence that θco is relatively insensitive to changes in pH[49]. When Roughton and Forster (1957) first introduced their method of estimating Vc and Dm, recommended values for α (temperature- and pH-dependent coefficient linked to kinetic reactions of CO with Hb) and β (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[22,33,50], ultimately affecting their respective Vc and Dm calculations. The assumption of pH = 8.0 was addressed by Forster (1987) in a later publication, providing a correction for α that accounts for a pH = 7.4. More recently, Ceridon *et al.* (2010) employed Forster's corrected α, and found highly unlikely values for Dm in all subjects. For this reason, the current study used α = 0.0058 and β = 0.73 as recommended by Ceridon *et al.* (2010), which likely provides the best estimation of Vc and Dm during exercise. Regardless of the assumptions for θ_{co} determination, the same value was used for both high and low fit groups, and therefore the calculated differences in Vc and Dm are unlikely to be due to calculated θ_{co}.

The American Thoracic Society (1995) guidelines for DLCO measurements recommend a 10-second single breath-hold technique. Our study employed a 6-second single breath-hold due to

our subjects having 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-second breath-hold has not been shown to affect single breath DLCO measurement in healthy individuals[51]. All participants in our study followed the same breathing technique, and thus the shortened breath-hold time would not likely affect between-group differences.

Every 1% increase in carbon monoxide backpressure (evaluated by % COHb) would diminish DLCO by 1%[52,53]. However, we did not adjust for CO backpressure, consistent with other reports in the literature[22,23]. We conducted additional pilot studies and determined that COHb increases less than 3% over the course of a typical study day, whereby subjects perform six DLCO maneuvers during exercise. Based on these results, and the results of others[50,53], the resultant effect on Vc and Dm calculation is less than 3% and likely inconsequential.

While we corrected DLCO for baseline (resting) hemoglobin concentration [Hb][21], there are reports that exercise causes an increase in [Hb], with the increase similar in both low fit and highly fit subjects[54]. In pilot studies, we found a 1.6% increase in [Hb] with exercise, which if unaccounted for, would underestimate Dm and Vc by 3% and 5% respectively. Importantly, there is no evidence that the [Hb] response to exercise is different with aerobic fitness[54].

Our study was designed to minimize the potential effect of increasing COHb and exercise [Hb] on DLCO, Vc, and Dm measurements; 1) the order of F_IO_2-DLCO maneuvers was randomized; 2) the order of exercise intensity was also randomized; 3) at least 2 minutes separated DLCO maneuvers; 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 a week between tests); 6) there is no evidence that the rise in COHb following multiple DLCO maneuvers is different in highly fit

compared to low fit subjects, and thus the between-group differences seen in the current study would not be obscured by an increasing COHb. Therefore, while 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 measurement of DLCO, Vc, and Dm were taken during the preliminary day, before the incremental exercise test. However, cardiac output and ventilation during baseline in both groups were greater than would be expected for true resting values. While we made considerations in providing a quiet, resting environment for all of our study participants, it is not certain if the pre-exercise levels of arousal were similar between fitness groups, and thus, the differences in DLCO and Vc at baseline may not reflect true resting values. However, the pre-exercise absolute values for DLCO and Vc are consistent with previous data showing that aerobic fitness is positively correlated with a greater resting Vc[14].

Finally, the cross-sectional design of the current investigation only allows for speculation of the effect of endurance training on diffusion capacity and pulmonary capillary blood volume 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[55]. Future studies should investigate changes in the pulmonary vascular with chronic exercise training of previously un-trained adult humans, and associated changes to pulmonary capillary blood volume and diffusing membrane capacity.

4.5 Conclusion

We examined the effect of aerobic fitness on diffusion capacity, pulmonary capillary blood volume, and diffusing membrane capacity response to exercise. We found that individuals with a high $\dot{V}O_{2max}$ have increased diffusion capacity during high intensity exercise, secondary to a greater diffusing membrane capacity compared to their low fit counterparts, These findings suggest that highly fit individuals appear to have differences within the alveolar-capillary membrane that facilitate increased O_2 demand required during high-intensity exercise.

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4.7 Figures and Tables

Table 4-1. Subject characteristics and pulmonary function

	LO-Fit		HI-Fit	
	Mean	% Predicted	Mean	% Predicted
n	14		15	
Age, yr	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	
$\dot{V}O_{2max}$, $L \cdot min^{-1}$	3.81±0.18	113.4±5.6	5.04±0.20*	152.4±7.4*
$\dot{V}O_{2max}$, $mL \cdot kg^{-1} \cdot min^{-1}$	45.0±1.2	99.0±3.1	64.6±1.8*	144.5±5.4*
TLC, L	6.99±0.24	105.3±2.6	7.69±0.25	113.4±2.8
FEV_1, L	4.38±0.14	96.6±2.8	5.04±0.18*	109.6±3.3*
FVC, L	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 mean ± SE. $\dot{V}O_{2max}$, maximal O_2 consumption; TLC, total lung capacity; FEV_1, forced expired volume in 1 second; FVC, forced vital capacity. *Significantly greater than LO-fit group $p < 0.05$.

Table 4-2. 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
$\dot{V}_E$, 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, L	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, b·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, L	6.68±0.26	7.55±0.31*	6.67±0.20	7.49±0.26*	6.87±0.22	7.64±0.29*
$\dot{Q}$, 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, b·min^{-1}	77±3	68±4*	147±5	159±4*	173±6	179±3
[Hb], g·dL^{-1}	15.6±1.1	14.8±1.4	15.5±1.1	15.1±1.1	15.5±1.1	15.2±1.4
Dyspnea	0.2±0.6	0.1±0.3	3.7±1.1	4.9±1.6*	6.1±1.6	7.2±1.4
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
Vc/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
Dm/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/$\dot{Q}$	4.6±0.3	5.4±0.3*	2.9±0.2	2.9±0.1	2.6±0.2	2.7±0.2
Vc/$\dot{Q}$	11.4±0.9	14.4±0.9*	7.21±0.5	7.36±0.6	7.3±0.6	6.9±0.4
Dm/$\dot{Q}$	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 means ± SE. PO, power output; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio; $\dot{V}_E$, minute ventilation; V_T, tidal volume; RR, respiratory rate, V_A, alveolar volume; $\dot{Q}$, cardiac output, SV, stroke volume; HR, heart rate; [Hb], hemoglobin concentration; Dyspnea, scale of breathlessness (1-10); DLCO/V_A, diffusing capacity relative to V_A; Vc/V_A, pulmonary capillary blood volume relative to V_A; Dm/V_A, diffusing membrane capacity relative to V_A. DLCO/$\dot{Q}$, diffusing capacity relative to $\dot{Q}$; Vc/$\dot{Q}$, pulmonary capillary blood volume relative to $\dot{Q}$; Dm/$\dot{Q}$, diffusing membrane capacity relative to $\dot{Q}$. *Significantly greater than LO-fit group, $P < 0.05$.

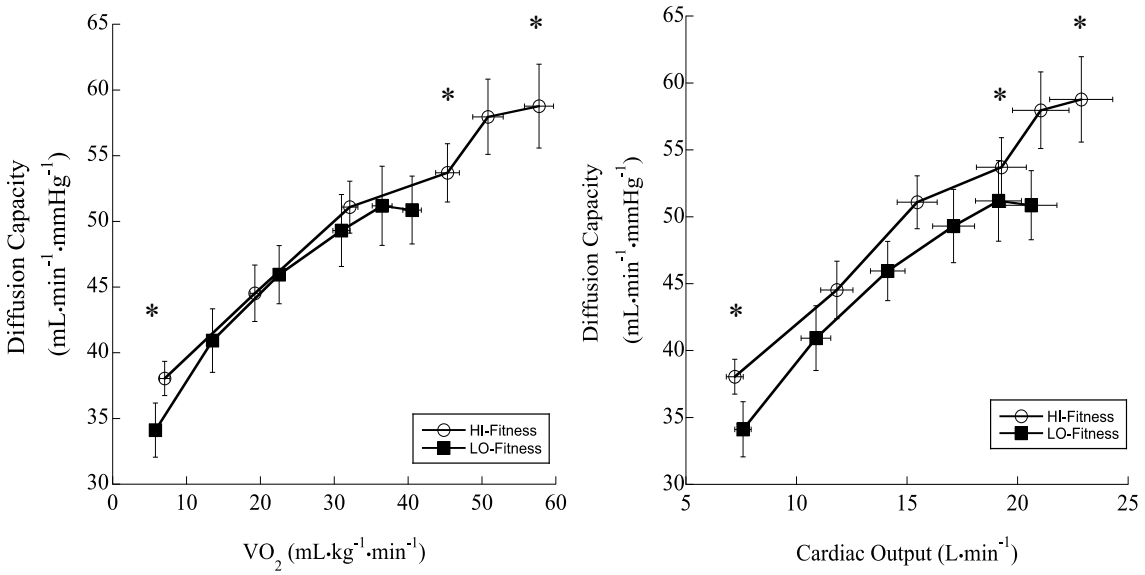

Figure 4-1: Diffusing Capacity Response to Exercise. *DLCO was greater in HI-fit subjects at baseline (P = 0.047), at 70% (P = 0.028) and 90% of $\dot{V}O_{2max}$ (P = 0.013) compared to LO-Fit subjects**.**

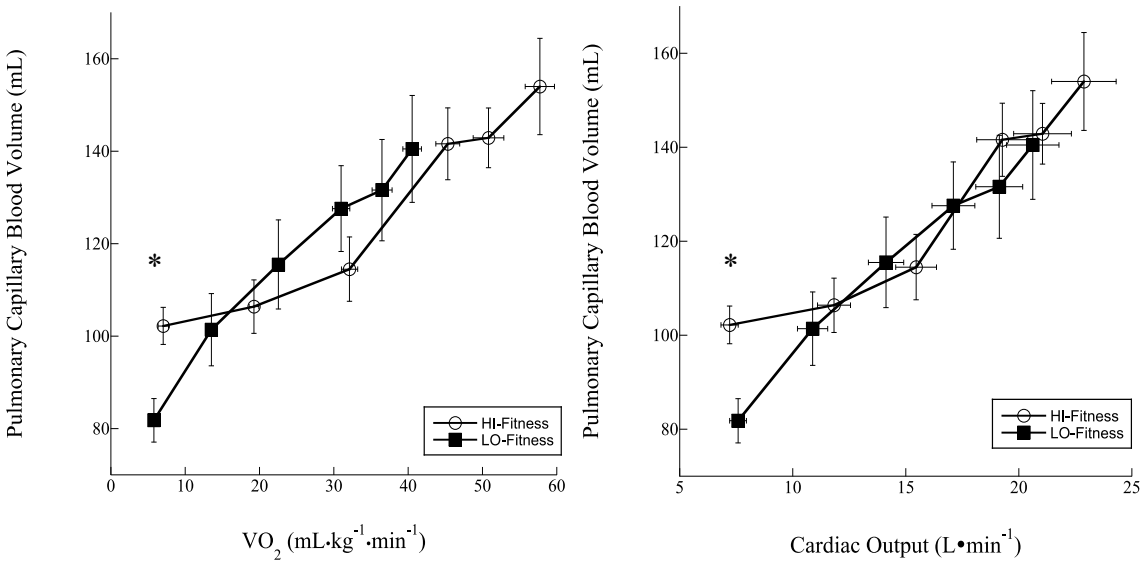

Figure 4-2. Pulmonary capillary blood volume response to exercise. *Vc was significantly higher in HI-fit subjects compared to LO fit subjects at rest ($P = 0.005$).

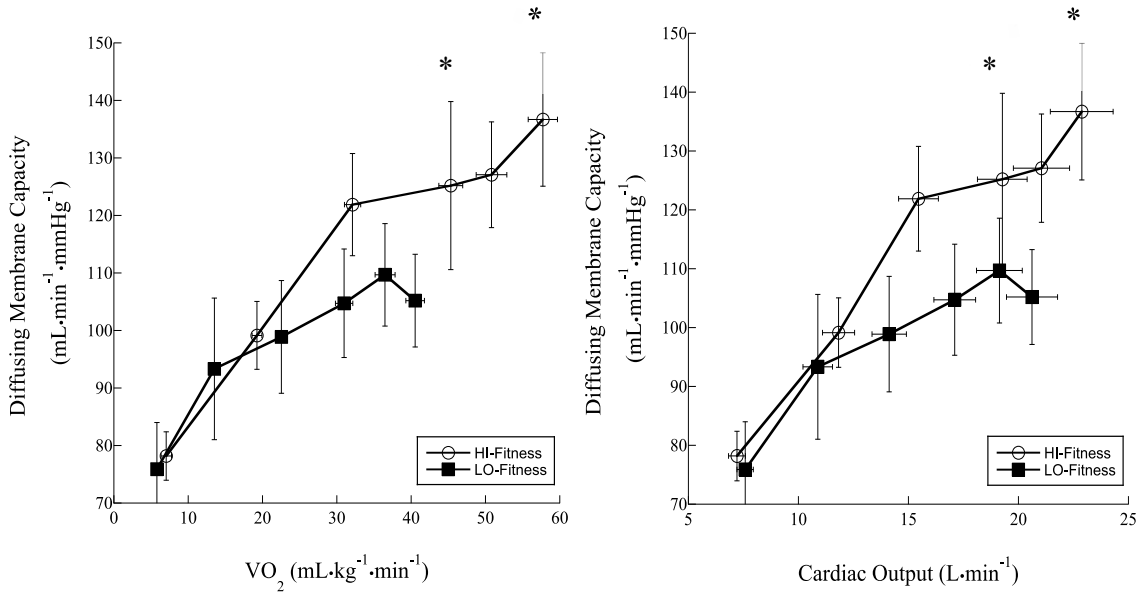

Figure 4-3: Diffusing membrane capacity during exercise. *Dm was significantly greater in HI-Fit subjects compared to LO-Fit subjects during exercise at 70% ($P = 0.035$) and 90% of $\dot{V}O_{2max}$ ($P = 0.006$).

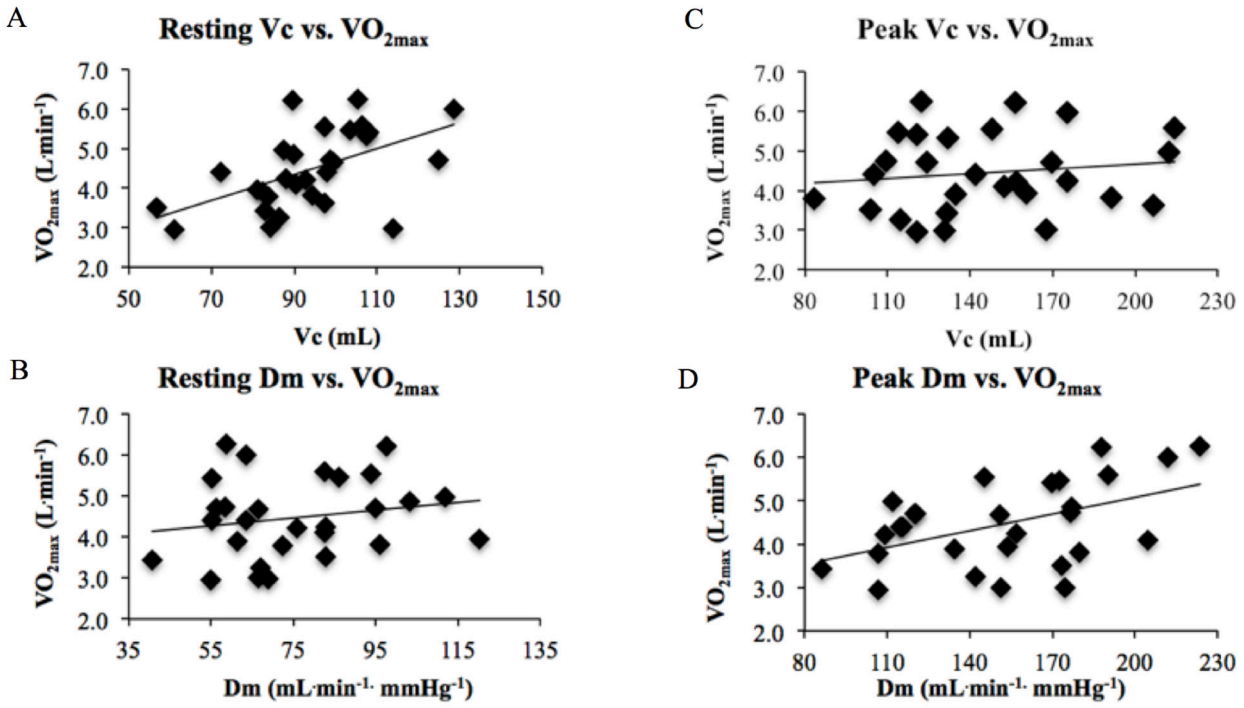

Figure 4-4: Correlations. A: Resting Vc is correlated to $\dot{V}O_{2max}$ r=0.57, *P*=0.001. B: Resting Dm is not correlated to $\dot{V}O_{2max}$ r=0.19, *P*=0.315. C: Vc at 90% workload is not correlated to $\dot{V}O_{2max}$ r=0.01, *P*=0.949. D: Dm at 90% workload is correlated to $\dot{V}O_{2max}$ r=0.38, *P*=0.043**.**

CHAPTER 5

Pulmonary Capillary Blood Volume Response to Exercise is Blunted in Mild COPD

5.1 Introduction

Chronic obstructive pulmonary disease (COPD) is a respiratory disorder largely caused by smoking, and is characterized by progressive, partially reversible airway obstruction[1]. Patients with COPD experience dyspnea during exertion, impairing their ability to complete day-to-day tasks and reducing quality of life[2]. While there are many studies examining dyspnea in severe COPD[1,3-5], evolving work in mild COPD (GOLD stage 1, $FEV_1/FVC < 70\%$, $FEV_1 \geq 80\%$ predicted[6]) have demonstrated greater impairment than previously appreciated[7,8]. These studies suggest that the impact of the disease is worse than is suggested by spirometry testing alone. Despite their relatively minor airway impairment, mild COPD patients demonstrate pronounced dyspnea with exercise, exaggerated ventilatory response to exercise (as evaluated by increased ventilatory inefficiency, $\dot{V}_E/\dot{V}CO_2$)[9,10], and reduced $\dot{V}O_{2peak}$ compared to controls. Importantly, increased ventilatory inefficiency (nadir $\dot{V}_E/\dot{V}CO_2 \geq 34$) has been shown to be predictive of mortality in COPD[9]. Some have suggested the increased $\dot{V}_E/\dot{V}CO_2$ during exercise in mild COPD results from greater deadspace, and may be secondary to pulmonary vascular dysfunction[11-15]. In keeping with this idea, pulmonary vascular dysfunction would reduce blood flow to the pulmonary microvasculature[13], decreasing surface area for gas exchange and increasing deadspace ventilation, thus requiring greater minute ventilation to maintain alveolar ventilation.

Previous research has shown that mild COPD patients have a resting diffusing capacity for carbon monoxide (DLCO) that is 20% below healthy age-matched controls[7]. During exercise, the healthy normal response is for diffusing capacity to increase in order to augment gas exchange[16], otherwise arterial hypoxemia will develop and exercise tolerance will be impaired[17-20]. The main components of DLCO are the membrane diffusing capacity (Dm), which reflects the surface area

and thickness of the alveolar membrane, and pulmonary capillary blood volume (Vc)[21]. Both Vc and Dm typically increase with exercise in healthy individuals to increase DLCO. In addition to facilitating an increase in DLCO, an increase in Dm and Vc reduces pulmonary vascular resistance by way of capillary recruitment and distension, thus blunting the rise in pulmonary artery pressure with exercise.

There is limited work showing that Vc and Dm are reduced in COPD at rest[22]. However, there have been no studies to date examining the DLCO, Vc, and Dm responses to exercise in mild COPD, and how this may be related to dyspnea and ventilatory inefficiency. Therefore, the purpose of this study is to determine if diffusion capacity, pulmonary capillary blood volume, and membrane diffusing capacity are blunted during exercise in mild COPD.

Given the decreased resting DLCO[7], and impaired pulmonary vascular function in mild COPD[11-15], we hypothesized that these patients would present with a reduced diffusing capacity, pulmonary capillary blood volume, and membrane diffusing capacity during incremental exercise compared to controls. To test this, we adapted the Roughton and Forster (1957) multiple-F_IO_2 (fraction of inspired oxygen) DLCO technique to determine Vc and Dm at rest and during exercise[21].

5.2 Methods

5.2.1 Ethical Approval

This study was approved by the Human Research Ethics Board of the University of Alberta (protocol #54658). All subjects gave written, informed consent to participate in the study.

5.2.2 Subjects

We recruited 15 patients with mild COPD (post-bronchodilator $FEV_1/FVC < 0.7$ and $FEV_1 \geq$

80% predicted, GOLD stage 1[6]) and 15 control subjects. Mean age, height, and weight were not different between groups, and we sex-matched control subjects to the COPD group. Fourteen COPD subjects used respiratory medications on a regular basis: short-acting β-agonist as needed (n=8), inhaled corticosteroid (n=8), and combined corticosteroid and long-acting β-agonist (n=6). Medical history of each participant was carefully reviewed by a study physician to ensure readiness to exercise. Subject characteristics and pulmonary function data are presented in Table 5-1.

5.2.3 Study Design Overview

Subjects completed a full pulmonary function test, followed by a medically supervised incremental cardiopulmonary exercise test to $\dot{V}O_{2peak}$ on a cycle ergometer. At least 48 h later, subjects returned to the laboratory for the exercise DLCO sessions. Subjects performed multiple-F_IO_2 DLCO maneuvers[21] while exercising at 40W, 50% and 80% of their previously determined $\dot{V}O_{2peak}$. Participants returned at least one week later for the echocardiography session to estimate cardiac output and pulmonary artery systolic pressure at rest and during exercise.

5.2.4 Preliminary Screening and Pulmonary Function Testing

Subjects were instructed to withhold from smoking 24 hours prior to testing day. Subjects reported to the laboratory and were screened for any cardiopulmonary disorders and/or medications by a physician. Participants completed a full pulmonary function test, including pre- and post-bronchodilator (400μg Salbutamol) spirometry, determination of lung volumes by plethysmography, and resting DLCO (V62J Body Plethysmograph, SensorMedics, Yorba Linda, CA) in accordance with the American Thoracic Society/European Respiratory Society guidelines[23-25].

5.2.5 Cardiopulmonary Exercise Test

Subjects then performed an incremental cycle (Ergoselect II 1200 Ergoline, Blitz, Germany) test to measure $\dot{V}O_{2peak}$. Initial power output was set to 0 W and was ramp-increased at a rate of 15 W per minute until volitional exhaustion. Standard expired gas measurements (oxygen consumption, carbon dioxide production, minute ventilation) were collected on a breath-by-breath basis (Encore229 Vmax, SensorMedics, Yorba Linda, CA). Heart rate was measured using electrocardiography (CardioSoft, GE Healthcare, Fairfield, CT, USA), and oxygen saturation was estimated using pulse oximetry (Radical 7, Masimo, Irvine, CA, USA). Inspiratory capacity measurements were performed at rest and every two minutes during the exercise test[26]. Participants rated the intensity of their "breathing discomfort" and "leg discomfort" at rest, and every two minutes during exercise using the modified 10-point Borg Scale[27,28]. Peak oxygen consumption was determined as the highest 30-second average achieved during the incremental exercise test.

Percent predicted $\dot{V}O_{2peak}$ was determined using the equation[29]:

$$\text{Absolute } \dot{V}O_{2peak\ predicted} = 4.2 - (0.033 \times \text{Age})$$

The relative exercise intensities of 50% and 80% of $\dot{V}O_{2peak}$ were determined by linear regression of work rate and $\dot{V}O_2$ obtained during the incremental exercise test.

5.2.6 Exercise Diffusion Capacity

Lung diffusion capacity for carbon monoxide (DLCO) was determined using the single-breath breath-hold technique[23,30-32] at baseline and during exercise (Encore V62J Autobox, SensorMedics, Yorba Linda, CA, USA). As hemoglobin concentration affects diffusion capacity[33] and increases with exercise intensity[34], hemoglobin concentration [Hb] was measured

at rest and during each exercise intensity from blood sampled at the finger tip (HemoCue 201+, HemoCue AB, Angelholm, Sweden). Accordingly, each DLCO measurement was corrected for [Hb] using the equation[33]:

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

Repeated tests of DLCO will increase the amount of carbon monoxide bound to hemoglobin, and DLCO decreases by 1% for every 1% increase in carboxyhemoglobin (COHb)[35]. Therefore, DLCO must be adjusted for increasing COHb. Carboxyhemoglobin was estimated in real-time using non-invasive pulse CO-oximetry (Radical 7, Masimo, Irvine, CA, USA), and DLCO was adjusted for COHb using the equation[35]:

$$DLCO_{COHb-adj} = DLCO_{meas} \times \left(1 + \left(\frac{COHb}{100}\right)\right)$$

To assess pulmonary capillary blood volume and membrane diffusing capacity, multiple-F_IO_2 DLCO breath holds were performed with three different F_IO_2 values (0.21, 0.40, 0.60) added to the standard test gas (0.3% carbon monoxide, 0.3% methane, balance nitrogen) during steady state exercise at 40W, 50% and 80% of $\dot{V}O_{2peak.}$ Methane was used in each gas mixture to measure alveolar volume (V_A), and determine adequate gas equilibration. Subjects exercised continuously for a minimum of three minutes to ensure steady state was achieved. Steady state was confirmed by a consistent heart rate (< 3 bpm change over one minute). Four minutes of washout time was given between trials. During exercise, the washout time was reduced to two minutes, as the clearance of CO gas from the lungs is faster with increased cardiac output and ventilation[36].

Prior to data collection, subjects were coached to avoid Müllerian or Valsalva maneuvers during the breath hold maneuver. To ensure alveolar PO_2 was stable, each subject was given five breaths of gas at the respective F_IO_2 for each DLCO test gas[18,37]. After pre-breathing, subjects exhaled to residual volume, and were instructed to inhale the respective DLCO test gas to total lung capacity and perform a breath hold for six seconds[18,37,38]. 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[39,40]. The DLCO trial was repeated if V_A was not within 5% of the previous trial. The V_A for each individual trial was similar at baseline and throughout exercise (Table 5-2). The order of the F_IO_2 was randomized to avoid any order effect. Pulmonary capillary blood volume (Vc) and membrane diffusing capacity (Dm) were determined using the equation[21]:

$$\frac{1}{DL} = \frac{1}{Dm} + \frac{1}{\theta_{co} \times Vc}$$

Theta (θ_{CO}) was calculated using the equation[21]:

$$\frac{1}{\theta_{co}} = \alpha \times P_AO_2 + \beta$$

In which α is a temperature- and pH-dependent coefficient linked to kinetic reactions of CO with Hb, and β is the ratio of red-cell membrane to red cell interior permeability. The values of α = 0.0058 and β = 0.73 were chosen based on previous research recommending these constants for studies during exercise, based on moderate red cell permeability[41]. We have used these values for α and β in previous exercise DLCO studies[18,37], and have obtained Dm and Vc data that are consistent with previous research[42,43].

Partial pressure of arterial carbon dioxide ($PaCO_2$) was estimated from end tidal CO_2 values, and partial pressure of alveolar oxygen (P_AO_2) was calculated using the alveolar gas equation[44]:

$$P_AO_2 = FIO_2 \left(P_{Bar} - P_{H_2O}\right) P_aCO_2 \times \frac{1 - FIO_2}{RER}$$

Where P_{BAR} is the barometric pressure in mmHg, P_{H2O} is the water vapor pressure at body temperature, F_IO_2 is the fraction of inspired oxygen, and RER is the respiratory exchange ratio, calculated as $\dot{V}CO_2$ to $\dot{V}O_2$ ratio. A regression equation was derived for the relationship between 1/DLCO and 1/θ for the three F_IO_2 values at each exercise intensity. The minimum acceptable r^2 value was set to 0.95, and DLCO maneuvers were repeated when r^2 values were outside of this range. Pulmonary capillary blood volume is calculated using the reciprocal of the slope (1/Vc) and membrane diffusing capacity was calculated using the reciprocal of the y-intercept (1/Dm)[21].

5.2.7 Echocardiography

At least one week after the exercise DLCO tests, participants returned for an exercise echocardiogram to determine cardiac output (Q) and pulmonary arterial systolic pressure at 40 W, 50%, and 80% of $\dot{V}O_{2peak}$. All echocardiograms were performed by one experienced sonographer (Vivid Q, GE Healthcare, Fairfield, CT USA) and were later evaluated by a cardiologist blinded to disease group and exercise intensity (ECHOPac, GE Healthcare, Fairfield, CT USA). Left ventricular outflow tract (LVOT) diameter was determined from the parasternal long axis view at the level of the aortic annulus, and measurements were taken at the end of systole. Cross sectional area (CSA) was calculated using the following formula:

$$CSA_{LVOT} = \pi \left(\frac{D}{2} \right)^2$$

Where D is the measured LVOT diameter in cm. Velocity-time integral (VTI) of the left ventricular outflow tract was obtained using pulsed wave Doppler sample volume within the aortic valve, from an apical five-chamber view of the heart. Cardiac output ($\dot{Q}$) was determined by the following formula:

$$\dot{Q} = VTI \times CSA_{LVOT} \times HR$$

Where HR is the heart rate in beats per minute. The pulmonary arterial pressure gradient was estimated from the peak tricuspid regurgitant jet velocity. The inferior vena cava diameter was measured from subcostal longitudinal images, and collapsibility index was calculated as percent difference between the minimal and maximal diameter of the inferior vena cava during a sniff maneuver. Right atrial pressure (RAP) was predicted using collapsibility index in accordance with the American Society of Echocardiography[45]. Pulmonary artery systolic pressure (PASP) was calculated using the following equation:

$$PASP = 4 \left(V_{TR} \right)^2 + RAP$$

Where V_{TR} is the peak tricuspid regurgitant jet velocity in meters per second, and RAP is right atrial pressure in mmHg.

5.2.8 Statistical Analysis

All pulmonary function variables are presented as percent predicted based on normative data established by the Canadian Respiratory Journal[46]. Additionally, spirometric data (FEV_1, FVC, FEV_1/FVC) are also presented as z-scores using the Global Lungs Initiative 2012 equations[47].

For all inferential analyses, the probability of a type I error was set at 0.05. Group data for each variable are expressed as mean ± standard error unless otherwise indicated. Statistical analysis was performed using two-way repeated measures ANOVA (SigmaPlot, v.13, Systat Software, Inc., San Jose, CA, USA) to evaluate the effect of mild COPD (COPD vs. control) on the diffusion capacity response (dependent variables: DLCO, Dm, Vc) to exercise (4 levels: baseline, 40W, 50%, and 80% of $\dot{V}O_{2peak}$). Where a main effect of disease state was found, a Tukey post-hoc test was performed to locate differences between groups and exercise intensities.

To further examine the mild COPD response, the group was subdivided by their nadir (lowest 30-second average value) ventilatory equivalent response during their incremental exercise test. Previous research has shown that a nadir $\dot{V}_E/\dot{V}CO_2 \geq 34$ is predictive of mortality[9]. The COPD group was also subdivided based on if they developed dynamic hyperinflation with incremental exercise, as defined by a decrease in their inspiratory capacity from baseline to peak exercise[3,48]. As the nadir $\dot{V}_E/\dot{V}CO_2$ was observed at 60 ± 10% of $\dot{V}O_{2peak}$ in controls, and 62 ± 16% of $\dot{V}O_{2peak}$ in mild COPD, Vc at 50% of $\dot{V}O_{2peak}$ was compared between these sub groups, as it was the closest data point to the nadir $\dot{V}_E/\dot{V}CO_2$. Unpaired Student's T-tests were used to determine differences between these subgroups. Pearson correlations were run to determine the relationship between nadir $\dot{V}_E/\dot{V}CO_2$ and pulmonary capillary blood volume, as well as membrane diffusing capacity during exercise at 50% of $\dot{V}O_{2peak}$.

5.3 Results

All subjects tolerated the study procedures well. Descriptive characteristics of all participants are provided in Table 5-1. Age, sex, and height were not significantly different between mild COPD and control subjects. As expected, mild COPD patients had lower FEV_1 and greater functional residual capacity compared to control, but there was no significant difference in total lung

capacity (Table 5-1). Mild COPD patients reported a higher score on the Medical Research Council (MRC) breathlessness scale (1-5) compared to control (Table 5-1).

5.3.1 Cardiopulmonary Responses to Exercise

Cardiopulmonary responses to steady state exercise at 40 W, 50%, and 80% of $\dot{V}O_{2peak}$ are given in Table 5-2. Oxygen consumption was not different between groups at rest, or at 40 W, but was lower in the COPD group at 50% and 80% of $\dot{V}O_{2peak}$ compared to control (Table 5-2). Similarly, heart rate and cardiac output were lower in mild COPD at 50 and 80% of $\dot{V}O_{2peak}$. The lower oxygen consumption and cardiac output at 50% and 80% was due to lower VO_{2peak} in mild COPD. Pulmonary artery systolic pressure was not significantly different between groups at rest or during exercise, although there were fewer comparisons due to a lack of subjects with sufficient quality of tricuspid regurgitant jet Doppler signal (control n = 10, mild COPD n = 9).

Dyspnea was not different between groups at rest (P = 0.595). During exercise, dyspnea was greater in the mild COPD group at 40 W (P = 0.001), but not at 50% (P = 0.650) or 80% of $\dot{V}O_{2peak}$ (P = 0.849, Figure 5-1). Minute ventilation was lower in mild COPD at 80% of VO_{2peak} (P = 0.009, Table 5-2) but not at lower exercise intensity or during baseline. The mild COPD group had reduced inspiratory capacity at 80% of $\dot{V}O_{2peak}$ compared to control (P = 0.038, Table 5-3). In the mild COPD group, 10 of 15 patients decreased their inspiratory capacity from rest to 80% of $\dot{V}O_{2peak}$ (mean change in IC from rest = -0.35 L). Ventilatory equivalent for CO_2 ($\dot{V}_E/\dot{V}CO_2$) was greater in mild COPD at rest (P < 0.001) and during exercise at 40 W (P = 0.005), 50% of $\dot{V}O_{2peak}$ (P < 0.001), and 80% of $\dot{V}O_{2peak}$ (P = 0.006, Figure 5-2). Mean nadir $\dot{V}_E/\dot{V}CO_2$ was significantly higher in mild COPD compared to control (Table 5-1). Ventilatory equivalent for O_2 ($\dot{V}_E/\dot{V}O_2$) was greater in mid COPD at 40W (P = 0.007) and 50% (P = 0.014) of VO_{2peak} compared to control (Table 5-2).

5.3.2 Effect of Mild COPD on Exercise Diffusing Capacity

Alveolar volume during the DLCO maneuvers was not statistically different between COPD and control groups ($P = 0.314$), and did not change with exercise intensity in either group.

At baseline, DLCO was lower in the COPD group compared to control ($P = 0.027$). With incremental exercise, both COPD and control groups increased DLCO with increasing oxygen consumption ($P < 0.001$). However, DLCO was higher in control subjects at 40 W ($P = 0.014$), 50% of $\dot{V}O_{2peak}$ ($P = 0.005$), and at 80% of $\dot{V}O_{2peak}$ ($P = 0.002$, Figure 5-3).

5.3.3 Pulmonary Capillary Blood Volume

At baseline, Vc was lower in the COPD group compared to control ($P = 0.017$). With incremental exercise, both COPD and control groups increased Vc with increasing oxygen consumption ($P < 0.001$). During exercise, the COPD group had significantly lower Vc, at all exercise intensities ($P = 0.022$, Figure 5-4).

5.3.4 Membrane Diffusing Capacity

With incremental exercise, both groups increased Dm with increasing oxygen consumption ($P < 0.001$). However, Dm was not significantly different between groups at rest or during exercise ($P = 0.112$, Figure 5-5).

5.3.5 Grouping the COPD Response by Ventilatory Inefficiency

Nine mild COPD patients had a nadir $\dot{V}_E/\dot{V}CO_2 \geq 34$, and six COPD patients had a nadir $\dot{V}_E/\dot{V}CO_2 < 34$. Of note, there were no control subjects who had a nadir $\dot{V}_E/\dot{V}CO_2 \geq 34$. Nadir $\dot{V}_E/\dot{V}CO_2$ was significantly correlated with pulmonary capillary blood volume at 50% of $\dot{V}O_{2peak}$ ($r = -0.41$, $P = 0.022$, Figure 5-6A), but not membrane diffusing capacity ($r = -0.11$, $P = 0.543$, Figure 5-6B). When grouped by high or low $\dot{V}_E/\dot{V}CO_2$, the high $\dot{V}_E/\dot{V}CO_2$ group had a lower

mean Vc during exercise at 50% of $\dot{V}O_{2peak}$ compared to control (P = 0.019, Figure 5-7A). Membrane diffusing capacity was not different between $\dot{V}_E/\dot{V}CO_2$ subgroups or control (P = 0.125, Figure 5-8A).

5.3.6 Grouping the COPD Response by Hyperinflation

Dynamic hyperinflation with exercise was observed in 10 mild COPD patients (mean change IC from rest = -0.36 L), while five did not hyperinflate with exercise (mean change IC from rest = +0.34 L). Of note, there were no control subjects who hyperinflated during exercise. Hyperinflators did not have significantly lower Vc during exercise at 50% of $\dot{V}O_{2peak}$ compared to non-hyperinflators or control (P = 0.057, Figure 5-7B). Also, Dm was not statistically different between groups (P = 0.196, Figure 5-8B).

Hyperinflators did not have significantly higher $\dot{V}_E/\dot{V}CO_2$ nadir (mean nadir $\dot{V}_E/\dot{V}CO_2$ = 33.9 ± 5.0) compared to non-hyperinflators (32.4 ± 3.4, P = 0.781).

5.3.7 Grouping the COPD Response by MRC breathlessness score

Six mild COPD patients reported a score of 1 (non-symptomatic), and nine reported a score of 2 (symptomatic) or higher on the MRC breathlessness score. Baseline DLCO was not different between symptomatic and non-symptomatic patients (P = 0.707). During exercise at 50% of $\dot{V}O_{2peak}$, DLCO was not different between symptomatic and non-symptomatic patients (P = 0.628).

5.4 Discussion

The present study examined the effect of mild COPD on diffusing capacity, pulmonary capillary blood volume, and membrane diffusing capacity response during exercise. Consistent with previous reports[7], resting DLCO was lower in mild COPD compared to control, secondary to a

diminished Vc. As expected, during exercise, DLCO was lower in mild COPD, and this was secondary to lower Vc compared to controls. The increase in pulmonary capillary blood volume by recruitment and distension[49] is vital to gas exchange and cardiac function during exercise[50]. Thus the decreased Vc during exercise suggests that some aspect of the lung vasculature is impaired in mild COPD.

It is possible that pulmonary capillary blood volume is reduced in COPD due to a blunting in microvascular blood flow. Reduced microvascular blood flow could be secondary to structural lung changes such as loss of radial traction or destruction of capillaries due to emphysema. Pulmonary vascular dysfunction could also reduce blood flow independent of emphysema. Also, hyperinflation may increase intrathoracic pressure, which could mechanically collapse pulmonary capillaries and reduce the volume of blood available for gas exchange.

5.4.1 The Pulmonary Vasculature in COPD

Previous research has observed elevated measured pulmonary artery pressure during exercise in mild COPD compared to control, and have attributed this to thickening of the smooth muscles in the pulmonary arteries[51]. These structural changes would increase vascular resistance, thus reducing blood flow to the pulmonary capillaries, and is consistent with recent work showing a reduction in blood flow to the pulmonary microvasculature[13]. Using contrast enhanced MRI, pulmonary microvascular blood flow was found to be reduced by 30% in non-emphysematous mild COPD[13]. Also, COPD patients with emphysema were found to have further reductions in microvascular blood flow[13], suggesting that dysfunction of the pulmonary microvasculature occurs prior to the pathogenesis of anatomical emphysema[9]. Pulmonary vascular dysfunction, in the form of decreased vascular compliance, would reduce pulmonary capillary perfusion, reducing available surface area for gas exchange and resulting in ventilation-perfusion ($\dot{V}_A/\dot{Q}$)

inequality. Thus, the decreased Vc during exercise in COPD may be due to a decreased blood flow to the capillaries resulting from pulmonary vascular dysfunction, and not necessarily destruction of capillary tissue secondary to emphysema.

5.4.2 Ventilatory Responses to Exercise in COPD

Ventilation was not statistically different between groups at rest or during exercise, likely due to the large variance within groups. However, the ventilatory equivalent to oxygen uptake ($\dot{V}_E/\dot{V}O_2$), and carbon dioxide ($\dot{V}_E/\dot{V}CO_2$), was elevated in the COPD group, suggesting inefficient ventilation (Figure 5-1 and Table 5-2). Of these two indices, $\dot{V}_E/\dot{V}CO_2$ has been shown to be a stronger indicator of ventilatory inefficiency[9]. This is consistent with previous research which has observed reduced ventilatory efficiency in mild COPD patients during exercise[27]. Importantly, increased nadir $\dot{V}_E/\dot{V}CO_2$ has been shown to be predictive of mortality in COPD[9]. The increased $\dot{V}_E/\dot{V}CO_2$ during exercise in COPD has been attributed to high $\dot{V}_A/\dot{Q}$ areas, or deadspace ventilation[11]. This is consistent with a previous study showing that the $\log SD_{\dot{V}}$ (an index of ventilation-perfusion inequality from reduced capillary blood flow) becomes worse with COPD severity more so than the $\log SD_{\dot{Q}}$ (which reflects $\dot{V}_A/\dot{Q}$ inequality as a result of local alveolar hypoventilation)[11]. Thus, it is possible that the blunted Vc observed in the current study would result in greater $\dot{V}_A/\dot{Q}$ mismatch and/or increased deadspace ventilation, and therefore contribute to the increased $\dot{V}_E/\dot{V}CO_2$ in mild COPD during exercise. When the COPD group is subdivided based on a nadir $\dot{V}_E/\dot{V}CO_2$ above or below 34, the group with a nadir $\dot{V}_E/\dot{V}CO_2 \geq 34$ has significantly lower exercise Vc (Figure 5-7A). A lower pulmonary capillary blood volume suggests a decreased capillary surface area for gas exchange. Therefore, the lower exercise Vc in mild COPD may contribute to ventilation-perfusion inequality, secondary to increased deadspace ventilation, and explain the elevated $\dot{V}_E/\dot{V}CO_2$ during exercise.

5.4.3 Hyperinflation in Mild COPD

The increased FRC observed in mild COPD in the current study provides some evidence of static lung hyperinflation (Table 5-1), which is the result of loss of the elastic recoil property of lung tissue in COPD[48,52,53]. During exercise, dynamic hyperinflation would increase alveolar pressure, and could mechanically collapse or reduce the transverse surface of pulmonary capillaries, thereby decreasing the volume of capillary blood available for diffusion[48]. In keeping with this idea, individuals who exhibit dynamic hyperinflation would be expected to have lower pulmonary capillary blood volume during exercise than those who do not hyperinflate. To answer this, the COPD group was split into dynamic hyperinflators (n=10) and non-hyperinflators (n=5) (Figure 5-7B), based on decreased inspiratory capacity at $\dot{V}O_{2peak}$ compared to rest[48]. Exercise Vc was not significantly lower in hyperinflators compared to non-hyperinflators, suggesting it is unlikely that the lower pulmonary capillary blood volume is a result of changes in operational lung volume. However, the current study is underpowered to detect differences in these sub-groups, and a larger trial would be needed to confirm this observation with at least 13 participants in each group.

Previous work has reduced dynamic hyperinflation in COPD patients during exercise using heliox, a low-density gas mixture of helium and oxygen that reduces airway resistance[54]. Despite a reduction in hyperinflation, $\dot{V}_E/\dot{V}CO_2$ did not change with heliox administration, and while they did not measure pulmonary capillary blood volume, these data are consistent with the notion that hyperinflation does not contribute to low exercise Vc. To test this, future studies should aim to recruit COPD patients who hyperinflate and administer heliox to determine if exercise Vc could be improved with a reduction in dynamic hyperinflation.

5.4.4 Implications for Dyspnea

Exertional dyspnea in COPD results from the imbalance between the central demand for breathing, and the mechanical ability of the lung to ventilate during exercise[1,55,56]. Multiple afferent signals contribute to the sensation of breathlessness[55,57], including lung stretch due to static and dynamic hyperinflation[7,48,55,58], ventilatory inefficiency[10], and juxtacapillary receptor (J-receptor) activation by vascular stretch[15,59]. Mean dyspnea score was greater in the COPD group at 40 W (Figure 5-1), and not at the relative comparisons of 50% and 80% of $\dot{V}O_{2peak}$. As discussed previously, it is possible that a reduction in inspiratory capacity (IC) or inspiratory reserve volume (IRV) could contribute to the increased dyspnea[48]. However, neither the IC nor IRV were significantly different between controls and COPD at 40 W (Table 5-3), which suggests that dynamic hyperinflation did not contribute to dyspnea at this submaximal exercise intensity. Also, ventilation was not significantly increased at 40 W (Table 5-2). Therefore, the greater dyspnea at 40 W in COPD is not explained by hyperinflation or increased ventilation.

Cardiac output was not different at 40 W (Table 5-2), which suggests that the lower Vc might reflect an increased pulmonary vascular resistance, leading to greater PAP. However, pulmonary artery systolic pressure was not significantly elevated in COPD compared to control. Unfortunately, we were only able to obtain high quality Doppler signals of the tricuspid regurgitant jet in 9 COPD and 10 controls, and thus we are likely underpowered to detect a significant difference in pulmonary artery systolic pressure in COPD compared to controls. Future studies could employ a Swan-Ganz pulmonary artery catheter to directly measure pulmonary pressures during exercise in mild COPD to determine if low Vc is consistent with high pulmonary artery pressure.

It might be expected that symptomatic mild COPD patients would have a reduced diffusing capacity at rest, and during exercise, compared to those who are asymptomatic. In the current study, we did not find a difference between symptomatic and non-symptomatic groups in diffusing capacity at rest or during exercise, indicating that the DLCO response is blunted regardless of symptomatic status in COPD. However, our sample size is not large enough to adequately address this question. Accordingly, a similar study could be conducted with additional mild COPD patients recruited on the basis of reported MRC scores below and above 2.

5.4.5 Study Limitations

An important limitation of this study concerns the calculation of Theta-CO (θ_{CO}). In our previous work[18,37], we substituted partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) for partial pressure of arterial CO_2 (P_ACO_2) in the alveolar gas equation (see methods section). There are reports that $P_{ET}CO_2$ can overestimate P_ACO_2 during exercise[60,61], which would affect the calculation P_AO_2, θ_{CO}, and ultimately Vc and Dm. Using arterial blood gas data[62] we determined that a ~3 mmHg overestimation of P_ACO_2 at peak exercise would change Vc by +0.5%, and Dm by -1.8% in young healthy individuals, and this systematic difference would have affected both groups equally and thus likely to be negligible. However, previous research has shown that the arterial-end tidal CO_2 difference ($P_{A\text{-}ET}CO_2$) is greater in mild COPD compared to healthy controls at rest and during exercise[63,64]. This would have a resultant effect of increasing the calculated P_AO_2, which would increase the calculated Theta-CO for each DLCO breath hold. Subsequently, the increased Theta-CO for each breath hold would reduce the slope of the regression, thus resulting in decreased Vc. In the current study, pulmonary capillary blood volume was found to be lower in COPD. Adjusting our calculations for the increased $P_{A\text{-}ET}CO_2$ in COPD would result in an

increase in the difference in Vc between groups. Therefore, the lower Vc observed in the COPD patients cannot be explained by any error in estimating P_AO_2.

Another potential methodological limitation to this study is the challenge of the breath hold maneuver to COPD patients. Exhalation to residual volume during exercise could be impeded with airway obstruction. Thus, gas trapping may decrease the available surface area for diffusion, which would be reflected in the measured V_A. However, alveolar volume did not change with increasing exercise intensity in either group (Table 5-2), indicating that the COPD group was able to reach residual volume prior to the TLC maneuver during exercise.

While we attempted to recruit control subjects with similar fitness to our mild COPD group, $\dot{V}O_{2peak}$ was significantly lower in the COPD group (Table 5-1). Our previous study showed that increased diffusing capacity at peak exercise in aerobically-trained individuals was due to greater membrane diffusing capacity, and not any differences in pulmonary capillary blood volume[18]. Also, during submaximal exercise, DLCO and Vc were not different between trained and untrained individuals for a given work rate. In contrast, the current study observed a lower diffusing capacity in COPD, secondary to lower pulmonary capillary blood volume at the same absolute work rate (40 W), while oxygen consumption was not different between groups (Table 5-2). This suggests that the relative difference in $\dot{V}O_{2peak}$ between COPD and control groups is unlikely to explain the difference in DLCO and Vc during submaximal exercise.

Future studies should compare DLCO, Vc, and Dm during exercise in COPD to traditional measures of ventilation-perfusion inequality using the multiple inert gas elimination technique (MIGET). If low pulmonary capillary blood volume during exercise were found concurrent with

increased areas of high $\dot{V}_A/\dot{Q}$ ratios, this would be consistent with the increased deadspace and ventilatory inefficiency observed in COPD during exercise.

5.5 Conclusion

We examined the effect of mild COPD on diffusing capacity, pulmonary Vc, and membrane diffusing capacity responses to exercise. Diffusing capacity was lower in mild COPD compared to control subjects, and this was due to a blunted pulmonary capillary blood volume, and not differences in membrane diffusing capacity. The lower Vc during exercise appeared to be associated with an increased $\dot{V}_E/\dot{V}CO_2$, which is consistent with previous research suggesting pulmonary vascular dysfunction in mild COPD. Thus, despite their relatively minor airway obstruction, mild COPD patients appear to have adverse changes to their pulmonary capillary response that affects their ability to increase DLCO during exercise.

5.6 References

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5.7 Tables and Figures

Table 5-1. Descriptive characteristics, pulmonary function, and resting lung volumes

	Control	Mild COPD	*P values*
Subjects (male/female)	6/9	6/9	
Age, yrs	65 ± 11	66 ± 10	0.703
Mass, kg	76.2 ± 13.0	70.5 ± 13.5	0.249
Height, m	1.68 ± 0.10	1.66 ± 0.08	0.554
Smoking history, pack-years	3 ± 4	38 ± 19	<0.001*
$\dot{V}O_{2peak}$, $L \cdot min^{-1}$	2.14 ± 0.58	1.69 ± 0.58	0.040*
$\dot{V}O_{2peak}$, % pred	116 ± 23	93 ± 20	0.007*
S_PO_2 rest, %	98.0 ± 1.0	96.5 ± 2.8	0.014*
S_PO_2 peak, %	96.9 ± 1.4	95.5 ± 3.0	0.117
$\dot{V}_E/\dot{V}CO_2$ nadir	28.1± 3.3	35.1 ± 5.4	<0.001*
FEV_1, L	2.85 ± 0.79	2.29 ± 0.43	0.026*
FEV_1, % pred	109 ± 14	94 ± 11*	0.004*
FEV_1, z-score	0.25 ± 0.96	-0.75 ± 0.53	0.002*
FVC, L	3.82 ± 1.01	3.56 ± 0.87	0.428
FVC, % pred	104 ± 15	105 ± 10	0.757
FVC, z-score	0.49 ± 0.90	0.34 ± 0.64	0.610
FEV_1/FVC, %	75 ± 5	64 ± 4	<0.001*
FEV_1/FVC, % pred	101 ± 7	83 ± 6	<0.001*
FEV1/FVC, z-score	-0.45 ± 0.67	-1.77 ± 0.54	<0.001*
TLC, L	5.94 ± 1.36	5.61 ± 1.53	0.530
TLC, % pred	102 ± 16	100 ± 14	0.486
FRC, L	3.13 ± 0.71	3.43 ± 0.73	0.271
FRC, % pred	102 ± 17	119 ± 24	0.008*
RV, L	1.94 ± 0.57	2.08 ± 0.84	0.576
RV, % pred	90 ± 28	101 ± 39	0.399
DLCO, $mL \cdot min^{-1} \cdot mmHg^{-1}$	22.3 ± 3.6	17.5 ± 5.7	0.011*
DLCO (% pred)	93 ± 10	74 ± 16	0.002*
MRC Dyspnea Scale (1-5)	1.0 ± 0.0	1.9 ± 0.8	0.001*

Values expressed as Means ± SD. $\dot{V}O_{2peak}$, peak oxygen consumption; S_PO_2, oxygen pulse saturation; $\dot{V}_E/\dot{V}CO_2$ nadir, ventilatory equivalent for CO_2 at lowest point during graded exercise test; FEV_1, forced expired volume in 1 second; FVC, forced vital capacity; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume assessed by plethysmography; DLCO, diffusion capacity at rest; MRC, Medical Research Council dyspnea scale. Z-score calculated using Global Lung Initiative 2012 equations[47]. *Significantly different from control.

Table 5-2. Cardiopulmonary responses to exercise

		Baseline	40W	50%	80%
PO,	Control		40±0	73±21	121±35
W	COPD		40±0	46±20	81±33
	P		N/A	0.003*	<0.001*
$\dot{V}O_2$,	Control	0.33±0.09	0.91±0.18	1.23±0.29	1.80±0.43
$L \cdot min^{-1}$	COPD	0.28±0.08	0.82±0.20	0.89±0.31	1.32±0.44
	P	0.655	0.397	0.004*	<0.001*
$\dot{V}CO_2$,	Control	0.30±0.08	0.80±0.19	1.13±0.27	1.88±0.43
$L \cdot min^{-1}$	COPD	0.24±0.05	0.70±0.21	0.75±0.29	1.31±0.41
	P				
$P_{ET}O_2$,	Control	100.9±6.1	95.3±3.1	95.1±4.7	99.0±4.1
mmHg	COPD	102.2±3.8	97.7±3.2	97.3±3.7	99.2±5.4
	P	0.373	0.128	0.153	0.739
$P_{ET}CO_2$,	Control	32.6±3.8	35.6±3.2	38.0±3.5	37.1±3.9
mmHg	COPD	29.8±1.7	33.4±2.8	34.1±3.2	35.0±4.0
	P	0.059	0.142	0.011*	0.175
$\dot{V}_E$,	Control	13.2± 2.9	25.8±6.4	33.7±7.9	55.5±14.0
$L \cdot min^{-1}$	COPD	12.1±2.2	26.2±6.7	27.9±9.1	45.2±12.7
	P	0.774	0.907	0.113	0.009*
$\dot{V}_E/\dot{V}O_2$	Control	40.8±7.7	28.2±2.8	28.0±2.6	31.2±3.6
	COPD	44.2±10.1	32.4±5.8	32.1±6.4	35.2±8.4
	P	0.284	0.021*	0.036*	0.123
S_PO_2,	Control	98.0±1.0	97.5±2.7	97.3±1.1	96.6±1.5
%	COPD	96.5±3.0	96.1±2.7	95.9±2.9	94.7±3.5
	P	0.014*	0.042*	0.002*	0.151
$\dot{Q}$,	Control	3.59±0.70	5.75±1.06	7.79±1.91	9.67±2.30
$L \cdot min^{-1}$	COPD	3.58 ±1.30	5.61±1.35	5.97±0.97	8.15±2.02
	P	0.730	0.648	0.015*	0.134*
HR,	Control	75±8	98±14	112±13	135±13
bpm	COPD	75±8	96±10	97±11	116±10
	P	0.920	0.306	0.003*	<0.001*
SV,	Control	48±9	58±13	66±16	68±18
mL	COPD	48±17	60±14	61±12	69±16
	P	0.949	0.759	0.366	0.918
PASP,	Control	22.8±10.4	34.4±15.7	42.4±18.5	50.0±19.1
mmHg	COPD	23.9±8.6	31.4±13.0	33.0±14.2	34.4±16.1
	P	0.819	0.678	0.348	0.141

Values means±SD. PO, power output; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; $P_{ET}O_2$, partial pressure of end tidal O_2; $P_{ET}CO_2$, partial pressure of end tidal CO_2; $\dot{V}_E$, minute ventilation; $\dot{V}_E/\dot{V}O_2$, ventilatory equivalent for oxygen; S_PO_2, oxygen pulse saturation; $\dot{Q}$, cardiac output; HR, heart rate; SV, stroke volume; PASP, pulmonary artery systolic pressure. *Significantly different from control.

Table 5-3. Operating lung volumes during exercise

		Baseline	40W	50%	80%
V_A,	Control	5.29±1.33	5.36±1.38	5.34±1.37	5.39±1.49
L	COPD	4.55±0.89	4.68±0.82	4.61±0.84	4.67±0.83
	P	0.054	0.086	0.082	0.092
V_T,	Control	0.78±0.19	1.19±0.27	1.50±0.34	2.01±0.51
L	COPD	0.68±0.16	1.20±0.37	1.28±0.45	1.72±0.40
	P	0.066	0.060	0.125	0.184
EELV/TLC,	Control	58±7	55±7	52±7	52±4
%	COPD	56±14	56±12	56±11	60±8
	P	0.764	0.819	0.217	<0.046*
IC,	Control	2.49±0.67	2.65±0.73	2.85±0.73	2.80±0.63
L	COPD	2.37±0.69	2.43±0.69	2.41±0.61	2.24±0.66
	P	0.590	0.348	0.089	0.038*
IC/TLC,	Control	42±7	45±7	48±7	48±4
%	COPD	44±14	44±12	44±11	40±8
	P	0.764	0.819	0.217	0.046*
IRV,	Control	1.71±0.57	1.47±0.62	1.38±0.50	0.78±0.25
L	COPD	1.69±0.61	1.23±0.70	1.13±0.48	0.57±0.40
	P	0.793	0.239	0.087	0.045*

V_A, alveolar volume; V_T, tidal volume; EELV/TLC, end expiratory lung volume as a percent of total lung capacity; IC, inspiratory capacity; IC/TLC, inspiratory capacity as a percent of total lung capacity; IRV, inspiratory reserve volume. *Significantly different from control.

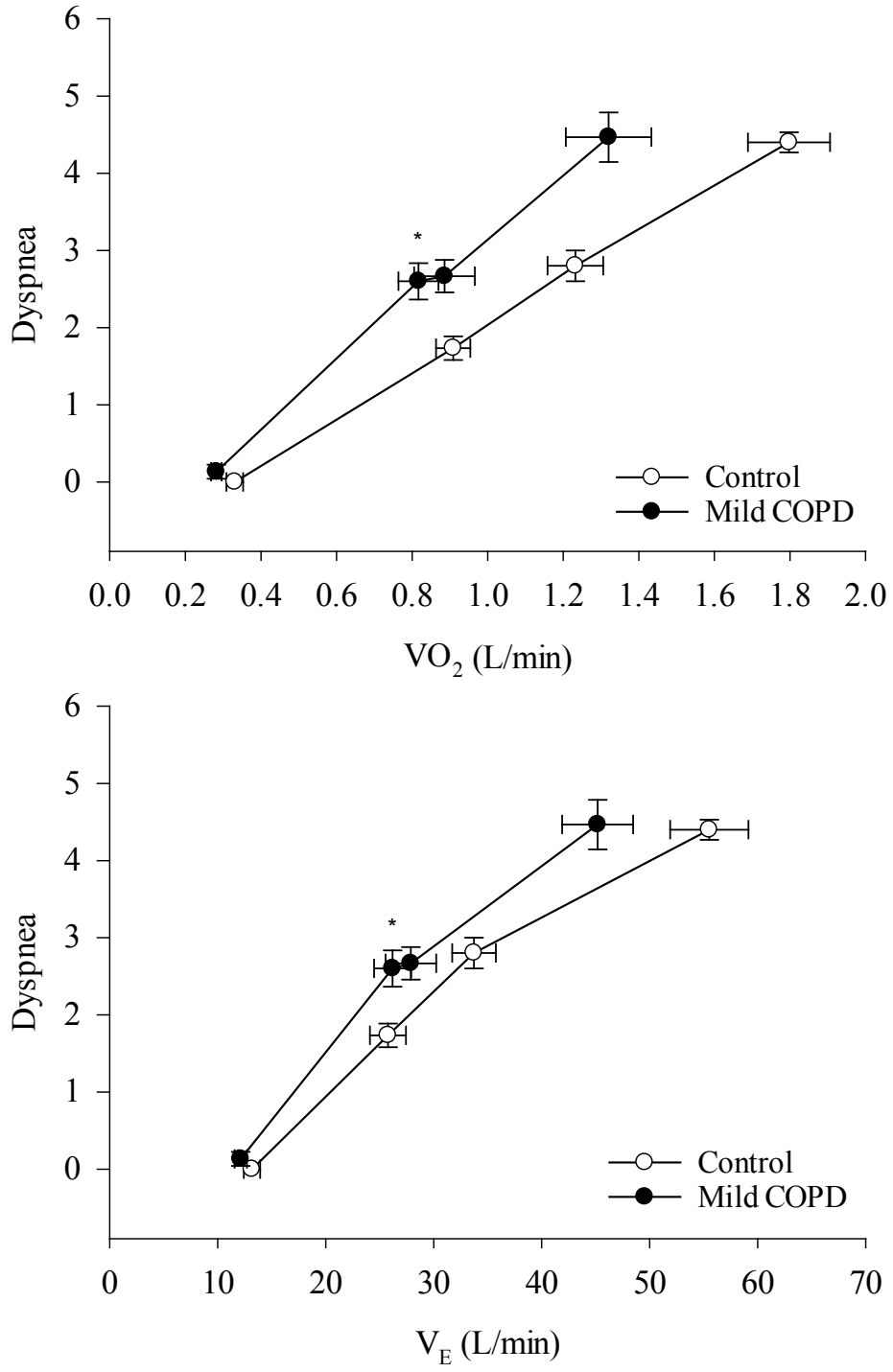

Figure 5-1. Dyspnea response to exercise. Dyspnea was greater in mild COPD during exercise at 40 W (*P < 0.05).

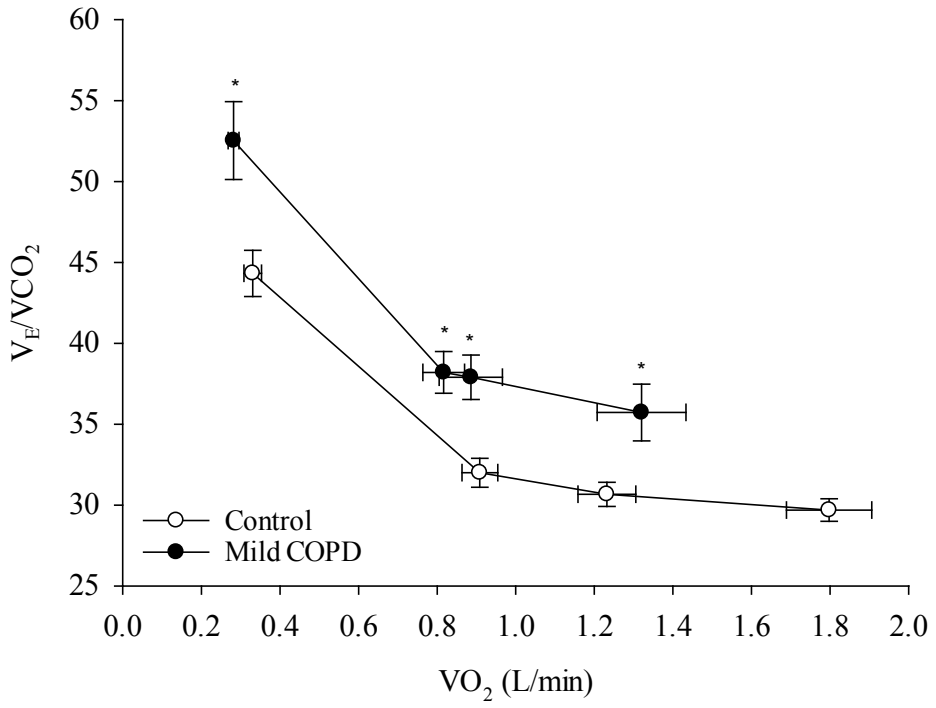

Figure 5-2. Ventilatory equivalent for CO_2 ($\dot{V}_E/\dot{V}CO_2$) during exercise. $\dot{V}_E/\dot{V}CO_2$ was greater in COPD at rest and during exercise at 40 W, 50%, and 80% of $\dot{V}O_{2peak}$ (*$P < 0.05$).

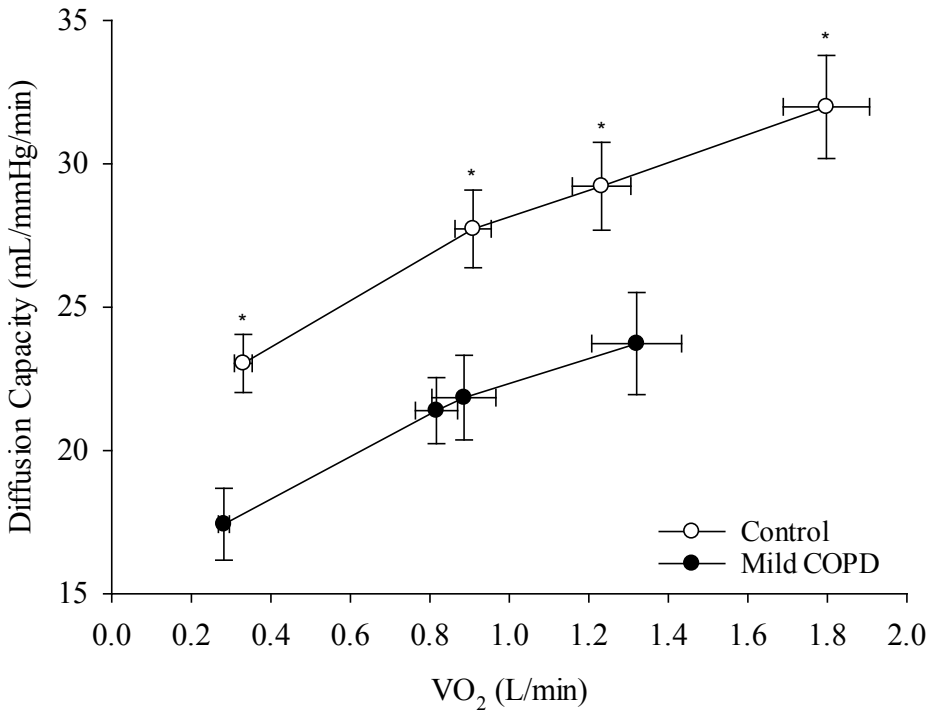

Figure 5-3. Diffusion capacity response to exercise. DLCO was lower in mild COPD subjects at baseline, and throughout exercise (*$P < 0.05$).

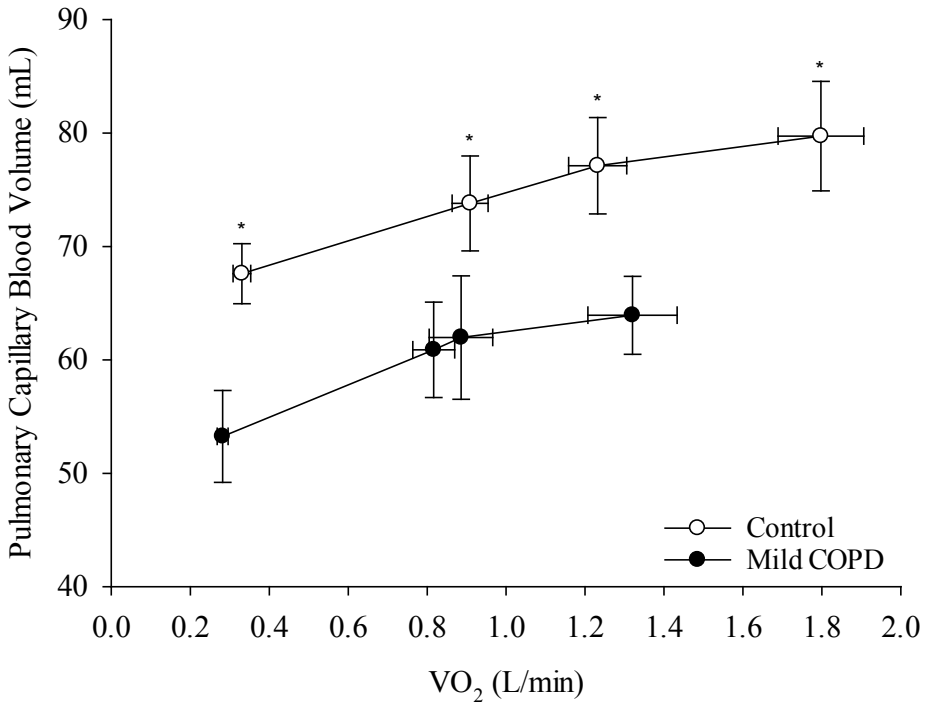

Figure 5-4. Pulmonary Vc response to exercise. Pulmonary capillary blood volume was lower in mild COPD at rest and during exercise at 40 W, 50%, and 80% of $\dot{V}O_{2peak}$ (*$P < 0.05$).

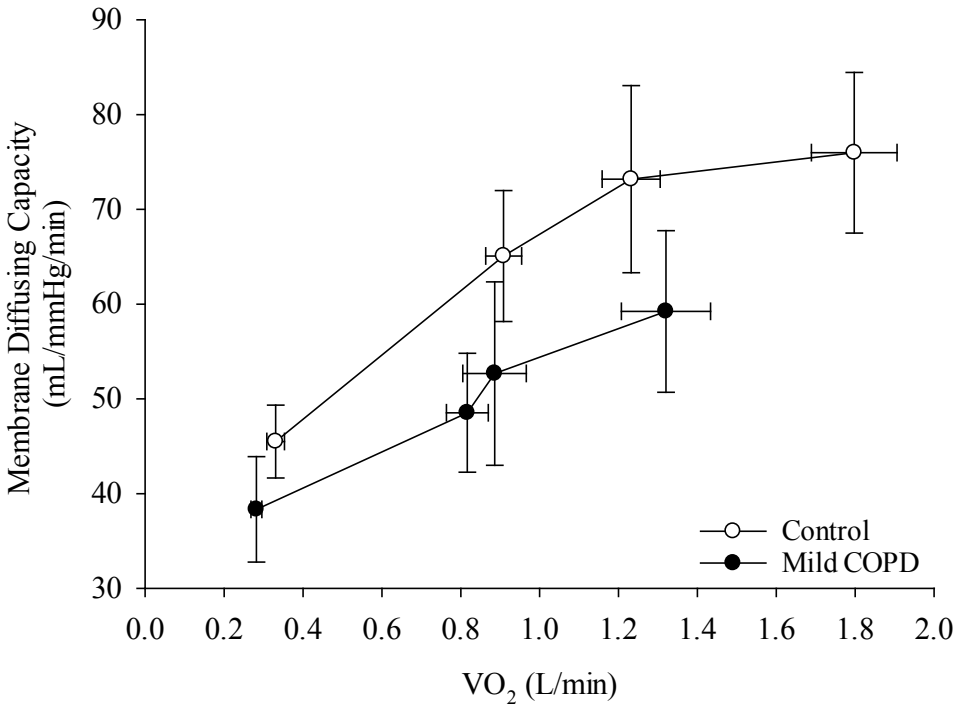

Figure 5-5. Membrane diffusing capacity response to exercise. Dm was not significantly different between groups at rest or during exercise ($P = 0.112$).

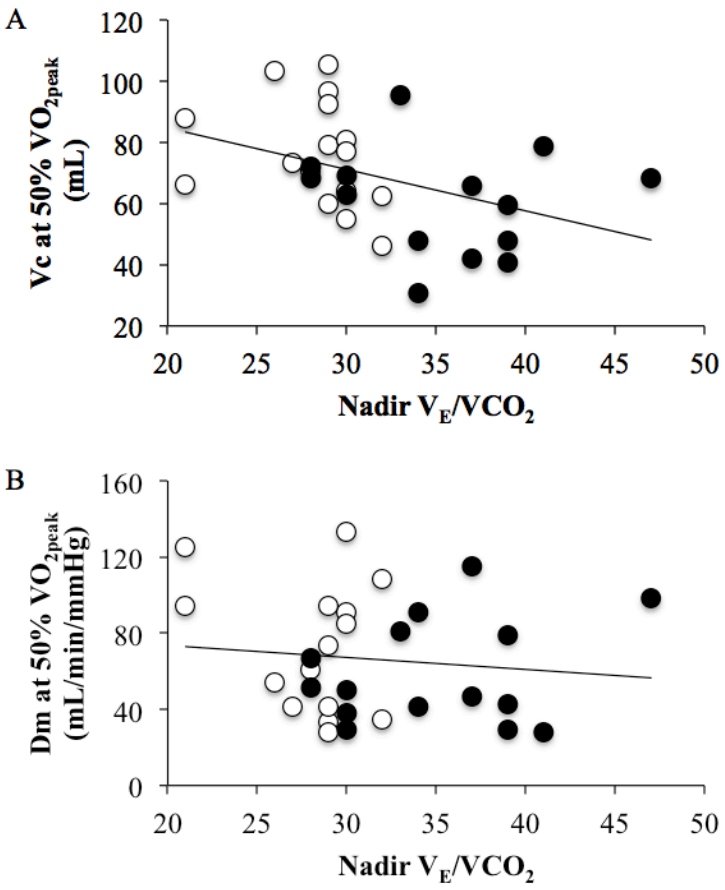

Figure 5-6. Correlation of individual nadir $\dot{V}_E/\dot{V}CO_2$ to components of diffusing capacity. **A:** Pulmonary capillary blood volume (r= -0.41, *P*=0.022). **B:** Membrane diffusing capacity at 50% $\dot{V}O_{2peak}$ (r= -0.11, *P*=0.543). White circles, control group; black circles, mild COPD group.

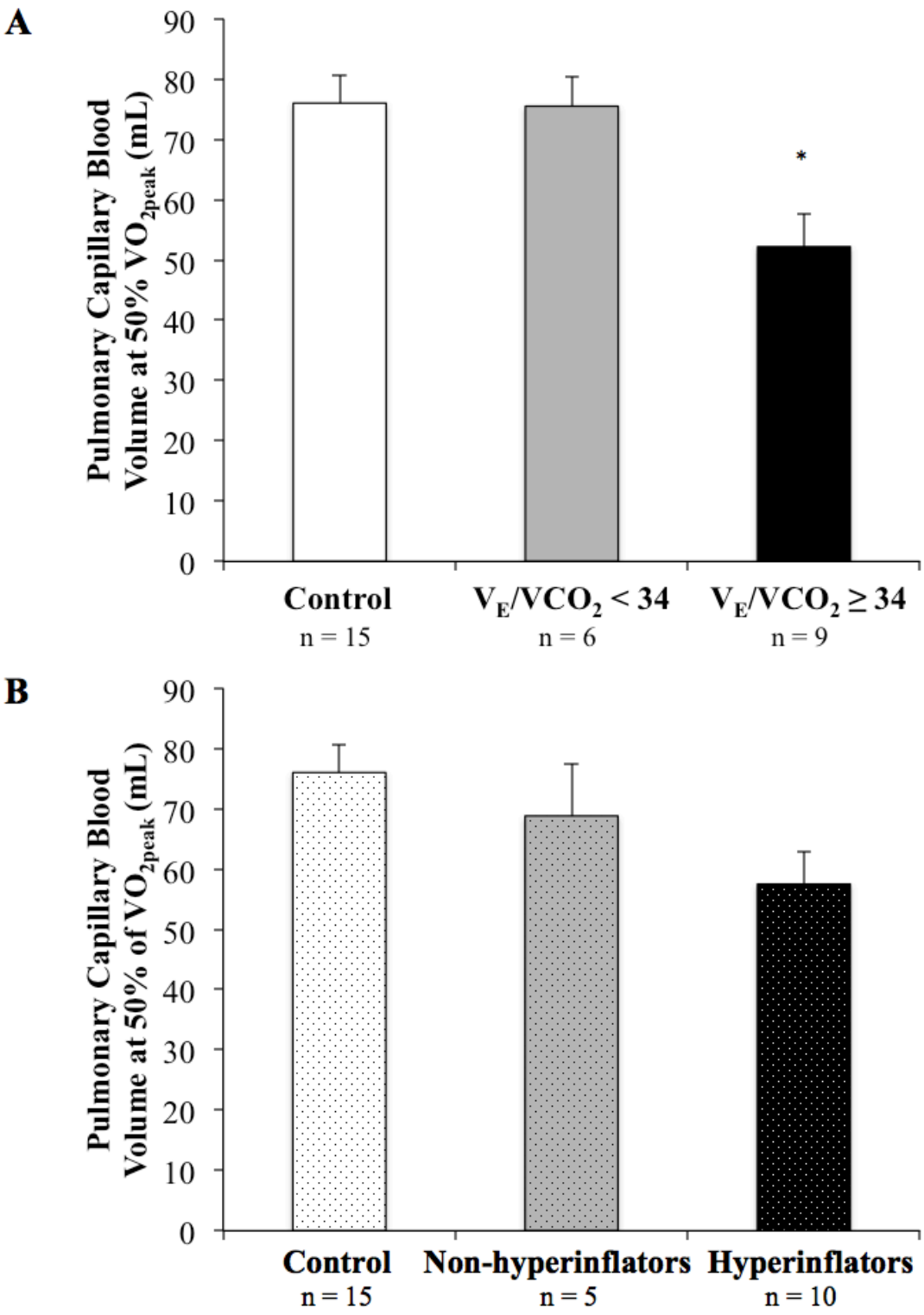

Figure 5-7. Subgroup pulmonary capillary blood volume at 50% of $\dot{V}O_{2peak}$. **A**. Nadir $\dot{V}_E/\dot{V}CO_2$ in mild COPD. **B**: Dynamic hyperinflators identified as decreased inspiratory capacity from rest to $\dot{V}O_{2peak}$. Error bars represent SEM. *Vc was significantly lower in the $\dot{V}_E/\dot{V}CO_2 \geq 34$ group compared to control ($P = 0.005$) and the $\dot{V}_E/\dot{V}CO_2 < 34$ group ($P = 0.038$). Vc was not different between hyperinflation groups ($P = 0.056$).

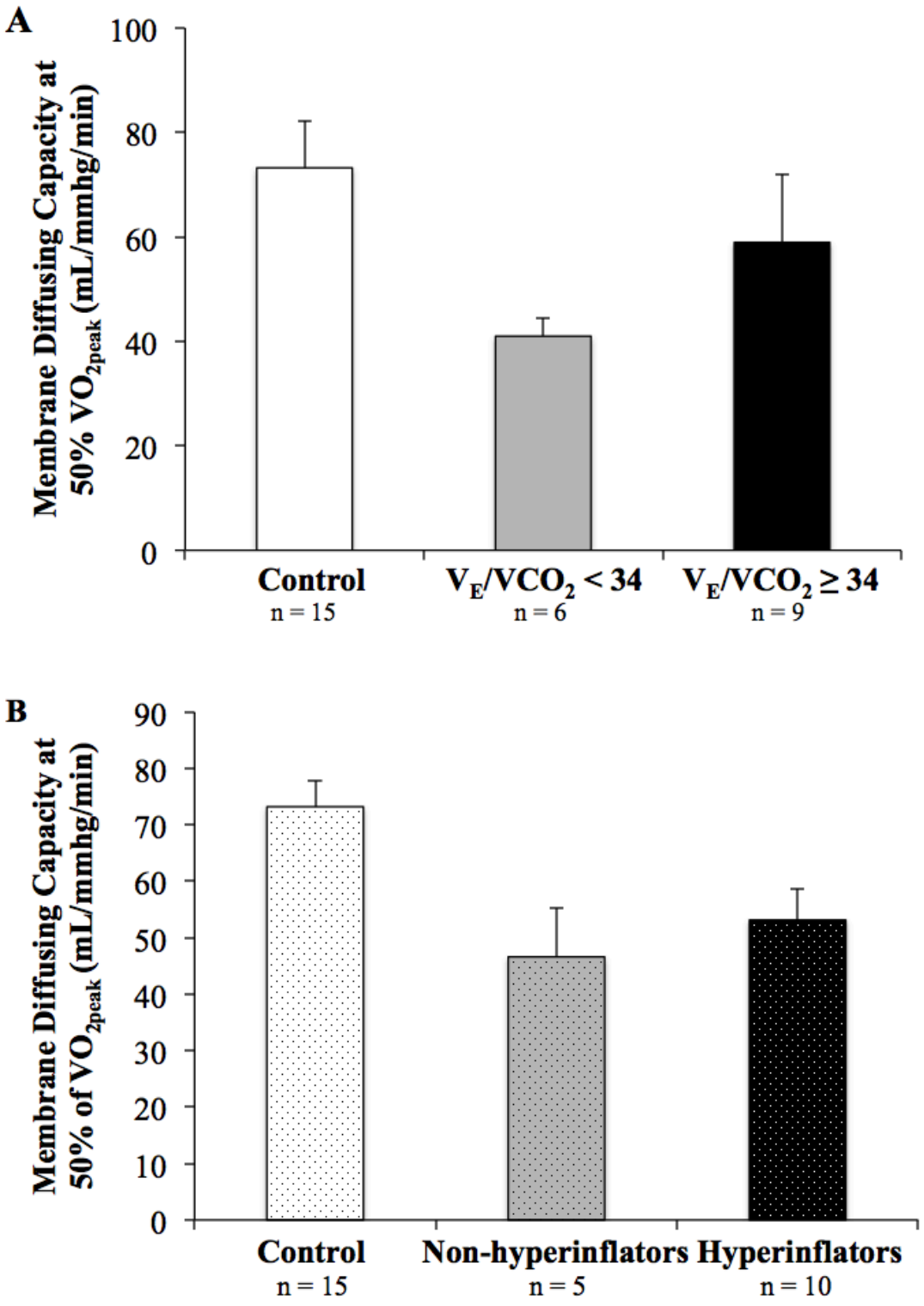

Figure 5-8. Subgroup membrane diffusing capacity at 50% of $\dot{V}O_{2peak}$. **A**: Nadir $\dot{V}_E/\dot{V}CO_2$ in mild COPD and **B**: Dynamic hyperinflation identified by decreased inspiratory capacity from rest to $\dot{V}O_{2peak}$. Error bars represent SEM. There was no difference in Dm between $\dot{V}_E/\dot{V}CO_2$ subgroups ($P = 0.315$) or hyperinflation subgroups ($P = 0.629$).

CHAPTER 6

General Discussion

6.1 Dissertation Overview

The purpose of this dissertation was to characterize the response of the pulmonary vasculature to exercise in health, including a pharmacological intervention, and in individuals with mild chronic obstructive lung disease. The first project studied the effect of dopamine receptor blockade on the gas exchange response during exercise[1]. Administration of metoclopramide improved indices of pulmonary gas exchange during exercise, as indicated by decreased alveolar-arterial oxygen difference ($A\text{-}aDO_2$) and increased arterial oxygen saturation, but reduced exercise performance.

Two subsequent projects examined the relative contributions of pulmonary capillary blood volume and membrane diffusing capacity to the overall increase in diffusing capacity during exercise. Endurance-trained athletes exhibit enhanced cardiovascular function compared to non-athletes, but it is accepted that exercise training does not enhance lung structure and function. The second study investigated differences between endurance-trained athletes and age- and height-matched sedentary controls. We found that the increased diffusing capacity (DLCO) at peak exercise in endurance-trained athletes is augmented by enhanced membrane diffusing capacity (Dm), while no difference in pulmonary capillary blood volume (Vc) was observed[2]. These data suggest that athletes appear to have differences within the pulmonary membrane that facilitate the increased oxygen consumption during high-level exercise.

In the third project, the determinants of pulmonary diffusing capacity were examined in mild chronic obstructive pulmonary disease (COPD) during exercise. We observed decreased DLCO at rest and during exercise, which can be explained by lower capillary blood volume, as there was no difference in membrane diffusing capacity. These results suggest that despite relatively minor airway obstruction, individuals with mild COPD exhibit some pulmonary vascular impairment at rest and during exercise.

6.2 Membrane Diffusing Capacity as an Index of Pulmonary Capillary Recruitment

As membrane diffusing capacity cannot be measured in alveoli that are not perfused, Dm is thought to reflect pulmonary capillary recruitment[2-4]. Membrane diffusing capacity increases with incremental exercise, but appears to plateau despite increasing oxygen uptake. Both studies examining aerobic athletes and mild COPD patients demonstrated an upper limit to Dm^2, prior to reaching peak exercise intensity. This suggests that low-intensity exercise fully recruits pulmonary capillaries. This is consistent with research demonstrating pulmonary capillary recruitment with moderate increases in blood flow, but no further capillary recruitment with any further increases in blood flow in the isolated canine lung[5]. Subsequently, any increases in pulmonary capillary blood volume beyond full recruitment would be due to capillary distension. This plateau in Dm during incremental exercise was first observed in a study dating back almost 50 years, which found a plateau in Dm during exercise in both training and non-training groups[6]. Another study found that both lowlanders and highlanders exposed to high altitude also demonstrate this plateau in Dm during incremental exercise at sea level and at altitude[7]. In our study examining sex differences, women and men alike demonstrated a similar plateau Dm with incremental exercise, regardless of differences in lung size[8]. Together, these studies suggest that a plateau in membrane diffusing capacity during exercise is likely a universal finding. As Dm could only increase with capillary recruitment, we would suggest that a plateau in membrane diffusing capacity might be considered a reliable index of full capillary recruitment.

6.3 IPAVA Recruitment to Protect the Lungs

It has been suggested that intrapulmonary arteriovenous anastomosis (IPAVA) recruitment acts as a ‘pop-off valve’ to protect the pulmonary vasculature from large increases in pulmonary artery pressure during incremental exercise[1,9-12]. Previous research from our group suggests that

individuals who do not recruit IPAVAs appear to have the lowest peak work, $\dot{V}O_{2max}$, cardiac output, and highest pulmonary artery pressure compared to those who recruit IPAVAs during exercise[12]. In the first study of this dissertation, we aimed to prevent IPAVA recruitment during incremental using a dopamine receptor blockade[1]. While we observed improvements in pulmonary gas exchange, IPAVA recruitment did not appear to be different with dopamine blockade. However, $\dot{V}O_{2max}$, cardiac output, and exercise performance were decreased compared to control[1].

While we were unsuccessful in determining pulmonary artery pressure using cardiac ultrasound, the increased ventilation at near-peak exercise (secondary to increased breathing frequency) and decreased stroke volume is consistent with that of juxta-capillary receptor stimulation in the lung secondary to a potentiated pulmonary artery pressure during exercise[13]. It is possible that the agitated saline contrast echocardiography method is not sensitive enough to detect changes in IPAVA recruitment with dopamine blockade. However, the decrease in cardiovascular function despite improved pulmonary gas exchange would be consistent with a decrease in IPAVA recruitment. Therefore, while speculative, our results would suggest that IPAVA recruitment may prevent excessively elevated vascular pressures[14] which may otherwise contribute to pulmonary edema or microvascular rupture[15].

Finally, our recent study examining the relationship of IPAVA recruitment to Vc and Dm during exercise provides insight into the time course of pulmonary vascular responses to exercise[16]. In a related study separate from this dissertation, IPAVA recruitment was detected at a Vc similar to that observed in the supine position[16]. The supine position translocates blood from the lower body to the thorax, and thus the supine position is thought to cause full pulmonary capillary recruitment[17-19]. In addition, recruitment of IPAVA was detected when the Dm response

appeared to plateau with incremental exercise, and this plateau in Dm is thought to represent the upper limit of capillary recruitment[16]. Taken together, these data would support the suggestion that IPAVA are opened during incremental exercise once the pulmonary capillaries are fully recruited.

6.4 Training the Lungs

Our finding of enhanced pulmonary membrane in endurance-trained athletes (Chapter 4) has generated some discussion on the ability to exercise train the lungs[20,21], prompting the question of an inherently greater lung diffusion in endurance-athletes as opposed to an endurance training regimen that facilitates these differences. Greater $\dot{V}O_{2max}$ has been linked to greater pulmonary capillary blood volume at rest[11], which was confirmed in our second study[2]. The lungs of athletes are larger than height-matched non-athletes[2], but previous research has not shown a significant difference in Vc or Dm with exercise training. Notably, Reuschlein *et al*. (1968) employed a 5-month aerobic and resistance exercise training program and found no difference in diffusing capacity, pulmonary capillary blood volume, or membrane diffusing capacity for a given $\dot{V}O_2$ between training and control groups. However, this study did not report any change to $\dot{V}O_{2max}$, and they did not examine the determinants of diffusing capacity at peak exercise[6], which is where the greatest changes were observed in our study[2]. Also, while not strictly considered exercise training, Dempsey *et al*. (1971) explored the effect of acute and chronic high altitude exposure on diffusing capacity, pulmonary capillary blood volume, and membrane diffusing capacity response to exercise[7], finding that 21-days of high altitude exposure did not change Vc or Dm during exercise in lowlanders. However, individuals living at high altitude had a greater Dm both at rest and during exercise. Together, these studies suggest that the differences reported

between the lungs of athletes and their sedentary counterparts are likely inherent, and not the result of chronic exercise training.

While exercise training has not been shown to change the Vc and Dm response in healthy individuals[6], training might bring about favorable changes to the pulmonary vasculature in COPD patients. Exercise training has been shown to improve exercise tolerance in COPD, with evidence of improved $\dot{V}O_{2peak}$ and dyspnea[22-24]. In the animal model, chronic exercise training improves pulmonary vascular function in rats with smoking-induced lung damage[25]. If chronic exercise training can improve pulmonary blood flow in COPD, it would be expected that Vc might improve with training, and improve pulmonary gas exchange during exercise.

As reviewed in Chapter 2, it is well known that in individuals with high $\dot{V}O_{2peak}$, exercise increases the $A\text{-}aDO_2$, leading to exercise-induced arterial hypoxemia[26,27]. Ventilation-perfusion inequality constitutes the majority of the measured $A\text{-}aDO_2$, but beyond a $\dot{V}O_2$ of 3.5 $L \cdot min^{-1}$ [28], a diffusion limitation contributes to the gas exchange impairment[26]. Study 2 found that highly fit individuals have increased diffusion capacity during peak exercise, secondary to greater membrane diffusing capacity compared to control[2]. Presumably, an expanded membrane diffusing capacity would prevent any diffusion limitation, and yet it is the highly fit who typically demonstrate a diffusion limitation with exercise. Although speculative, it is possible that the expanded membrane diffusing capacity in endurance-trained athletes is not enough to overcome the reduced pulmonary transit time[29]. Regardless, despite having greater diffusion capacity, the demand for diffusion of oxygen is much greater in highly fit individuals. Therefore, the imbalance between the diffusion capacity for oxygen and oxygen demand likely explains the diffusion limitation observed during high intensity exercise in the highly fit.

6.5 Pulmonary Vascular Dysfunction in Mild COPD

There is evidence of vascular disease that affects the smooth muscle of pulmonary arteries in mild COPD[30-32], which is consistent with the reduced pulmonary capillary blood volume during exercise observed in Study 3. Previous work has observed inflammation and remodeling of the microvasculature[31,33], as well as damage to the pulmonary capillary endothelium in mild COPD[34,35]. Individuals with the emphysema sub-type of COPD exhibit increased total lung capacity due to alveolar and capillary destruction[36]. However, total lung capacity was not different between mild COPD and controls in Study 3, suggesting they did not have significant emphysema. Additionally, research using contrast-enhanced MRI has shown reduced resting microvascular blood flow in non-emphysematous mild COPD patients[37]. Taken together, these studies suggest that the pulmonary vasculature appears to be dysfunctional in mild COPD, and thus the reduced capillary blood available for pulmonary gas exchange may be due to upstream changes in blood flow preceding destruction of the alveolar-capillary interface in emphysema. An exercise training study in mild COPD may help clarify if vascular dysfunction or capillary destruction explains the reduced capillary blood volume during exercise. Exercise training might improve pulmonary vascular function in non-emphysematous COPD, which would subsequently increase pulmonary Vc during exercise. In contrast, exercise training would not be expected to improve exercise Vc in patients with emphysema, as they would have irreversible capillary destruction.

6.6 Sex Differences in Diffusing Capacity

The first two studies in this dissertation examined only men because it was not known if sex might be a confounding factor in the pulmonary vascular response to exercise. A number of studies have examined sex differences in pulmonary physiology during exercise[38-40]. Women

may develop greater gas exchange impairment during exercise due to their smaller lungs compared to men, and thus may be at higher risk of exercise induced arterial hypoxemia, even at a lower $\dot{V}O_{2max}$[40,41]. In a related study separate from this dissertation, we found that women demonstrate lower diffusing capacity/cardiac output ratio, pulmonary capillary blood volume, and membrane diffusing capacity compared to height-matched men during exercise[8]. However these differences disappear after correcting for the larger alveolar volume in men. Additionally, the drop in DLCO/$\dot{Q}$ was proportionally lower in women, and pulmonary transit times did not drop below 0.3 s in either sex. Thus, there is no compelling evidence of intrinsic sex difference in the diffusing capacity, Vc, or Dm response to exercise after accounting for the difference in alveolar volume[8]. In light of these findings, the third study in this dissertation examined both men and women to minimize confounding factors outside of the disease state.

6.7 Diffusing Capacity for Nitric Oxide

Diffusing capacity for nitric oxide (DLNO) has been proposed as an alternate method to determine pulmonary capillary blood volume and membrane diffusing capacity[42-45]. Nitric oxide is added to standard DLCO test gas, and the diffusing capacity for nitric oxide and carbon monoxide are assessed simultaneously. The benefit of this technique is the ability to measure Vc and Dm in a single breath, which would reduce carbon monoxide exposure and considerably reduce experimental time. The DLNO technique to assess Vc and Dm relies on similar principles as the multiple-F_IO_2 DLCO method used in our studies, but uses two gases with different binding rates. The reaction rate of nitric oxide binding to hemoglobin is 280 times faster than that of carbon monoxide, and therefore the rate of NO uptake by blood (θ_{NO}) is extremely fast and assumed to be infinite[44]. Thus, DLNO is assumed to be approximately equal to the diffusing membrane capacity for nitric oxide. However, nitric oxide is a potent vasodilator, particularly in

the lung vasculature[46,47]. Prior research has shown that administration of nitric oxide at rest and during exercise reduces pulmonary hypertension in COPD and affects ventilation-perfusion inequality[48]. Notably, the amount of nitric oxide administered as a therapeutic in that study was 40 ppm[48], which is the same concentration used in the DLNO method to measure Vc and Dm[44]. Therefore, the use of DLNO in patients with COPD would likely alter pulmonary circulation, ventilation-perfusion matching, and lead to inaccurate calculations.

6.8 Future Research

Dopamine is a pulmonary vasodilator, and the first experiment of this dissertation showed that dopamine blockade reduced stroke volume and increased ventilation, secondary to increased breathing frequency during exercise[1]. While considered indirect evidence, these data are consistent with an increased pulmonary artery pressure during exercise[13,49-51]. Thus, dopamine blockade administration could be used as an experimental model of pulmonary hypertension during exercise in future studies. Sustained elevated pulmonary microvascular pressures could cause stress failure and capillary rupture[14,15], which could be assessed using broncho-alveolar lavage before and after exercise with, and without dopamine blockade.

As previously discussed, IPAVA recruitment has been theorized to protect the lung against possible microvascular rupture[1,10-12,52]. An alternate experiment could target individuals who do not recruit IPAVAs. If a lack of IPAVA recruitment during exercise were accompanied with low peak cardiac output, low alveolar-arterial oxygen difference, and increased pulmonary artery pressure, this would be consistent with the notion that IPAVA recruitment is beneficial to the normal cardiopulmonary response to exercise.

The second experiment (Chapter 4) offered several explanations for increased DLCO at peak exercise in endurance-trained athletes[2]. Aerobic athletes have a greater blood volume compared to non-athletes, which may facilitate capillary recruitment and distension at high cardiac outputs[53]. Thus, it would be possible to determine the effect of greater blood volume or plasma volume in athletes compared to untrained individuals on the DLCO response to exercise. Exercise diffusing capacity would be measured before and after the removal of a unit of blood in athletes, to determine if increased blood volume affects diffusing capacity. Additionally, replacement of the volume of blood lost using saline or lactated ringer solution would determine the contribution of hemoglobin in an isovolumic fashion.

The third and final experiment (Chapter 5) attributed lower diffusing capacity during exercise in mild COPD to a blunted pulmonary capillary blood volume. In secondary analysis, it was theorized that dynamic hyperinflation might reduce pulmonary capillary blood volume by way of mechanically compressing pulmonary capillaries. To better answer this question, additional patients should be recruited on the basis of dynamic hyperinflation to compare with patients who do not exhibit a change in their inspiratory capacity with exercise.

To determine the effect of blunted pulmonary capillary blood volume on pulmonary gas exchange, this study would benefit from the addition of measures of ventilation-perfusion inequality using the multiple inert gas elimination technique. This would clarify the connection between low Vc and increased dead space ventilation due to areas of high ventilation-perfusion ratios, and would provide more information on early pulmonary vascular dysfunction in mild COPD.

Finally, an additional study could be conducted in patients with pulmonary arterial hypertension (PAH). Pulmonary arterial hypertension is a primary elevation of pressure in the pulmonary artery, which has the effect of reducing cardiac output, increasing right heart strain, and presents with resting and exertional dyspnea, profound exercise intolerance, and a 7-year survival rate of only 49%[54]. Previous research has shown that resting diffusing capacity is decreased in PAH, due to reductions in both Vc and Dm[55], and patients often demonstrate exercise-induced arterial hypoxemia of unknown etiology. It would be expected that diffusing capacity would be similarly reduced during exercise, and thus investigation of exercise Vc and Dm would contribute to the understanding of pulmonary gas exchange in pulmonary hypertension.

6.9 Summary

This dissertation aimed to describe the response of the pulmonary vasculature to exercise in a number of physiological conditions. Study 1 (Chapter 3) administered a dopamine blockade to highlight the importance of dopamine receptors in the pulmonary vasculature, which may present as a potential target for future research. Study 2 (Chapter 4) provided evidence that aerobically-trained athletes have an enhanced pulmonary membrane, which is some of the first evidence that some aspect of the lungs are enhanced in highly trained individuals. Study 3 (Chapter 5) observed a blunted pulmonary capillary blood volume response to exercise in mild COPD, which is consistent with previous work suggesting that pulmonary vasculature dysfunction precedes changes to the alveolar interface, such as in emphysema. Together, this dissertation contributes to the understanding of the importance of the pulmonary vasculature to cardiovascular function and pulmonary gas exchange during exercise in health and mild chronic obstructive lung disease.

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Appendix A

Diffusing Capacity Response to Exercise in Moderate COPD

Both components of diffusing capacity: pulmonary capillary blood volume, and membrane diffusing capacity were assessed during exercise in moderate COPD (n=9) to further describe the progression of impaired diffusion with disease severity.

Table A-1. Descriptive characteristics of moderate COPD patients

Subjects (male/female)	5/4
Age	70 ± 6
Weight	80.0 ± 18.6
BMI	28.2 ± 4.8
Smoking history, pack-years	46 ± 23
$\dot{V}O_{2peak}$, L·min^{-1}	1.44 ± 0.70
$\dot{V}O_{2peak}$, % pred	77 ± 25
S_PO_2 rest, %	95.9 ± 2.1
S_PO_2 peak, %	90.6 ± 3.6
$\dot{V}_E/\dot{V}CO_2$ nadir	36.0 ± 5.3
FEV_1, L	1.77 ± 0.38
FEV_1, % pred	66 ± 7
FEV_1, z-score	-2.01 ± 0.42
FVC, L	3.61 ± 0.83
FVC, % pred	99 ± 13
FVC, z-score	0.25 ± 0.81
FEV_1/FVC, %	50 ± 9
FEV_1/FVC, % pred	68 ± 13
FEV1/FVC, z-score	-3.05 ± 0.90
TLC, L	6.67 ± 1.74
TLC, % pred	111 ± 18
FRC, L	3.83 ± 1.24
FRC, % pred	126 ± 22
RV, L	2.67 ± 0.97
RV, % pred	124 ± 26
DLCO, mL·min^{-1}·mmHg^{-1}	15.0 ± 6.4
DLCO, % pred	62 ± 15

Values expressed as Means ± SD. $\dot{V}O_{2peak}$, peak oxygen consumption; S_PO_2, oxygen pulse saturation; $\dot{V}_E/\dot{V}CO_2$ nadir, ventilatory equivalent for CO_2 at lowest point during graded exercise test; FEV_1, forced expired volume in 1 second; FVC, forced vital capacity; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume assessed by plethysmography; DLCO, diffusion capacity at rest. Z-score calculated using Global Lung Initiative 2012 equations.

Table A-2. Cardiopulmonary responses to exercise in moderate COPD (n=9)

	Baseline	40 W	50% $\dot{V}O_{2peak}$	80% $\dot{V}O_{2peak}$
PO, W		40 ± 0	43 ± 25	73 ± 42
$\dot{V}O_2$, L·min^{-1}	0.38 ± 0.11	0.91 ± 0.14	1.05 ± 0.30	1.34 ± 0.46
$\dot{V}CO_2$, L·min^{-1}	0.24 ± 0.05	0.70 ± 0.21	0.75 ± 0.29	1.31 ± 0.41
$P_{ET}O_2$, mmHg	102.7 ± 5.3	98.5 ± 4.3	98.3 ± 5.5	99.8 ± 5.3
$P_{ET}CO_2$, mmHg	29.2 ± 3.1	33.2 ± 3.5	33.8 ± 4.5	35.2 ± 5.6
RER	0.90 ± 0.08	0.90 ± 0.06	0.91 ± 0.10	0.98 ± 0.06
S_PO_2, %	95.9 ± 2.1	94.6 ± 2.0	94.2 ± 2.0	93.4 ± 1.7
$\dot{V}_E/\dot{V}CO_2$	47.8 ± 10.6	37.9 ± 4.0	38.3 ± 6.1	36.2 ± 5.5
HR, b·min^{-1}	79 ± 13	100 ± 15	101 ± 12	114 ± 11
V_A	5.4 ± 1.6	5.3 ± 1.6	5.3 ± 1.6	5.3 ± 1.5
$\dot{V}_E$, L·min^{-1}	14.1 ± 2.9	31.0 ± 6.2	31.6 ± 7.1	44.9 ± 11.4
V_T, L	0.76 ± 0.22	1.24 ± 0.20	1.25 ± 0.42	1.56 ± 0.61
EELV/TLC, %	60 ± 7	66 ± 8	63 ± 7	68 ± 9
IC, L	2.61 ± 0.68	2.22 ± 0.72	2.44 ± 0.73	2.13 ± 0.73
IC/TLC, %	40 ± 7	34 ± 8	37 ± 7	32 ± 9
IRV, L	1.85 ± 0.67	0.98 ± 0.57	1.18 ± 0.49	0.57 ± 0.34

Values are means ± SD. PO, power output; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; $P_{ET}O_2$, partial pressure of end tidal oxygen; $P_{ET}CO_2$, partial pressure of end tidal CO_2; RER, respiratory exchange ratio; S_PO_2, oxygen pulse saturation; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for CO_2; HR, heart rate; V_A, alveolar volume; $\dot{V}_E$, minute ventilation; V_T, tidal volume; EELV/TLC, end expiratory lung volume as a percent of total lung capacity; IC, inspiratory capacity; IC/TLC, inspiratory capacity as a percent of total lung capacity; IRV, inspiratory reserve volume.

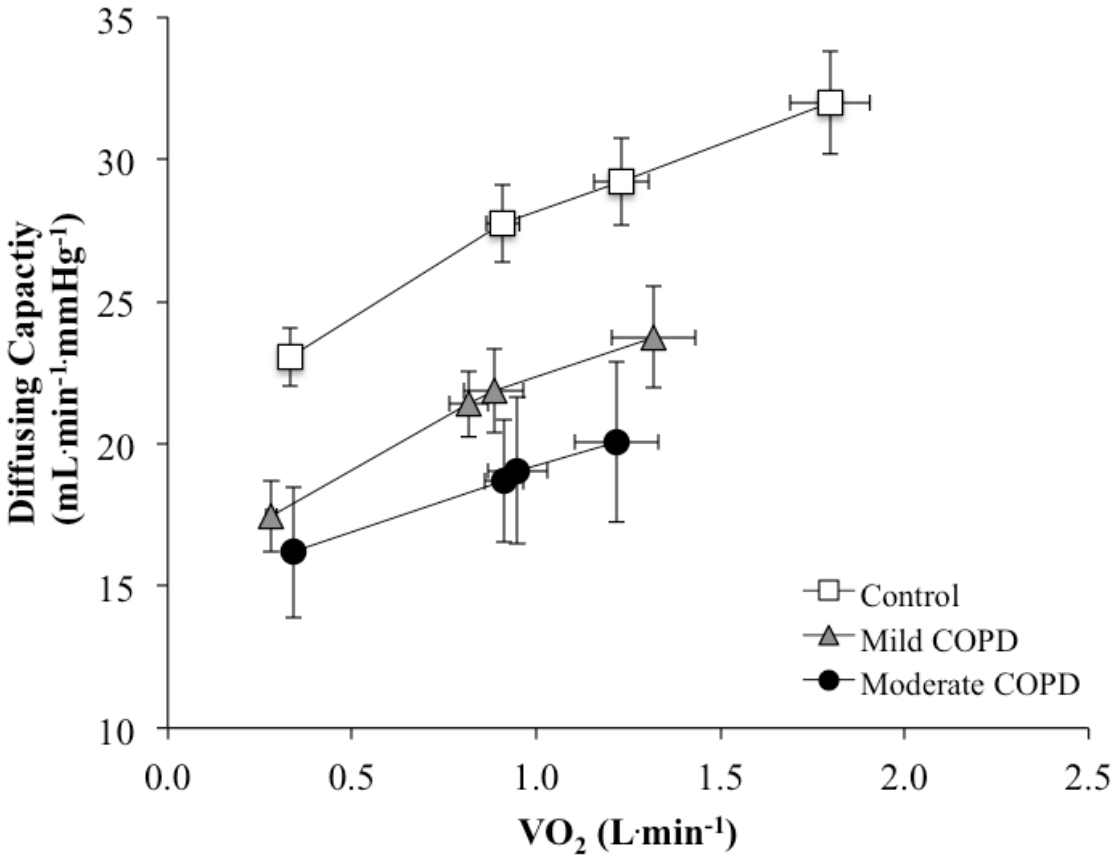

Figure A-1. Diffusing capacity during exercise in mild (n=15) and moderate COPD (n=9).

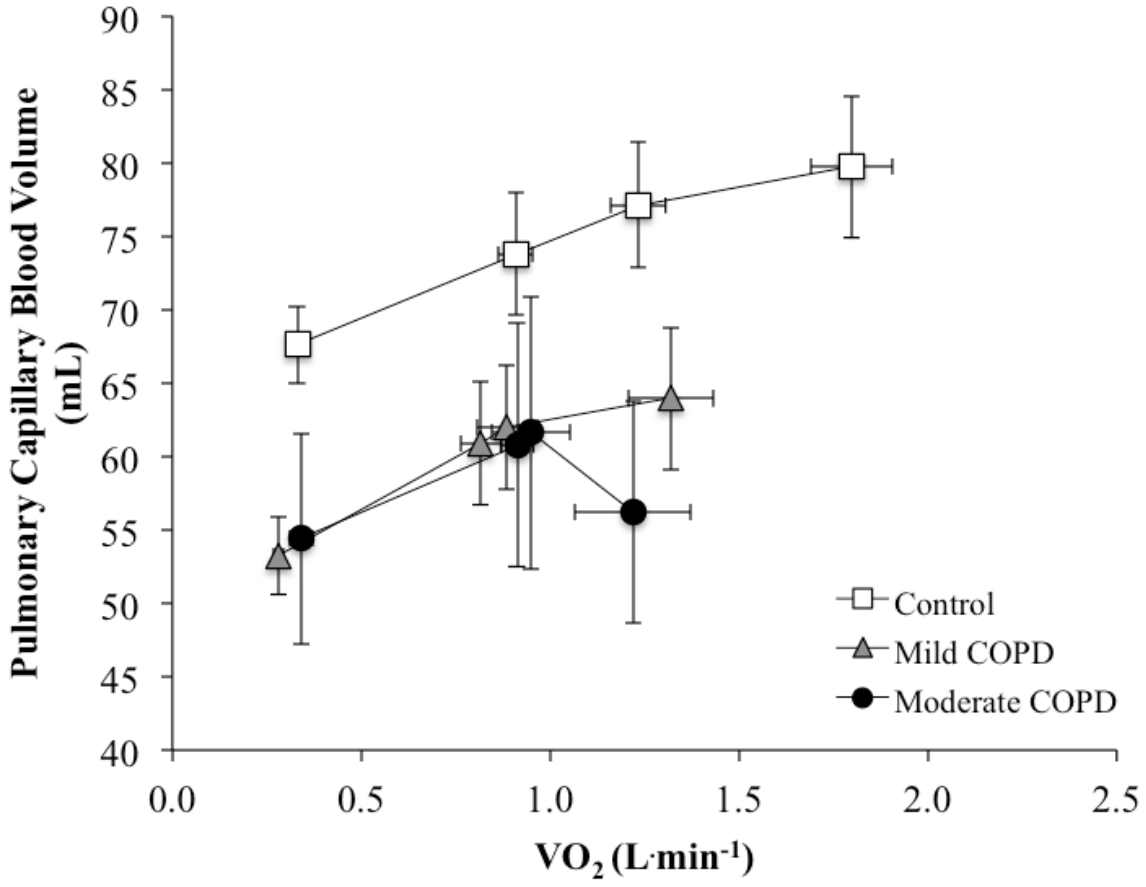

Figure A-2. Pulmonary capillary blood volume response to exercise in mild (n=15) and moderate COPD (n=9).

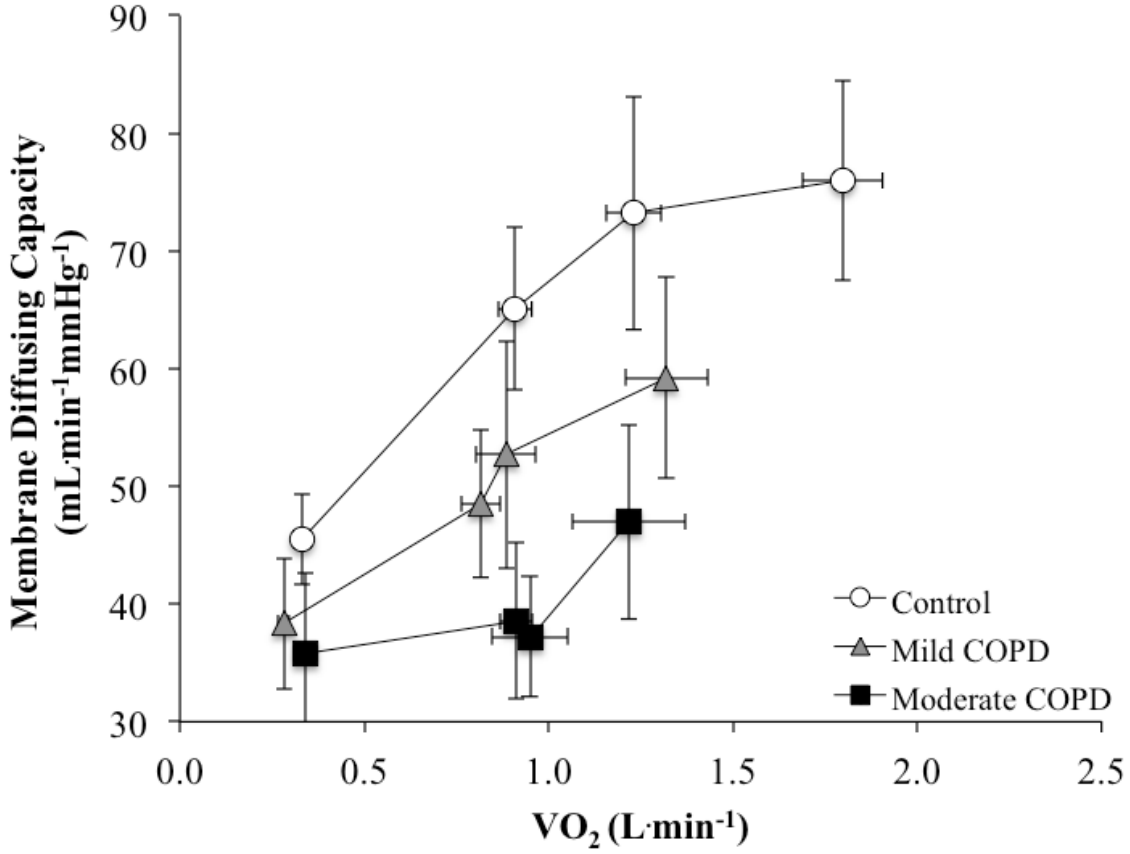

Figure A-3. Membrane diffusing capacity response to exercise in mild (n=15) and moderate COPD (n=9).

Appendix B

Diffusing Capacity Response to Exercise With Dopamine Blockade

The effects of dopamine blockade administration on the determinants of diffusing capacity (Vc and Dm) were assessed in a subset of mild COPD (n=9) and healthy controls. (n=10). Dopamine blockade (20 mg oral metoclopramide) was administered one hour before diffusing capacity measurements.

Table B-1. *Cardiopulmonary responses to exercise with dopamine blockade in healthy controls (n=10)*

	Baseline		40 W		50% $\dot{V}O_{2peak}$		80% $\dot{V}O_{2peak}$	
	Placebo	Blockade	Placebo	Blockade	Placebo	Blockade	Placebo	Blockade
$\dot{V}O_2$, $L \cdot min^{-1}$	0.35±0.10	0.32±0.06	0.92±0.16	0.94±0.17	1.29±0.34	1.32±0.23	1.91±0.40	1.93±0.36
$\dot{V}CO_2$, $L \cdot min^{-1}$	0.32±0.10	0.27±0.07	0.83±0.17	0.75±0.19	1.20±0.27	1.14±0.17	2.01±0.31	2.01±0.33
$P_{ET}O_2$, mmHg	103.2±6.4	100.3±4.2	95.6±2.7	94.0±3.1	95.7±5.2	95.1±3.3	100.1±4.5	98.7±3.5
$P_{ET}CO_2$, mmHg	31.3±3.7	30.2±2.4	35.3±2.2	34.1±2.7	37.3±2.5	36.1±1.8	36.4±3.4	36.5±4.2
RER	0.92±0.15	0.86±0.10	0.90±0.06	0.80±0.09	0.94±0.07	0.87±0.07	1.07±0.09	1.04±0.06
S_PO_2, %	98.1±1.0	97.6±1.3	97.5±1.2	96.3±0.71	97.3±1.3	96.3±0.46	96.5±1.9	95.8±1.16
$\dot{Q}$, $L \cdot min^{-1}$	4.22±1.1	3.6±1.5	6.2±1.7	6.1±1.9	8.5±2.3	8.0±2.4	10.6±2.3	10.7±2.7
HR, $b \cdot min^{-1}$	78±6	70±10	95±12	94±11	113±14	110±14	140±11	140±10
SV, mL	52±12	48±17	63±15	60±14	74±18	61±12	76±19	69±13
PASP, mmHg	19.4±10.2	25.8±1.8	31.2±10.5	35.8±9.4	39.3±6.2	34.5±9.8	46.0±10.1	42.8±12.2
$\dot{V}_E$, $L \cdot min^{-1}$	13.6±3.1	12.0±2.7	26.9±6.0	26.5±5.3	36.3±9.2	35.6±6.8	60.7±12.5	59.6±16
V_T, L	0.88±0.18	0.77±0.31	1.20±23	1.12±0.22	1.59±0.33	1.47	2.21±0.44	2.07±0.57
RR, $b \cdot min^{-1}$	16±3	14±4	22±4	23±1	24±3	24±2	29±3	29±6
$\dot{V}_E/\dot{V}CO_2$	43.5±5.8	45.3±10.4	32.5±4.1	35.2±2.4	30.2±1.7	32.3±1.9	30.0±2.2	31.1±2.1

Values are means ± SD. PO, power output; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; $P_{ET}O_2$, partial pressure of end tidal oxygen; $P_{ET}CO_2$, partial pressure of end tidal CO_2; RER, respiratory exchange ratio; S_PO_2, oxygen pulse saturation; $\dot{Q}$, cardiac output; HR, heart rate; SV, stroke volume; PASP, pulmonary artery systolic pressure; $\dot{V}_E$, minute ventilation; V_T, tidal volume; RR, respiratory rate; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for CO_2.

Table B-2. Cardiopulmonary responses to exercise with dopamine blockade in mild COPD (n=9)

	Baseline		40 W		50% $\dot{V}O_{2peak}$		80% $\dot{V}O_{2peak}$	
	Placebo	Blockade	Placebo	Blockade	Placebo	Blockade	Placebo	Blockade
$\dot{V}O_2$, $L \cdot min^{-1}$	0.29±0.08	0.33±0.07	0.85±0.21	0.93±0.16	0.96±0.33	0.99±0.33	1.38±0.48	1.47±0.59
$\dot{V}CO_2$, $L \cdot min^{-1}$	0.26	0.29±0.07	0.76±0.23	0.80±0.18	0.85±0.31	0.85±0.33	0.1.37	1.44±0.63
$P_{ET}O_2$, mmHg	102.0±4.3	103.5±5.0	97.7±4.0	97.3±2.4	96.8±4.5	98.2±4.0	99.2±6.1	101.1±3.3
$P_{ET}CO_2$, mmHg	30.0±1.8	29.5±3.0	33.8±2.6	33.2±2.8	34.4±3.1	33.7±3.4	35.1±3.8	34.6±3.6
RER	0.88±0.09	0.86±0.10	0.88±0.07	0.85±0.09	0.88±0.08	0.85±0.08	1.00±0.05	0.97±0.06
S_PO_2, %	96.3±3.0	97.0±2.0	95.7±2.9	94.2±3.4	95.6±3.0	94.0±3.2	94.6±3.9	92.8±4.1
Q, $L \cdot min^{-1}$	3.6±1.5	4.3±2.1	6.1±2.0	6.3±2.1	8.0±2.4	7.4±3.8	10.7±2.7	9.7±5.0
HR, $b \cdot min^{-1}$	77±9	77±8	96±11	96±11	98±6	98±6	116±11	117±11
SV, mL	53±16	55±21	65±12	68±23	65±12	73±32	73±21	81±40
PASP, mmHg	23.9±8.6	39.3±10.8	33.7±13.3	33.0±18.6	33.0±14	41.9±13.9	34.4±16	31.1±8.7
$\dot{V}_E$, $L \cdot min^{-1}$	13.4±3.7	13.6±2.3	28.7±7.0	29.5±4.4	31.4±7.3	30.1±7.8	48.2±9.3	46.2±16.3
V_T, L	0.7±0.2	1.1±0.2	1.3±0.2	1.4±0.3	1.4±0.4	1.5±0.5	1.8±0.3	1.8±0.5
RR, $b \cdot min^{-1}$	19±3	14±4	23±6	23±5	23±5.1	23±5	27±4	30±6
$\dot{V}_E/\dot{V}CO_2$	53.0±9.0	54.0±7.4	38.5±4.6	38.6±3.9	38.1±5.1	38.2±4.0	35.9±6.6	36.0±5.9

Values are means ± SD. PO, power output; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; $P_{ET}O_2$, partial pressure of end tidal oxygen; $P_{ET}CO_2$, partial pressure of end tidal CO_2; RER, respiratory exchange ratio; S_PO_2, oxygen pulse saturation; $\dot{Q}$, cardiac output; HR, heart rate; SV, stroke volume; PASP, pulmonary artery systolic pressure; $\dot{V}_E$, minute ventilation; V_T, tidal volume; RR, respiratory rate; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for CO_2.

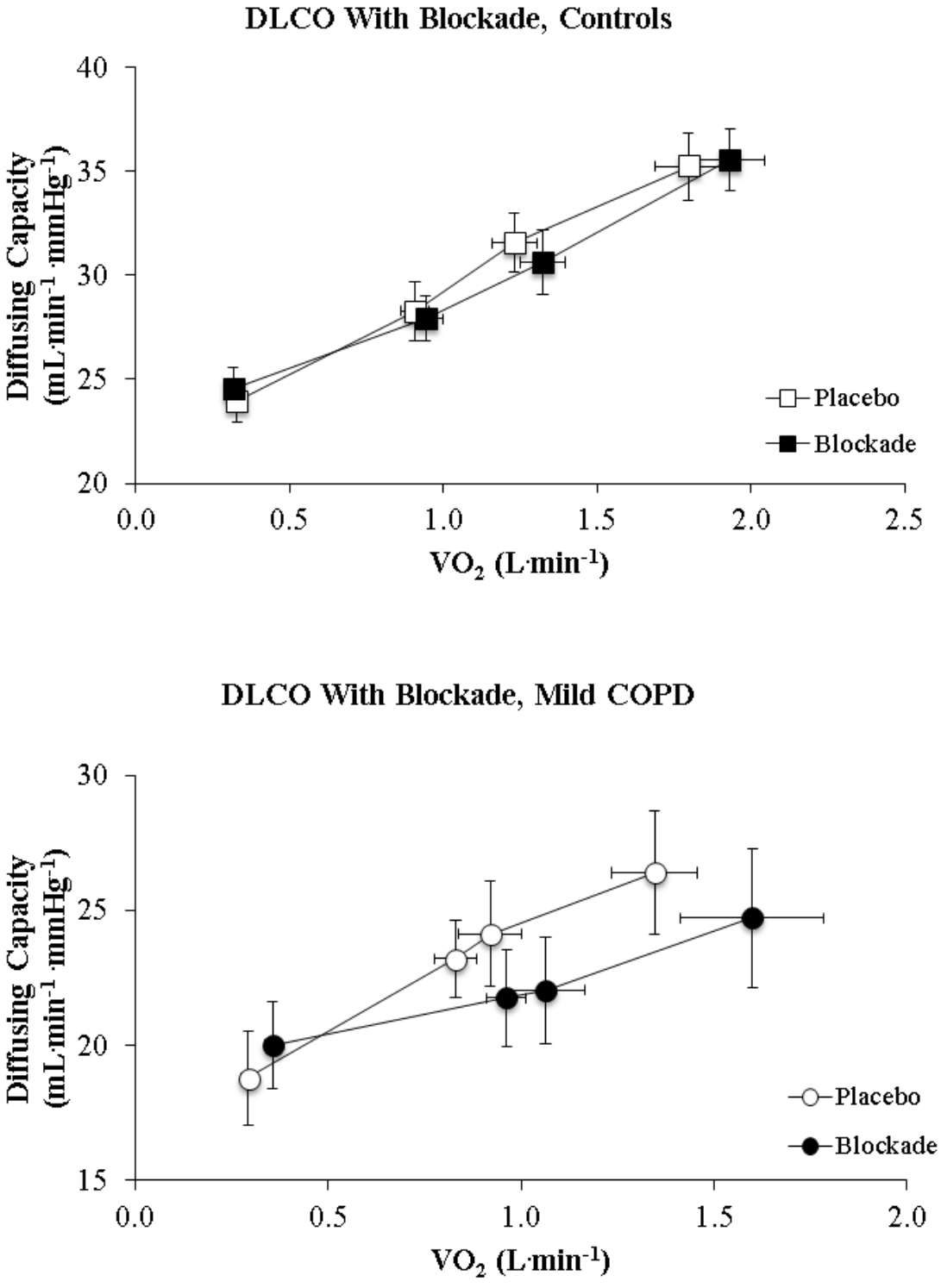

Figure B-1. Diffusing capacity response to exercise with dopamine blockade in controls (n=10) and mild COPD (n=9).

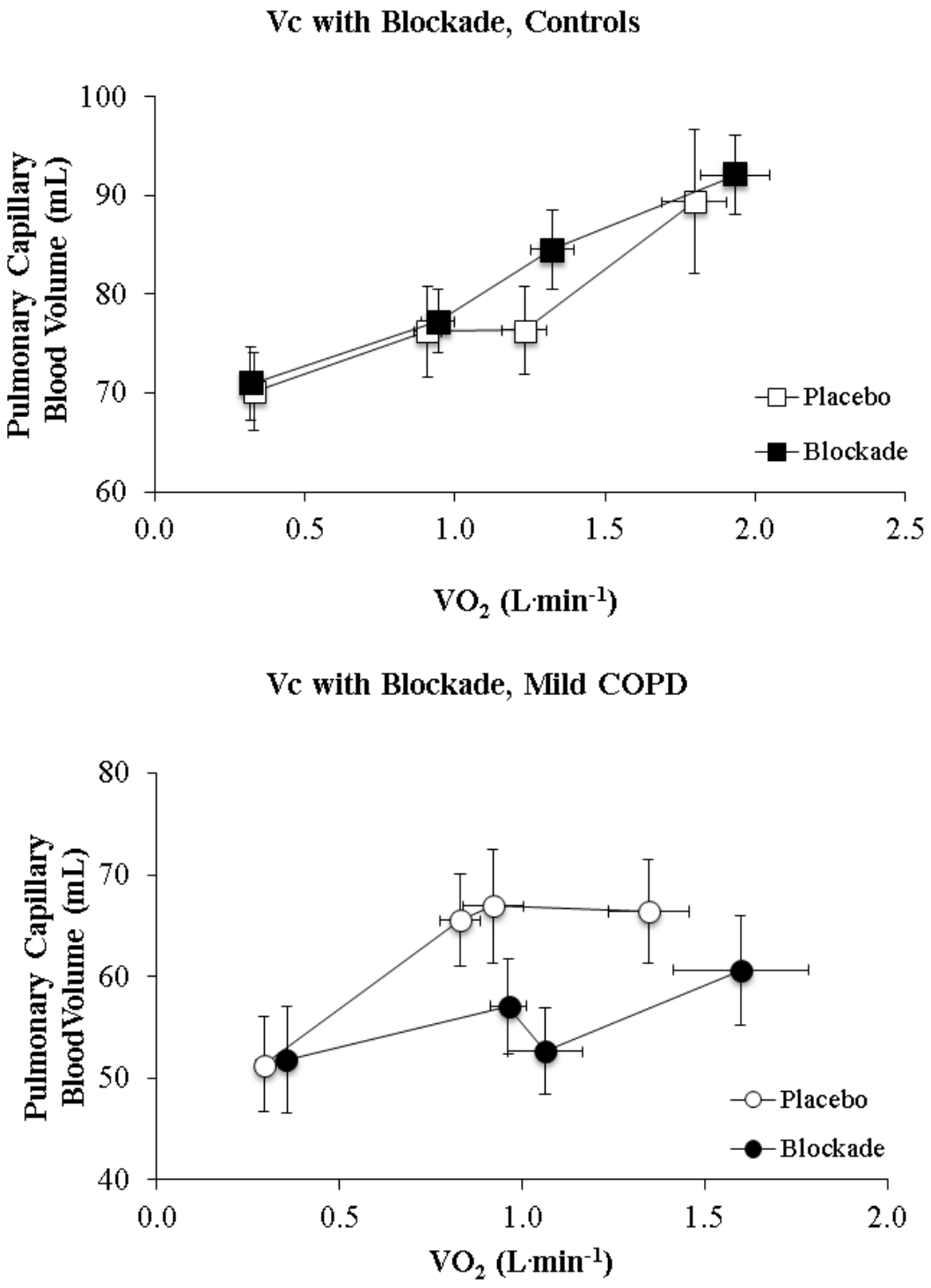

Figure B-2. Pulmonary capillary blood volume response to exercise with dopamine blockade in controls (n=10) and mild COPD (n=9).

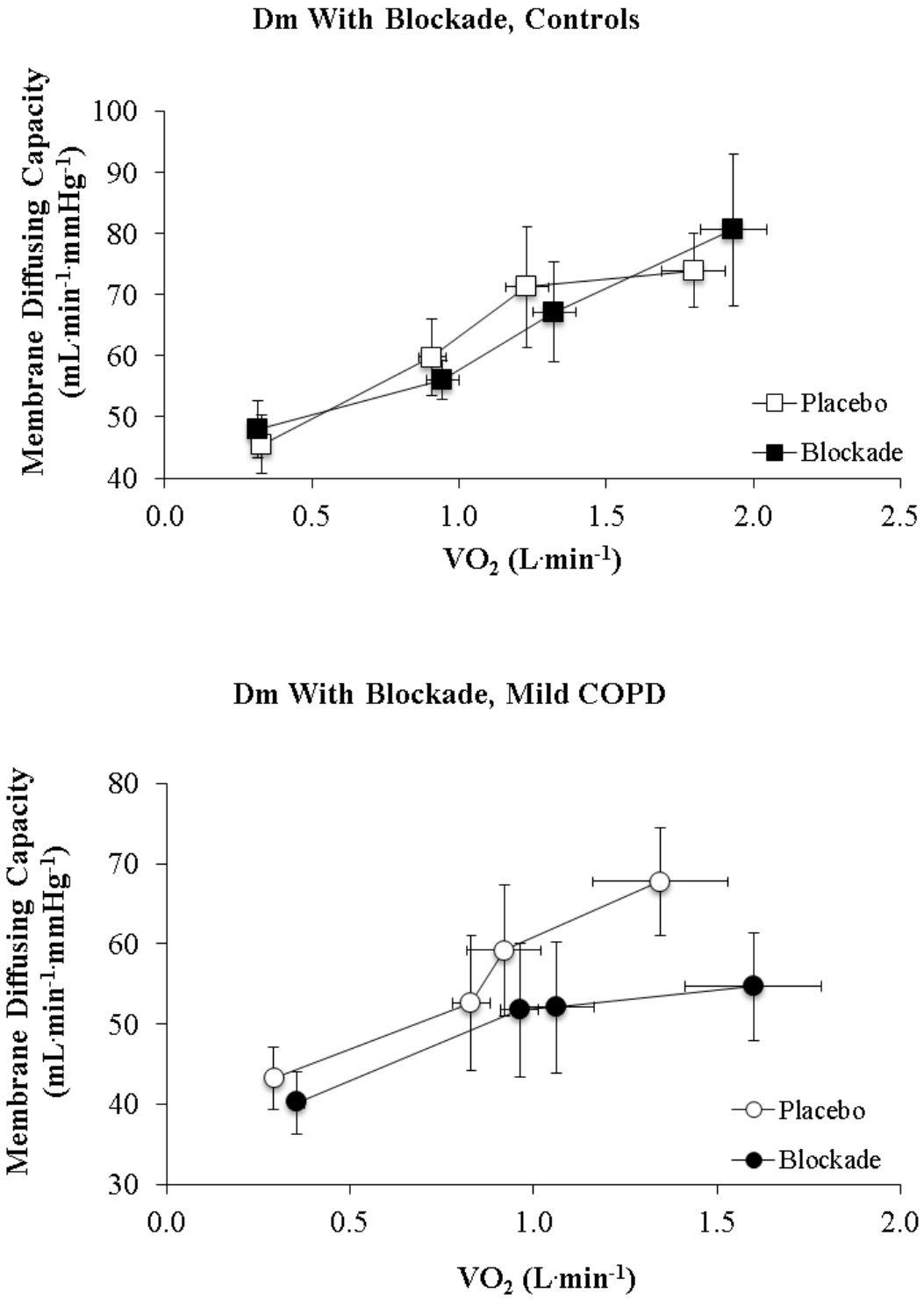

Figure B-3. Membrane diffusing capacity response to exercise with dopamine blockade in controls (n=10) and mild COPD (n=9).