Cardiovascular and Ventilatory Regulation in Chronic Obstructive Pulmonary Disease

by

Devin Brent Phillips

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Faculty of Kinesiology, Sport, and Recreation
University of Alberta

Abstract

The purpose of this dissertation was to investigate cardiovascular function and ventilatory regulation at rest and during exercise in patients with chronic obstructive pulmonary disease (COPD). This dissertation consisted of three separate experiments that aimed to provide insight into the pathophysiology of COPD and examine potential therapeutic interventions to improve dyspnea and exercise capacity in patients with COPD.

The first study examined the effect of carotid chemoreceptor (CC) inhibition on cardiovascular and ventilatory regulation at rest in patients with mild to moderate COPD. Thirteen mild-moderate COPD patients and thirteen age- and risk-matched controls completed resting cardiovascular function measurements with either intravenous saline or dopamine (2 $\mu g \cdot kg^{-1} \cdot min^{-1}$) while breathing normoxia or hyperoxia (100% O_2). On a separate day, a subset of seven COPD patients and seven controls completed muscle sympathetic nervous activity (MSNA) measurements while breathing normoxia or hyperoxia (100% O_2). Arterial stiffness was determined by pulse-wave velocity (PWV) and MSNA was measured by microneurography. Brachial blood flow was determined using Doppler ultrasound, cardiac output was estimated by impedance cardiography, and vascular conductance was calculated as flow/mean arterial pressure (MAP). Carotid chemoreceptor inhibition with dopamine decreased PWV, and MAP ($p<0.05$) while increasing vascular conductance in COPD. No change in cardiovascular function was observed with dopamine in controls. CC inhibition with hyperoxia decreased peripheral PWV and MSNA ($p<0.05$) in COPD but not controls.

The second study examined the effect of CC on ventilatory and cardiovascular regulation, dyspnea and exercise tolerance in patients with mild to moderate COPD. Twelve COPD patients and twelve age- and risk-matched healthy controls completed two time-to-symptom limitation

(T_{LIM}) constant load exercise tests at 75% peak power output with the order randomized to either intravenous saline or low-dose dopamine (2 $\mu g \cdot kg^{-1} \cdot min^{-1}$) to inhibit the CC. Ventilatory responses were evaluated using expired gas data and dyspnea was evaluated using a modified Borg scale. Inspiratory capacity maneuvers were performed to determine operating lung volumes. Cardiac output was estimated using impedance cardiography and vascular conductance was calculated as cardiac output/MAP. At a standardized exercise time of 4-minutes and at T_{LIM}, ventilation, operating volumes and dyspnea were not different between saline and dopamine conditions in COPD or controls. Mean arterial blood pressure was decreased with dopamine, secondary to increased vascular conductance, in COPD, while no change was observed in controls. There was no change in time to exhaustion in either group with dopamine.

The third study examined the effect of inhaled nitric oxide (iNO) on ventilation, dyspnea and exercise capacity in patients with mild COPD. In a randomized-control crossover study, fifteen patients with mild COPD and fifteen healthy age- and risk-matched controls completed symptom-limited cardiopulmonary exercise tests (CPET) while breathing either normoxia (placebo) or 40 parts per million iNO. During the CPET, detailed ventilatory, hemodynamic and perceptual response data were obtained. Inhaled NO increased peak oxygen uptake ($p<0.05$) in COPD, compared to placebo, while no effect was observed in controls. At the highest equivalent work rate of 60 Watts, iNO reduced ventilation and dyspnea (both $p=0.05$) in COPD, while no effect was observed in controls.

When combined, the experimental results suggest three major findings. First, CC inhibition decreased PWV, MSNA and improved vascular conductance in COPD, suggesting that tonic CC activity is elevated at rest and contributes to the elevated arterial stiffness in mild to moderate COPD. Second, CC inhibition with low-dose dopamine improved exercise vascular conductance

in COPD, however, ventilation, dyspnea and exercise tolerance were unaffected by CC inhibition in mild to moderate COPD patients. The results suggest that the CC appears to be a modulator of vasoconstrictor outflow during exercise in COPD patients, however, the exaggerated ventilatory response typically observed in COPD was not explained by heightened activity and sensitivity of the CC (*i.e*. autonomic dysfunction). Lastly, iNO increased exercise capacity in mild COPD, secondary to reduced ventilation and dyspnea. These data suggest that mild COPD patients demonstrated pulmonary vascular dysfunction that contributed to exercise intolerance, secondary to inefficiencies in gas exchange. Further, these data help to explain why patients with mild COPD demonstrate disproportionately greater dyspnea relative to the degree of airway obstruction. The results of this dissertation help to identify therapeutic targets to lower cardiovascular risk, reduce dyspnea and increase exercise capacity in patients with COPD.

Preface

This doctoral dissertation is the original work by myself, Devin, B. Phillips. All of the work presented was conducted in the Clinical Physiology Laboratory at the University of Alberta. The research projects in Chapters Three and Four received ethical approval from the University of Alberta Research Ethics Board (Biomedical Panel Protocol #00043106). The research project in Chapter Five received ethical approval from Health Canada and the University of Alberta Health Research Ethics Board (Biomedical Panel Protocol Pro00078715).

A version of the research project in Chapter Five has been published in the Journal of Physiology, as Phillips DB, Steinback CD, Collins SE, Bryan TL, Wong EYL, Tedjasaputra V, Bhutani M and Stickland MK. (2018). The carotid chemoreceptor contributes to the elevated arterial stiffness and vasoconstrictor outflow in COPD. *J Physiol.* 596, 3233-3244. I was responsible for all parts of the study, including design, subject recruitment, data collection and analysis, and manuscript preparation. Steinback CD, Collins SE, Bryan TL, Wong EYL, Tedjasaputra V and Bhutani M assisted with data collection and manuscript preparation. Stickland MK was the principal investigator and oversaw all aspects of the study.

A version of the research project in Chapter Four has been published in Respiratory Medicine, as Phillips DB, Collins SE, Bryan TL, Wong EYL, McMurtry MS, Bhutani M and Stickland MK. (2019). The effect of carotid chemoreceptor inhibition on exercise tolerance in chronic obstructive pulmonary disease: a randomized-controlled crossover trial. *Resp Med*. E-pub ahead of print. I was responsible for all parts of the study, including design, subject recruitment, data collection and analysis, and manuscript preparation. Collins SE, Bryan TL, Wong EYL, McMurtry MS and Bhutani M assisted with data collection and manuscript preparation. Stickland MK was the principal investigator and oversaw all aspects of the study.

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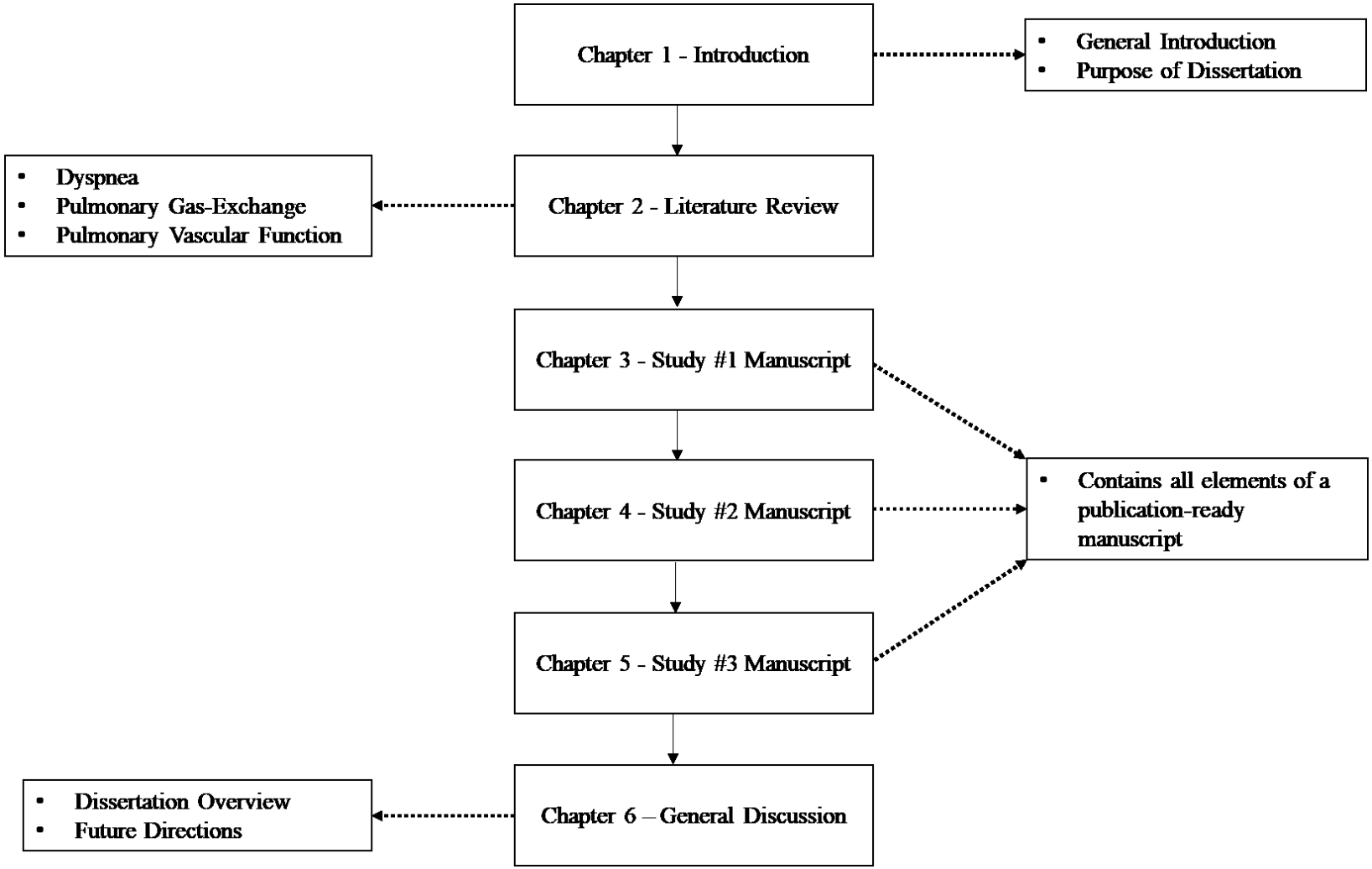
Abbreviations

A-aPO_2	Alveolar to arterial difference in partial pressure of oxygen
ANOVA	Analysis of variance
BMI	Body mass index
CC	Carotid chemoreceptor
cfPWV	Carotid-femoral pulse wave velocity
CHF	Chronic heart failure
CO	Cardiac Output
COPD	Chronic obstructive pulmonary disease
CPET	Cardiopulmonary exercise test
crPWV	Carotid-radial pulse wave velocity
DH	Dynamic hyperinflation
DLCO	Diffusing capacity for carbon monoxide
DM	Diffusing membrane thickness
ECG	Electrocardiogram
EDV	End-diastolic volume
EELV	End-expiratory lung volume
EF	Ejection fraction
EFL	Expiratory flow limitation
EILV	End-inspiratory lung volume
EMGdi	Diaphragmatic electromyography
ESV	End-systolic volume
f_B	Breathing frequency
FEV_1	Forced expired volume in 1 second
F_IO_2	Fraction of inspired oxygen

FRC	Functional residual capacity
FVC	Forced vital capacity
HR	Heart rate
I.V.	Intravenous
IC	Inspiratory capacity
iNO	Inhaled nitric oxide
IRV	Inspiratory reserve volume
LAP	Left atrial pressure
LV	Left ventricle
MAP	Mean arterial pressure
MIGET	Multiple inert gas elimination technique
mPAP	Mean pulmonary arterial pressure
MSNA	Muscle sympathetic nerve activity
NO	Nitric oxide
P_ACO_2	Partial pressure of alveolar carbon dioxide
P_aCO_2	Partial pressure of arterial carbon dioxide
PAH	Pulmonary arterial hypertension
P_AO_2	Partial pressure of alveolar oxygen
P_aO_2	Partial pressure of arterial oxygen
P_B	Barometric pressure
PCO_2	Partial pressure of carbon dioxide
PDE5	Phosphodiesterase five
P_ECO_2	Partial pressure of expired carbon dioxide
$P_{ET}CO_2$	Partial pressure of end-tidal carbon dioxide
$P_{ET}O_2$	Partial pressure of end-tidal oxygen

PFT	Pulmonary function test
P_{H20}	Partial pressure of water
PO_2	Partial pressure of oxygen
Ppm	Parts per million
PVR	Pulmonary vascular resistance
PWV	Pulse wave velocity
R	Respiratory quotient
RER	Respiratory exchange ration
RV	Right ventricle
RVSP	Right ventricular systolic pressure
S_PO_2	Oxygen saturation using pulse oximetry
SV	Stroke volume
TAPSE	Tricuspid annular plane systolic excursion
TLC	Total lung capacity
TLIM	Time to symptom limitation
$\dot{V}_A$	Alveolar ventilation
$\dot{V}_A/\dot{Q}$	Ventilation to perfusion equivalent
Vc	Pulmonary capillary blood volume
$\dot{V}CO_2$	Volume of carbon dioxide production
$\dot{V}_D$	Deadspace ventilation
$\dot{V}_E/\ \dot{V}CO_2$	Ventilatory equivalent to carbon dioxide production
$\dot{V}_E$	Minute ventilation
$\dot{V}O_2$	Volume of oxygen consumption
V_T	Tidal volume

Dissertation Roadmap



Chapter One
Introduction

1.1 General introduction

Chronic Obstructive Pulmonary Disease (COPD) is a respiratory disorder characterized by progressive, partially reversible airway obstruction. It is estimated that over 700,000 Canadian adults have COPD [65, 135], and COPD is currently the 4th leading cause of death in Canada [189]. Patients with COPD experience significant breathlessness (dyspnea) during exertion, which has been shown to profoundly reduce patient quality of life, physical activity, and impair the ability to complete normal daily activities. The maintenance of physical activity in COPD is vital, as a low level of physical activity in COPD is related to poor quality of life, and increased risk of hospitalization, and mortality [4, 93, 183]. Much of the previous research has studied the mechanisms of dyspnea in moderate and severe COPD (forced expired volume in 1 second (FEV_1) = 30-80% predicted) [137, 153], but the reasons for dyspnea in mild COPD (FEV_1 >80% predicted), whose symptoms appear disproportionate to their relatively preserved airway function [27], are poorly understood [49, 70]. Recent research has shown that the exaggerated ventilatory response to exercise (also termed ventilatory inefficiency) observed in mild COPD is an important contributor to exertional dyspnea [49, 70] and is predictive of mortality, independent of disease severity [131]. Recently, our laboratory has demonstrated that the exaggerated ventilatory response to exercise in COPD may be explained by elevated activity and sensitivity of the carotid chemoreceptor, which would be suggestive of autonomic dysfunction [191]. Alternatively, Elbehairy *et al.* [49] suggested that the increased ventilatory response is secondary to increased deadspace and ventilation/perfusion inequality. Further, the authors suggested that the increased deadspace was likely the result of pulmonary vascular dysfunction. Despite these evidence-based hypotheses, the mechanisms for the increased exercise ventilatory inefficiency in mild COPD are largely unknown.

1.2 Purpose of dissertation

This dissertation consisted of three separate interventional studies designed to investigate cardiovascular function and ventilatory regulation in patients with COPD. The first study (Chapter Three) was a single-blind, randomized control, crossover trial that examined the effect of carotid chemoreceptor inhibition on cardiovascular and ventilatory regulation at rest in patients with mild to moderate COPD. The second study was a double-blind, randomized control, crossover trial that examined the effect of carotid chemoreceptor inhibition on cardiovascular and ventilatory regulation, exertional dyspnea and exercise tolerance in mild-moderate COPD. The goal of the first two studies was to determine if autonomic dysfunction (*i.e.* elevated carotid chemoreceptor activity and sensitivity) was a key contributor to abnormal ventilatory regulation (*i.e.* heightened ventilation relative to metabolic demand) and cardiovascular dysfunction in COPD. The third study (Chapter Five) was a double-blind, randomized control, crossover trial that examined the effect of inhaled nitric oxide on ventilatory regulation, exertional dyspnea and exercise capacity in mild COPD. The goal of the third study was to determine if abnormal pulmonary gas exchange, secondary to pulmonary vascular dysfunction, contributed to the elevated ventilatory response to exercise, exertional dyspnea and reduced exercise capacity in mild COPD. The overarching goal of the dissertation was to better understand cardiovascular function and ventilatory regulation in patients with COPD. This body of work provides insight into the pathophysiology of COPD, and examines potential therapeutic interventions to reduce dyspnea and increase exercise capacity in patients with COPD.

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Chapter Two
Review of the Literature

2.1 Chronic Obstructive Pulmonary Disease

Chronic Obstructive Pulmonary Disease (COPD) is a respiratory disorder characterized by progressive partially reversible airway obstruction. It is estimated that over 4% (700,000 adults) of Canadians have COPD [135], and COPD is currently the 4th leading cause of death in Canada [189]. Despite standard lung function testing being a key contributor to COPD diagnosis and management, exertional dyspnea (perceived breathlessness) and exercise intolerance are the most commonly reported symptoms [8, 33, 146].

As dyspnea progressively worsens, individuals with COPD often decrease their physical activity to avoid symptoms, which leads to large reductions in cardiorespiratory fitness and poor health status [35, 197]. Conversely, previous research has shown that increasing physical activity levels can improve long-term outcomes (*i.e.* lower risk of hospitalization, reduced rate of lung function decline) in COPD patients [62, 63]. When combined, it becomes clear that the maintenance of physical activity and management of dyspnea in COPD is a vital strategy to maintain and improve quality of life and long-term health outcomes (*i.e.* hospitalization, mortality).

Much research has been done studying the mechanisms of dyspnea and reduced exercise tolerance and capacity in moderate and severe COPD [137, 140, 153], but the mechanisms for dyspnea in mild COPD (forced expired volume in 1 second (FEV_1) >80% predicted), whose symptoms appear disproportionate to the degree of airway obstruction [27], are poorly understood [49, 70]. Recent research suggests that the exaggerated ventilatory response to exercise (also termed ventilatory inefficiency) observed in mild COPD is an important contributor to exertional dyspnea [49]. The reasons for the increased exercise ventilatory inefficiency in COPD, as well as potential therapies to mitigate this physiological abnormality, are largely unknown, and are the

focus of this dissertation. The purpose of Chapter Two is to review 1) the ventilatory response to exercise in healthy individuals and patients with COPD, 2) the mechanisms of ventilatory inefficiency and dyspnea in COPD, and, 3) interventions to reduce exercise ventilation and dyspnea in patients with COPD.

2.2 Exertional dyspnea and exercise intolerance in COPD

Exertional dyspnea is a hallmark of COPD and, despite mild airflow obstruction, dyspnea is the primary reason for exercise intolerance in mild COPD [49, 70, 146]. Previous research in mild COPD has shown, that despite a mild reduction in lung function, exercise capacity (as measured by peak power output and oxygen uptake) is reduced by more than 20% and dyspnea ratings are significantly higher for a given work rate, when compared to healthy controls [70, 146]. Recent studies suggests that the observed dyspnea, in mild COPD, is the result of increased work of breathing and diaphragmatic activity during incremental exercise [70]. Previous research has demonstrated that the increased work of breathing and thus dyspnea in COPD comes from 1) airflow limitation (*i.e.* expiratory flow limitation and resulting dynamic hyperinflation)[49, 73, 112, 131, 144], and 2) an exaggerated ventilatory response to exercise (*i.e.* increased minute ventilation relative to carbon dioxide production, $\dot{V}_E/\dot{V}CO_2$)[49, 131, 132]. A great deal of research has focused on improving airflow limitation in COPD; however, very little has been done to understand and treat the exaggerated ventilatory response to exercise in COPD [142].

2.3 Ventilatory mechanics during exercise

2.3.1 Ventilatory mechanics in health

During incremental exercise, minute ventilation increases to raise alveolar ventilation in proportion to oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) to regulate arterial blood gases. The progressive rise in minute ventilation is accomplished by increased tidal

volume (V_T) and breathing frequency (f_B). During mild exercise (<50% $\dot{V}O_{2peak}$), increased minute ventilation is predominantly a result of increased V_T. In healthy humans, increased V_T is a combination of reduced end-expiratory lung volume (EELV) and increased end-inspiratory lung volume (EILV). At higher exercise intensities, at approximately 50% of vital capacity, there is a plateau in V_T and minute ventilation is further increased by f_B [120]. This change in operating lung volume and breathing pattern helps minimize deadspace ventilation and both resistive and elastic components of the work of breathing [83, 181]. However, at near-maximal exercise intensities, EELV can increase above resting values in an attempt to achieve higher expiratory flow in health [74]. Although the increase in EELV may be a sign of expiratory flow limitation, it is commonly assumed that, in healthy populations, the respiratory system is overbuilt and mechanical constraint is rarely the limiting factor when determining exercise capacity and cardiopulmonary fitness (*i.e.* $\dot{V}O_{2peak}$)[41, 126]. However, a large body of research has provided compelling evidence that fit young individuals are susceptible to exercised-induced hypoxemia, which would infer some sort of respiratory (*i.e.* gas exchange) impairment [43]. This will be further discussed in detail in the upcoming gas-exchange sections.

2.3.2 Ventilatory mechanics in COPD

In patients with COPD, the presence of expiratory flow limitation (airway obstruction) and reduced lung elastic recoil (emphysema) can result in abnormal mechanical responses during exercise, ultimately leading to increased dyspnea and early cessation of exercise [58, 141]. In health, the increased expiratory effort progressively decreases EELV and expiratory flow is sufficient to allow complete exhalation in time for the next inhalation. In patients with COPD, the combined effect of increased airway resistance (obstruction) and loss of elastic recoil (emphysema) increases the mechanical time constant for lung emptying and, at higher expiratory

flow rates, can lead to insufficient exhalation of V_T [58]. As a result, exhalation may be incomplete, causing an increase EELV and progressive gas trapping, also termed dynamic hyperinflation (DH). Dynamic hyperinflation is a key mechanistic consequence of airflow limitation and is a major cause of exertional dyspnea in COPD [140, 146]. It is important to note that DH can occur, independent of static hyperinflation, and would not be detected using standard pulmonary function tests in resting states (*i.e.* spirometry, plethysmography). Dynamic hyperinflation can limit V_T expansion during exercise, which can lead to mechanical constraint and a critically low inspiratory reserve volume (IRV) or V_T plateau [111, 140]. At this plateau, V_T becomes fixed on the upper extreme of the lung pressure volume curve, resulting in increased elastic loading and reduced dynamic lung compliance. This is an important mechanical event that can mark the onset of an increased uncoupling between respiratory muscle effort and volume displacement achieved, also termed neuromechanical uncoupling [70, 111]. Guenette *et al*. [70] demonstrated that although mild COPD patients only present with minor spirometric abnormalities and minimal lung hyperinflation at rest, these subjects demonstrated DH, greater resistive and elastic loading of the inspiratory muscles and an increased total work of breathing during exercise, when compared to healthy controls. The increase in total work of breathing was secondary to a greater inspiratory resistive work of breathing. In the same study, diaphragm function was assessed invasively with diaphragmatic electromyography (EMGdi). At rest and during exercise, EMGdi was elevated in mild COPD. The authors concluded that, despite mild airway obstruction, the respiratory drive to breathe was elevated and was a key contributor to elevated dyspnea and reduced exercise capacity in mild COPD [70]. The key findings from this study highlight that, relying solely on lung function tests to assess severity of obstruction may underestimate the clinically important physiological impairment in mild COPD.

2.3.3 Relationship between ventilatory mechanics and dyspnea

Multiple studies have shown that mechanical abnormalities are key contributors to elevated dyspnea in mild COPD [70, 112, 139, 143, 146]. It is likely that DH is advantageous early in exercise to attenuate flow limitation and gas trapping. However, as ventilatory demand increases, the increased operating lung volume (*i.e.* IC and IRV) may constrain V_T expansion and increase neural drive (*i.e.* neuromechanical uncoupling), forming the basis for perceptions of greater respiratory difficulty and increased ratings of dyspnea.

2.4 Pulmonary gas exchange at rest and during exercise

2.4.1 Ventilation

The purpose of alveolar ventilation ($\dot{V}_A$) is to deliver fresh gas (O_2) to the gas exchanging portions of the lungs and remove metabolic accumulation (CO_2) in order to maintain arterial blood gas homeostasis. Total ventilation is the total volume of gas entering or exiting the lung and consists of $\dot{V}_A$ and deadspace ventilation ($\dot{V}_D$):

$$\dot{V}_E = \dot{V}_A + \dot{V}_D$$

At rest and during exercise, $\dot{V}_A$ is closely linked to $\dot{V}CO_2$ and the healthy lung has fairly good matching of ventilation to perfusion [202, 232]. A portion of gas remains in the conducting airways and does not participate in gas-exchange, and is termed anatomical deadspace. Additionally, alveoli that are poorly perfused relative to their ventilation are termed alveolar deadspace. When combined, these two forms of deadspace are termed physiologic deadspace, or deadspace ventilation. Minute ventilation can be easily measured using expired gas analysis, however, $\dot{V}_A$ and $\dot{V}_D$ are more difficult to determine. Physiologic deadspace can be estimated using the Bohr

equation [11]. This formula assumes that deadspace CO_2 is zero and all expired CO_2 comes from alveolar ventilation. The equation can be written as follows;

$$\dot{V}_D/\dot{V}_E = (P_ACO_2 - P_ECO_2) / (P_ACO_2)$$

In this equation, P_ACO_2 and P_ECO_2 refer to alveolar and mixed expired partial pressures of CO_2, respectively. Due to the difficulty of estimating P_ACO_2, P_aCO_2 (as measured by arterial blood gas sampling) is often used as a substitute in the Bohr equation, as P_ACO_2 and P_aCO_2 are often assumed to be identical [53, 231]. In patients with lung disease and gas-exchange abnormalities (*i.e.* ventilation/perfusion mismatch, diffusion limitation, shunt), P_aCO_2 would be increased relative to P_ACO_2 which could increase the calculated dead space fraction [174, 222].

Once deadspace is quantified, $\dot{V}_A$ can be determined using the following equation:

$$\dot{V}_A/\dot{V}_E = 1 - \dot{V}_D/\dot{V}_E$$

Further, the relationship between $\dot{V}_A$, $\dot{V}CO_2$ and PCO_2 (P_ACO_2 or P_aCO_2) is well displayed in the following equation [168]:

$$PCO_2 = (\dot{V}CO_2 / \dot{V}_A) \times K$$

In this equation, K is a conversion factor and is used to adjust $\dot{V}CO_2$ to body temperature and pressure. As previously mentioned, $\dot{V}_A$ and $\dot{V}CO_2$ are closely linked, however, to better understand the effectiveness of $\dot{V}_A$, measurements of P_ACO_2 (or P_aCO_2) is required.

2.4.2 Ventilatory efficiency

Well matched alveolar ventilation to perfusion is critical to maximize ventilatory efficiency and minimize deadspace or "wasted" ventilation [225, 232, 233]. Measuring ventilatory efficiency has been a widely-used tool to assess functional impairment and disease severity in both heart

[104, 171, 214] and lung diseases [131, 132, 191] . The most common clinical marker of measuring ventilatory efficiency at rest and during exercise is non-invasively, using a metabolic measurement system and recording expired gas (*i.e.* $\dot{V}_E/\dot{V}CO_2$). In conditions of increased deadspace during exercise (*i.e.* chronic heart failure or COPD), it is logical that $\dot{V}_E$ must increase in order to maintain adequate $\dot{V}_A$ and blood gas homeostasis (*i.e.* P_aCO_2). Assuming a normal P_aCO_2 (~36-40 mmHg), the increased $\dot{V}_D$ and $\dot{V}_E$ would be distinguishable using simple expired gas analysis and displaying $\dot{V}_E/\dot{V}CO_2$. Although an elevated $\dot{V}_E/\dot{V}CO_2$ may be an index of ventilatory inefficiency, without measurements of arterial blood CO_2, it is extremely difficult to conclude that elevated $\dot{V}_E/\dot{V}CO_2$ is the direct result of increased deadspace.

2.4.3 Ventilatory response during exercise in healthy aging

The effects of healthy aging on the respiratory system have been well identified and general consensus is that the gradual age-related worsening of pulmonary gas-exchange and ventilatory mechanics does not compromise arterial blood gas homeostasis during exercise [39, 100]. However, multiple studies have demonstrated that healthy aging is associated with ventilatory mechanical constraint, worsened gas-exchange efficiency and elevated $\dot{V}_E/\dot{V}CO_2$, when compared to younger healthy individuals [38, 55, 128, 147]. Thus, it is important, in context of this dissertation, to review the known effects of heathy aging on the respiratory system in order to differentiate these responses with the pathological effects that may arise from the development of COPD.

Age-related changes in pulmonary connective tissue result in reduced elastic recoil and driving pressure for expiratory flow [56, 99, 216]. Further, the increased lung compliance predisposes older individuals to expiratory flow limitation when ventilatory demand is elevated during exercise [99, 100, 128, 235]. Additionally, resting IC is also diminished in older individuals,

likely due to the reduction in diaphragmatic strength [147, 159]. The combination of reduced baseline IC and expiratory flow limitation can result in dynamic hyperinflation during exercise, which would 1) increase the elastic work of breathing, 2) rapidly decline the IRV, and 3) increase the sensation of dyspnea. In addition to changes in respiratory mechanics, aging is associated with a progressive reduction in alveolar-capillary surface area, which leads to reduced diffusing capacity, ventilation-perfusion mismatch and ultimately, increased deadspace [20, 55, 101, 147]. The increased deadspace, typically observed in healthy older individuals, results in a higher ventilatory requirement for any given metabolic demand (*i.e.* elevated $\dot{V}_E/\dot{V}CO_2$), when compared to young individuals. The reduced ventilatory efficiency in the face of ventilatory constraint can lead to increased sensations of dyspnea [55, 147, 235]. Although the physiological changes in respiration may not be a primary cause of exercise limitation in older healthy individuals, the derangements of pulmonary gas-exchange and respiratory mechanics are exaggerated in patients with COPD. Further, this underlines the importance of including age-matched controls when studying exercise pathophysiology in COPD.

2.4.4 Elevated ventilatory response and dyspnea during exercise in mild COPD

Several previous studies in mild COPD have consistently demonstrated an elevated ventilatory response (*i.e.* greater $\dot{V}_E/\dot{V}CO_2$) during exercise [49, 70, 146, 191]. The elevated $\dot{V}_E/\dot{V}CO_2$ response to exercise appears to be clinically important, as it independently predicts mortality in COPD and indicates that physiological abnormalities beyond airflow obstruction are important in determining disease severity, dyspnea and risk of death [131, 137, 211].

Previous research by Ofir *et al.* [146] investigated the mechanisms of dyspnea in mild COPD patients and concluded that abnormal ventilatory mechanics and, importantly, elevated ventilatory demand (*i.e.* $\dot{V}_E/\dot{V}CO_2$) were the key contributors to elevated exertional dyspnea and

diminished exercise capacity. These findings were confirmed in a follow up study by Guenette *et al.* [70], who concluded that the observed ventilatory inefficiency, as determined by an exaggerated $\dot{V}_E/\dot{V}CO_2$ during exercise was responsible for earlier onset of DH, mechanical constraint, neuromechanical dissociation between the drive to breathe and V_T expansion, greater dyspnea and earlier cessation of exercise.

Using directly measured arterial blood gases, Elbehairy *et al.* [49] found that the increased exercise $\dot{V}_E/\dot{V}CO_2$ in mild COPD was secondary to increased deadspace. The increased deadspace ventilation resulted in a compensatory increase in total minute ventilation (*i.e.* increased $\dot{V}_E/\dot{V}CO_2$) to maintain effective alveolar ventilation and arterial blood gas homeostasis. Importantly, arterial blood gas analysis allowed for determination of deadspace (Bohr equation) using the classic Riley three-compartment model of alveolar ventilation/perfusion ($\dot{V}_A/\dot{Q}$) matching [173] (shunt, 'ideal', and deadspace); however, the lung is not functionally three compartments. Rather, individual segments within the lung function as a continuum of $\dot{V}_A/\dot{Q}$ ratios, with shunt (blood flow but no ventilation) and deadspace (ventilation but no blood flow) being the extremes, with lung segments operating within these two limits. Thus, it is unclear if the deadspace ventilation observed in mild COPD represents pure deadspace, or rather regions of very high $\dot{V}_A/\dot{Q}$ (*i.e.* high ventilation with some perfusion).

When looking at the mechanical abnormalities and the increased ventilatory demand in tandem, it could be interpreted that the erosion of the ventilatory capacity (mechanical constraint) and the elevated demand ($\dot{V}_E/\dot{V}CO_2$) can both negatively influence the perception of dyspnea in mild COPD [49, 146].

2.4.5 Gas-exchange in health

The matching of alveolar ventilation to pulmonary capillary perfusion is a critical process in the O_2 transport chain and extreme precision is required to ensure efficient gas exchange and avoid arterial hypoxemia. Gas-exchange is commonly evaluated by the alveolar-to-arterial PO_2 difference (A-aPO_2). The A-aPO_2 (P_AO_2 – P_aO_2) can be estimated, if systemic arterial blood samples (P_aO_2, and P_aCO_2) and expired gas ($\dot{V}O_2$ and $\dot{V}CO_2$) data are obtained. P_AO_2 is estimated using the alveolar gas equation, seen below:

$$P_AO_2 = [(P_B – PH_20) \times F_IO_2] – (P_ACO_2/R) + [P_ACO_2 \times F_IO_2 \times (1-R)/R]$$

In this equation, P_ACO_2 is estimated by P_aCO_2 (assuming P_ACO_2=P_aCO_2), and R is the respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$).

At rest in healthy individuals, the A-aPO_2 is relatively small (~2-5 mmHg), however, A-aPO_2 progressively worsens during exercise (up to 30 mmHg at $\dot{V}O_{2max}$) and can reach values up to 40 mmHg in highly trained endurance athletes [91, 198]. Key determinants of A-aPO_2 include $\dot{V}_A/\dot{Q}$ mismatch, diffusion limitation and shunt [194] and will be discussed further.

2.4.6 Ventilation-perfusion matching in health

At rest in normal healthy individuals, alveolar ventilation is approximately 5 L/min and CO is around the same. In theory, this would suggest that the $\dot{V}_A/\dot{Q}$ is perfectly matched. However, the lung is not a uniform organ and previous research has clearly shown some mild degree of $\dot{V}_A/\dot{Q}$ mismatch at rest [233]. Glenny *et al.* [67] demonstrated that common mechanisms of $\dot{V}_A/\dot{Q}$ mismatching at rest include gravitational hydrostatic gradients, pleural pressure gradients, lung compressibility, and the geometry of the airway and vascular trees. Despite these mechanisms of mismatching, the distribution of $\dot{V}_A$ and $\dot{Q}$ are relatively uniform in normal healthy individuals at

rest and gas-exchange limitations (*i.e.* increased A-aPO_2) at rest are seldom the result of $\dot{V}_A/\dot{Q}$ mismatching [43, 224].

Previous research has provided evidence of $\dot{V}_A/\dot{Q}$ mismatching during exercise in health, which has been shown to increase A-aPO_2 at all levels of exercise [91, 223]. The precise mechanism for increased $\dot{V}_A/\dot{Q}$ mismatch during exercise is not certain, however, previous research suggests that interstitial pulmonary edema, a direct result of increase pulmonary artery pressure, is the likely cause [43, 59]. Gale *et al.* [59] suggested that the observed $\dot{V}_A/\dot{Q}$ mismatch during exercise is interstitial edema acting to compress small airways and blood vessels. Further, Burnham *et al.* [19] evaluated magnetic resonance images (MRI) and demonstrated the observed $\dot{V}_A/\dot{Q}$ mismatch with exercise was associated with increased heterogeneity of pulmonary blood flow and not ventilation, which would suggest that the exercise effect on $\dot{V}_A/\dot{Q}$ mismatch is more likely on the pulmonary vascular side and not the airways. To summarize, $\dot{V}_A/\dot{Q}$ mismatch is observed in healthy individuals and can account for up to 50% of the elevated A-aPO2 in individuals with moderate to severe gas exchange limitations at rest or during exercise.

2.4.7 Diffusion limitation

At heavy levels of exercise, a diffusion limitation begins to develop, which increases A-aPO_2 further and can have negative consequences (*i.e.* exercise induced arterial hypoxemia). However, this is usually only observed in high oxygen uptakes (> 40 ml/kg/min) in trained endurance athletes [90, 91]. The mechanism of diffusion limitation is poorly understood, however, it has been hypothesized that diffusion limitation may be due to the development of edema (see above), and/or inadequate pulmonary transit time. During heavy exercise (CO >24 L/min), pulmonary capillary blood volume expands via capillary recruitment and distension and is thought to reach its morphological limit [227]. If blood flow (*i.e.* CO) were to increase further, this could

result in a decreased amount of time available for gas exchange, which could lead to a diffusion limitation. The minimum transit time required for O_2 equilibrium between alveolar gas and end-capillary blood is approximately 0.3 seconds [40]. There is evidence of decreased transit time during exercise, however, no published work to date has shown clear evidence of pulmonary transit time as a key mechanism for diffusion limitation.

2.4.8 Shunt

Shunts are defined as blood that enters systemic arterial circulation without contacting ventilated areas of the lung [86, 231]. Shunts can occur within the lungs or between atria (termed intra-pulmonary), and outside of the lungs (termed extra-pulmonary). Extra-pulmonary shunts are the result of a small portion of oxygenated blood leaving the left ventricle to the bronchiole circulation and returning to the left atrium via the thebesian veins. Previous research from Torre-Buone [212] demonstrated that extrapulmonary shunt, as measured by the change in arterial PO_2 while breathing 100% O_2, accounts for less than 1% of total CO and is not major contributor to increase A-aPO_2 during exercise in healthy individuals. Stickland *et al.* [198] provided evidence of intra-pulmonary shunts during exercise, as evaluated by the agitated saline contrast echocardiography technique. The results from this study showed that intrapulmonary shunts were associated with elevated A-aPO_2 and the authors hypothesized that intrapulmonary shunts may contribute to the impairment of gas exchange (increased A-aPO_2) during exercise in healthy young males [198]. Despite these findings, it remains controversial as to whether true intrapulmonary shunts exist, as there is no evidence of intrapulmonary shunt during exercise when using the multiple inert gas elimination technique (MIGET)[43, 91]. However, it has been hypothesized that pre-capillary gas-exchange may impact the ability to detect intrapulmonary shunts using MIGET. Specifically, pre-capillary (or shunt) vessels may excrete inert gas (infused in venous circulation

to obtain MIGET) which would result in the failure to detect intrapulmonary shunt with MIGET [117, 198].

2.4.9 Gas-exchange in COPD

Patients with COPD often present with hypoxemia and or/ hypercapnia at rest and during exercise. Hypoxemia in COPD is usually explained by increased A-aPO_2 as a result of greater perfusion of low $\dot{V}_A/\dot{Q}$ regions (*i.e.* $\dot{V}_A/\dot{Q}$ mismatch)[222]. Hypercapnea is typically explained by expiratory flow limitation and decreased effective alveolar ventilation, secondary to areas of high $\dot{V}_A/\dot{Q}$ and subsequent alveolar dead space [222]. In 1977, Wagner *et al.* [222] were the first to explore ventilation and perfusion relationships in moderate-severe COPD patients (FEV_1=22–58% predicted) using MIGET. From this study, 3 distinct patterns were observed. First, some patients demonstrate a pattern of high $\dot{V}_A/\dot{Q}$ with no evidence of low $\dot{V}_A/\dot{Q}$ or shunt. The authors concluded that this pattern fits with the known alveolar destruction in patients with emphysema, which would reduce capillary blood flow to ventilated alveoli, resulting in regions of increased $\dot{V}_A/\dot{Q}$ or deadspace. The second group demonstrated large areas of low $\dot{V}_A/\dot{Q}$, with minimal shunt and no regions of high $\dot{V}_A/\dot{Q}$. These groups would be suggestive of airway dysfunction and under-ventilated areas of the lungs. A third group demonstrated a combination of both low and high $\dot{V}_A/\dot{Q}$ areas. The authors noted that distributions with high $\dot{V}_A/\dot{Q}$ was observed in patients with emphysematous changes in lung compliance and that the reduced perfusion, or hypoperfusion, is likely the result of impaired blood-flow due to capillary destruction [222]. Conversely, areas of low $\dot{V}_A/\dot{Q}$ were seen mostly in patients with chronic coughing and sputum and impaired ventilation, which is likely secondary to airway obstruction and dysfunction. Interestingly, the $\dot{V}_A/\dot{Q}$ patterns observed within the study accounted for all the increased A-aPO_2 in the COPD patients. There was no evidence of diffusion-limitation induced hypoxemia (increased A-aPO_2) and patients with

similar arterial blood gases often had different $\dot{V}_A/\dot{Q}$, which provides evidence that $\dot{V}_A/\dot{Q}$ and mechanisms of hypoxemia cannot be precisely determined from measuring arterial blood and expired gases alone.

In 2009, Rodriguez-Roisin *et al.* [174] completed a follow up study to Wagner's work from 1977. Rodriguez-Roisin examined the relationship between $\dot{V}_A/\dot{Q}$ inequality and COPD severity, as determined by the Global Initiative for COPD (GOLD) spirometric staging classifications [167]. The key finding was that $\dot{V}_A/\dot{Q}$ inequality was greater than expected in patients with mild airflow obstruction (GOLD Stage 1). Further, the observed $\dot{V}_A/\dot{Q}$ inequality in mild COPD affected blood flow more than ventilation, as evident by observed regions with high $\dot{V}_A/\dot{Q}$ ratios (ventilation with low blood flow), which is suggestive of elevated deadspace. Barbera *et al.* [6] investigated the relationship between morphological changes in pulmonary muscular arteries and $\dot{V}_A/\dot{Q}$ matching in patients with mild COPD. The key finding was that narrower lumens and thicker intimal wall layer was associated with a higher degree of $\dot{V}_A/\dot{Q}$ inequality ($r=0.51$, $p<0.05$) in mild COPD. When the findings from these studies are combined, it becomes clear that the observed gas-exchange impairment in mild COPD patients may partially be the result of early changes in pulmonary vascular function negatively affecting perfusion. The link between $\dot{V}_A/\dot{Q}$ inequality, pulmonary vascular dysfunction and ventilatory inefficiency in COPD will be discussed, in detail, in the upcoming *pulmonary vascular function* section of the review.

2.5 Pulmonary vascular function

2.5.1 Pulmonary vascular function in health

Under normal physiological conditions, the pulmonary circulation is characterized by low vascular pressure and resistance [203, 237]. During exercise, the increased oxygen demand from the working muscles requires an increase in oxygen delivery, and thus, increased cardiac output.

As blood flow increases, mean left atrial pressure (LAP) and subsequently, mean pulmonary arterial pressure (mPAP) also increase. Increased mPAP can have negative effects on gas exchange if pressure becomes too high, as the capillaries are susceptible to stress failure [234]. Large increases in mean LAP have been directly linked to increased development of pulmonary edema [77]. Therefore, it is imperative that LAP and mPAP remain relatively low in the face of increased blood flow. The relationship between mPAP, LAP and cardiac output is defined by the pulmonary vascular resistance (PVR) equation.

$$PVR = (mPap - LAP) / \text{cardiac output})$$

In healthy individuals, it has been shown that exercise is normally associated with decreased PVR and this drop in resistance is thought to be secondary to pulmonary vascular recruitment of the capillaries and increased dispensability (reduced vascular tone) of the normal pulmonary resistance vessels [105, 129, 203, 233]. Additionally, previous research has demonstrated that recruitment of anatomical intrapulmonary shunts may also decrease PVR during exercise [198]. When combined, these three key mechanisms (recruitment, dispensability and shunt) assist to prevent large increases in pulmonary arterial pressure during exercise.

2.5.2 Pulmonary vascular function in COPD

It has been shown that PVR can either remain constant or increase during exercise in patients with cardiac or pulmonary diseases, which would suggest some sort of dysfunction with pulmonary circulation [13, 203, 210]. Abnormal pulmonary gas exchange is a characteristic of COPD and these patients often present with pulmonary vascular dysfunction and develop pulmonary hypertension [9, 84, 121, 162]. Both structural and functional factors have been suggested to contribute to pulmonary vascular dysfunction in COPD. The structural changes have been associated with the loss of capillary surface area [179, 184, 241] and vascular remodeling of

the intimal layer in the pulmonary artery smooth muscle [6, 121]. Importantly, research has been done in experimental models of COPD that suggest the pulmonary vascular structural changes may precede the development of emphysema [229, 239]. Functional changes that are associated with pulmonary vascular dysfunction include increased vascular tone, which may be in part due to poorly ventilated lung units and local hypoxia, also termed hypoxic pulmonary vasoconstriction. Using invasive surgical specimens procedures, Magee *et al.* [121] showed that patients with mild to moderate COPD (FEV_1=73% predicted), with minimal emphysema, have a thickened intimal layer of the pulmonary artery, when compared to non-smoking healthy controls. In a similar study by the same group [240], mild COPD patients ($FEV_1/FVC = 0.63$) showed no effect on pulmonary hemodynamics when breathing 100% oxygen at rest and during exercise, despite a thickened intimal layer of the pulmonary artery. Collectively, the authors suggest that the observed increased in pulmonary artery thickness was not because of hypoxic pulmonary vasoconstriction, but rather chronic maladaptation of the pulmonary vasculature, likely due to smoking history. Endothelial cells are important for regulating pulmonary vascular function, reducing vascular tone and allowing increased blood flow to the capillaries for gas exchange [57, 233]. Smoking-induced damage to the endothelium may result in both systemic and pulmonary circulatory issues and likely plays a large role in the development of pulmonary vascular dysfunction in COPD [121, 154] . Using in vitro specimen techniques, Peinado *et al.* [154] evaluated structural abnormalities and endothelium-dependent relaxation mediated by nitric oxide in pulmonary arteries of mild-moderate COPD patients (FEV_1=72 ± 15% predicted), smokers with normal lung function, and non-smokers with normal lung function. Maximal relaxation was lower in COPD, when compared to non-smoking controls. These results provided evidence of pulmonary vascular dysfunction in COPD patients, second to the functional impairment of endothelium-dependent relaxation.

Interestingly, both COPD patients and smokers with normal lung function demonstrated thickened intima's, compared to non-smokers. These results suggest that pulmonary vascular abnormalities might originate prior to airflow obstruction. Using contrast enhanced magnetic resonance imaging, Hueper *et al.* [92] demonstrated reduced pulmonary microvascular blood flow in nonemphysematous lung regions in patients with mild COPD. The authors concluded that the low pulmonary perfusion in a nonemphysematous intact pulmonary vascular bed was likely the result of pulmonary vascular dysfunction and may help to explain the work by Rodriguez-Roision demonstrating high $\dot{V}_A/\dot{Q}$ ratios (ventilation with no blood flow) in patients with mild COPD. When these studies are combined, it is evident that pulmonary circulation can be abnormal in patients with mild COPD and it is plausible that early pulmonary vascular dysfunction, prior to the development of emphysema, may be a key contributor to $\dot{V}_A/\dot{Q}$ inequality in COPD.

2.5.3 Pulmonary vascular function as a potential mechanism for the increased ventilatory response to exercise

Patients with mild COPD exhibit an elevated $\dot{V}_E/\dot{V}CO_2$, while adequately preserving arterial blood gas homeostasis and effective alveolar ventilation during exercise, which logically infers that deadspace is increased. The underlying mechanism(s) for the increased deadspace ventilation and $\dot{V}_E/\dot{V}CO_2$ in mild COPD is currently unclear, however, mounting evidence suggest that pulmonary microvascular abnormalities and its potential effect on $\dot{V}_A/\dot{Q}$ matching may be a key contributor.

As previously discussed above, Rodriguez-Roisin *et al.* [174] found that $\dot{V}_A/\dot{Q}$ inequality (using MIGET) was greater than expected in mild COPD patients at rest with relatively preserved spirometry, leading the authors to conclude that the $\dot{V}_A/\dot{Q}$ inequality in COPD was the result of reduced pulmonary vascular perfusion. Barbera *et al.* [6] showed that mild COPD patients have

pulmonary vascular remodeling, as demonstrated by narrower lumens and thicker walls of the pulmonary arteries, and that these morphologic changes were associated with greater $\dot{V}_A/\dot{Q}$ inequality secondary to a skewed ventilation distribution towards regions of high $\dot{V}_A/\dot{Q}$. Consistent with hypoperfusion, our recent work has shown a blunted pulmonary capillary blood volume response to exercise in mild COPD, when compared to age- and height-matched non-smoking controls [206]. Importantly, the low pulmonary capillary blood volume was associated with increased $\dot{V}_E/\dot{V}CO_2$ during incremental exercise, suggesting that low pulmonary perfusion leads to deadspace and/or regions of high $\dot{V}_A/\dot{Q}$. Collectively, these findings suggest that pulmonary vascular dysfunction likely occurs in mild COPD, independent of emphysema, and that this leads to $\dot{V}_A/\dot{Q}$ mismatch, specifically regions of the lung with high $\dot{V}_A/\dot{Q}$ lung units, resulting in ventilatory inefficiency.

2.6 Modulations of the ventilatory response to exercise in COPD

2.6.1 Airway dysfunction and ventilatory inefficiency in mild COPD

It has been suggested that increased intrathoracic pressure secondary to dynamic hyperinflation and/or a mechanical constraint during exercise in COPD may contribute to reduce pulmonary blood flow by compressing the capillaries and restricting blood flow [79, 188, 240]. This capillary compression would reduce pulmonary capillary blood volume, and lead to increased $\dot{V}_A/\dot{Q}$ mismatch/deadspace (*i.e.* regions with less perfusion and therefore high relative $\dot{V}_A/\dot{Q}$ ratios). However, when airway resistance and dynamic hyperinflation were reduced in severe COPD patients by breathing helium, no change in $\dot{V}_E/\dot{V}CO_2$ during exercise was observed [151]. Similarly, reducing hyperinflation with a short acting beta-agonist did not reduce $\dot{V}_E/\dot{V}CO_2$ during exercise in moderate-severe COPD [151, 157]. These results would suggest that reducing

intrathoracic pressure secondarily to reducing dynamic hyperinflation and/or a mechanical constraint does not affect $\dot{V}_A/\dot{Q}$ matching or $\dot{V}_E/\dot{V}CO_2$ during exercise in mild COPD.

2.6.2 Carotid chemoreceptor control of ventilation in COPD

The central nervous system is responsible for the control of ventilation at rest and during exercise. The central nervous system generates a motor pattern, which is transmitted to the respiratory muscles to contract and initiate ventilation. Although the control of ventilation is complex, it is well accepted that afferent feedback from the carotid chemoreceptors are key influences in central motor output [200, 201].

There are multiple studies that have used high oxygen delivery (*i.e.* hyperoxia) as a physiological intervention aimed at reducing ventilation and dyspnea, and improving exercise tolerance in non-hypoxemic COPD patients [54, 136, 157, 185]. Peters *et al.* [157] demonstrated a significant improvement (3.1 ± 1.1 min, p=0.002) in exercise endurance time (constant-load cycle ergometry at 75% peak power output) when non-hypoxemic patients breathed hyperoxia (F_IO_2 = 0.5). The authors attributed the improved exercise tolerance to a reduction in ventilatory drive and dyspnea. In this study, breathing hyperoxia reduced blood lactate and increased base excess, which suggests that the reduction in ventilation was linked to reduced metabolic acidosis [157]. Importantly, $\dot{V}_E/\dot{V}CO_2$ was also reduced with hyperoxia, which suggests that the decrease in ventilation may be independent of metabolic factors (*i.e.* acidosis) and is potentially influenced by the carotid chemoreceptors. Breathing hyperoxia would suppress the carotid chemoreceptors and potentially reduce ventilation [37], however, hyperoxia would also increase the arterial oxygen content and thus decrease blood flow (*i.e.* cardiac output) to maintain appropriate oxygen delivery [192]. Because of the potential autoregulatory (*i.e.* cardiac output) and metabolic (*i.e.* acidosis and oxidative capacity) effects of oxygen, it is difficult to determine the contribution of the carotid

chemoreceptors on both ventilatory and cardiovascular regulation during exercise in COPD patients, using a hyperoxia intervention.

Recently it has been shown that, in addition to the exaggerated ventilatory response to exercise, non-hypoxemic mild to moderate COPD patients have elevations in baseline carotid chemoreceptor activity (as demonstrated by the large reduction in ventilation with breathing 100% O_2), and carotid sensitivity (as demonstrated by a potentiated ventilatory response to hypoxia), which appears to be related to elevated cardiovascular risk in these patients [191]. This elevated hypoxic ventilatory response suggests the carotid chemoreceptor is sensitized in COPD, which is similar to previous work in patients with heart failure [47, 195]. Our recent investigation examined whether inhibition of the carotid chemoreceptors with low-dose dopamine reduced the ventilatory response to exercise in mild-moderate COPD patients. We found that while carotid chemoreceptor inhibition reduces resting minute ventilation slightly and improves cardiovascular function at rest (See study one in Chapter Three), chemoreceptor inhibition did not reduce the potentiated ventilatory response to exercise or improve exercise endurance time during a constant-load exercise test (See study two in Chapter Four). Together, these data suggest that the elevated carotid chemoreceptor activity and sensitivity was not a key contributor to the exaggerated ventilatory response to exercise in patients with COPD.

2.6.3 Exercise training and pulmonary vascular function in COPD

While it is generally assumed that lung function does not adapt to exercise training, there is evidence of training-induced changes within the pulmonary vasculature. Exercise training in rabbits has been shown to improve pulmonary vascular function [25]. While in an animal model of COPD, exercise training decreases the amount of pulmonary arterial smooth muscle [80]. Muscularization of the pulmonary artery is associated with increased vessel tone and reduced

perfusion, indicating that exercise training may reverse vascular dysfunction and improve perfusion in COPD. Similarly, exercise training has been shown to completely prevent small pulmonary artery muscularization that occurs in hypoxia-induced pulmonary arterial hypertension [229]. Our recent research has shown that resting and exercise pulmonary capillary blood volume is elevated in endurance-trained subjects as compared to less fit healthy controls, suggesting that chronic exercise training may improve pulmonary perfusion [205]. Consistent with the finding, diffusion capacity (DLCO), which is primarily determined by pulmonary capillary blood volume and pulmonary membrane diffusing capacity, has been shown to be correlated with peak oxygen consumption in healthy subjects [242]. Exercise training through pulmonary rehabilitation has been well-documented to improve exercise tolerance, quality of life and dyspnea [125, 186], while also reducing the $\dot{V}_E/\dot{V}CO_2$ response to exercise in COPD [21, 22, 161]. Exercise training improves systemic vascular function in COPD [60, 220]; however it is unclear if chronic exercise improves pulmonary vascular function. While chronic exercise training does not appear to improve airflow obstruction or lung volumes in COPD patients, no study has specifically examined pulmonary perfusion in COPD. Should COPD patients have improvements in pulmonary vascular function with chronic exercise training, then we would expect an increase in resting and exercise pulmonary capillary blood volume, leading to better overall $\dot{V}_A/\dot{Q}$ matching and a reduction in $\dot{V}_E/\dot{V}CO_2$ during exercise.

2.6.4 Pulmonary vascular dysfunction, nitric oxide bioavailability and gas exchange in COPD

Inhaled nitric oxide (iNO) is a therapy used in conditions of pulmonary vascular dysfunction, such as pulmonary arterial hypertension (PAH)[106, 156, 230], to increase NO bioavailability and improve pulmonary vascular function. Previous research in PAH and congestive heart failure (CHF) patients has shown that standard doses (20–40 parts per million

(ppm)) of iNO can reduce pulmonary vascular resistance [106] and increase exercise capacity ($\dot{V}O_{2peak}$) [81, 123].

Despite emerging evidence that COPD is associated with pulmonary vascular dysfunction, there is limited research using iNO in COPD. Roger *et al.* [175] found that iNO during exercise improved arterial PO_2 and reduced pulmonary vascular resistance in a small sample (n=7) of severe COPD patients (mean FEV1=39% predicted). However, iNO did not significantly improve $\dot{V}_A/\dot{Q}$ matching during exercise. Importantly, these severe COPD patients had resting hypoxemia, and had evidence of substantial pulmonary vascular destruction (*i.e.* emphysema), as demonstrated by a very low resting diffusion capacity (63% predicted), static lung hyperinflation and an elevated residual volume. In the presence of vascular destruction, iNO is likely ineffective at greatly improving pulmonary microvascular perfusion and thus $\dot{V}_A/\dot{Q}$ matching. It stands to reasons that, should mild COPD patients have pulmonary vascular dysfunction in an otherwise intact pulmonary vasculature, then the pulmonary circulation should be responsive to increasing nitric oxide bioavailability, delivered by iNO, which would in turn improve pulmonary vascular blood flow, $\dot{V}_A/\dot{Q}$ matching, and reduce $\dot{V}_E/\dot{V}CO_2$, and lower dyspnea and improve exercise capacity. To date, minimal research has been conducted to better understand the relationship between pulmonary vascular dysfunction, $\dot{V}_A/\dot{Q}$ matching and $\dot{V}_E/\dot{V}CO_2$ at rest and during exercise in mild COPD patients.

2.7 Evaluating exercise tolerance and capacity in COPD

From both a research and clinical perspective, measuring performance and associated pathophysiological and perceptual responses (*i.e.* $\dot{V}_E/\dot{V}CO_2$, operating lung volume and dyspnea) during standardized exercise tests are critical in the evaluation of COPD [164]. Exercise testing is considered a fundamental component for accurately quantifying cardiorespiratory fitness and

identifying potential mechanisms that cause reductions in exercise tolerance and capacity in clinical populations such as COPD. Within the scope of this dissertation, exercise tolerance is defined as the ability to sustain a given physical task. Exercise capacity is often used interchangeably with exercise tolerance, however, this can be misleading, as exercise capacity can infer one or all of the following: 1) peak power output 2) aerobic power ($\dot{V}O_{2peak}$ and $\dot{V}O_{2max}$) or 3) aerobic capacity. For this reason, exercise capacity will be defined as the peak oxygen uptake ($\dot{V}O_{2peak}$) that a patient or healthy control obtains at their individual symptom limitation to incremental exercise. Examples of symptom limitation would be perceived dyspnea, leg fatigue or discomfort, or an inability to maintain power output (*i.e.* point at which an individual is unable to maintain a target cadence on a cycle ergometer).

In clinical research settings, exercise tests are often used as a baseline characterization of exercise tolerance and exercise capacity, and cardiopulmonary fitness [164]. Additionally, they are commonly used to measure the functional effect of a rehabilitative or pharmacological intervention [164]. The most common laboratory-based exercise tests are incremental exercise tests (also termed a cardiopulmonary exercise test (CPET)) and constant-load exercise tests [148, 164, 165]. Much of the previous research in COPD (all levels of severity) have used both incremental and constant-load exercise tests.

Quite often, incremental exercise tests are used as a baseline characterization in clinical studies, as they allow the evaluation of submaximal and peak-exercise responses, which often provides valuable outcomes for each individual patient. The following are a few key examples: 1) peak power output and $\dot{V}O_2$ expressed as a % of predicted using specific prediction equations, 2) the dynamic change in operating lung volumes (*i.e.* DH and critically low IRV), 3) ventilatory efficiency (*i.e.* Nadir $\dot{V}_E/\dot{V}CO_2$) and, 4) the relationship between ventilatory drive and volume

displacement (*i.e.* neuromechanical uncoupling). Incremental exercise tests have been used, in patients with COPD, to assess changes in exercise capacity following non-pharmacological interventions (*i.e.* pulmonary rehabilitation) with the reported changes in $\dot{V}O_{2peak}$ ranging from 10-40% (0.1–0.5 $L \cdot min^{-1}$)[213]. However, pharmacological interventions (*i.e.* short- and long- acting bronchodilator therapy, and hyperoxia) have typically demonstrated no change or small changes in $\dot{V}O_{2peak}$ (0.04 – 0.18 $L \cdot min^{-1}$)[113]. Results of incremental exercise tests have been shown to be extremely reproducible in patients with respiratory disease, with no learning effect and small coefficients of variation in $\dot{V}O_{2peak}$ (~3%)[1].

Constant-load exercise tests are commonly used to examine exercise tolerance (or endurance) following a rehabilitative or pharmacological intervention. These tests have grown in popularity over the past decade as they have shown to be more sensitive to physiological changes (*i.e.* reduced exercise ventilation or DH) than incremental exercise tests. Constant-load exercise tests are typically administered at an intensity corresponding to 75-85% of the peak power output, determined from a baseline incremental exercise test. The primary outcome in these tests is time to symptom limitation (T_{LIM}) and has been shown to be responsive to rehabilitative interventions and pharmacological interventions aimed at reducing exercise ventilation, dynamic hyperinflation and dyspnea [164]. Although these tests are highly sensitive to post-intervention changes in physiological function, the determined exercise tolerance from the test is limited to one exercise intensity. Furthermore, there is often large variability in the test duration in homogenous samples of COPD patients, which often requires participants to repeat tests at varying intensities to properly assess symptom limitation exercise tolerance. The increase variability in constant-load exercise test duration may be the result of poor test-retest reliability [164]. A recent review by Puente-Masestu *et al.* (ERS task force) recommended limiting target test durations for a pre-intervention

constant work-rate exercise test to between 180 and 480 seconds [164]. Exercise durations within this range are usually limited by abnormal ventilatory-mechanical (*i.e.* $\dot{V}_E/\dot{V}CO_2$, DH) or sensory-mechanical (*i.e.* dyspnea) responses, not other reasons (*i.e.* boredom, temperature, dry mouth or seat discomfort) [36, 164]. For invasive randomized-control crossover trials (*i.e.* drug intravenous infusion or arterial blood gas sampling), the practical use of a constant load exercise test is questioned as repeat tests are often not feasible.

Both incremental and constant-load exercise tests are useful to assess the effects of experimental interventions on cardiopulmonary fitness, and exercise tolerance and capacity in patients with COPD. Constant-load exercise tests are considered more sensitive to detect an effect than incremental exercise tests. However, incremental tests permits the evaluation of physiological and perceptual variables (*i.e.* $\dot{V}_E/\dot{V}CO_2$ and dyspnea) at submaximal and maximal exercise intensities and are considered a stronger index of exercise capacity. Further, references values are available for incremental tests, but not constant-loaded exercise tests [164]. If the primary outcome is the effect of an intervention on exercise tolerance in COPD patients, a constant-load exercise test, at approximately 75% peak power output, should be utilized [36, 164]. If the primary outcome is comprised of both submaximal and peak cardiopulmonary variables (*i.e.* Nadir $\dot{V}_E/\dot{V}CO_2$, $\dot{V}O_{2peak}$), an incremental exercise test should be utilized. In studies that are designed to include a control group, where between-group comparisons are to be made at absolute exercise intensities, an incremental exercise test should also be utilized [164].

2.8 Summary

Patients with COPD experience significant exertional dyspnea, which has been shown to reduce quality of life, physical activity, and increase risk of mortality. A large majority of previous research has focused on studying the mechanisms of dyspnea in moderate and severe COPD, but

the reasons for dyspnea in mild COPD, whose symptoms appear disproportionate to the degree of airway obstruction, are not well understood. Mild COPD patients show an increased ventilatory inefficiency during exercise (demonstrate by greater $\dot{V}_E/\dot{V}CO_2$), which is a key contributor to dyspnea and is predictive of mortality. Recently research demonstrated the increased $\dot{V}_E/\dot{V}CO_2$ during exercise in mild COPD was secondary to increased deadspace; however, it is unclear if this increased deadspace ventilation represents pure deadspace, or rather alveolar $\dot{V}_A/\dot{Q}$ inequality (*i.e.* regions of high $\dot{V}_A$ with some pulmonary perfusion). It has been suggested that this increased deadspace is secondary to pulmonary vascular dysfunction and hypoperfusion of the pulmonary capillaries; however, no study to-date has specifically examined if improving pulmonary vascular function acutely will improve perfusion, $\dot{V}_A/\dot{Q}$ inequality and $\dot{V}_E/\dot{V}CO_2$ during exercise.

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Chapter Three

The carotid chemoreceptor contributes to the elevated arterial stiffness and vasoconstrictor outflow in COPD

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3.1 Introduction

Chronic obstructive pulmonary disease (COPD) is a respiratory disorder characterized by progressive, partially reversible airway obstruction, dyspnea and marked exercise intolerance. Although COPD is considered a disease of the lung, there is evidence of substantial systemic manifestations, such as cardiovascular disease, that develop as a consequence of COPD [238]. Previous research has shown elevated sympathetic nervous activity (SNA) and central arterial stiffness in both hypoxemic and non-hypoxemic COPD patients, both of which would contribute to cardiovascular deterioration, and are linked to increased cardiovascular events/mortality [3, 178, 221].

The mechanism(s) causing elevated SNA and central arterial stiffness in COPD are poorly understood, however, recent work suggests that greater activity of the carotid chemoreceptors (CC) may play a role [191]. The CCs are located within the carotid body at the level of the carotid sinus and are sensitive to circulating stimuli including O_2, CO_2, inflammation (IL-6, TNF-α) and reactive oxygen species, and are key mediators of ventilatory control and sympathetic vasoconstrictor outflow [44, 76, 163]. Importantly, the CCs have been shown to be tonically active and sensitized in conditions such as chronic heart failure (CHF), leading to elevations in SNA [122, 201]. Recently we have shown enhanced resting CC activity and sensitivity in non-hypoxemic non-hypercapnic COPD patients [191]. CC sensitivity was related to peripheral pulse-wave velocity (PWV), and CC inhibition by breathing 100% O_2 normalized PWV in COPD patients [191]. These findings are consistent with previous published experiments in hypoxemic COPD patients with severe respiratory failure demonstrating that breathing 100% O_2 reduced resting SNA [82]. Combined with previous work in CHF, these data suggest that the CCs may play an important role in cardiovascular regulation and may help explain the increased sympathetic outflow and central arterial stiffness even in stable non-hypoxemic COPD patients. Therefore, the aim of the present

study was to examine the effect of CC inhibition on central arterial stiffness and cardiovascular function at rest in COPD patients. It was hypothesized that CC inhibition with either 100% inspired O_2, or intravenous (I.V.) dopamine would reduce central arterial stiffness, secondary to reduced sympathetic vasoconstrictor outflow in COPD patients, while no change would be observed in controls.

3.2 Methods

3.2.1 Ethical approval

The present study was a randomized, single-blind, placebo controlled design and was approved by the University of Alberta Health Research Ethics Board (Biomedical Panel Protocol #00043106). Participant written informed consent was obtained prior to any research procedures. The study conformed to the standards set by the latest revision of the *Declaration of Helsinki*.

3.2.2 Participants and Procedure

Thirteen non-hypoxemic mild-moderate COPD patients (GOLD Stage I and II, post-bronchodilator (forced expired volume in 1 s (FEV_1)/forced vital capacity (FVC) ratio <0.7, FEV_1 ≥50% predicted) with a smoking history >10 pack-years and thirteen age- and risk-matched controls with minimal smoking history (<10 pack-years) were recruited. Participants were carefully screened to exclude supplemental oxygen therapy, diabetes, known cardiovascular disease, body mass index ≥35, severe inflammatory disorders (such as connective tissue disease), severe sleep apnea (STOP-Bang score >3, Apnea-Hypopnea Index >30 as evaluated by overnight sleep monitoring with ApneaLink Plus, ResMed Ltd., Bella Vista, Australia) and recent respiratory exacerbations (<6 months). Medication use (not including respiratory medication) was similar between the control and COPD groups (Table 1). The lack of cardiovascular disease was also

confirmed by a normal resting blood pressure (systolic <140 mmHg and diastolic <95 mmHg) and 12-lead ECG, as well as normal blood pressure and ECG responses to maximal exercise.

Three sessions were completed over a 3-week period in the following order: 1) participant enrollment and medical history, standard pulmonary function and cardiopulmonary exercise test as previously described [47, 191], 2) basal chemoreceptor reflex assessment, and, 3) the experimental trial. Prior to all study procedures, participants were asked to abstain from caffeine, smoking, vigorous exercise, alcohol and respiratory medications for at least 6 hours preceding testing.

3.2.3 Basal chemoreceptor assessment

Prior to data collection, participants rested quietly in the supine position while breathing normoxic air on a mouthpiece for 10 min to obtain baseline data. Basal activity of the CCs were then evaluated by examining the transient reduction in minute ventilation in response to 2 minutes of hyperoxia (F_IO_2=1.0). The difference between the baseline and nadir (15 s) ventilation was used as the index of basal CC activity [37, 191]. Following a minimum 10 minute break, a new baseline ventilation was obtained, and participants then completed a standard hyperoxic hypercapnic rebreathe test to determine central medullary chemoreceptor sensitivity [170]. A 4 L rebreathing bag was filled with a specialized gas mixture (7% CO_2, 50% O_2 and 43% N_2). Following a brief bout of hyperoxic breathing (F_IO_2 =0.5), participants then began rebreathing until the end-tidal CO_2 values reached 55 mmHg or participants requested to stop. After another minimum 10 minute break, baseline ventilation was obtained again, and sensitivity of the CCs were evaluated by measuring the hypoxic ventilatory response using a stepwise method, as previously described [47, 191]. Briefly, participants breathed normoxic air for 3 min followed by 3 min at a target SpO_2 of 90% and then 3 min at a target SpO_2 of 85%. Arterial saturation was analyzed as opposed to end-

tidal O_2 due to the uncertainty regarding the accuracy of estimating arterial blood gas from end-tidal expired gas in patients with lung disease [102]. Sufficient rest was given between trials to ensure that ventilation and arterial blood pressure returned to the initial baseline before continuing to the next test.

3.2.4 Experimental trial

Following instrumentation, participants breathed freely on the mouthpiece for 15 min to obtain baseline data. Interventions were then conducted in random order: 1) saline and normoxia, 2) saline and hyperoxia (F_IO_2=1.0), 3) dopamine and normoxia and, 4) dopamine and hyperoxia. Each intervention was separated by 10 minutes. For the dopamine trials, one minute steady-state mean data were recorded following a 10 min wash-in. Two-minute hyperoxia interventions were conducted with either saline or dopamine infusion and measurements were analyzed in the second minute [192]. On a separate day, a subset of seven COPD patients and seven controls completed resting muscle SNA (MSNA) measurements while breathing normoxia or hyperoxia.

3.2.5 Dopamine

Prior to the experimental trial, an I.V. catheter was inserted into the antecubital vein of the left arm. Participants received either isotonic I.V. saline or I.V. dopamine hyrdochloride (2 $\mu g \cdot kg^{-1} \cdot min^{-1}$; Hospira, Lake Forest, IL, USA) via an automated constant-infusion pump (Alaris, San Diego, CA, USA). Dopamine does not cross the blood brain barrier and would not affect the central chemoreceptors [243]. Previous research has shown that this specific dose effectively inhibits the carotid chemoreceptors in humans [107, 195].

3.2.6 Hyperoxia

During both the chemoreceptor assessment and the experimental trials, 2-minute hyperoxia interventions (F_IO_2=1.0) were conducted. Transient hyperoxia has been previously shown to

rapidly inhibit the CC [134, 195]. Prolonged bouts of hyperoxia were avoided as long-term hyperoxia can act as a central stimulant, which would confound results from the current study [34].

3.2.7 Cardiorespiratory measures

For both the chemoreceptor reflex assessment and the experimental trial, all data were recorded and integrated with a data acquisition system (Powerlab 16/30; ADInstruments, New South Wales, Australia) and stored for subsequent analysis using associated software (LabChart 8.0 Pro; ADInstruments). Minute ventilation was measured by a pneumotachometer (3700 series, Hans Rudolph, Kansas City, MO, USA) and expired CO_2 and O_2 were measured (CD-3A and S-3A; AEI Technologies, Naperville, IL, USA) continuously from a small sample port off the mouthpiece to obtain end-tidal CO_2 and O_2. Inspired gas was humidified (HC 150; Fisher and Paykel Healthcare, Auckland, New Zealand) and delivered continuously using a flow-through system to prevent rebreathing of expired gas. Inspired gas was modulated using air-oxygen (normoxia to hyperoxia) or air-nitrogen (normoxia to hypoxia) blender systems. Heart rate was determined from the R-R interval with a single lead ECG (lead II, Dual Bio Amp; ADInstruments). Cardiac output was estimated with impedance cardiography (Physioflow, Manatec, Paris, France), which has been previously validated [48]. Arterial oxygen saturation (SpO_2) was estimated with pulse oximetry (N-595; Nellcor Oximax, Boulder, CO, USA) using a left ear-lobe sensor. Blood pressure was determined using finger plethysmography (Finometer Midi, Amsterdam, Netherlands) and was calibrated to brachial artery pressure at regular intervals using manual auscultation.

3.2.8 Doppler ultrasound

Similar to previous research [47], brachial arterial mean blood velocity was obtained from the right arm using pulse-wave Doppler ultrasound (GE, Vivid-7, 4-5 MHz, < 60deg angle of

insonation). Briefly, the average velocity was multiplied by the cross-sectional area of the brachial artery and then multiplied by 60 to determine brachial flow ($ml \cdot min^{-1}$)[47, 192]. Brachial conductance was calculated as brachial flow/mean arterial pressure.

3.2.9 Arterial stiffness

Arterial stiffness was determined by pulse wave velocity. Pulse waves were gathered simultaneously using applanation tonometry (Mikro-tip Catheter Transducers model SPT-301, Millar Instruments, Inc., Houston, Texas) from the carotid, radial and femoral arteries. Pulse wave velocity was determined using the following formula:

$$PWV = D \cdot \Delta t^{-1}$$

In the formula, D was the distance (m) between sites and Δt was the time difference(s) between pulse waves using the foot-to-foot method [110]. Carotid-femoral (cfPWV) and carotid-radial (crPWV) were used as indices of central and peripheral arterial stiffness, respectively [3, 191]. Distance was measured on the skin using a tape measure beginning at the sternal notch and extending to the recording sites of the cfPWV and crPWV. At least 10 consecutive beats were averaged to represent PWV over a complete respiratory cycle [110].

3.2.10 Muscle sympathetic nerve activity

MSNA was recorded via peroneal microneurography as previously described [23]. Briefly, the raw sympathetic signal was rectified and integrated to produce a neurogram with a characteristic bursting pattern. MSNA was then analyzed using a semi-automated peak detection algorithm (Chart 8.1.3; ADInstruments) and expressed as the change in burst frequency ($bursts \cdot min^{-1}$) and incidence ($bursts \cdot 100\ heart\ beats^{-1}$) from baseline to hyperoxia.

3.2.11 Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM) unless otherwise stated. For all inferential analysis, statistical significance was set *a priori* at $p<0.05$. Primary endpoints were carotid-femoral pulse wave velocity and brachial artery conductance. A multi-factorial analysis of variance (ANOVA) was used to evaluate the effect of CC inhibition with either dopamine or hyperoxia (factor A) on key dependent variables (repeated factor) in COPD and controls (fixed factor). If a significant change or interaction effect was found, a Bonferroni multiple comparisons test was used to locate the differences. Unpaired t-analysis was used to evaluate participant characteristics and chemoreceptor reflexes between groups. All statistical analyses were performed using Sigma Plot Software version 13 (Systat Software Inc., San Jose, CA, USA).

3.3 Results

3.3.1 Participants

Descriptive characteristics for the COPD and control groups are displayed in Table 3.1. There were no between-group differences in age, height, body mass or BMI. As expected, the COPD group had a significantly greater smoking history, airway obstruction, and greater functional residual capacity and residual volume compared to controls (Table 3.1). COPD patients had significantly lower peak oxygen uptake but there was no evidence of oxygen desaturation at rest or peak exercise in either group (Table 3.1).

3.3.2 Chemoreceptor reflex

The transient reduction in minute ventilation during hyperoxia and the slope of the ventilatory response to hypoxia was greater in COPD patients, when compared to controls (Table 3.2). These data suggest that COPD patients have greater CC activity and sensitivity. There was

no difference in the hyperoxic hypercapnic ventilatory responses, between groups, indicating that central chemoreceptor sensitivity is similar between COPD and controls (Table 3.2).

3.3.3 Respiratory responses to carotid chemoreceptor inhibition

The cardiorespiratory data for both groups is presented in Table 3.3. Minute ventilation was similar between groups during saline infusion while breathing room air (Table 3.3). CC inhibition with hyperoxia and low-dose dopamine, significantly reduced minute ventilation in COPD, but had no effect in controls. When given both dopamine and hyperoxia, there was no further reduction in minute ventilation, suggesting that the interventions were not additive and the dopamine was likely a stronger CC inhibitor than inhaled O_2.

3.3.4 Cardiovascular responses to carotid chemoreceptor inhibition

See Table 3.3 and Figures 3.1 and 3.2 for grouped cardiovascular data. Both central (cfPWV) and peripheral (crPWV) pulse-wave velocity were elevated in COPD during saline infusion while breathing room air, when compared to controls (Fig. 3.1). Carotid-radial PWV was reduced with breathing 2 minutes of 100% O_2 in COPD, but not controls. Dopamine reduced both cfPWV and crPWV in COPD, but not controls (Fig. 3.1). MAP and pulse pressure were significantly reduced with dopamine in COPD, but not with hyperoxia (Table 3.3). No change in arterial pressure was observed in controls between conditions. Cardiac output, stroke volume, heart rate and brachial artery blood flow were unaffected by either hyperoxia or dopamine, in both groups (Table 3.3, Fig. 3.2). Both brachial and total vascular conductance were significantly increased with dopamine in COPD, but unchanged in controls, while conductance was unaffected with hyperoxia in either group (Table 3.3, Fig. 3.2). Brachial artery diameter was similar between groups with saline (Table 3.3). Dopamine increased brachial artery diameter in COPD, while no change was observed in controls (Table 3.3).

In the subset of participants with MSNA recordings, there were no differences in resting burst frequency (42 ± 4 vs 43 ± 8 bursts·min^{-1}, p=0.61) or burst incidence (64 ± 9 vs 65 ± 13 bursts·100 heart beats^{-1}, p=0.79) between COPD patients and controls, respectively. CC inhibition with hyperoxia significantly decreased MSNA in COPD, but not controls (Fig. 3.3).

3.4 Discussion

Carotid chemoreceptor inhibition with low-dose dopamine reduced central and peripheral arterial stiffness and increased vascular conductance in COPD patients, while no change was observed in risk-matched controls. Similarly, carotid chemoreceptor inhibition with inspired hyperoxia reduced both peripheral arterial stiffness and MSNA in COPD, while no effect was observed in controls. These data demonstrate the importance of the CC in contributing to the elevated arterial stiffness in COPD. There are multiple studies documenting elevated central PWV in COPD patients, however, the potential mechanism(s) for the increased PWV in COPD remain unclear [119, 124, 178]. This is the first study to demonstrate that central PWV can be reduced acutely in COPD. Our findings, combined with previous work examining peripheral PWV responses to CC inhibition demonstrates that the CCs have an important influence on PWV and cardiovascular risk in mild-moderate COPD patients.

The CC has been shown to be a significant sensor for both the control of breathing and cardiovascular function [195, 201]. Importantly, it is unlikely that central chemoreception contributed to the observed reduction in PWV with dopamine, as dopamine does not cross the blood brain and would not stimulate the rostral ventrolateral medulla [243]. It is often assumed that low-dose dopamine causes vasodilation through stimulation of dopamine-1 vascular receptors. However, low-dose dopamine does not cause vasodilation in healthy controls [47, 192], but

vasodilation is seen typically in conditions of high CC activity and sensitivity such as CHF [47, 195] and at present COPD. It is acknowledged that the vasodilation observed with dopamine in COPD could be due to the direct peripheral vasodilatory actions of dopamine (via direct stimulation of dopamine-1 vascular receptors); however, it would be assumed that any direct peripheral vascular effects of dopamine would have also been observed in risk-matched controls. Results from the current study show both CC activity and sensitivity was elevated in COPD, and dopamine reduced ventilation and caused vasodilation in these patients, while controls showed no response to dopamine. These results strongly suggest that the cardiovascular effects of low-dose dopamine in COPD are secondary to CC inhibition and not a central chemoreceptor or peripheral vascular effect.

3.4.1 Cardiovascular responses to carotid chemoreceptor inhibition

Arterial stiffness is determined by arterial wall material properties (*e.g.* elastin and collagen) and vessel diameter [145]. Neither short-term hyperoxia nor dopamine would acutely affect arterial wall properties. Rather, our data demonstrate vasodilation (increased brachial artery diameter and vascular conductance) with CC inhibition, indicating that the reduction in PWV with CC inhibition is likely secondary to increased vessel diameter. Changes in vessel diameter are highly influenced by MSNA [110, 204] and increased MSNA has been linked with increased PWV, independent of age, body composition or blood pressure [204]. It is well documented that afferent carotid body discharge contributes to elevated SNA in health and CHF [46, 78, 217], and inhibition of the CC reduces SNA and improves cardiovascular function in CHF [47, 195, 201]. MSNA is chronically elevated in COPD, compared to healthy individuals [3, 169], and in the current study, CC inhibition significantly reduced both peripheral and central arterial stiffness, and MSNA in COPD patients. Based on well-established research linking the CC to MSNA, and

MSNA to PWV, our data would indicate that the observed reduction in PWV with CC inhibition is likely the result of reduced MSNA.

Carotid-radial PWV reflects stiffness of the peripheral arteries, which are generally considered resistive vessels and thus under more smooth muscle sympathetic control, compared to carotid-femoral PWV (*i.e.* central arterial stiffness). It is therefore not surprising that carotid-radial PWV had a larger reduction with dopamine than carotid-femoral PWV (0.9±0.3 vs 0.6±0.3 $m \cdot sec^{-1}$, respectively) in COPD patients. A meta-analysis concluded that a 1 $m \cdot sec^{-1}$ increase in carotid-femoral PWV elevated cardiovascular mortality risk by 15% [221]. Based on these data, the 0.6 $m \cdot sec^{-1}$ reduction in carotid-femoral PWV observed with CC inhibition in COPD patients in the current study would suggest an 8-9% reduction in risk of mortality with CC inhibition in COPD.

3.4.2 Chemoreflex

The reflex actions of both the central and peripheral (CC) chemoreceptors have been well characterized in other clinical conditions, however, there are limited studies in patients with COPD. Most of the previous research in COPD has focused on severe COPD patients who have complex physiological responses due to additional co-morbidities (*e.g,* heart failure), severe air-way obstruction and chronic hypoxemia [16, 82, 160]. Importantly, the patients in our study had mild-moderate COPD, were non-hypoxemic and were compared to risk matched controls without COPD. While the ventilatory response to hyperoxia (CC activity) and hypoxia (CC sensitivity) were significantly greater in COPD patients, there was no difference in the hyperoxic hypercapnic ventilatory response, compared to risk-matched controls. These data suggest that while the peripheral chemoreceptors (*i.e.* CCs) appear sensitized and hyperactive in COPD, central chemoreception appears normal.

3.4.3 Limitations

Minute ventilation and peripheral arterial stiffness were reduced while breathing 100% O_2 in COPD, and these findings are similar to previous work in COPD [191]. Despite this evidence of CC inhibition, inhaled hyperoxia did not have a similar effect to dopamine on central arterial stiffness and vascular conductance in COPD patients. It is important to note that arterial stiffness and blood flow measurements were taken in the second minute of the 2 min hyperoxic bout. It is possible that, consistent with previous research in healthy participants [192], there was an acute vasodilatory response (<60 s) and a subsequent return to baseline, and that collecting data in the second minute resulted in us missing the transient vasodilation. The lack of vasodilation with hyperoxia may also be explained by autoregulation as the increased oxygen content with hyperoxia would reduce blood flow to maintain O_2 delivery [192]. While the current results indicate that chronic suppression of CC activity may be an important target to reduce PWV and cardiovascular risk, care should be taken regarding the use of long-term hyperoxia, as this may have important deleterious secondary consequences [7].

At moderate-to-high doses (*i.e.* 5-20 $\mu g \cdot kg^{-1} \cdot min^{-1}$), dopamine can stimulate alpha- and beta-receptors [29, 87]. Beta-receptor stimulation would be expected to increase heart rate; however, heart rate was unaffected in both groups with 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ dopamine infusion, suggesting an absence of beta-adrenergic stimulation. Alpha-adrenergic stimulation would result in vasoconstriction, reducing conductance and increasing blood pressure; however, the opposite was observed in COPD patients. Therefore, the cardiovascular responses observed with dopamine in COPD patients are unlikely to be explained by alpha- or beta- adrenergic stimulation.

Our groups were not matched for smoking history, and therefore we cannot separate the independent effect of smoking history vs. chronic airflow obstruction on chemoreception and vascular regulation.

While previous research has shown elevated resting MSNA in COPD [3, 169], there was no between-group difference in baseline MSNA in our small subset of participants with MSNA data (n=7 each group). Unfortunately, we were not able to obtain MSNA data in a larger sample of COPD patients, because of technical difficulties in obtaining and maintaining the MSNA signal. Based on the previous research demonstrating neurohumoral activation in COPD [2, 3, 88, 169] and the variability in baseline MSNA observed in our controls, we were likely underpowered to detect a difference in baseline MSNA. Although no between-group difference in baseline MSNA was observed, our results combined with previous research examining carotid chemoreception and neurohumoral activation, support the conclusion that the reduction in arterial stiffness was likely secondary to a reduction in MSNA.

3.5 Conclusions

CC inhibition reduced arterial stiffness and MSNA, and improved conductance in COPD. These data suggest that CC activity is increased at rest and contributes to elevated arterial stiffness in COPD. Our findings provide a potential mechanism for the increased PWV typically observed in COPD and demonstrate that the CC plays an important role in resting cardiovascular regulation in COPD. Interventions aimed at chronically reducing CC activity may be effective strategies to reduce cardiovascular risk in COPD.

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Table 3.1. Participant characteristics

	Control	COPD	P value
Participants	13	13	
Male/Female	8/5	10/3	
Age (years)	64 ± 14	61 ± 14	0.67
Height (cm)	168.7 ± 9.3	170.2 ± 5.5	0.63
Mass (kg)	78.0 ± 15.1	74.4 ± 14.7	0.55
BMI ($kg \cdot m^{-2}$)	27.2 ± 3.7	25.6 ± 3.8	0.26
Smoking history (pack years)	3.9 ± 4.5	41.8 ± 29.7	<0.001
Current smoker (n)	0	9	
Modified MRC dyspnea (scale 0-4)	0.0 ± 0.0	1.3 ± 0.5	<0.001
Medication use (n)			
SABA	0	8	
ICS	0	8	
Combined ICS/LABA	0	6	
β-blocker	1	2	
ACE-ARB	0	1	
Diuretics	1	0	
Statins	4	3	
Pulmonary function			
FEV_1 (L)	3.0 ± 0.8	2.4 ± 0.6	<0.001
FEV_1 (% predicted)	106.9 ± 13.9	83.1 ± 18.0	<0.001
FEV_1 (z-score)	0.13 ± 0.86	-1.41 ± 1.26	<0.001
FEV_1/FVC (% predicted)	97.2 ± 5.7	79.9 ± 10.1	<0.001
FEV_1/FVC (z-score)	-0.75 ± 0.47	-2.28 ± 1.17	<0.001
FRC (% predicted)	98.4 ± 17.6	118.1 ± 20.5	0.015
RV (% predicted)	87.8 ± 18.9	113.9 ± 21.4	0.004
TLC (% predicted)	100.3 ± 15.3	104.5 ± 15.0	0.555
DLCO (% predicted)	93.4 ± 10.5	65.1 ± 21.6	<0.001
Cardiopulmonary peak exercise responses			
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	33.3 ± 9.3	24.9 ± 8.9	0.028
$\dot{V}O_2$ (% predicted)	130 ± 25	88 ± 26	0.002
$\dot{V}CO_2$ ($L \cdot min^{-1}$)	2.83 ± 0.86	1.98 ± 0.71	0.011
$\dot{V}_E$ ($L \cdot min^{-1}$)	92.6 ± 29.3	68.2 ± 17.3	0.017
$P_{ET}CO_2$ (mmHg)	34.1 ± 4.8	32.8 ± 4.9	0.489
HR ($beats \cdot min^{-1}$)	162 ± 20	144 ± 22	0.065
$SpO_{2baseline}$ (%)	95.8 ± 4.2	97.0 ± 1.5	0.412
SpO_{2peak} (%)	95.6 ± 3.2	95.4 ± 2.5	0.864
Nadir $\dot{V}_E/\dot{V}CO_2$	29.2 ± 2.2	34.9 ± 5.5	<0.001
Change in IC baseline to peak exercise (L)	0.1 ± 0.4	-0.4 ± 0.4	0.007

Data are presented as n or mean ± SD. COPD: chronic obstructive pulmonary disease; MRC: medical research council; BMI: body mass index; SABA: short-acting β-agonist; ICS: inhaled corticosteroid; angiotensin converting enzyme; ARB, angiotensin receptor blocker; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; FRC: functional residual capacity; RV; residual volume; TLC: total lung capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; $\dot{V}O_2$: oxygen uptake; $\dot{V}CO_2$: carbon dioxide production; $\dot{V}_E$: minute ventilation; $P_{ET}CO_2$: partial pressure of carbon dioxide; HR: heart rate; SpO_2: oxygen saturation measured by pulse oximeter; IC: inspiratory capacity.

Table 3.2. Central and carotid chemoreceptor reflex responses

	Control	COPD
Transient hyperoxia		
Baseline $\dot{V}_E$ ($L \cdot min^{-1}$)	7.88 ± 0.51	8.41 ± 0.83
Nadir (15 s) $\dot{V}_E$ ($L \cdot min^{-1}$)	7.76 ± 0.61	6.16 ± 0.61
Nadir (15 s) change in $\dot{V}_E$ from baseline ($L \cdot min^{-1}$)	0.12 ± 0.46	2.25 ± 0.59*
Hyperoxic hypercapnic ventilatory response		
$\Delta\dot{V}_E/\Delta P_{ET}CO_2$ slope ($L \cdot min^{-1} \cdot mmHg^{-1}$)	1.22 ± 0.27	0.93 ± 0.18
Hypoxic ventilatory response		
$\Delta\dot{V}_E/\Delta SpO_2$ slope ($L \cdot min^{-1} \cdot \%^{-1}$)	0.04 ± 0.03	0.23 ± 0.08*

Data are presented as mean ± SEM. $\dot{V}_E$: minute ventilation; SpO_2: oxygen saturation measured by pulse oximeter; $P_{ET}CO_2$: partial pressure of carbon dioxide.
* $p<0.05$ between groups

Table 3.3. Respiratory and cardiovascular responses with either saline or dopamine while breathing room air or 2 minutes of hyperoxia (100% O_2) in controls and COPD

	Group	Saline Normoxia	Saline Hyperoxia	Dopamine Normoxia	Dopamine Hyperoxia
$\dot{V}_E$ (L·min^{-1})	Control	7.3 ± 0.4	7.9 ± 0.4	8.0 ± 0.7	8.2 ± 0.8
	COPD	8.5 ± 0.8	7.7 ± 0.7*	7.0 ± 0.6*ǂ	7.3 ± 0.5*ǂ
$P_{ET}CO_2$ (mmHg)	Control	37.1 ± 1.3	34.8 ± 1.3	37.9 ± 1.6	34.9 ± 1.5
	COPD	34.0 ± 2.2	32.0 ± 2.1	36.2 ± 1.4	33.9 ± 1.7
$P_{ET}O_2$ (mmHg)	Control	103.5 ± 1.9	576.8 ± 11.2#	103.5 ± 2.1	562.6 ± 18.6#
	COPD	110.5 ± 3.2	553.3 ± 19.2*	105.5 ± 2.2	567.7 ± 21.4*
SpO_2 (%)	Control	95.5 ± 0.7	98.7 ± 0.5#	95.5 ± 0.6	99.0 ± 0.3#
	COPD	94.3 ± 0.5	97.2 ± 0.7*	93.4 ± 0.6	97.6 ± 0.6*
Q (L·min^{-1})	Control	4.8 ± 0.3	5.0 ± 0.4	5.0 ± 0.4	5.1 ± 0.5
	COPD	4.5 ± 0.3	4.7 ± 0.3	4.6 ± 0.3	4.9 ± 0.3
HR (beats·min^{-1})	Control	62.3 ± 2.4	62.1 ± 2.4	67.3 ± 2.8	65.1 ± 2.7
	COPD	62.2 ± 2.7	58.9 ± 2.7	64.8 ± 2.5	64.0 ± 2.7
SV (ml·beat^{-1})	Control	78.8 ± 6.7	80.9 ± 6.4	75.1 ± 6.1	79.3 ± 6.5
	COPD	73.5 ± 5.0	80.6 ± 5.9	72.6 ± 6.3	83.3 ± 8.8
MAP (mmHg)	Control	91.0 ± 2.3	93.7 ± 2.5	91.9 ± 2.8	92.4 ± 2.9
	COPD	93.2 ± 2.1	92.7 ± 2.2	87.0 ± 2.9*ǂ	85.7 ± 3.3*ǂ
PP (mmHg)	Control	45.6 ± 3.3	45.5 ± 3.0	47.8 ± 3.3	47.5 ± 2.4
	COPD	48.9 ± 3.5	46.5 ± 2.7	42.4 ± 2.4*ǂ	43.6 ± 3.0*ǂ
Q/MAP (ml·min^{-1}·mmHg^{-1})	Control	53.9 ± 4.7	53.7 ± 4.9	54.9 ± 4.6	56.0 ± 5.2
	COPD	47.7 ± 3.1	49.5 ± 3.0	53.8 ± 3.8*	57.6 ± 4.1*
Brachial artery diameter (mm)	Control	3.79 ± 0.18	3.78 ± 0.19	3.85 ± 0.17	3.83 ± 0.16
	COPD	3.97 ± 0.19	4.03 ± 0.18	4.08 ± 0.17*ǂ	4.10 ± 0.19*ǂ

Data are presented as mean ± SEM. $\dot{V}_E$: minute ventilation; $P_{ET}CO_2$: partial pressure of carbon dioxide; $P_{ET}O_2$: partial pressure of oxygen; SpO_2: oxygen saturation measured by pulse oximeter; Q: cardiac output; HR: heart rate; SV: stroke volume; MAP: mean brachial arterial pressure; PP: brachial artery pulse pressure; Q/MAP: vascular conductance.
# $p<0.05$ vs control saline normoxia, * $p<0.05$ vs COPD saline normoxia, ǂ $p<0.05$ vs control within condition

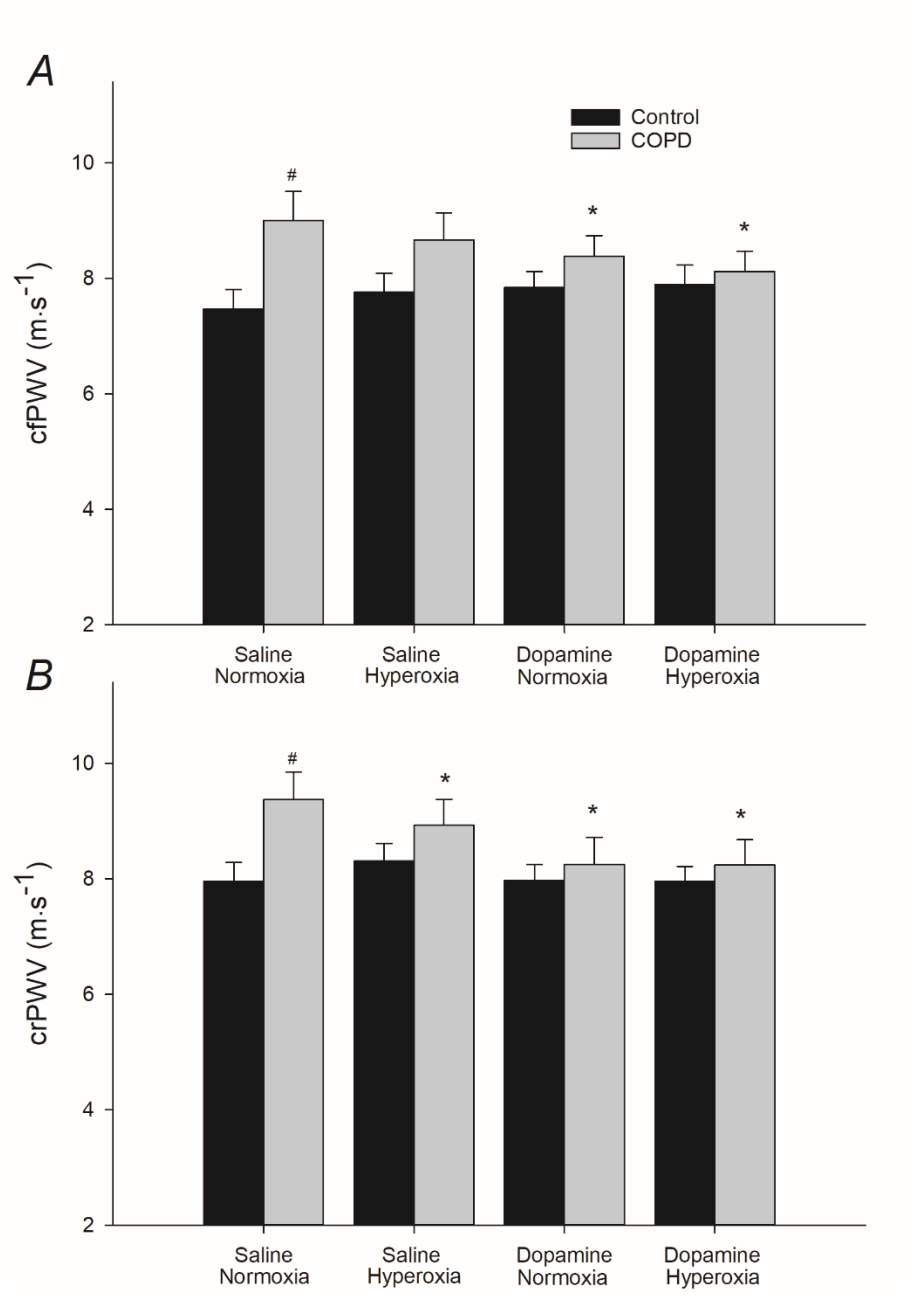

Figure 3.1. Carotid-femoral (cfPWV)(*A*) and carotid-radial (crPWV)(*B*) pulse-wave velocity with either saline or dopamine while breathing room air or 2 minutes of hyperoxia (100% O_2) in controls and COPD. Both cfPWV and crPWV were elevated in COPD, compared to control, in the saline and normoxia condition. Dopamine reduced both cfPWV and crPWV in COPD, while hyperoxia reduced crPWV in COPD. Data are presented as mean ± SEM.
# $p < 0.05$ vs control saline normoxia, * $p<0.05$ vs COPD saline normoxia

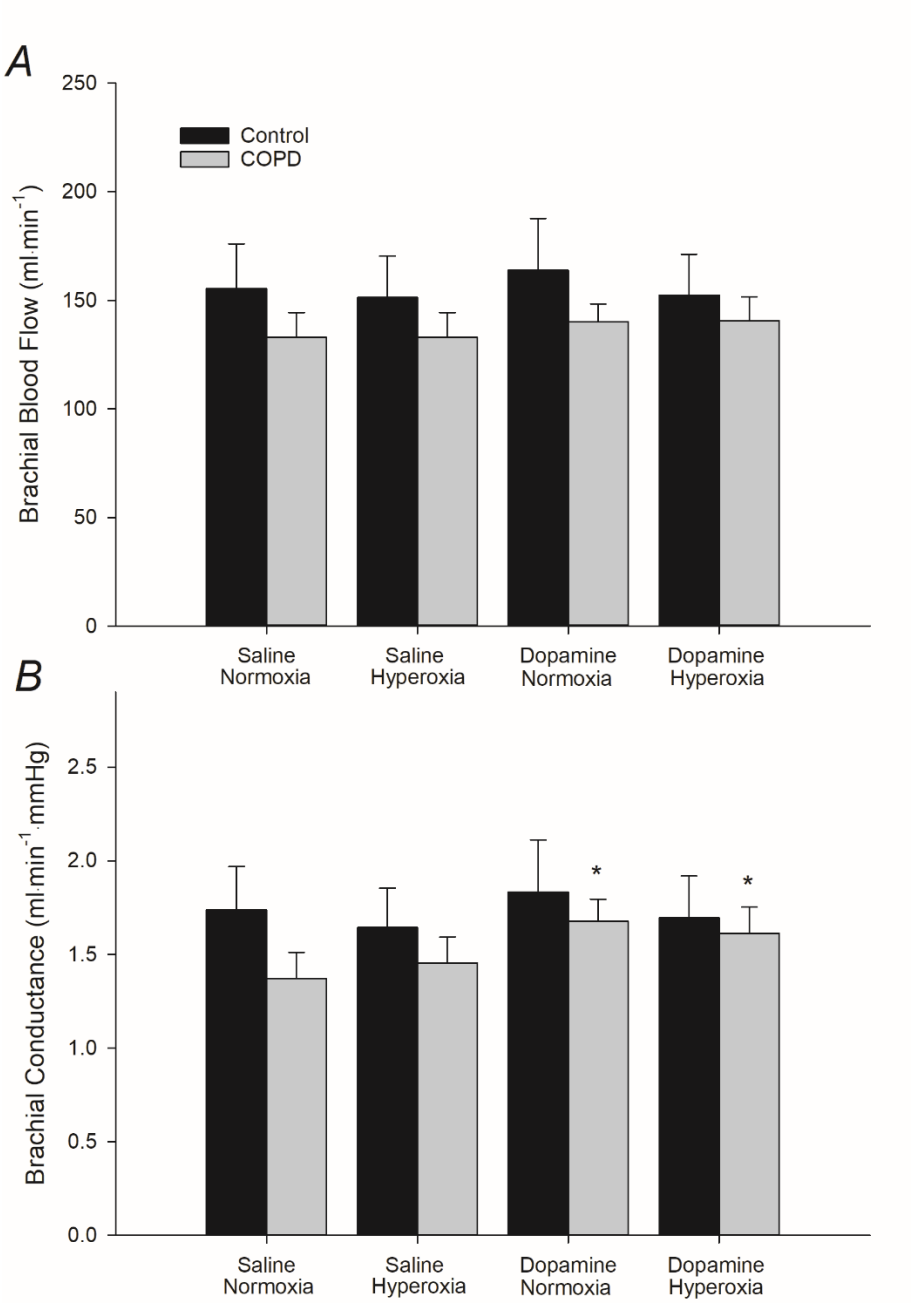

Figure 3.2. Brachial blood flow (*A*) and conductance (*B*) with either saline or dopamine while breathing room air or 2 minutes of hyperoxia (100% O_2) in controls and COPD. Dopamine increased conductance in COPD. Data are presented as mean ± SEM.
* $p<0.05$ vs COPD saline normoxia

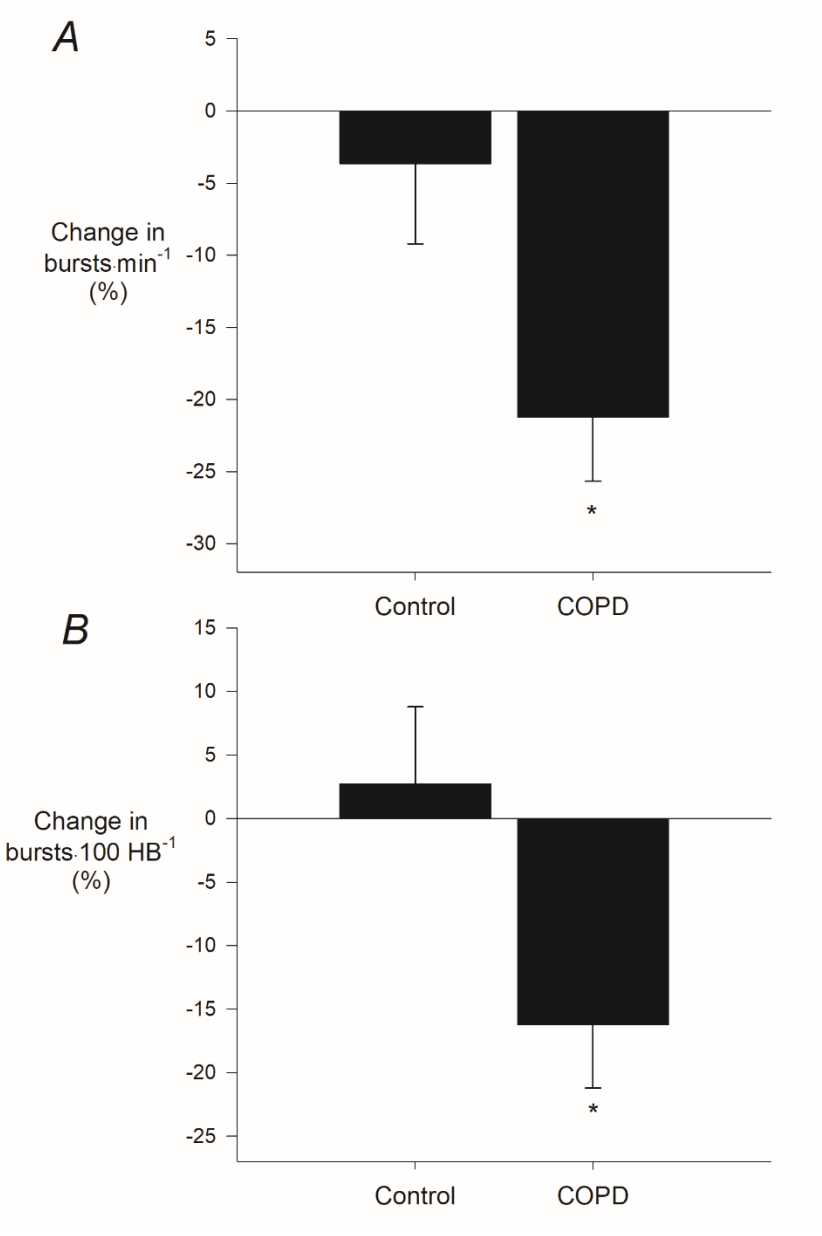

Figure 3.3. Change in muscle sympathetic nerve activity (MSNA) burst frequency (*A*) and burst incidence (*B*) from normoxia to hyperoxia in controls and COPD. MSNA was reduced with hyperoxia in COPD. Data are presented as mean ± SEM.
* $p<0.05$ between groups

Chapter Four

The effect of carotid chemoreceptor inhibition on exercise tolerance in COPD: a randomized-controlled crossover trial

A version of this chapter has been published in Respiratory Medicine, November 2019

4.1 Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a respiratory disorder characterized by progressive, partially reversible airway obstruction. Patients with COPD experience significant dyspnea during exertion, which has been shown to profoundly reduce exercise tolerance, physical activity, and impair the ability to complete day-to-day tasks [49, 70, 146]. The maintenance of physical activity in COPD is vital, as low physical activity in COPD is not only related to poor quality of life, but is also related to increased mortality [228]. Previous research has suggested that the observed dyspnea during incremental exercise in COPD is the result of increased work of breathing and diaphragmatic activity [70]. It has been shown that the increased work of breathing and thus dyspnea in COPD comes from 1) expiratory flow limitation and resulting dynamic hyperinflation [49, 72, 73, 111, 112, 131, 140, 144], and 2) an exaggerated ventilatory response to exercise (*i.e.* increased minute ventilation relative to carbon dioxide production, $\dot{V}_E/\dot{V}CO_2$) [49, 132, 146, 191]. A great deal of work has focused on improving airflow limitation in COPD; however, very little has been done to understand and treat the exaggerated ventilatory response to exercise in COPD [142]. The elevated $\dot{V}_E/\dot{V}CO_2$ response to exercise appears to be clinically important, as it independently predicts mortality in COPD and indicates that physiological abnormalities beyond airflow obstruction are important in determining disease severity, dyspnea and risk of death [131, 137, 211].

Recently, it has been shown that, in addition to the exaggerated ventilatory response to exercise, non-hypoxemic mild to moderate COPD patients have elevated baseline carotid chemoreceptor (CC) activity (as demonstrated by the large reduction in ventilation with breathing 100% O_2), and sensitivity (as demonstrated by a potentiated ventilatory response to hypoxia)[158, 191]. Our recent work has demonstrated that CC inhibition with low-dose dopamine infusion

reduced minute ventilation and improved cardiovascular function at rest in non-hypoxemic COPD patients [158]. Previous research has also found that the CCs can play a role in ventilation as well as the sympathetic control of cardiovascular function during exercise in health [10, 192, 196] and in chronic heart failure (CHF) [47, 195]. Specifically, CC inhibition during exercise reduced minute ventilation and muscle sympathetic nervous activity (MSNA), and increased vascular conductance in health [192, 196]. In CHF, CC inhibition during exercise resulted in vasodilation and improved blood flow to working muscles in CHF [195].

It is unclear if the enhanced activity and sensitivity of the CCs observed at rest 1) contributes to the exaggerated ventilatory response observed during exercise in COPD, and 2) affects cardiovascular regulation during exercise in COPD. Therefore, the purpose of this study was to examine whether CC inhibition improved ventilatory and cardiovascular regulation, dyspnea and exercise tolerance in COPD. It was hypothesized that CC inhibition would improve exercise tolerance, secondary to a reduction in minute ventilation and dyspnea, and improved cardiovascular function.

4.2 Methods

4.2.1 Study design

The present study was a randomized, double-blind, placebo-controlled crossover design and was approved by the University of Alberta Health Research Ethics Board (Biomedical Panel Pro00043106). This study is part of a larger research program examining the CC in health and disease. Data from nine of the control participants were included in a separate manuscript examining the effect of CC inhibition on exercise tolerance in patients with CHF [32].

After providing written informed consent, participants completed four sessions over a 4-week period. Session 1 included a thorough medical history screening, a complete pulmonary

function test and a symptom-limited incremental (20 W·2 min^{-1}) cardiopulmonary cycle exercise test to determine maximal work rate. Session 2 consisted of a basal chemoreflex assessment, which included tests to determine the ventilatory responses to hyperoxia, hypoxia and hypercapnia. Sessions 3 and 4 consisted of two constant work-rate cycle exercise tests to symptom limitation (T_{LIM}) at 75% of the maximal work rate, on separate days, while receiving either intravenous (I.V.) saline or low-dose I.V. dopamine (order randomized). Prior to all study procedures, participants were asked to abstain from caffeine, smoking, vigorous exercise, alcohol and respiratory medications for at least 12 hours preceding the testing.

4.2.2 Participants

Twelve non-hypoxemic mild-moderate COPD patients (GOLD Grade I and II, with post-bronchodilator (forced expired volume in 1 second (FEV_1)/forced vital capacity (FVC) ratio <0.7, FEV_1 ≥50% predicted) with a smoking history >10 pack-years and twelve age- and sex-matched controls with normal lung function, with minimal smoking history (<10 pack-years) were recruited. We chose to include non-hypoxemic GOLD 1 and II patients, as these types of patients previously demonstrated elevated CC activity and sensitivity [158, 191], which may contribute to the increased ventilation and dyspnea typically observed during exercise. Patients with severe COPD were excluded, as they are prone to exercise-induced arterial hypoxemia and hypercapnea, which may have influenced CC activity [132]. Participants were carefully screened to exclude supplemental oxygen therapy, diabetes, existing cardiovascular disease, body mass index ≥35, severe inflammatory disorders (such as connective tissue disease), severe risk of sleep apnea (STOP-Bang score > 3 [28]), and none of the patients with COPD had a recent exacerbation (< 6 months).

4.2.3 Pulmonary function

Spirometry, plethysmography and diffusing capacity for carbon monoxide (D_{LCO}) measurements were completed as per current guidelines [118, 127, 226]. Measurements were expressed as percent of predicted normal values [75]. Spirometric data were also presented as z-scores using the Global Lung Initiative 2012 equations [166].

4.2.4 Cardiopulmonary incremental exercise testing

Incremental exercise tests (Session 1) consisted of a 3-minute steady state resting period followed by 1-minute of unloaded pedaling then a 20 watt increase in work rate every 2-minutes to symptom limitation. Peak power output was defined as the highest work rate that the subject was able to maintain for $\geq$30 seconds [97]. During the exercise tests, participants rated their perceived breathing and leg discomfort using the modified Borg scale [12] at steady-state rest, within the last 30 seconds of every 2-minute exercise interval and at the end of exercise, followed by an inspiratory capacity (IC) maneuver. All ventilatory and cardiovascular measurements were collected over the first 30 seconds of every second minute during the exercise test and were linked with the perceptual ratings and IC measurements collected in the final 30 seconds of the respective minute to avoid contamination of the expired gas data from the IC maneuvers [98]. At symptom limitation, participants completed an IC maneuver immediately followed by the Borg ratings of breathing and leg discomfort, and ventilatory and cardiovascular measurements were averaged over the last 30 seconds of exercise.

All exercise trials were performed on an electronically braked cycle ergometer (Ergoselect II 1200 Ergoline, Blitz, Germany) and cardiorespiratory data were collected using a metabolic measurement system (Encore229 Vmax, SensorMedics, Yorba Linda, CA, USA). Operating lung volumes were calculated from IC maneuvers [71]. Arterial O_2 saturation (S_pO_2) was estimated

using finger pulse oximetry (N-595; Nellcor Oximax, Boulder, CO, USA). Heart rate was measured using electrocardiography (CardioSoft, GE Medical Systems, Milwaukee, WI, USA). Arterial blood pressure was taken by manual auscultation.

4.2.5 Basal chemoreceptor assessment

Basal chemoreceptor assessments were completed as previously described [158]. Briefly, CC activity was evaluated by examining the transient reduction in minute ventilation in response to 2-minutes of hyperoxia ($F_IO_2 = 1.0$) [37]. Following a minimum 10-minute break, participants completed a standard hyperoxic hypercapnic rebreathe test to determine central medullary chemoreceptor sensitivity [170]. A 4 L rebreathing bag was filled with a specialized gas mixture (7% CO_2, 50% O_2 and 43% N_2). Following a brief bout of hyperoxic breathing ($F_IO_2 = 0.5$), participants then began rebreathing until the end-tidal CO_2 values reached 55 mmHg or participants requested to stop. A 10-minute break was given to ensure that ventilation and arterial blood pressure returned to baseline prior to initiating the next test. CC sensitivity was then evaluated by measuring the hypoxic ventilatory response using a stepwise method [47, 158, 191]. Briefly, participants breathed normoxic air for 3-minutes followed by 3-minutes at a target SpO_2 of 90% and then 3-minutes at a target SpO_2 of 85%. Arterial saturation was analyzed as opposed to end-tidal O_2 due to the uncertainty regarding the accuracy of estimating arterial blood gas from end-tidal expired gas in patients with lung disease [102]. For the hypoxia/hyperoxia trials, inspired gas was modulated using air-oxygen (normoxia to hyperoxia) or air-nitrogen (normoxia to hypoxia) blender systems.

For the chemoreceptor assessment, all data were recorded and integrated with a data acquisition system (Powerlab 16/30; ADInstruments, New South Wales, Australia) and stored for subsequent analysis using associated software (LabChart 8.0 Pro; ADInstruments). Minute

ventilation was measured by a pneumotachometer (3700 series, Hans Rudolph, Kansas City, MO, USA) and expired CO_2 and O_2 were measured (CD-3A and S-3A; AEI Technologies, Naperville, IL, USA) continuously from a small sample port off the mouthpiece to obtain end-tidal CO_2 and O_2. Inspired gas was humidified (HC 150; Fisher and Paykel Healthcare, Auckland, New Zealand) and delivered continuously using a flow-through system to prevent rebreathing of expired gas. Heart rate was determined from the R-R interval with a single lead ECG (lead II, Dual Bio Amp; ADInstruments). SpO_2 was estimated with ear-lobe pulse oximetry (N-595; Nellcor Oximax, Boulder, CO, USA).

4.2.6 Constant work-rate exercise tests

The constant work-rate tests (Sessionss 3 and 4) consisted of a 3-minute steady-state resting period and a 1-minute period of unloaded pedaling followed by an immediate increase in work rate corresponding to 75% of the peak work rate. Exercise endurance time was recorded from the onset of loaded cycle exercise to the point of symptom limitation. The measurement procedures for the constant work-rate tests were identical to the incremental exercise tests mentioned above, however, additional parameters were also collected. Cardiac output was continuously monitored beat-by-beat and recorded in 30-second averages with impedance cardiography (PhysioFlow, Manatec, Paris, France). Impedance cardiography provides an accurate determination of cardiac output at rest and during exercise [24, 116], and is preferred over other non-invasive techniques such as inert gas rebreathing as these can be inaccurate in lung disease. The system is used regularly in COPD [85, 116, 150, 158] and is unaffected by lung hyperinflation [48]. Haemoglobin concentration was estimated using venous sampling (HemoCue 201+, HemoCue, Angelholm, Sweden). Estimations of oxygen delivery were determined using cardiac output, arterial O_2 saturation and haemoglobin concentration data. Vascular conductance was calculated as cardiac

output/mean arterial pressure (MAP). Using continuous-wave near infrared spectroscopy (NIRS; Oxymon MK III, Artinis Medical Systems, The Netherlands) oxyhaemoglobin and deoxyhaemoglobin were determined by measuring light attenuation at 760 and 864 nm wavelengths using the modified Beer-Lambert law [14, 68, 180]. Briefly, optodes were placed directly on the skin over the left thigh at the vastus lateralis muscle, 10 cm above the patella. The optodes were held in place using double-sided tape on the optode holder and, additionally, opaque adhesive tape was placed over the entire optode probe, which also aided with light shielding. Optodes were separated by 30 mm allowing for a depth of penetration of 15 mm [89]. Prior to optode placement, skinfold thickness of the vastus lateralis was determined manually using a skinfold caliper (Trimcal 4000, Sequoia Fitness Products, USA) to confirm the adipose tissue thickness covering the muscle was less than 10 mm [89, 180]. Care was taken to ensure consistent optode placement between trials to standardize tissue area sampled and minimize measurement variability. NIRS has been shown to accurately measure the change in tissue oxygenation (index of local muscle blood flow and O_2 extraction) in skeletal muscle during exercise [15, 68, 236]. In the current study, NIRS-derived tissue oxygenation was quantified by the change in total oxyhemoglobin from baseline, during constant load exercise.

4.2.7 Dopamine and saline intervention

Prior to the experimental trials (Day 3 and 4), an intravenous (I.V.) catheter was inserted into the antecubital vein of the left arm. Participants received either isotonic I.V. saline (Day 3) or I.V. dopamine hydrochloride (2 $\mu g \cdot kg^{-1} \cdot min^{-1}$; Hospira, Lake Forest, IL, USA, Day 4) via an automated constant-infusion pump (Alaris, San Diego, CA, USA). The order of the experimental days was randomized. Only the research coordinator and supervising physician were aware of the

condition (dopamine or saline). Neither the study participant nor the lead researcher directly conducting the exercise test were aware of the testing condition. Previous research has shown that this specific dose effectively inhibits the carotid chemoreceptors in humans [107, 192]. Dopamine does not cross the blood brain barrier and would not affect the central chemoreceptors [243].

4.2.8 Analysis

The primary study outcome was the effect of dopamine on exercise tolerance, defined as the time to symptom limitation, when compared to placebo. Secondary outcomes included the effect of dopamine on physiological and perceptual responses at rest and during exercise. Our previous work in mild to moderate patients with COPD demonstrated a 21 ± 24 % reduction in resting ventilation with dopamine [158]. Assuming a similar effect size, we determined *a priori* that 12 COPD patients would be sufficient to detect a significant effect of dopamine on exercise tolerance (α=0.05, power=0.8).

Data are presented as mean ± standard deviation unless otherwise stated. For all inferential analysis, statistical significance was set *a priori* at $p < 0.05$. Unpaired t-tests were used to compare pulmonary function, CC activity and sensitivity, and CPET data between groups. Three-way repeated-measures ANOVA were used to evaluate the effect of saline vs. dopamine (factor A) during exercise on key dependent variables (repeated factor) in COPD and controls (fixed factor). To interpret the condition by time interaction within each group, a two-way repeated-measures ANOVA was used. Four main time points were used for evaluation of exercise parameters: pre-exercise baseline, a standard time of 2- and 4- minutes (isotime) and T_{LIM}. If a significant change or interaction effect was found, a multiple comparison Bonferroni T-test was completed to locate the differences for all ANOVA's. All statistical analyses were performed using Sigma Plot Software version 13.0 (Systat Software Inc., San Jose, CA, USA).

4.3 Results

4.3.1 Participants

Descriptive characteristics for the COPD and control groups are displayed in Table 4.1. There were no between-group differences in age, height or BMI. The COPD group had a significantly greater smoking history than controls, and all but three COPD patients were ex-smokers. No individuals in the control group were current smokers. Medication use (not including respiratory medication) was similar between the control and COPD groups (Table 4.1).

4.3.2 Lung function and cardiopulmonary exercise test

Pulmonary function and CPET data are presented in Table 4.2. As expected, the COPD group had airflow obstruction, as well as increased functional residual capacity and residual volume compared to controls. Resting IC and D_{LCO} were lower in COPD. Exercise data show that COPD patients had significantly lower peak oxygen uptake ($\dot{V}O_2$), $\dot{V}_E$ and work rate; however, there was no evidence of arterial hypoxemia at rest or peak exercise in either group (Table 4.2). COPD patients demonstrated a reduction in IC from baseline to peak exercise, which is suggestive of dynamic hyperinflation (Table 4.2). COPD patients had greater ventilatory inefficiency than controls, as shown by the elevated $\dot{V}_E/\dot{V}CO_2$ slope and $\dot{V}_E/\dot{V}CO_2$ Nadir (lowest 30 second average).

4.3.3 Basal chemoreflex

Consistent with previous research [158, 191], the transient reduction in minute ventilation during hyperoxia was greater in COPD, when compared to controls (2.2 ± 1.4 vs 0.2 ± 1.4 $L \cdot min^{-1}$, p=0.012). The slope of the ventilatory response to hypoxia was also greater in COPD patients, when compared to controls (0.27 ± 0.15 vs 0.07 ± 0.11 $L \cdot min^{-1} \cdot S_pO_2\%^{-1}$, p=0.001). These data suggest that COPD patients have greater resting CC activity and sensitivity. There was no difference in the hyperoxic hypercapnic ventilatory responses, between groups, indicating that

central chemoreceptor sensitivity is similar between COPD and controls (1.2 ± 1.3 vs 1.0 ± 0.7 $L \cdot min^{-1} \cdot mmHg^{-1}$, p=0.500).

4.3.4 Effect of dopamine on exercisee endurance time

There was no significant improvement in endurance time with dopamine, in either group, when compared to saline (Control: saline=10.9 ± 4.8 vs dopamine=11.6 ± 4.0 min, p=0.31; COPD: saline=8.5 ± 4.7 vs dopamine=10.0 ± 6.1 min, p=0.417). The reasons for stopping the exercise tests are displayed in Table 4.3. In controls, the predominant reason for stopping was leg discomfort, regardless of condition. In COPD patients, the predominant reason for stopping was dyspnea, also regardless of condition.

4.3.5 Effect of dopamine on ventilation and dyspnea during exercise

Ventilatory responses to exercise are displayed in Tables 4.4 and 4.5 and Figure 4.1. As COPD patients exercised at a lower absolute intensity, $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$, were significantly lower during exercise in COPD, when compared to controls, independent of condition. As compared to controls, $\dot{V}_E/\dot{V}CO_2$ was significantly higher in COPD, independent of condition, (p<0.001). At isotime (2- and 4- minutes) and T_{LIM}, there was no significant difference in $\dot{V}O_2$, $\dot{V}CO_2$, breathing pattern or operating lung volume between dopamine and saline conditions, in either group. In both groups, there was a condition x time interaction for $\dot{V}_E$ (p=0.049 Control, p=0.016 COPD). Post-hoc analysis revealed that at 2-minute isotime, $\dot{V}_E$ (p=0.015 Control, p=0.028 COPD) and $\dot{V}_E/\dot{V}CO_2$ (p=0.017 Control, p=0.011 COPD), were significantly lower with dopamine, in both groups (Fig. 4.1). However, there was no difference in either $\dot{V}_E$ (p=0.412 Control, p=0.214 COPD) or $\dot{V}_E/\dot{V}CO_2$ (p=0.391 Control, p=0.519 COPD), at 4 minutes or T_{LIM} between saline and dopamine, in either group (Fig 4.1). Dopamine significantly increased $P_{ET}CO_2$ at 4-minute isotime in both groups (p=0.016 Control, p=0.036 COPD), but not at T_{LIM} (p=0.785

Control, p=0.685 COPD) (Tables 4.4 & 4.5). S_PO_2 was unaffected by dopamine throughout exercise, in either group.

At rest, isotime, and T_{LIM}, there was no between-group differences in mean dyspnea ratings (p=0.103) (Tables 4.4 & 4.5, Fig. 1). Further, there was no main effect for condition (dopamine vs saline) in either group at rest, isotime or T_{LIM} (p=0.182 control, p=0.448 COPD).

4.3.6 Effect of dopamine on hemodynamic responses to exercise

Hemodynamic responses to exercise are displayed in Tables 4.4 and 4.5 and Figure 2. During exercise, cardiac output was lower in COPD (p<0.001), likely because of the significantly lower absolute work rate. During exercise, vascular conductance was significantly lower in COPD patients as compared to controls (main effect p=0.04). A group by condition interaction for vascular conductance was observed at 4-minutes (p=0.04). With dopamine, vascular conductance increased and MAP decreased at all time points in COPD (main effect p<0.001, main effect p<0.003, respectively). Vascular conductance was only increased at T_{LIM} in controls (p=0.048). These data suggest that dopamine had a greater effect on vascular conductance in COPD patients than controls. Despite improved vascular conductance with dopamine, there was no effect for condition (saline vs dopamine) in cardiac output (p=0.661 control, p=0.607 COPD), vastus-lateralis total oxygenation (p=0.719 control, p=0.464 COPD) or perceived leg discomfort (p=0.460 control, p=0.461 COPD) at rest, isotime and T_{LIM}. Estimated O_2 delivery was not different between conditions at T_{LIM} (p=0.334) in COPD, however, there was a small but significant increase in controls at T_{LIM} (p=0.04). In controls, the increased O_2 delivery, with dopamine, was secondary to a larger increase in venous hemoglobin concentration, from baseline to T_{LIM}, when compared to the saline condition (dopamine: 1.4 ± 1.0 vs. saline: 0.6 ± 0.8 $g \cdot dL^{-1}$, p=0.007).

4.4 Discussion

This is the first known study to examine the effect of carotid chemoreceptor inhibition during whole-body exercise in non-hypoxemic patients with mild-moderate COPD and the major findings are twofold. First, carotid chemoreceptor inhibition with low-dose dopamine increased vascular conductance during exercise in COPD patients and, to a lesser extent, healthy controls. These data demonstrate the importance of the CCs in contributing to vascular regulation during whole-body exercise in health and in patients with COPD, however, this did not translate into an improvement in exercise tolerance. Second, no consistent reduction in exercise ventilation (*i.e.* lower $\dot{V}_E/\dot{V}CO_2$ or $\dot{V}_E$), was observed in COPD patients with dopamine and, as a result, dyspnea was unaffected. These findings indicate that the exaggerated ventilatory response to exercise typically observed in COPD patients is likely not explained by the heightened activity and sensitivity of the CC.

4.4.1 Ventilatory responses to exercise with dopamine

Although the control of ventilation is complex, it is well accepted that afferent feedback from the CC and central medullary chemoreceptors are key influences at rest and during exercise [42]. Boetger and Ward demonstrated a blunted ventilatory response to cycle ergometry exercise in young, apparently healthy individuals when the CCs were inhibited with 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ I.V. dopamine [10]. Specifically, the authors found that, although the transient ventilatory response was slower, (as evident by a lower $\dot{V}_E$ in the first 90-seconds of exercise), the steady-state ventilatory response (>2-minutes of exercise) was unaffected by dopamine, when compared to placebo. Although COPD patients in the current study demonstrated elevated resting CC activity and sensitivity, as well as an exaggerated ventilatory response to exercise, CC inhibition did not consistently affect ventilation (*i.e.* $\dot{V}_E$ and $\dot{V}_E/\dot{V}CO_2$) during constant-load cycle exercise. While a

reduction in $\dot{V}_E$ at 2-minutes of exercise was observed with dopamine in both groups, there was no consistent reduction in $\dot{V}_E$ throughout the exercise tests (4-minute isotime and T_{LIM}) in either group. The observed reduction in $\dot{V}_E$ 2-minutes into exercise parallels the findings of Boetger and Ward, which suggests that CC inhibition slows the initial ventilatory response to whole-body exercise in both COPD and controls [10]. As exercise ventilation was largely unaffected by dopamine, it is not surprising that operating lung volume and dyspnea were not different between saline and dopamine conditions in COPD or controls. The findings from our study suggest that the elevated basal activity and sensitivity of the CCs in COPD does not explain the exaggerated ventilatory response to exercise typically observed in mild to moderate non-hypoxemic patients with COPD [49, 191]. Although our intervention (dopamine) did not translate to improvements in exercise tolerance, the current study helps to better understand the role of the CC in ventilatory control during exercise in patients with COPD.

4.4.2 Cardiovascular responses to exercise with dopamine

During exercise, sympathetic nervous activity (SNA) increases, which causes vasoconstriction in the kidneys and GI tract, and competes with local vasodilatory influences to manage blood pressure and locomotor muscle blood flow [18, 103]. It is generally assumed that the increased MSNA during exercise is the result of feed-forward mechanisms such as feedback from muscle metaboreceptors, central command and the resetting of systemic baroreceptors [176]. However, emerging research has clearly demonstrated that the CC is a key contributor to increased SNA and vasoconstrictor mediated restraint of exercising muscle blood flow, such that CC inhibition during exercise resulted in reduced SNA, vasodilation and increased blood flow to working muscle in health [192, 196] and CHF [195].

Recently, we have demonstrated that CC inhibition reduced MSNA and central arterial stiffness, and improved vascular conductance, at rest, in mild to moderate non-hypoxemic COPD patients [158]. In the present study, as compared to the saline control condition, CC inhibition with dopamine increased vascular conductance and reduced MAP during exercise in COPD. A small but significant increase in vascular conductance was observed in controls at T_{LIM}, however, MAP was unaffected by dopamine throughout exercise. Despite evidence of vasodilation, there was no effect of dopamine on cardiac output, total oxygen delivery or locomotor muscle (*i.e.* vastus lateralis) tissue oxygenation, as assessed by NIRS, in COPD. Further, the observed vasodilation did not translate to improved exercise tolerance. These data suggest that, although CC inhibition may not acutely improve oxygen delivery and blood flow to working muscles, the CCs are key contributors to vascular regulation during exercise in patients with mild to moderate COPD.

4.4.3 Considerations and limitations

Despite evidence of improved vascular conductance during exercise with dopamine, we did not observe a significant reduction in minute ventilation and dyspnea during exercise with dopamine in patients with COPD. Multiple studies have used high oxygen (*i.e.* breathing hyperoxia) as a physiological intervention aimed at reducing ventilation and dyspnea and improving exercise tolerance in non-hypoxemic COPD patients [54, 136, 157, 185]. A limitation of breathing hyperoxia during exercise is that any potential effect of reducing CC activity on ventilation cannot be distinguished from the secondary effects of hyperoxia such as the increase in arterial O_2 content and resulting increase in O_2 delivery. For this reason, the present study used a low-dose dopamine intervention, as it has been shown to be an effective method to inhibit the CC, without the autoregulatory and metabolic effects typically observed with hyperoxia [107, 158, 192].

It is often assumed that low-dose dopamine causes vasodilation through stimulation of dopamine-1 vascular receptors. However, low-dose dopamine does not cause vasodilation in healthy controls [47, 158, 192], while vasodilation is observed in conditions of high CC activity and sensitivity such as CHF [47, 195] and COPD [158]. It is acknowledged that the vasodilation observed with dopamine in COPD could be due to the direct peripheral vasodilatory actions of dopamine (via direct stimulation of dopamine-1 vascular receptors); however, any direct peripheral vascular effects of dopamine would have also been consistently observed in controls at rest and during exercise. Further, dopamine reduced ventilation at rest in COPD patients, and at the onset of exercise in both controls and COPD. These observations strongly suggest that dopamine inhibited the CCs. Further, direct stimulation of vascular dopamine-1 receptors would not be expected to affect ventilation. When combined, these results suggest that the cardiovascular effects observed in the current study are secondary to CC inhibition and not a peripheral vascular effect.

Patients with COPD demonstrate elevated deadspace during whole-body exercise, even in patients with mild airflow obstruction [49]. In the current study, it is unclear if deadspace was affected by dopamine, as arterial blood gas data were not obtained. However, no consistent changes in breathing pattern, operating lung volume or $P_{ET}CO_2$ were observed during exercise in COPD and it is unlikely that deadspace was affected by dopamine. Further these data would suggest that the elevated deadspace, typically observed in COPD, is not a direct result of elevated activity and sensitivity of the CCs.

In the current study, dopamine was infused at 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ as this dose has been previously shown to inhibit the CC in humans [107, 192]. Limberg *et al*. showed substantial variability in the dose-specific effect of dopamine on CC sensitivity in young healthy individuals,

as evaluated by the hypoxic ventilatory response (HVR) at varying infusion rates (1-4 $\mu g \cdot kg^{-1} \cdot min^{-1}$) [114]. The authors concluded that no uniform dose is ideal to inhibit the CCs and reduce HVR, and suggested that individual dosing (between 1-4 $\mu g \cdot kg^{-1} \cdot min^{-1}$) should be used when designing experiments focused on the independent contribution of the CCa on integrative physiological effects. It is plausible that, had individualized dosing of dopamine been used in the current study, a consistent reduction in exercise $\dot{V}_E$ and dyspnea may have been observed. Importantly, moderate dose dopamine (*i.e.* ~5 $\mu g \cdot kg^{-1} \cdot min^{-1}$), can stimulate alpha-adrenergic receptors, which would result in vasoconstriction, reducing conductance and would confound the current results [29, 87]. Although individual dosing may have provided a more effective intervention to inhibit the CCs, we chose a single, conservative dose of I.V. dopamine *a priori* because of the complexity of physiological experiments in COPD and the risk of alpha-adrenergic stimulation.

Despite the mean change in exercise endurance (94 ± 400 s) nearly achieving the minimal clinically important difference of 101 seconds [165], only 2 of the 12 COPD patients within the study achieved a 101 second improvement in exercise endurance time with dopamine. Based on our observed effect size and variability in the change in exercise endurance time from saline to dopamine, 158 COPD patients would be required to detect a significant effect of CC inhibition on exercise endurance time (α=0.05, β=0.2, power=80%, Effect Size= 0.235). We acknowledge the small sample size of our study, however, the inability to detect a significant effect on the primary outcome is largely due to the small observed effect size and not an inadequate sample size.

4.5 Conclusion

Inhibition of the CC with dopamine improved vascular conductance during exercise in COPD, although no consistent changes in ventilation, dyspnea or exercise tolerance were observed. Our findings suggest that the exaggerated ventilatory response typically observed in

COPD is not explained by heightened activity and sensitivity of the CCs. However, the CCs appear to modulate vasoconstrictor outflow during exercise in non-hypoxemic COPD patients.

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Table 4.1. Participant characteristics

	Control	COPD	P value
Participants	12	12	
Male/Female	7/5	7/5	
Age (years)	63 ± 11	67 ± 8	0.297
Height (cm)	169.0 ± 7.3	168.8 ± 7.8	0.936
Mass (kg)	77.4 ± 14.1	76.8 ± 11.7	0.910
BMI ($kg \cdot m^{-2}$)	27.0 ± 4.1	27.2 ± 4.0	0.917
Smoking history (pack years)	5 ± 5	33 ± 13	<0.001
Current smoker (n)	0	3	
MRC dyspnea scale (1-5)	1.0 ± 0.0	2.0 ± 0.7	0.001
Medication use n			
SABA	0	8	
LABA	0	9	
ICS	0	9	
Combined ICS/LABA	0	8	
β-blocker	2	2	
ACE-ARB	0	4	
Statins	5	4	

Data are presented as n or mean ± SD. BMI: body mass index; MRC: medical research council; SABA: short-acting β-agonist; LABA: long-acting β-agonist; ICS: inhaled corticosteroid; ACE: angiotensin converting enzyme; ARB, angiotensin receptor blocker.

Table 4.2. Pulmonary function and cardiopulmonary exercise test peak responses

	Control	COPD	P value
Post-Bronchodilator Lung Function			
FEV_1 (L)	3.28 ± 0.70	2.32 ± 0.55	<0.001
FEV_1 (% predicted)	112 ± 13	83 ± 15	<0.001
FEV_1 (z-score)	0.58 ± 0.92	-0.88 ± 0.74	<0.001
FVC (L)	4.41 ± 0.87	3.88 ± 0.86	0.129
FVC (% predicted)	112 ± 13	105 ± 14	0.136
FVC (z-score)	0.95 ± 0.97	0.60 ± 0.86	0.669
FEV_1/FVC	0.74 ± 0.04	0.5 ± 0.08	<0.001
FEV_1/FVC (% predicted)	98.1 ± 4.7	80.7 ± 13.3	0.004
FEV_1/FVC (z-score)	-0.60 ± 0.49	-2.00 ± 0.90	<0.001
VC (% predicted)	112 ± 13	105 ± 13	0.226
IC (% predicted)	123 ± 19	102 ± 20	0.016
FRC (% predicted)	103 ± 14	121 ± 20	0.030
RV (% predicted)	87 ± 15	110 ± 18	0.003
TLC (% predicted)	102 ± 11	102 ± 15	0.976
DLCO (% predicted)	91 ± 11	66 ± 22	0.002
Cardiopulmonary peak exercise responses			
Work rate (W)	191 ± 64	102 ± 34	<0.001
Work rate (% predicted)	132 ± 45	89 ± 21	<0.001
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	36.2 ± 10.3	21.5 ± 6.1	<0.001
$\dot{V}O_2$ ($L \cdot min^{-1}$)	2.70 ± 0.90	1.59 ± 0.42	0.003
$\dot{V}O_2$ (% predicted)	134 ± 37	86 ± 20	<0.001
$\dot{V}CO_2$ ($L \cdot min^{-1}$)	2.94 ± 0.9	1.71 ± 0.44	0.002
$\dot{V}_E$ ($L \cdot min^{-1}$)	97.5 ± 35.2	61.6 ± 18.9	0.015
$\dot{V}_E$ (% predicted)	95 ± 31	70 ± 10	0.006
$\dot{V}_E/\dot{V}CO_{2peak}$	32.5 ± 3.1	35.2 ± 6.3	0.013
Nadir $\dot{V}_E/\dot{V}CO_2$	29.0 ± 1.7	35.6 ± 5.9	0.001
Slope $\dot{V}_E/\dot{V}CO_2$	25.3 ± 6.61	32.6 ± 6.2	0.013
$P_{ET}CO_2$ (mmHg)	33.0 ± 2.6	32.9 ± 3.8	0.953
HR ($beats \cdot min^{-1}$)	160 ± 20	127 ± 13	<0.001
$SpO_{2baseline}$ (%)	96.3 ± 2.8	96.4 ± 2.1	0.841
SpO_{2peak} (%)	94.3 ± 3.7	93.9 ± 5.0	0.723
Dyspnea (Borg)	6.2 ± 2.0	5.7 ± 1.4	0.555
Leg Discomfort (Borg)	6.8 ± 1.7	6.6 ± 2.3	0.614
Dyspnea/$\dot{V}_E$ slope	0.08 ± 0.03	0.11 ± 0.05	0.046
Δ IC baseline to peak exercise (L)	0.25 ± 0.47	-0.34 ± 0.43	0.006

Data are presented as mean ± SD. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; VC: vital capacity; IC: inspiratory capacity; FRC: functional residual capacity; RV; residual volume; TLC: total lung capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; $\dot{V}O_2$: oxygen uptake; $\dot{V}CO_2$: carbon dioxide production; $\dot{V}_E$: minute ventilation; $P_{ET}CO_2$: partial pressure of carbon dioxide; HR: heart rate; SpO_2: oxygen saturation measured by pulse oximeter.

Table 4.3. Selection frequency of reasons for stopping constant load exercise tests

		Control	COPD
Reasons for stopping (n)			
Dyspnea	Saline	1	6
	Dopamine	1	6
Leg Discomfort	Saline	6	3
	Dopamine	7	1
Both	Saline	5	3
	Dopamine	4	5

In controls, the majority of individuals stopped because of leg discomfort. In COPD, the majority of patients stopped because of dyspnea. The selection frequency was similar between saline and dopamine conditions in each group

Table 4.4. Physiological and perceptual parameters at isotime (4-minutes) during a constant-load cycle exercise test with saline or dopamine in controls and COPD

	Control		COPD		Main Effects *p* value		Interaction *p* value
Variable	Saline	Dopamine	Saline	Dopamine	Group	Condition	Group x Condition
Work Rate (W)	143 ± 48	143 ± 48	76 ± 26	76 ± 26			
$\dot{V}O_2$ (L·min^{-1})	2.2 ± 0.7	2.2 ± 0.7	1.5 ± 0.4	1.4 ± 0.4	**0.02**	0.45	0.85
$\dot{V}CO_2$ (L·min^{-1})	2.3 ± 0.7	2.4 ± 0.7	1.4 ± 0.4	1.4 ± 0.4	**0.02**	0.75	0.85
$\dot{V}_E$ (L·min^{-1})	72.4 ± 20.4	68.7 ± 18.8	47.5 ± 10.8	45.2± 10.3	**0.008**	0.24	0.58
$\dot{V}_E/\dot{V}CO_2$	30.6 ± 2.6	29.4 ± 2.4	37.3 ± 6.9	36.5 ± 7.1	**0.001**	0.15	0.64
$P_{ET}CO_2$ (mmHg)	35.4 ± 3.1	37.1 ± 2.8*	33.0 ± 3.9	34.6 ± 4.4*	0.17	0.04	0.95
SpO_2 (%)	97.6 ± 1.3	96.3 ± 1.3	93.8 ± 3.2	92.7 ± 3.7	0.07	0.14	0.69
V_T (L)	2.50 ± 0.51	2.53 ± 0.58	1.71 ± 0.50	1.84 ± 0.48	**0.02**	0.39	0.68
f_B (breaths·min^{-1})	28.1 ± 7.6	27.4 ± 5.8	29.4 ± 8.0	27.3 ± 5.8	0.82	0.25	0.35
IC (L)	3.20 ± 0.76	3.22 ± 0.70	2.40 ± 0.66	2.36 ± 0.45	**0.04**	0.35	0.93
IRV (L)	0.85 ± 0.76	0.74 ± 0.38	0.59 ± 0.38	0.59 ± 0.38	0.45	0.24	0.52
Q (L·min^{-1})	12.5 ± 2.4	12.8 ± 2.7	8.9 ± 2.7	8.9 ± 2.4	**0.01**	0.30	0.21
HR (beats·min^{-1})	146 ± 17	146 ± 17	119 ± 17	120 ± 21	**0.02**	0.68	0.82
SV (ml·beat^{-1})	85 ± 14	88 ± 17	75 ± 17	74 ± 17	0.26	0.35	0.18
MAP (mmHg)	118 ± 10	113 ± 7	105 ± 15	91 ± 15*	**0.02**	**<0.001**	**0.01**
Q/MAP (ml·min^{-1}·mmHg^{-1})	107 ± 17	114 ± 20	85 ± 21	101 ± 27*	**0.04**	**0.01**	**0.04**
Dyspnea (Borg)	4.3 ± 1.7	3.8 ± 1.4	3.8 ± 1.4	3.4 ± 1.0	0.85	0.60	0.86
Leg Discomfort (Borg)	4.9 ± 1.7	4.8 ± 1.7	4.6 ± 1.4	4.0 ± 1.4	1.00	0.18	1.00

Data are presented as mean ± SD. $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; $\dot{V}_E$, minute ventilation; $P_{ET}CO_2$, pressure of end-tidal carbon dioxide; SpO_2: oxygen saturation measured by pulse oximeter; V_T, tidal volume; f_B, breathing frequency; IC, inspiratory capacity; IRV, inspiratory reserve volume Q, cardiac output; HR, heart rate; SV, stroke volume; MAP, mean brachial arterial pressure; Q/MAP, vascular conductance. * p <0.05 between conditions within group

Table 4.5. Physiological and perceptual parameters at time to symptom limitation (T_{LIM}) during a constant-load cycle exercise test with saline or dopamine in controls and COPD

	Control		COPD		Main Effects *p* value		Interaction *p* value
Variable	Saline	Dopamine	Saline	Dopamine	Group	Condition	Group x Condition
Work Rate (W)	143 ± 166	143 ± 166	76 ± 80	76 ± 80			
Endurance Time (Min)	10.9 ± 4.8	11.6 ± 4.0	8.5 ± 4.7	10.0 ± 6.1	0.26	0.30	0.64
$\dot{V}O_2$ (L·min^{-1})	2.5 ± 0.7	2.5 ± 1.0	1.5 ± 0.4	1.4 ± 0.4	**<0.001**	0.89	0.97
$\dot{V}CO_2$ (L·min^{-1})	2.6 ± 1.0	2.6 ± 1.0	1.5 ± 0.4	1.4 ± 0.4	**0.002**	0.30	0.56
$\dot{V}_E$ (L·min^{-1})	84.8± 27.0	86.6 ± 28.3	52.0 ± 10.8	55.0 ± 15.2	**0.004**	0.15	0.52
$\dot{V}_E/\dot{V}CO_2$	35.1 ± 4.1	35.1 ± 3.4	39.1 ± 5.2	39.1 ± 5.2	0.05	0.98	0.98
$P_{ET}CO_2$ (mmHg)	31.0 ± 3.4	30.8 ± 3.4	31.6 ± 4.5	31.3 ± 4.5	0.88	0.74	0.73
SpO_2 (%)	95.1 ± 2.4	94.8 ± 2.8	93.0 ± 6.2	91.7 ± 5.8	0.22	0.22	0.48
V_T (L)	2.43 ± 0.48	2.35 ± 0.51	1.76 ± 0.51	1.75 ± 0.42	**0.03**	0.36	0.60
f_B (breaths·min^{-1})	36.6 ± 7.3	36.9± 7.3	31.0 ± 8.3	32.5 ± 6.9	0.16	0.08	0.78
IC (L)	3.24 ± 0.73	3.27 ± 0.63	2.30 ± 0.73	2.43 ± 0.55	**0.03**	0.58	0.35
IRV (L)	0.82 ± 0.35	0.90 ± 0.45	0.69 ± 0.69	0.62 ± 0.42	0.41	1.00	0.50
Q (L·min^{-1})	13.9 ± 2.2	14.8 ± 3.3	9.5 ± 2.5	9.6 ± 2.9	**0.02**	0.18	0.21
HR (beats·min^{-1})	155 ± 14	157 ± 19	125 ± 19	131 ± 2	**0.02**	0.17	0.51
SV (ml·beat^{-1})	90 ± 14	96 ± 2	75 ± 17	73 ± 19	0.06	0.38	0.17
MAP (mmHg)	120 ± 14	114 ± 14	106 ± 19	92 ± 19*	**0.02**	**0.004**	0.19
Q/MAP (ml·min^{-1}·mmHg^{-1})	118 ± 27	128 ± 41*	92 ± 31	108 ± 42*	0.08	**0.01**	0.77
O_2 Delivery (mlO_2·min^{-1})	2837 ± 826	3098 ± 1062*	1855 ± 557	1883 ± 689	**0.006**	0.16	**0.03**
Dyspnea (Borg)	7.8 ± 1.9	7.9 ± 1.9	6.8 ± 1.8	7.1 ± 2.2	0.62	0.84	0.85
Leg Discomfort (Borg)	8.8 ± 1.7	9.0 ± 1.7	7.1 ± 1.9	7.6 ± 2.0	0.38	1.00	1.00

Data are presented as mean ± SD. $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; $\dot{V}_E$, minute ventilation; $P_{ET}CO_2$, pressure of end-tidal carbon dioxide; SpO_2: oxygen saturation measured by pulse oximeter; V_T, tidal volume; f_B, breathing frequency; IC, inspiratory capacity; IRV, inspiratory reserve volume Q: cardiac output; HR: heart rate; SV: stroke volume; MAP: mean brachial arterial pressure; Q/MAP: vascular conductance. * p <0.05 between conditions within group

0

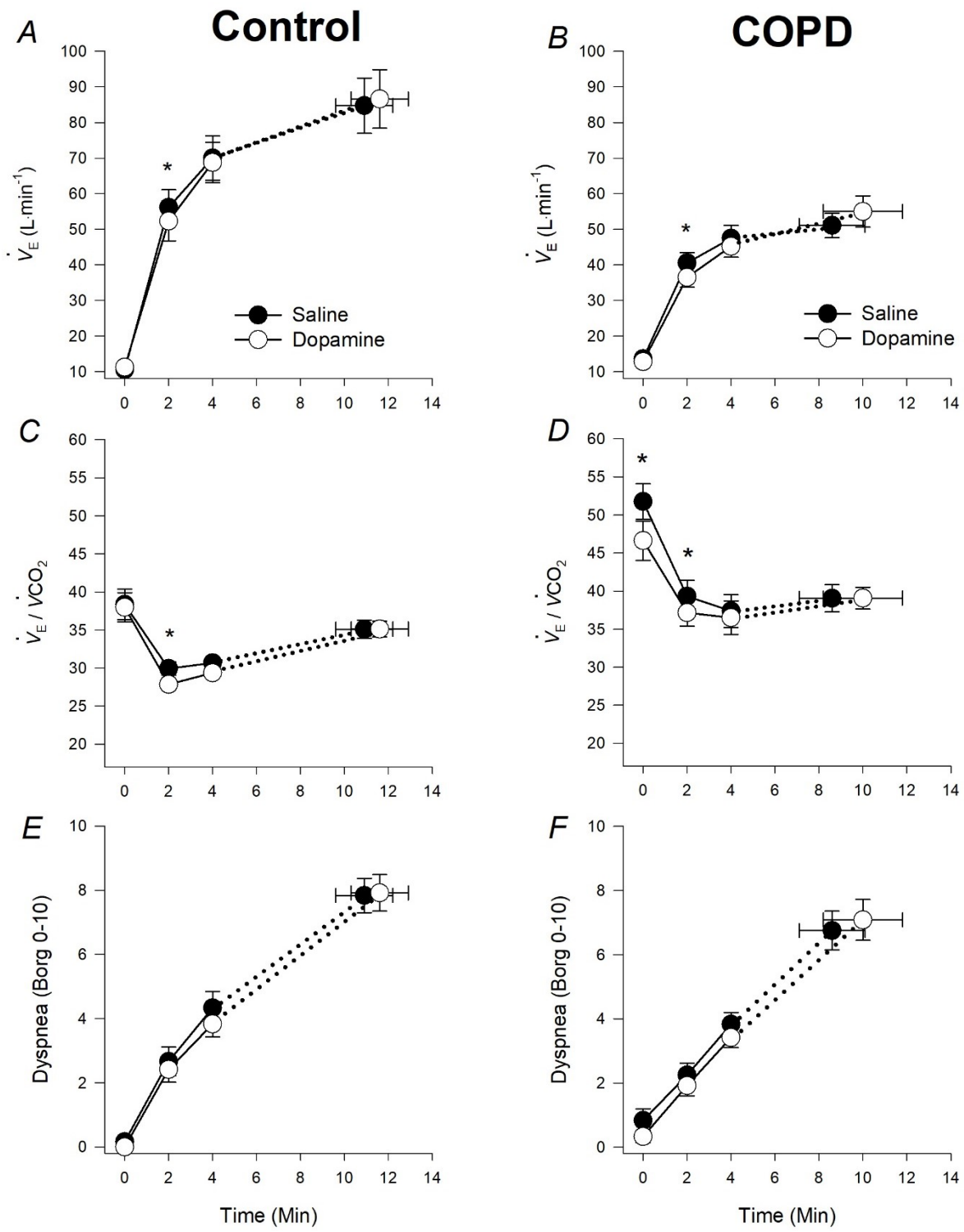

Figure 4.1. Mean ± SEM minute ventilation ($\dot{V}_E$), ventilatory equivalent to carbon dioxide production ($\dot{V}_E/\dot{V}CO_2$) and exertional dyspnea at rest and during constant-load cycle ergometry in controls (*left column*) and COPD (*right column*).
* $p<0.05$ saline vs dopamine within group

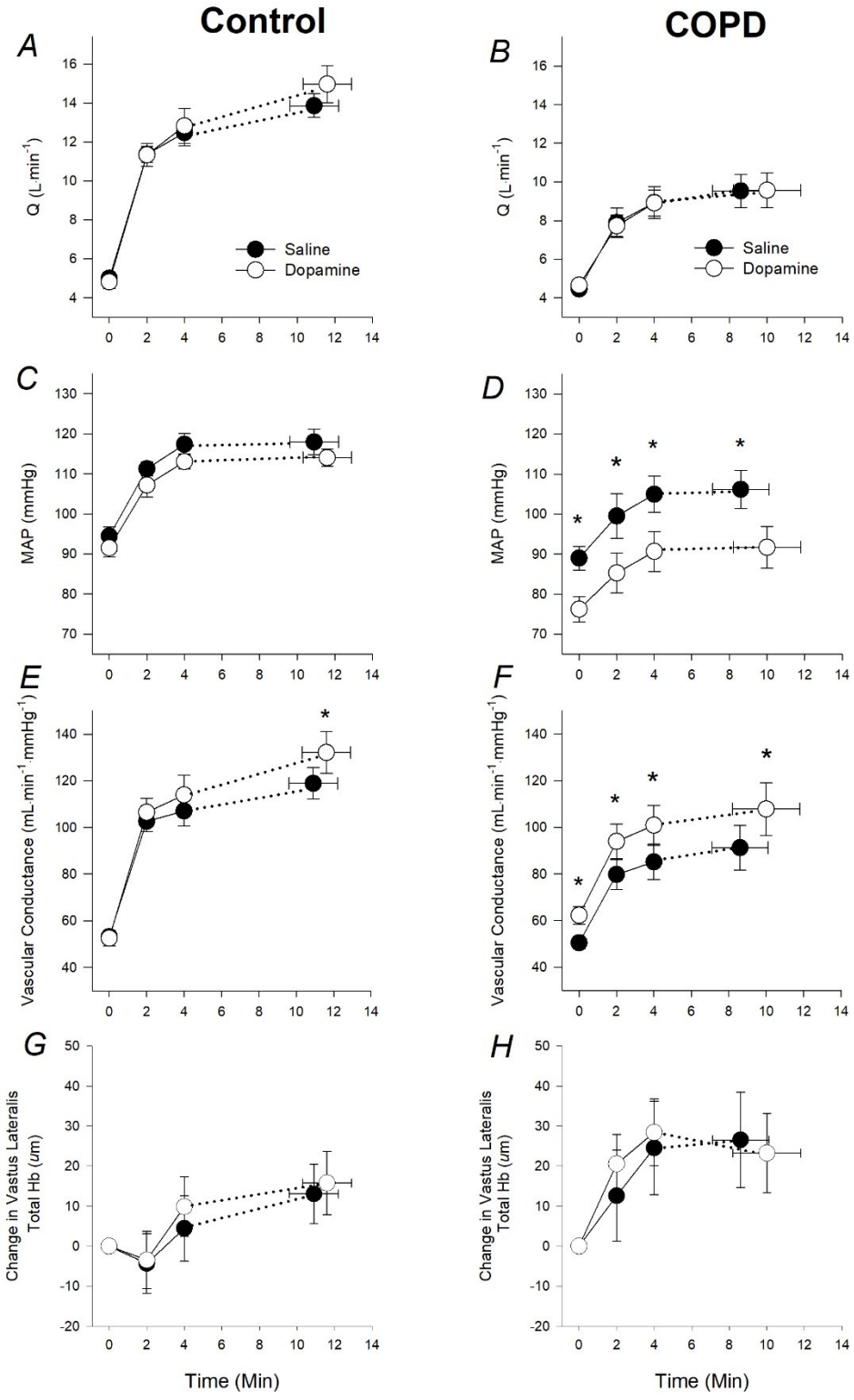

Figure 4.2. Mean ± SEM cardiac output (*Q*), mean arterial pressure (MAP), vascular conductance and vastus lateralis tissue oxygentation (Total Hemoglobin (Hb)) at rest and during constant-load cycle ergometry in controls (*left column*) and COPD (*right column*).
* $p<0.05$ saline vs dopamine within group

Chapter Five

Inhaled nitric oxide improves exercise capacity in patients with mild chronic obstructive pulmonary disease: a randomized-control crossover trial.

5.1 Introduction

Exertional dyspnea (perceived breathlessness) is a hallmark of chronic obstructive pulmonary disease (COPD) regardless of severity and is the primary reason for exercise intolerance even in patients with mild COPD (defined as a forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) ratio below the lower limit of normal (LLN) and a $FEV_1 \geq 80\%$ predicted)[49, 70, 146]. Dyspnea has been shown to profoundly reduce physical activity and quality of life in COPD, both of which are associated with increased mortality [4, 93, 183]. Previous research in mild COPD has demonstrated that exertional dyspnea is the result of increased work of breathing during exercise [70], and that this increased work of breathing comes from: 1) an exaggerated ventilatory response to exercise (*i.e.* increased minute ventilation relative to carbon dioxide production, $\dot{V}_E/\dot{V}CO_2$), and 2) airflow limitation (*i.e.* dynamic hyperinflation)[49, 73, 112, 131, 144]. A great deal of research has focused on improving airflow limitation in COPD; however, very little has been done to understand and treat the exaggerated ventilatory response to exercise in mild COPD.

Previous studies in mild COPD have consistently shown an elevated $\dot{V}_E/\dot{V}CO_2$ response during exercise [49, 70, 146, 191] and it is an independent predictor of mortality [131]. This increased $\dot{V}_E/\dot{V}CO_2$ in mild COPD appears to be secondary to increased deadspace ventilation [49], which results in a compensatory increase in total minute ventilation to maintain alveolar ventilation and arterial blood gas homeostasis. The reasons for the increased deadspace and ventilatory demand during exercise in mild COPD are currently unclear, however, pulmonary microvascular abnormalities and hypoperfusion of pulmonary capillaries are potential mechanisms [92, 174, 206]. Patients with mild COPD have reduced pulmonary microvascular blood flow in nonemphysematous lung regions, suggesting that the low pulmonary perfusion in an intact

capillary bed is secondary to pulmonary vascular dysfunction [92]. Recently, Tedjasaputra *et al.* demonstrated a blunted pulmonary capillary blood volume response to exercise in patients with mild COPD which was associated with increased $\dot{V}_E/\dot{V}CO_2$ during exercise [206]. Collectively, these findings suggest that pulmonary vascular dysfunction in mild COPD leads to pulmonary capillary hypoperfusion and elevated deadspace during exercise, resulting in a greater $\dot{V}_E/\dot{V}CO_2$.

Inhaled nitric oxide (iNO) is a selective pulmonary vasodilator and has been shown to improve pulmonary vascular function and peak oxygen consumption in patients with pulmonary arterial hypertension [81, 94, 156] and heart failure [123]. Despite emerging evidence that COPD is associated with pulmonary vascular dysfunction [92, 206], limited research has focused on experimentally modulating the pulmonary vasculature during exercise in mild COPD. Therefore, using iNO as a novel vascular intervention, the current study tested the hypotheses that: 1) iNO would improve ventilatory efficiency (*i.e.* lower $\dot{V}_E/\dot{V}CO_2$) in mild COPD; and 2) this reduction in ventilation would be associated with improvements in exertional dyspnea and exercise capacity. Should iNO improve ventilatory efficiency and exertional dyspnea in mild COPD, these findings would help identify a vascular target to improve exercise capacity in mild COPD.

5.2 Materials and methods

5.2.1 Participants

The study included fifteen participants with mild COPD (FEV_1/FVC < lower limit of normal (LLN) and $FEV_1 \geq 80\%$ predicted [166]) with a smoking history (>10 pack-years) and fifteen age- and risk-matched healthy controls with minimal smoking history (<10 pack-years) and normal lung function. Individuals with cardiovascular, metabolic, neuromuscular or any other disease that could contribute to dyspnea or abnormal cardiopulmonary responses to exercise were excluded. Individuals that were currently using oral steroids, phosphodiesterase type 5 (PDE5)

inhibitors or supplemental O_2 were excluded. All participants were non-hypoxemic and non-hypercapnic. Individuals with evidence of cardiac dysfunction (ejection fraction <50%) or pulmonary hypertension (right ventricular systolic pressure > 40 mmHg) were excluded.

5.2.2 Study design

The study (Figure 5.1) was a single-centre, randomized, double-blind, placebo controlled, crossover trial (ClinicalTrials.gov Identifier: NCT03679312) and received ethical approval from Health Canada and the University of Alberta Health Research Ethics Board (Biomedical Panel Pro00078715).

After providing written informed consent, all participants completed four sessions over a 4-week period. Session 1 included medical history screening, post-bronchodilator (400ug salbutamol) pulmonary function testing and a symptom-limited incremental cardiopulmonary cycle exercise test (CPET) to determine peak work rate. Session 2 included a resting echocardiography session to evaluate cardiac function. On sessions 3 and 4, participants completed, on separate days, two randomly ordered CPETs (1:1 ratio) while breathing either normoxia (placebo, Visit 3) or 40 ppm iNO (Visit 4). Participants were block randomized in a 1:1 allocation ratio. Participants were asked to abstain from their short- and long-acting bronchodilators for 24 hours prior to testing and, avoid caffeine, smoking, vigorous exercise and alcohol for at least 6 hours prior to testing. Additionally, a subset of 7 mild COPD patients consented to complete two constant work-rate cycle exercise tests to symptom limitation at 75% of the maximal work rate, on separate days, while breathing either placebo (Session 5) or iNO (Session 6), with the order of testing days randomized.

5.3.3 Intervention

A 40 ppm dose of iNO was given using a customized NO delivery system (SoKINOX, Vitalaire, Ontario, Canada). Briefly, the device consisted of a non-rebreathing circuit connected to a flow sensor and NO was delivered after dilution with oxygen. The placebo condition consisted of participants breathing medical grade normoxic gas (21% O_2 and balance N_2) delivered by the same non-rebreathing system (soKINOX) as the NO condition. Both NO and placebo tanks were setup in tandem, all identifying information was removed and both cylinder tanks appeared identical. A 5-minute wash-in was completed prior to all experimental exercise trials, regardless of condition. Only the research assistant and supervising physician were aware of the condition (placebo or iNO). Neither the study participant nor the lead researcher directly conducting the exercise trials were aware of the condition.

Formation of nitric dioxide (NO_2) during NO breathing can potentially lead to pulmonary edema, however, it is extremely uncommon while breathing NO at doses of $\leq$80 ppm[190]. Throughout the trial, inspired O_2, NO and NO_2 were closely monitored with gas analyzers (soKINOX, Vitalaire, Ontario, Canada). Additionally, Methemoglobin (METHb) was continuously monitored using carbon-monoxide oximetry (Radical 7, Masimo, Irvine, CA, USA). NO_2 and METHb did not rise above 2 ppm and >5%, respectively, during any trials.

5.2.4 Pulmonary function

Spirometry, plethysmography and diffusing capacity for carbon monoxide (D_{LCO}) measurements were completed as per current guidelines [118, 127, 226]. Measurements were expressed as percent of predicted normal values [75] or as z-scores [166, 187].

5.2.5 Resting echocardiography

Echocardiographic images were collected to allow the assessment of cardiac structure and function in accordance with current guidelines [109]. Images were collected using a commercially available ultrasound system (Vivid Q, GE Healthcare, Fairfield, CT, USA) and 1.5 – 3.6 MHz phased array transducers with the participant in a semi-supine position. A single-lead electrocardiograph (ECG) was attached to the participant and connected to the ultrasound system for heart rate monitoring. Participants then rested quietly for 10 min to obtain baseline data. Following baseline, images were collected while participants breathed either placebo or iNO. Although the washout time for NO is very quick (*i.e*. less than 1-minute)[94], each intervention was separated by at least 5-minutes to ensure all physiological parameters returned to baseline before continuing to the next condition [156]. All echocardiograms were performed by an experienced sonographer and were acquired within a range of 70-90 frames per second. All echocardiogram analysis was completed by the same sonographer, blinded to the experimental condition (EchoPAC, GE Healthcare, Fairfield, CT, USA).

Resting left ventricular (LV) stroke volume was estimated using continuous-wave Doppler ultrasound of the aortic blood velocity time integral tracing of five heartbeats from a 4-chamber view. The cross-sectional area of the left ventricular outflow tract (LVOT) was determined using two-dimensional ultrasound in the parasternal short-axis view. The average velocity was then multiplied by the cross-sectional area of the LVOT to determine stroke volume. Cardiac output was calculated as a product of stroke volume and heart rate measured on the ECG at the time of the velocity time integral. Using two-dimensional echocardiography (apical 4- and 2-chamber images), left (Simpson's biplane) and right (single plane) was used to calculate end-diastolic

volume (EDV) and end-diastolic volume (ESV). Both left and right ventricular ejection fraction (EF) were calculated using the following formula:

$$EF = (EDV\text{-}ESV)/EDV \quad [109]$$

Right ventricular systolic pressure (RVSP) was calculated using tricuspid regurgitant peak velocity measured, using continuous wave Doppler in the apical 4-chamber view, and estimated right atrial pressure (RAP), in the following formula:

$$RVSP = 4(V_{TR})^2 + RAP \quad [177]$$

Right atrial pressure was estimated through imaging of the inferior vena cava (IVC) from a subcostal view at rest and during an inspiratory sniff. The RAP was estimated to be 3 mmHg if the diameter of the IVC was < 2.1 cm and collapsed >50% with a sniff test. If IVC diameter was > 2.1 cm and collapsed <50% with a sniff test, RAP value was estimated at 15 mmHg. If either IVC diameter was >2.1 or the collapsibility was less than 50%, an intermediate value of 8 mmHg was used as the RAP estimate [109, 177]. Global right ventricular systolic function was assessed by the tricuspid annular plane systolic excursion (TAPSE) using an M-mode apical 4-chamber image with the cursor placed on the tricuspid lateral annulus. During the echocardiography trials, systemic arterial blood pressure (manual auscultation of brachial artery) and arterial saturation (pulse oximetry) data were collected simultaneously.

5.2.6 Cardiopulmonary exercise testing

Exercise tests consisted of a 5-minute resting period followed by 2-minutes of unloaded pedaling then a 20 W increase in work rate every 2-minutes to symptom limitation. Peak work rate was defined as the highest work rate that the participant was able to maintain for $\geq$30 seconds. Participants rated their perceived breathing and leg discomfort using the modified Borg scale [12]

at steady-state rest, within the last 30 seconds of every 2-minute exercise interval and at the end of exercise, followed by an inspiratory capacity (IC) maneuver. All ventilatory and cardiovascular measurements were collected over the first 30 seconds of every second minute during the exercise test and were linked with the perceptual ratings and IC measurements collected in the final 30 seconds of the respective minute to avoid contamination of the expired gas data from the IC maneuvers. At symptom limitation, participants completed an IC maneuver immediately followed by the Borg ratings of breathing and leg discomfort, and ventilatory and cardiovascular measurements were averaged over the last 30 seconds of exercise. Participants were asked to select qualitative phrases that best described how their breathing felt at peak exercise [182].

All exercise tests were performed on an electronically braked cycle ergometer (Ergoselect II 1200 Ergoline, Blitz, Germany) using a cardiorespiratory metabolic measurement system (Encore229 Vmax, SensorMedics, Yorba Linda, CA, USA). Arterial O_2 saturation (S_pO_2) was estimated using finger pulse oximetry (N-595; Nellcor Oximax, Boulder, CO, USA). Heart rate was measured using electrocardiography (CardioSoft, GE Medical Systems, Milwaukee, WI, USA). Arterial blood pressure was taken by manual auscultation. Exercise cardiac output (Q) was continuously monitored beat-by-beat and recorded in 30-second averages with impedance cardiography (PhysioFlow, Manatec, Paris, France). Impedance cardiography provides an accurate determination of cardiac output at rest and during exercise [116], and is preferred over other non-invasive techniques such as inert gas rebreathing as these can be inaccurate in lung disease. Systemic vascular conductance was calculated as Q/mean arterial pressure.

5.2.7 Constant work-rate exercise tests

Constant work-rate exercise tests were completed in a subset of 7 mild COPD patients. Following a 5-minute steady-state resting period, participants completed a 1-minute period of

unloaded pedaling followed by an immediate increase in work rate corresponding to 75% of the peak work rate. Exercise endurance time was recorded from the onset of loaded cycle exercise to the point of symptom limitation. All measurement procedures were identical to the incremental exercise test.

5.2.8 Outcome variables

The primary study outcome was the effect of iNO on exercise capacity, defined as the peak oxygen uptake ($\dot{V}O_{2peak}$), compared to placebo. Secondary outcomes included the effect of iNO on physiological and perceptual responses at rest and during exercise.

5.2.9 Analysis

Previous research in patients with pulmonary hypertension demonstrated an 18 ± 16 % increase in $\dot{V}O_{2peak}$ with iNO [81]. Assuming a similar effect size in mild COPD, 15 patients would be sufficient to detect an effect of iNO on $\dot{V}O_{2peak}$ (α=0.05, power=0.8).

Data are presented as mean ± standard deviation (SD) unless otherwise stated. For all inferential analysis, statistical significance was set *a priori* at $p<0.05$. Unpaired t-analysis was used to evaluate subject characteristics and pulmonary function between groups. A multifactorial repeated measures analysis of variance (ANOVA) was used to analyze the effects of placebo vs iNO at rest and during exercise (repeated factor) in mild COPD and controls (fixed factor) in all primary and secondary outcomes. If a significant change or interaction effect was found, a multiple comparison Bonferroni T-test was completed to locate the differences. Qualitative descriptors of dyspnea were analyzed using Fisher's exact test. All statistical analyses were performed using Sigma Plot Software version 13.0 (Systat Software Inc., San Jose, CA, USA).

5.3 Results

5.3.1 Participants

Descriptive characteristics and resting pulmonary function data are displayed in Table 5.1. Mild COPD patients had a significantly greater smoking history than controls, and all but three mild COPD patients were ex-smokers. No individuals in the control group were current smokers, and 10 were never smokers. As expected, all mild COPD patients were below the LLN for FEV_1/FVC, while all controls were within normal range. Mild COPD patients had greater residual volume, compared to controls. There were no between-group differences in total lung capacity, vital capacity, functional residual capacity or inspiratory capacity. Mild COPD patients had a significantly reduced D_{LCO}, despite no between-group differences in alveolar volume.

Resting echocardiography data are displayed in Table 5.2. All mild COPD patients and controls had a left ventricular ejection fraction above 50% and a right ventricular systolic pressure (RVSP) below 40 mmHg. RVSP and tricuspid annular plane systolic excursion were not different between groups (p=0.091, p=0.524, respectively). Inhaled NO reduced RVSP in both groups (COPD p=0.017, Control p=0.035), while ultrasound-derived stroke volume and cardiac output, systemic arterial blood pressure and SpO_2 were unaffected.

5.3.2 Primary outcomes

Compared with placebo, iNO significantly increased $\dot{V}O_{2peak}$ by 19 ± 19 % (placebo: 1.53 ± 0.10 vs. iNO: 1.80 ± 0.14 $L \cdot min^{-1}$, effect size (ES) =0.997, p<0.001) in mild COPD (Table 5.3, Figure 5.2), while no effect was observed in healthy controls (placebo: 2.57 ± 0.20 vs iNO 2.58 ± 0.19 $L \cdot min^{-1}$, p=0.93, ES=0.01) (Table 5.3, Figure 5.3).

5.3.3 Secondary outcomes

5.3.3.1 Physiological responses

Selected physiological responses for both groups are displayed in Table 5.3. Additionally, selected physiological responses to incremental exercise in mild COPD are displayed in Figure 5.4 and an identical Figure for controls is available in Figure 5.5. During exercise, $\dot{V}_E/\dot{V}CO_2$ was higher ($p<0.001$) in COPD, compared to controls. $\dot{V}_E/\dot{V}CO_2$ was significantly reduced with iNO at all sub-maximal exercise intensities in COPD ($p<0.001$), when compared to placebo, while no effect was observed in controls ($p=0.470$). At the highest equivalent work rate of 60 Watts, iNO reduced $\dot{V}_E/\dot{V}CO_2$ by 3.8 ± 4.2 in COPD, while no effect was observed in controls (Table 5.2). The $\dot{V}_E/\dot{V}CO_2$ nadir, slope and y-intercept were reduced in COPD ($p<0.001$, $p=0.042$, $p=0.049$, respectively) and unaffected in controls (Figure 5.6). Although $\dot{V}_E/\dot{V}CO_2$ was reduced with iNO in mild COPD, it remained significantly elevated when compared to the healthy control placebo condition (Figure 5.7). An interaction was observed for minute ventilation ($p=0.010$) wherein $\dot{V}_E$ was reduced at both 40 and 60 W ($p=0.027$, $p=0.041$, respectively) and increased at peak exercise ($p=0.015$) with iNO in COPD, while no effect was observed in controls.

The lower $\dot{V}_E$, in COPD, was secondary to a reduction in breathing frequency ($p<0.01$). Tidal volume was unaffected by iNO in both groups ($p=0.776$). During exercise, COPD patients breathed at a higher EELV and EILV than controls (both $p<0.001$). However, EELV and EILV were unaffected by iNO ($p=0.472$, $p=0.776$, respectively) in both groups.

During exercise, P_{ETCO2} was lower in COPD compared to controls ($p<0.01$)(Table 5.3). Further, P_{ETCO2} was significantly greater with iNO at 20 and 40 W in COPD ($p=0.034$, $p=0.035$, respectively), while no effect was observed in controls ($p=0.703$, $p=0.747$). During exercise, SpO_2

was lower in COPD compared to controls ($p<0.001$), however, SpO_2 was unaffected by iNO in either group ($p=0.249$)(Table 5.3).

A main effect for group was observed for impedance-cardiography derived Q ($p=0.002$), and post-hoc analysis revealed that Q was lower in COPD at peak exercise ($p<0.001$), while no between-group differences were observed at all submaximal intensities. Further, Q was unaffected by iNO in either group ($p=0.917$). Total vascular conductance was similar between groups ($p=0.447$) and unaffected by iNO ($p=0.905$) during exercise (Table 5.3).

5.3.3.2 Perceptual responses

A group by condition interaction was observed for exertional dyspnea ($p=0.007$). Dyspnea was reduced at 40 and 60 W (both $p<0.001$) with iNO in COPD (Figure 5.8), while no effect was observed in controls ($p=0.392$). At the highest equivalent work rate of 60 W, iNO reduced dyspnea by 1.1 ± 1.2 Borg units in COPD, while no effect was observed in controls (Table 5.3). Dyspnea relative to ventilation was unaffected by iNO in mild COPD (Figure 5.8). The qualitative descriptors of dyspnea at peak exercise are displayed in Figure 5.9. The dominant qualitative descriptor was the sensation of increased work/effort in both COPD and controls. The qualitative descriptors of unsatisfied inspiration and inspiratory difficulty were selected more frequently in COPD *versus* controls at peak exercise ($p=0.028$, $p=0.035$, respectively). However, iNO had no effect on selection frequency of the qualitative descriptors of dyspnea at peak exercise, within each group. During exercise, perceived leg discomfort was higher in COPD compared to controls ($p<0.026$), however, leg discomfort was unaffected by iNO in either group ($p=0.236$).

5.3.3.3 Constant work-rate exercise tests

Results from the constant work-rate exercise tests are displayed in Figure 5.10 and Table 5.4. Compared with placebo, iNO significantly increased exercise endurance time by 169 ± 137 s

(p=0.017) in COPD patients. At a standard exercise time of 4-minutes, iNO reduced $\dot{V}_E$ by 4.5 ± 3.8 L·min^{-1} (p=0.020), $\dot{V}_E/\dot{V}CO_2$ by 2.6 ± 1.9 (p=0.011) and dyspnea by 0.6 ± 0.5 Borg units (p=0.017).

5.4 Discussion

The main findings from the current study are twofold. First, iNO improved exertional dyspnea and exercise capacity in patients with mild COPD, while no effect was observed in healthy age-matched controls. Second, the lowered dyspnea ratings, in mild COPD, were secondary to significant reductions in $\dot{V}_E/\dot{V}CO_2$, $\dot{V}_E$ and f_B at sub-maximal exercise intensities, without significant changes in operating lung volume, the dyspnea/$\dot{V}_E$ relationship or arterial O_2 saturation. As iNO increased nitric oxide bioavailability, we observed a reduction in RVSP, $\dot{V}_E/\dot{V}CO_2$ and dyspnea and a corresponding increase in $\dot{V}O_{2peak}$, these data suggest that pulmonary vascular dysfunction is a key contributor to heightened dyspnea and exercise limitation in patients with mild airflow obstruction.

5.4.1 Clinical importance

Compared with placebo, iNO reduced dyspnea during exercise at 40 and 60 W by 1.0 and 1.1 Borg units, respectively, in mild COPD. The magnitude of these improvements exceeded the minimal clinically importance difference (MCID) of 1 Borg unit [172]. Inhaled NO reduced the nadir $\dot{V}_E/\dot{V}CO_2$ by 2.9 units and increased $\dot{V}O_{2peak}$ by 0.27 L·min^{-1} in mild COPD. Although there is no established MCID for nadir $\dot{V}_E/\dot{V}CO_2$ or $\dot{V}O_{2peak}$, both variables (*i.e.* high $\dot{V}_E/\dot{V}CO_2$ and low $\dot{V}O_{2peak}$) are independent predictors of mortality in COPD [131, 149]. In our subset of COPD patients (n=7) that completed the constant-load exercise test, iNO improved exercise endurance time by 169 seconds, which exceeded the MCID (101 seconds) in COPD [164]. When combined, these data show clear evidence that acute improvements in pulmonary vascular function with iNO

resulted in clinically meaningful improvements in dyspnea and exercise capacity in patients with mild COPD. Further, these data highlight new areas of exploration for therapeutic interventions to improve ventilatory efficiency, exercise capacity and ultimately quality of life in COPD.

5.4.2 Physiological and perceptual response to iNO in mild COPD

Multiple studies have demonstrated an elevated $\dot{V}_E/\dot{V}CO_2$ during exercise in mild COPD [49, 70, 146, 206], however, the current study is one of the first to experimentally reduce $\dot{V}_E/\dot{V}CO_2$ without affecting airways mechanics or arterial O_2 saturation. The underlying mechanism of elevated $\dot{V}_E/\dot{V}CO_2$ in mild COPD is not well understood, however, Elbehairy *et al*. demonstrated that the increased exercise $\dot{V}_E/\dot{V}CO_2$ in mild COPD was secondary to increased deadspace ventilation [49]. It is possible that pulmonary microvascular abnormalities may be a key contributor to the increased deadspace ventilation and $\dot{V}_E/\dot{V}CO_2$ during exercise in mild COPD. Dinh-Xuan *et al*. found that patients with advanced COPD have impaired pulmonary vascular function [45]. Using contrast enhanced magnetic resonance imaging, Hueper *et al*. recently demonstrated reduced resting pulmonary microvascular blood flow in nonemphysematous lung regions in mild COPD patients [92]. Rodriguez-Roisin *et al*. found that ventilation/perfusion ($\dot{V}_A/\dot{Q}$) inequality was greater than expected in mild COPD patients at rest, leading the authors to conclude that the $\dot{V}_A/\dot{Q}$ inequality in mild COPD was the result of reduced pulmonary vascular perfusion [174]. Tedjasaputra *et al*. recently demonstrated a blunted pulmonary capillary blood volume response to exercise in mild COPD compared to age- and height-matched controls [206]. The low pulmonary capillary blood volume was associated with increased $\dot{V}_E/\dot{V}CO_2$ during exercise, suggesting that low pulmonary perfusion leads to deadspace and/or regions of high $\dot{V}_A$ relative to $\dot{Q}$ [206]. Collectively, these findings suggest that pulmonary vascular dysfunction likely occurs in mild COPD, independent of emphysema, leading to $\dot{V}_A/\dot{Q}$ mismatch and increased

alveolar deadspace, resulting in ventilatory inefficiency (*i.e.* greater $\dot{V}_E/\dot{V}CO_2$) and dyspnea. Iyer *et al.* demonstrated an improvement in pulmonary microvascular blood flow following administration of sildenafil (PDE5 inhibitor) in smokers with preserved spirometry and early emphysema [95]. In the current study, the iNO-mediated reduction in $\dot{V}_E/\dot{V}CO_2$ during exercise suggests that iNO increased pulmonary microvascular perfusion, leading to improved $\dot{V}_A/\dot{Q}$ matching, reduced deadspace ventilation and therefore reduced ventilation for a given metabolic demand. Although the nadir $\dot{V}_E/\dot{V}CO_2$ (global index of ventilatory efficiency) was reduced by 2.9 units with iNO in mild COPD, it remained 3.2 units higher than controls. This would suggest that 48% of the elevated $\dot{V}_E/\dot{V}CO_2$ observed during exercise in mild COPD could be attributed to pulmonary microvascular dysfunction, while the remaining 52% is likely due to other factors including pulmonary microvascular destruction (*i.e.* emphysema and capillary bed obliteration). Our results build on the findings of Iyer *et al.* [95] and provide novel evidence that partially-reversible pulmonary microvascular dysfunction is a contributor to pulmonary-gas exchange abnormalities and exercise intolerance in mild COPD patients.

Previous research by Guenette *et al*. demonstrated elevated respiratory neural drive and total work of breathing during incremental cycle exercise in patients with mild COPD [70]. Further, the increased sensation of dyspnea observed in the study was secondary to a widened dissociation between respiratory neural drive and the mechanical response of the respiratory system [70]. The authors concluded that the observed neuromechanical dissociation was secondary to a combination of increased ventilatory demand and mechanical constraint [70]. In the current study, the observed reduction in ventilation and dyspnea in mild COPD took place in the absence of any changes in operating lung volume. When combined with the lack of change in airway mechanics (*i.e.* operating lung volume), and the similar dyspnea/ventilation relationship between

conditions (iNO vs placebo), these data provide strong evidence that the reduction in dyspnea with iNO is secondary to reduced ventilatory demand. The reduction in ventilation was secondary to reduced breathing frequency and not tidal volume or operating lung volume. It is unlikely that iNO affected the position of tidal volume on the sigmoid pressure-volume relationship which suggests no change in mechanical constraint on tidal volume expansion, between conditions, during exercise in mild COPD. It is likely that the reduced tachypnea decreased the velocity of shortening of inspiratory muscles, leading to a reduction in respiratory neural drive and improvement in neuromechanical coupling, and thus, a lower sensation of dyspnea [70]. Our data suggest that the iNO-mediated reduction in dyspnea at sub-maximal exercise intensities, likely the result of reduced respiratory neural drive, caused mild COPD patients to delay their symptom limitation to exercise which allowed for exercise at a higher metabolic load (*i.e.* higher $\dot{V}CO_2$) despite no changes in airway mechanics. However, without detailed measurements of respiratory mechanics and neural drive, these comments are speculative. To our knowledge, the current study is the first study to demonstrate that experimentally modulating the pulmonary microvasculature, and not the airways, can positively impact dyspnea and exercise capacity in mild COPD.

5.4.3 Considerations

The current study had a small sample size and the generalizability of our results are restricted to a small relatively homogenous group of patients with mild COPD. However, the sample size (n=15 COPD) and effect size (ES=0.96, pre/post correlation=0.91) were large enough to detect a significant difference in $\dot{V}O_{2peak}$, with 99.7% power (α=0.05).

Although we observed a reduction in $\dot{V}_E/\dot{V}CO_2$ with iNO, arterial blood gas data were not obtained, and therefore our conclusion that reduced $\dot{V}_E/\dot{V}CO_2$ was secondary to reduced deadspace is speculative. Future experiments need to evaluate pulmonary gas exchange using the multiple

inert gas elimination technique, which allows for quantification of pure deadspace (ventilation with no perfusion) and high $\dot{V}_A/\dot{Q}$ regions of the lung (ventilation with low relative perfusion). Should iNO improve $\dot{V}_A/\dot{Q}$ and reduce deadspace, this would clearly establish that partially-reversible vascular dysfunction contributes to the heightened ventilatory demand and dyspnea during exercise in mild COPD.

It is unclear whether iNO would improve ventilatory efficiency, dyspnea and exercise capacity in more severe COPD. With advancing COPD, there would be capillary destruction, pulmonary arterial hypertension, and hypoxemia. In the presence of microvascular destruction, iNO is likely ineffective at improving pulmonary capillary perfusion and thus $\dot{V}_A/\dot{Q}$ matching. Further, with advanced disease, hypoxic pulmonary vasoconstriction (HPV) occurs in an attempt to optimize $\dot{V}_A/\dot{Q}$ matching[174], and release of HPV may actually worsen $\dot{V}_A/\dot{Q}$ matching, resulting in hypoxemia, greater ventilation and dyspnea, and further impaired exercise capacity.

When inhaled, NO diffuses from the airway epithelium into the subjacent vascular smooth muscle, where it binds with guanylate cyclase and produces cyclic guanosine monophosphate (cGMP), resulting in smooth muscle relaxation [94]. Free NO in the blood binds to hemoglobin and is rapidly inactivated, and therefore has minimal effects on systemic smooth muscle[66]. Although we did not directly measure pulmonary vascular function (*i.e.* smooth muscle relaxation or NO concentration within the pulmonary arteries, we observed an iNO-mediated reduction in RVSP, while no changes in systemic vascular regulation, airway function or cardiac function were observed. As cardiac output and systemic vascular conductance were unaffected by iNO, it is unlikely that the improvement in $\dot{V}O_{2peak}$, in the current study, is the result of improved central hemodynamics. These data strongly suggest that our intervention successfully targeted the pulmonary vascular smooth muscle to reduce pulmonary vascular resistant and improve perfusion.

5.5 Conclusions

Inhaled NO increased exercise capacity in mild COPD, secondary to reduced ventilation and dyspnea. These data suggest that mild COPD patients demonstrate pulmonary microvascular dysfunction that contributes to increased $\dot{V}_E/\dot{V}CO_2$, dyspnea and exercise intolerance. Our findings help to explain why patients with relatively preserved airways demonstrate disproportionately greater dyspnea relative to the degree of airway obstruction and help to identify a pulmonary vascular target to improve dyspnea, exercise capacity, and by extension quality of life in mild COPD.

5.6 References

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Table 5.1 Participant characteristics

Parameter	Control	Mild COPD
Subjects (n)	15	15
Males/females (n)	8/7	11/4
Age (years)	61 ± 13	65 ± 10
Height (cm)	172.4 ± 9.0	171.3 ± 7.5
Mass (kg)	80.5 ± 15.3	76.4 ± 11.6
Body mass index ($kg \cdot m^{-2}$)	27.1 ± 4.4	27.2 ± 4.1
Smoking history (pack-years)	6 ± 3	32 ± 13
mMRC score (0-4)	0.1 ± 0.3	1.0 ± 0.8**
Peak incremental $\dot{V}O_2$ (% pred)	119 ± 25	82 ± 20***
Peak incremental work rate (% pred)	117 ± 29	78 ± 22**
Dyspnea/$\dot{V}O_2$ slope (Borg unit/$ml \cdot kg^{-1} \cdot min^{-1}$)	0.20 ± 0.02	0.37 ± 0.04**
Medication use n		
SABA	0	10
LABA	0	10
ICS	0	8
Combined ICS/LABA	0	10
Pulmonary function		
Post-bronchodilator		
FEV_1 (L)	3.42 ± 0.98	2.46 ± 0.6***
FEV_1 (% pred)	110 ± 16	89 ± 11***
FEV_1 (z-score)	0.60 ± 1.18	-1.12 ± 0.66***
FEV_1/FVC (%)	75 ± 5	60 ± 8***
FEV_1/FVC (% pred)	99 ± 6	82 ± 9***
FEV_1/FVC (z-score)	-0.14 ± 0.63	-2.10 ± 0.56***
Pre-bronchodilator		
FEV_1 (L)	3.33 ± 0.93	2.43 ± 0.62***
FEV_1 (% pred)	107 ± 14	87 ± 11***
FEV_1/FVC (%)	75 ± 5	60 ± 8***
FEV_1/FVC (% pred)	99 ± 6	82 ± 9***
SVC (% pred)	106 ± 18	98 ± 32
IC (% pred)	109 ± 25	107 ± 16
FRC (% pred)	107 ± 17	118 ± 23
RV (% pred)	94 ± 23	121 ± 31**
TLC (% pred)	102 ± 17	112 ± 23
D_{LCO} ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	26.3 ± 6.1	17.9 ± 7.3**
D_{LCO} (% pred)	96 ± 15	68 ± 21**
D_{LCO} (z-score)	0.22 ± 0.96	-2.05 ± 1.74***
V_A (L)	5.95 ± 1.61	5.35 ± 1.26
V_A (% pred)	92 ± 15	85 ± 14
V_A (z-score)	-0.01 ± 1.33	-0.72 ± 1.13
TLC - V_A (L)	0.63 ± 0.55	1.54 ± 0.92**

Values are mean ± SD. mMRC: modified medical research council; $\dot{V}O_2$: oxygen uptake; SABA: short-acting β-agonist; LABA: long-acting β-agonist; ICS: inhaled corticosteroid; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; SVC: slow vital capacity; IC: inspiratory capacity; FRC: functional residual capacity; RV; residual volume; TLC: total lung capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; V_A: alveolar volume. * p<0.05; **p<0.01; ***P<0.001

Table 5.2. Resting echocardiographic and cardiovascular data with either placebo or inhaled nitric oxide (iNO) in controls and mild COPD.

	Control		Mild COPD	
Parameter	**Placebo**	**iNO**	**Placebo**	**iNO**
LV-EDV (ml)	89 ± 22	84 ± 17	82 ± 14	79 ± 13
LV-ESV (ml)	30 ± 11	32 ± 6	32 ± 6	30 ± 8
LV-EF (%)	68 ± 3	66 ± 2	61 ± 2	61 ± 7
RV-EDV (ml)	36 ± 8	34 ± 14	43 ± 8	34 ± 7
RV-ESV (ml)	14 ± 5	13 ± 5	20 ± 4	17 ± 4
RV-EF (%)	61 ± 8	61 ± 7	53 ± 5	50 ± 4
TAPSE (mm)	21. 8 ± 2.2	22.4 ± 1.8	21.0 ± 3.0	20.2 ± 3.0
RVSP (mmHg)	18.0 ± 5.8	14.5 ± 4.7*	24.4 ± 9.4	20.7 ± 8.0*
MAP (mmHg)	89.0 ± 11.0	90.2 ± 10.8	89.4 ± 8.2	88.8 ± 7.0
HR (beats·min^{-1})	66 ± 10	62 ± 7	72 ± 12	72 ± 12
SV (ml·beat^{-1})	48 ± 8	49 ± 9	48 ± 7	49 ± 9
Q (L·min^{-1})	3.4 ± 0.5	3.4 ± 0.3	3.4 ± 0.5	3.4 ± 0.3
S_pO2 (%)	96.1 ± 1.2	95.4 ± 1.1	94.7 ± 2.3	95.3 ± 2.6
FiNO (ppm)	0.0 ± 0.0	39.9 ± 2.6	0.0 ± 0.0	40.4 ± 0.7
$FiNO_2$ (ppm)	0.00 ± 0.00	0.19 ± 0.33	0.00 ± 0.00	0.03 ± 0.05
FiO_2	0.21 ± 0.00	0.21 ± 0.00	0.21 ± 0.00	0.21 ± 0.00
MET Hb (%)	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.2 ± 0.3

Data are presented as mean ± SD. iNO: inhaled nitric oxide; COPD: chronic obstructive pulmonary disease; LV-EDV: left ventricle end-diastolic volume; LV-ESV: left ventricle end-systolic volume; LV-EF: left ventricle ejection fraction; RV-EDV: right ventricle end diastolic volume; RV-ESV: right ventricle end-systolic volume; RV-EF: right ventricle ejection fraction; TAPSE: tricuspid annular plane systolic excursion TR_{max}: peak velocity of tricuspid regurgitant jet; RVSP: right ventricular systolic pressure; MAP: mean arterial systemic blood pressure; HR: heart rate; SV: stroke volume; Q_{ECHO}: echocardiography-derived cardiac output; SpO_2: oxygen saturation estimated by pulse oximetry; FiNO: fraction of inspired nitric oxide; $FiNO_2$: fraction of inspired nitrogen dioxide; FiO_2: fraction of inspired oxygen; MET Hb: Methemoglobin estimated by pulse oximetry.

* $p<0.05$ between condition within group.

Table 5.3 Effect of inhaled nitric oxide (iNO) versus placebo on physiological and perceptual responses at rest, the highest equivalent work rate, and peak exercise in patients with mild COPD and healthy controls.

		Rest		HEWR (60 W)		Peak	
Parameter	**Group**	**Placebo**	**iNO**	**Placebo**	**iNO**	**Placebo**	**iNO**
Work Rate (W)	Control					172 ± 60	169 ± 58
	COPD					**101 ± 37**	**116 ± 41*****
Dyspnea Borg (0-10)	Control	0.1 ± 0.3	0.1 ± 0.3	1.7 ± 1.0	1.7 ± 1.1	6.3 ± 2.3	6.9 ± 2.2
	COPD	0.3 ± 0.6	0.3 ± 0.6	**3.3 ± 1.1**	**2.2 ± 0.8*****	5.6 ± 2.2	5.1 ± 2.2
Leg discomfort (Borg 0-10)	Control	0.0 ± 0.0	0.0 ± 0.0	1.9 ± 1.2	1.8 ± 1.1	6.8 ± 2.0	6.7 ± 2.0
	COPD	0.3 ± 0.7	0.2 ± 0.4	3.5 ± 1.4	2.7 ± 1.2	6.0 ± 2.0	5.9 ± 2.2
$\dot{V}O_2$ (L·min^{-1})	Control	0.38 ± 0.11	0.40 ± 0.11	1.15 ± 0.14	1.21 ± 0.16	2.57 ± 0.81	2.58 ± 0.80
	COPD	0.37 ± 0.10	0.38 ± 0.07	1.04 ± 0.14	1.11 ± 0.13	**1.53 ± 0.56**	**1.80 ± 0.65*****
$\dot{V}CO_2$ (L·min^{-1})	Control	0.36 ± 0.11	0.33 ± 0.10	1.05 ± 0.10	1.10 ± 0.15	2.92 ± 0.89	2.92 ± 0.92
	COPD	0.33 ± 0.09	0.34 ± 0.05	1.00 ± 0.16	1.08 ± 0.16	**1.67 ± 0.66**	**1.95 ± 0.74****
$\dot{V}_E$ (L·min^{-1})	Control	15.5 ± 4.5	13.9 ± 2.4	32.0 ± 4.1	32.5 ± 3.4	89.1 ± 30.5	89.1 ± 30.6
	COPD	15.7 ± 3.7	14.2 ± 0.8	**36.5 ± 7.0**	**35.1 ± 6.1***	**55.5 ± 15.7**	**62.8 ± 18.6***
$\dot{V}_E/\dot{V}CO_2$	Control	43.9 ± 6.2	44.3 ± 6.2	30.4 ± 2.7	29.7 ± 2.2	31.4 ± 2.2	31.3 ± 2.7
	COPD	49.2 ± 8.8*	41.8 ± 8.8	**36.4 ± 4.3**	**32.6 ± 3.5****	34.4 ± 4.6	33.3 ± 4.7
V_T (L)	Control	1.01 ± 0.38	0.96 ± 0.26	1.53 ± 0.29	1.62 ± 0.39	2.49 ± 0.82	2.48 ± 0.72
	COPD	1.03 ± 0.39	1.08 ± 0.30	1.70 ± 0.34	1.71 ± 0.28	1.99 ± 0.38	2.05 ± 0.41
f_B (breaths·min^{-1})	Control	16.2 ± 3.9	15.2 ± 3.6	21.4 ± 3.7	20.9 ± 4.2	35.9 ± 6.5	36.1 ± 8.7
	COPD	16.5 ± 4.8	14.1 ± 5.5	**22.8 ± 6.2**	**21.0 ± 5.5***	28.5 ± 7.1	30.5 ± 5.7
EELV (% TLC)	Control	55.1 ± 7.3	55.9 ± 7.2	54.3 ± 6.7	53.8 ± 6.1	55.4 ± 8.0	53.6 ± 6.9
	COPD	57.6 ± 7.3	56.3 ± 8.5	59.7 ± 7.4	57.7 ± 8.2	61.7 ± 7.9	60.4 ± 7.3
EILV (% TLC)	Control	70.6 ± 7.2	70.8 ± 5.9	78.5 ± 7.7	79.1 ± 6.9	92.9 ± 5.3	91.0 ± 3.6
	COPD	73.5 ± 7.7	73.1 ± 8.8	86.1 ± 5.9	84.1 ± 5.9	92.5 ± 5.1	92.2 ± 5.7
SpO_2 (%)	Control	96 ± 1	96 ± 1	96 ± 2	96 ± 1	94 ± 1	95 ± 2
	COPD	95 ± 2	95 ± 2	94 ± 3	94 ± 3	93 ± 3	94 ± 3
P_{ETCO2} (mmHg)	Control	34.6 ± 2.4	34.1 ± 2.4	38.2 ± 2.2	39.0 ± 2.6	35.5 ± 4.1	35.5 ± 4.1
	COPD	30.6 ± 3.7	32.3 ± 4.3	34.3 ± 4.8	35.3 ± 4.0	33.7 ± 4.4	34.9 ± 4.3
Q (L·min^{-1})	Control	5.1 ± 0.6	5.0 ± 1.0	8.1 ± 1.7	7.8 ± 1.7	14.0 ± 3.9	14.6 ± 2.8
	COPD	5.3 ± 0.6	5.3 ± 1.0	7.9 ± 0.9	7.8 ± 1.4	10.2 ± 1.7	10.6 ± 1.9
Q/MAP (ml·min^{-1}·mmHg^{-1})	Control	63 ± 13	55 ± 13	83 ± 15	83 ± 13	115 ± 31	132 ± 29
	COPD	65 ± 9	66 ± 15	88 ± 15	86 ± 17	105 ± 14	110 ± 18

Data are presented as mean ± SD. HEWR: highest equivalent work rate; COPD: chronic obstructive pulmonary disease; $\dot{V}O_2$: oxygen consumption; $\dot{V}CO_2$: carbon dioxide production; $\dot{V}_E$: minute ventilation; V_T: tidal volume; f_B: breathing frequency; EELV: end-expiratory lung volume; EILV: end-inspiratory lung volume; SpO_2: oxygen saturation estimated by pulse oximetry; P_{ETCO2}: partial pressure of end-tidal CO_2; Q: cardiac output; MAP, mean arterial pressure; Q/MAP, vascular conductance. * p<0.05; **p<0.01; ***P<0.001 between condition within group.

Table 5.4. Physiological and perceptual parameters at rest, isotime (4-minutes), and time to symptom limitation (T_{LIM}) during a constant-load cycle exercise test at 80 ± 25 W with placebo or iNO in mild COPD. n=7

	Rest		**Isotime**		**T_{LIM}**	
Parameter	**Placebo**	**iNO**	**Placebo**	**iNO**	**Placebo**	**iNO**
Exercise endurance time (min)			4.0 ± 0.0	4.0 ± 0.0	**10.4 ± 8.0**	**13.2 ± 7.3***
Dyspnea (Borg 0-10)	0.3 ± 0.8	0.4 ± 0.8	**3.5 ± 0.5**	**2.9 ± 0.7***	5.4 ± 2.1	5.6 ± 2.6
Leg discomfort (Borg 0-10)	0.3 ± 0.8	0.4 ± 0.8	4.0 ± 0.6	3.5 ± 0.8	5.9 ± 1.6	6.3 ± 2.4
$\dot{V}O_2$ ($L \cdot min^{-1}$)	0.40 ± 0.15	0.33 ± 0.08	1.50 ± 0.62	1.52 ± 0.59	1.66 ± 0.72	1.71 ± 0.66
$\dot{V}CO_2$ ($L \cdot min^{-1}$)	0.30 ± 0.11	0.30 ± 0.05	1.63 ± 0.70	1.60 ± 0.64	1.77 ± 0.78	1.73 ± 0.59
$\dot{V}_E$ ($L \cdot min^{-1}$)	15.6 ± 3.9	13.0 ± 2.6	**52.7 ± 18.0**	**48.2 ± 18.9***	58.2 ± 19.3	56.0 ± 16.0
$\dot{V}_E/\dot{V}CO_2$	46.9 ± 7.4	44.3 ± 9.3	**33.7 ± 5.4**	**31.1 ± 4.3***	34.3 ± 4.7	33.1 ± 4.2
V_T (L)	1.17 ± 0.46	1.02 ± 0.46	2.19 ± 0.44	2.25 ± 0.40	2.17 ± 0.33	2.16 ± 0.28
f_B ($breaths \cdot min^{-1}$)	13.9 ± 2.8	14.1 ± 5.4	24.0 ± 6.0	21.6 ± 5.7	26.6 ± 6.6	25.7 ± 4.9
EELV (% TLC)	59.5 ± 9.1	57.6 ± 2.8	59.5 ± 5.3	58.6 ± 8.2	59.1 ± 9.0	59.8 ± 8.0
EILV (% TLC)	77.3 ± 10.7	73.4 ± 8.7	93.4 ± 8.3	92.6 ± 6.9	92.7 ± 6.0	93.4 ± 4.6
SpO_2 (%)	95.6 ±	96.4 ± 2.3	95.4 ± 1.6	95.7 ± 2.2	94.4 ± 2.2	94.3 ± 2.6
P_{ETCO2} (mmHg)	31.2 ± 2.8	31.3 ± 3.1	35.3 ± 5.1	37.2 ± 4.8	33.9 ± 4.2	33.8 ± 2.7

Data are presented as mean ± SD. COPD: chronic obstructive pulmonary disease; $\dot{V}O_2$: oxygen consumption; $\dot{V}CO_2$: carbon dioxide production; $\dot{V}_E$: minute ventilation; V_T: tidal volume; f_B: breathing frequency; EELV: end-expiratory lung volume; EILV: end-inspiratory lung volume; SpO_2: oxygen saturation estimated by pulse oximetry; P_{ETCO2}: partial pressure of end-tidal CO_2. * $p<0.05$ between condition.

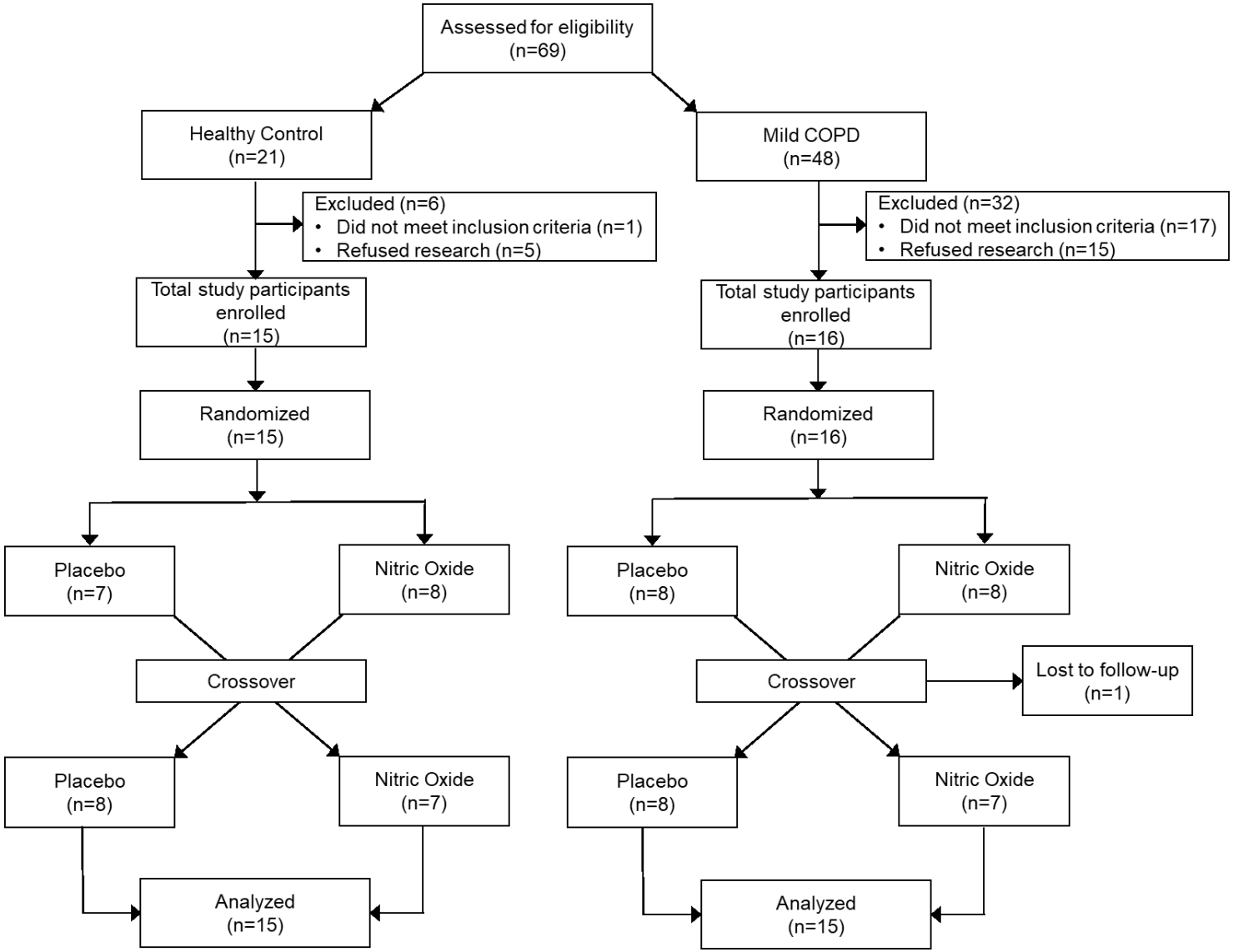


Figure 5.1. CONSORT diagram of study participants.

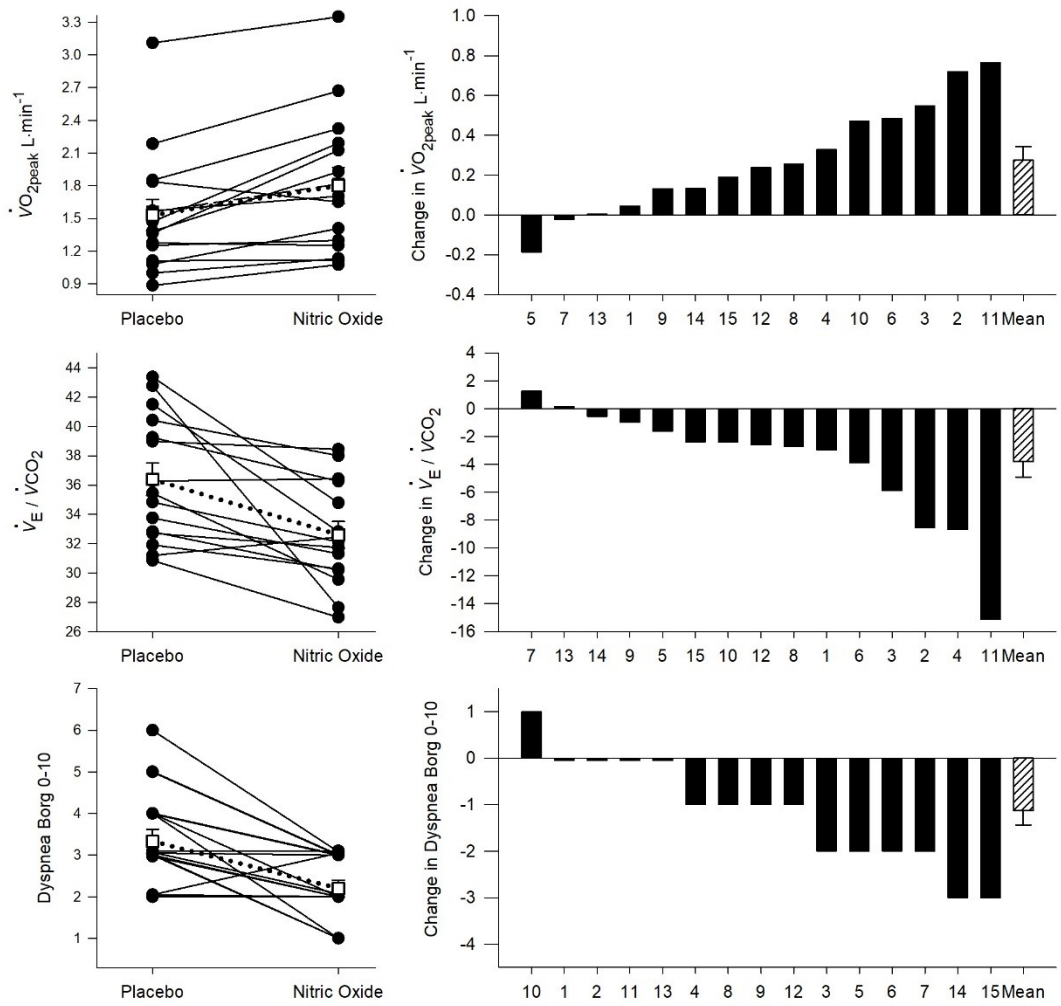


Figure 5.2. Individual values (left column) and post-dose differences (right column) in peak oxygen uptake ($\dot{V}O_{2peak}$), and ventilatory equivalent to carbon dioxide production ($\dot{V}_E/\dot{V}CO_2$) and exertional dyspnea at the highest equivalent work rate of 60 W in patients with mild chronic obstructive pulmonary disease (COPD). On left column, open square symbols with dashed horizontal lines denotes mean ± SD. On right column, change in outcomes reflects the post-dose difference (*i.e.* inhaled nitric oxide *minus* placebo).

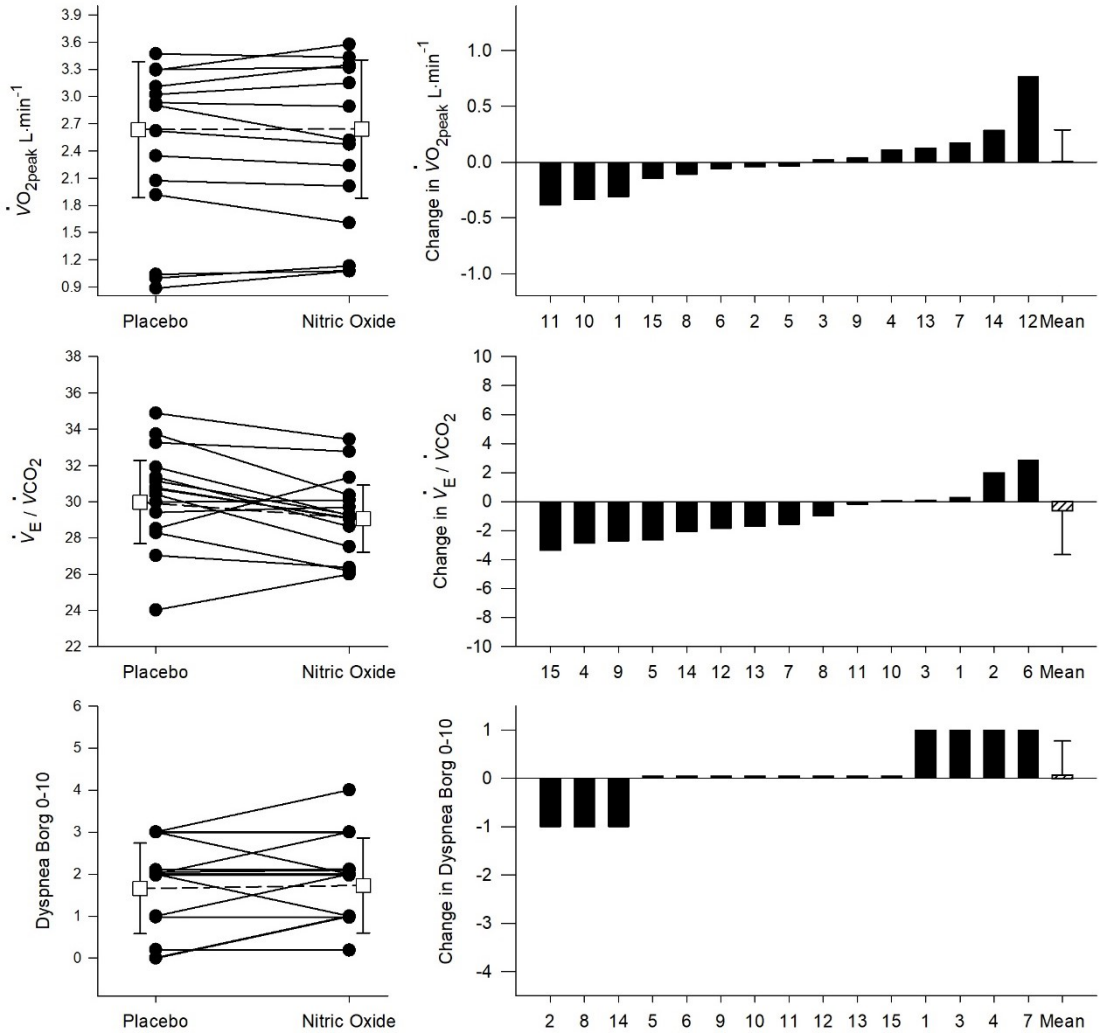


Figure 5.3. Individual values (left column) and post-dose differences (right column) in peak oxygen uptake ($\dot{V}O_{2peak}$), and ventilatory equivalent to carbon dioxide production ($\dot{V}_E/\dot{V}CO_2$) and exertional dyspnea at the highest equivalent work rate of 60 W healthy controls. On left column, open square symbols with dashed horizontal lines denotes mean ± SD. On right column, change in outcomes reflects the post-dose difference (*i.e.* inhaled nitric oxide *minus* placebo).

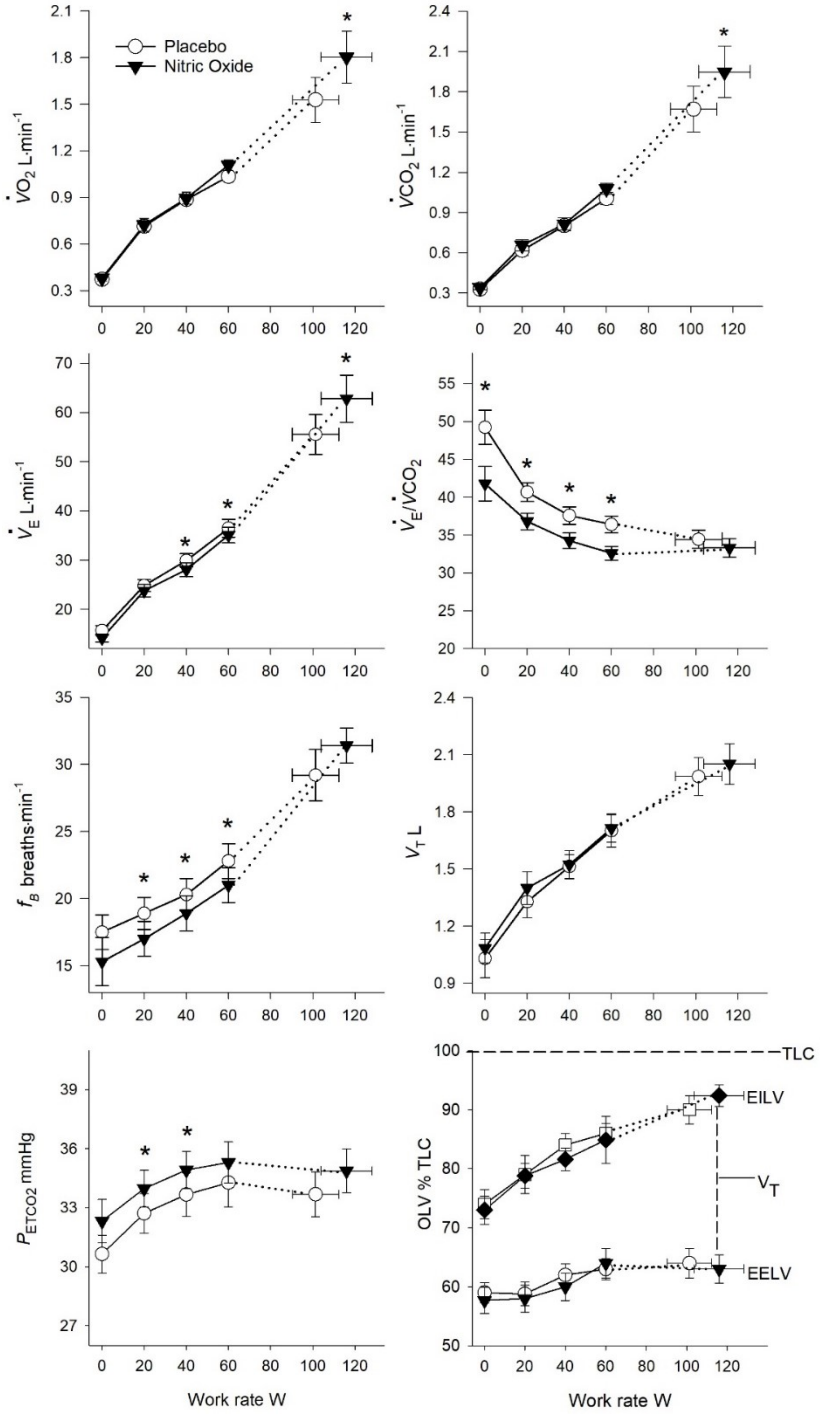


Figure 5.4. Mean ± SEM oxygen uptake ($\dot{V}O_{2peak}$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), ventilatory equivalent to carbon dioxide production ($\dot{V}_E/\dot{V}CO_2$), breathing frequency (f_B), tidal volume (V_T), partial pressure of end-tidal carbon dioxide (P_{ETCO2}) and operating lung volumes (OLV) at rest and during incremental exercise to symptom limitation in patients with mild chronic obstructive pulmonary disease (COPD). End-expiratory lung volume (EELV); end-inspiratory lung volume (EILV); total lung capacity (TLC).
* $p<0.05$ between condition.

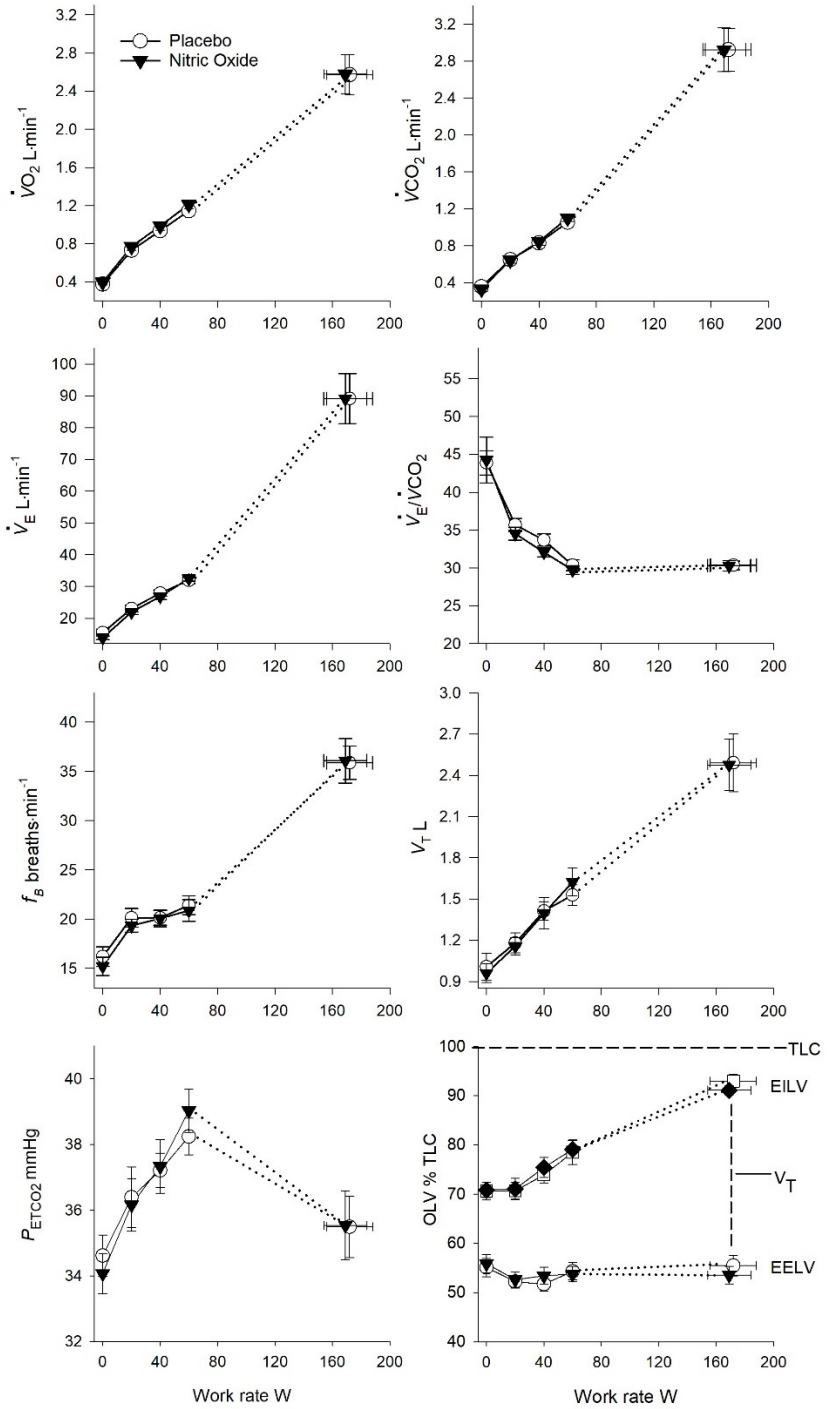


Figure 5.5. Mean ± SEM oxygen uptake ($\dot{V}O_{2peak}$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), ventilatory equivalent to carbon dioxide production ($\dot{V}_E/\dot{V}CO_2$), breathing frequency (f_B), tidal volume (V_T), partial pressure of end-tidal carbon dioxide (P_{ETCO2}) and operating lung volumes (OLV) at rest and during incremental exercise to symptom limitation in healthy controls. End-expiratory lung volume (EELV); end-inspiratory lung volume (EILV); total lung capacity (TLC).

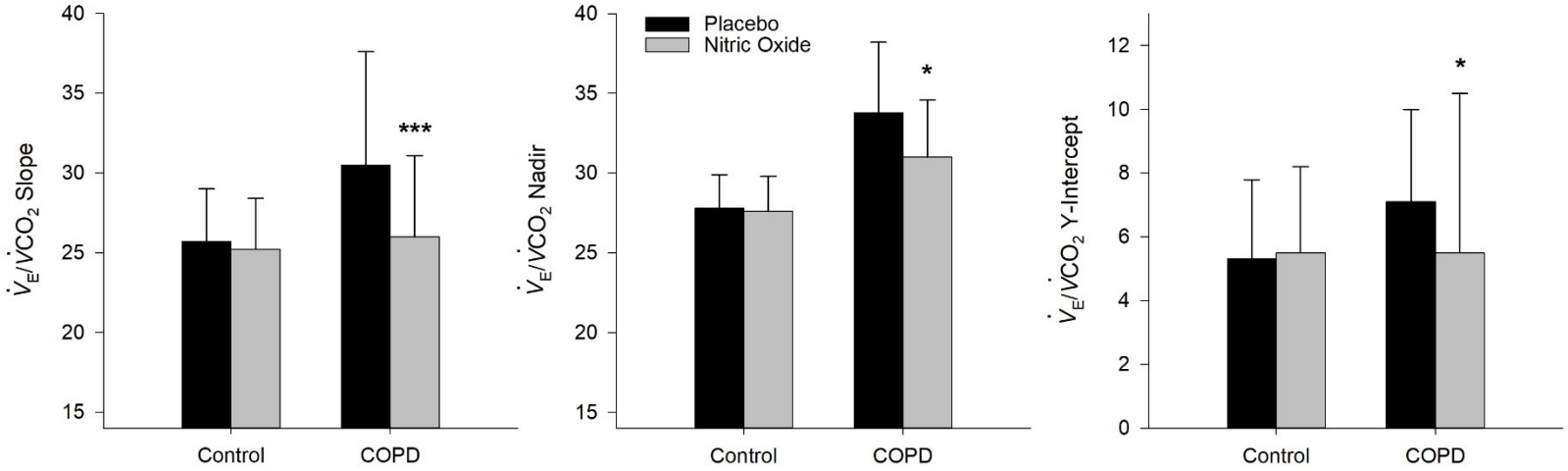


Figure 5.6. Mean ± SD measures of ventilatory efficiency (ventilatory equivalent to carbon dioxide production ($\dot{V}_E/\dot{V}CO_2$)) in either placebo or iNO (inhaled nitric oxide) conditions in healthy controls and patients with mild chronic obstructive pulmonary disease (COPD).
* p<0.05; ***P<0.001 between condition within group.

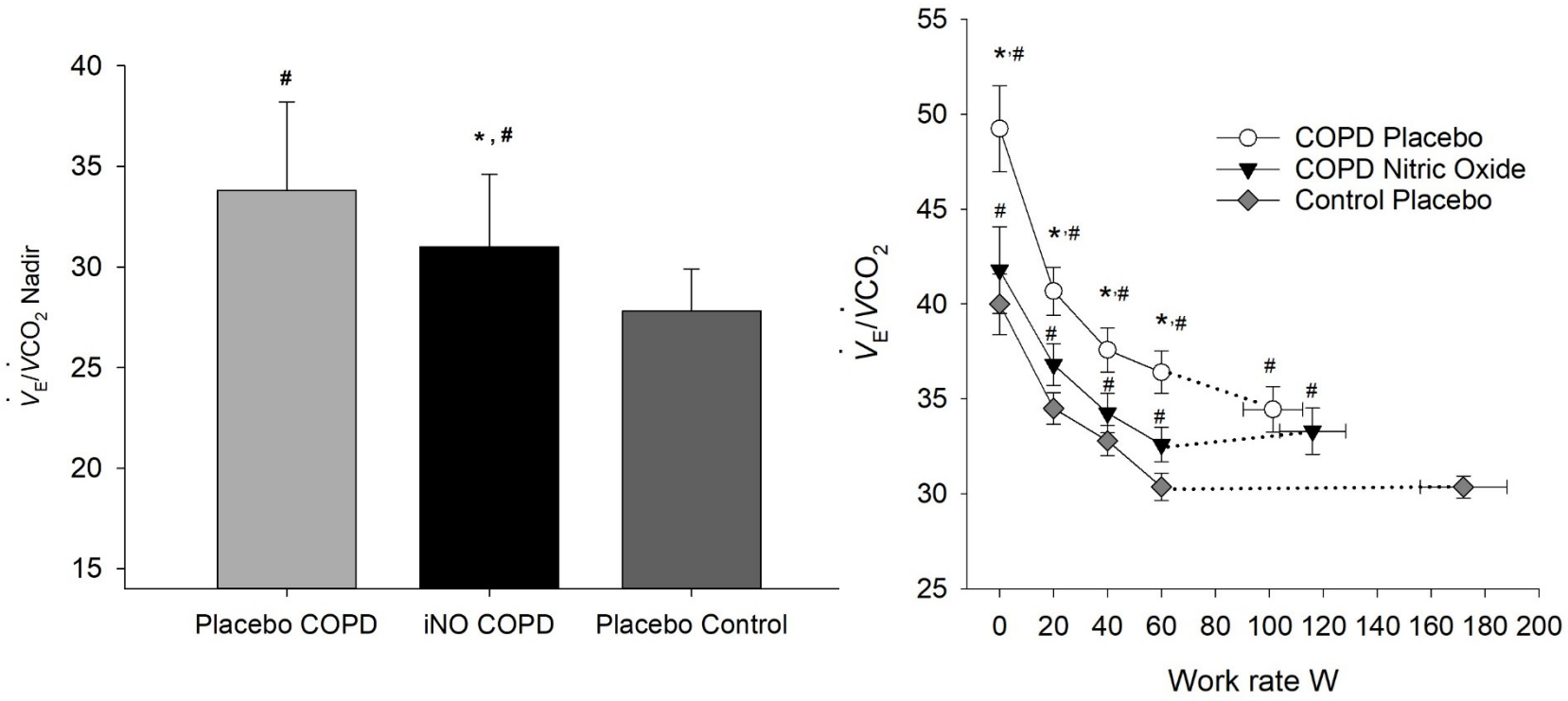


Figure 5.7. Left: Mean ± SD nadir ventilatory equivalent to carbon dioxide production ($\dot{V}_E/\dot{V}CO_2$). Right: Mean ± SEM $\dot{V}_E/\dot{V}CO_2$ during incremental exercise in placebo and iNO (inhaled nitric oxide) conditions in mild chronic obstructive pulmonary disease (COPD) in comparison to the placebo condition within healthy controls.
* $p<0.05$ COPD placebo vs COPD nitric oxide, # $p<0.05$ compared to placebo control.

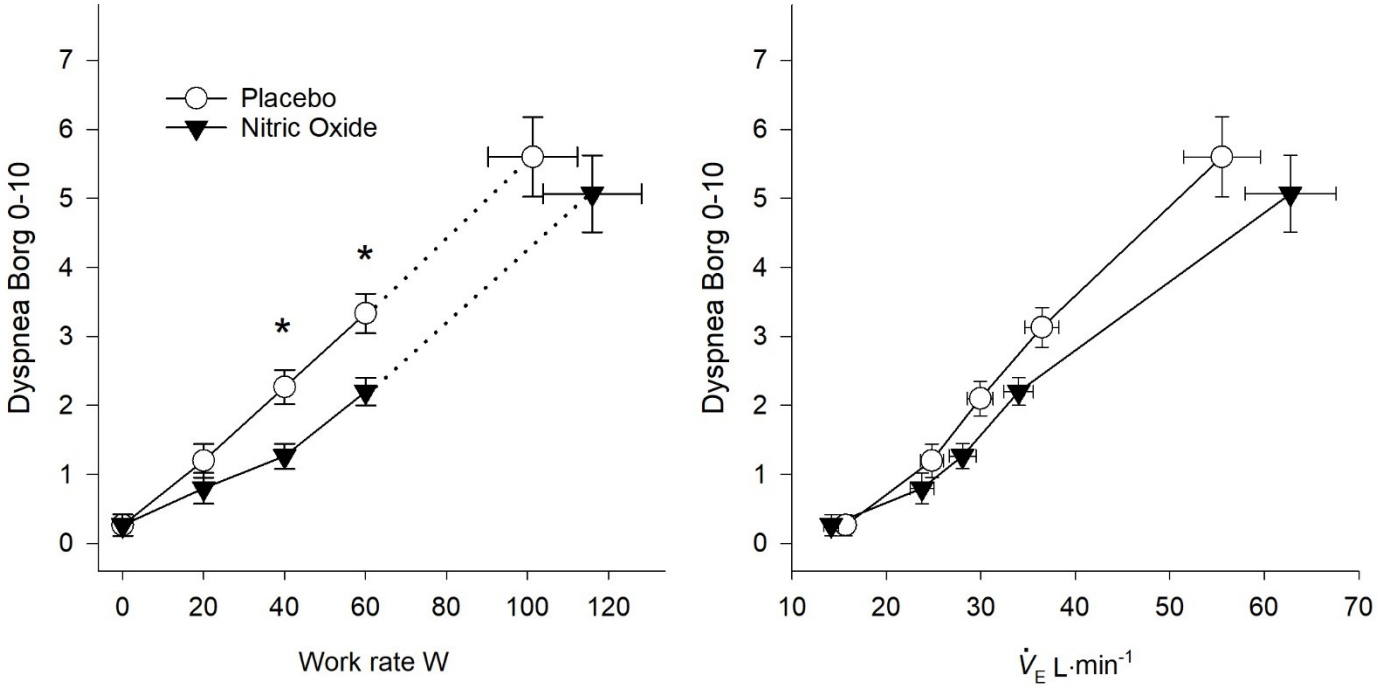
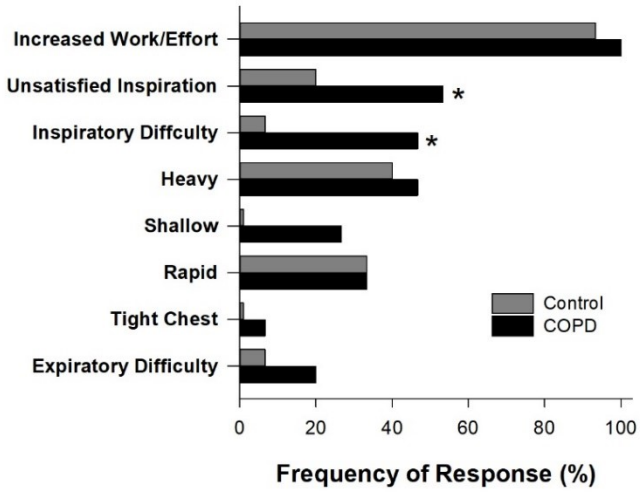
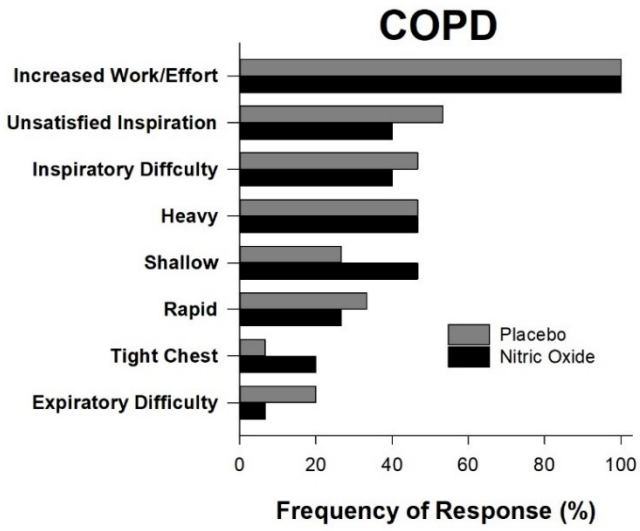


Figure 5.8. Mean ± SEM exertional dyspnea relative to work rate (left) and minute ventilation (right) during incremental exercise to symptom limitation in patients with mild chronic obstructive pulmonary disease (COPD).
* $p<0.05$ between condition.





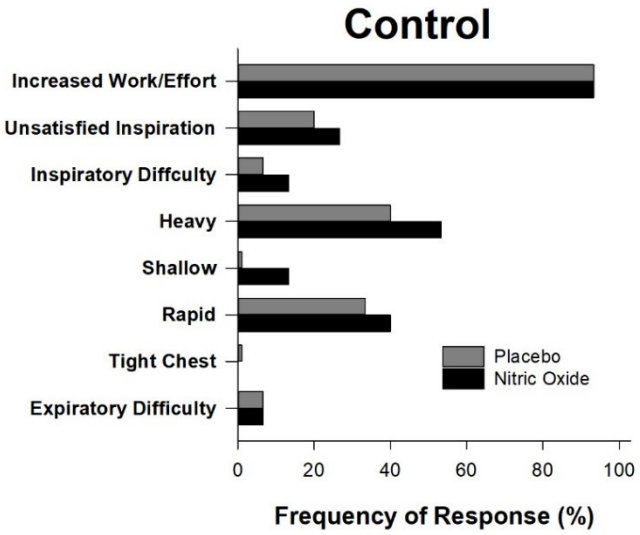


Figure 5.9. The selection frequency of qualitative descriptors of exertional dyspnea at peak exercise show in mild chronic obstructive pulmonary disease (COPD) *versus* controls in placebo condition (top), placebo *versus* inhaled nitric oxide (iNO) within the mild COPD group (middle), and placebo versus iNO with the healthy control group (bottom). * $p<0.05$ between groups.

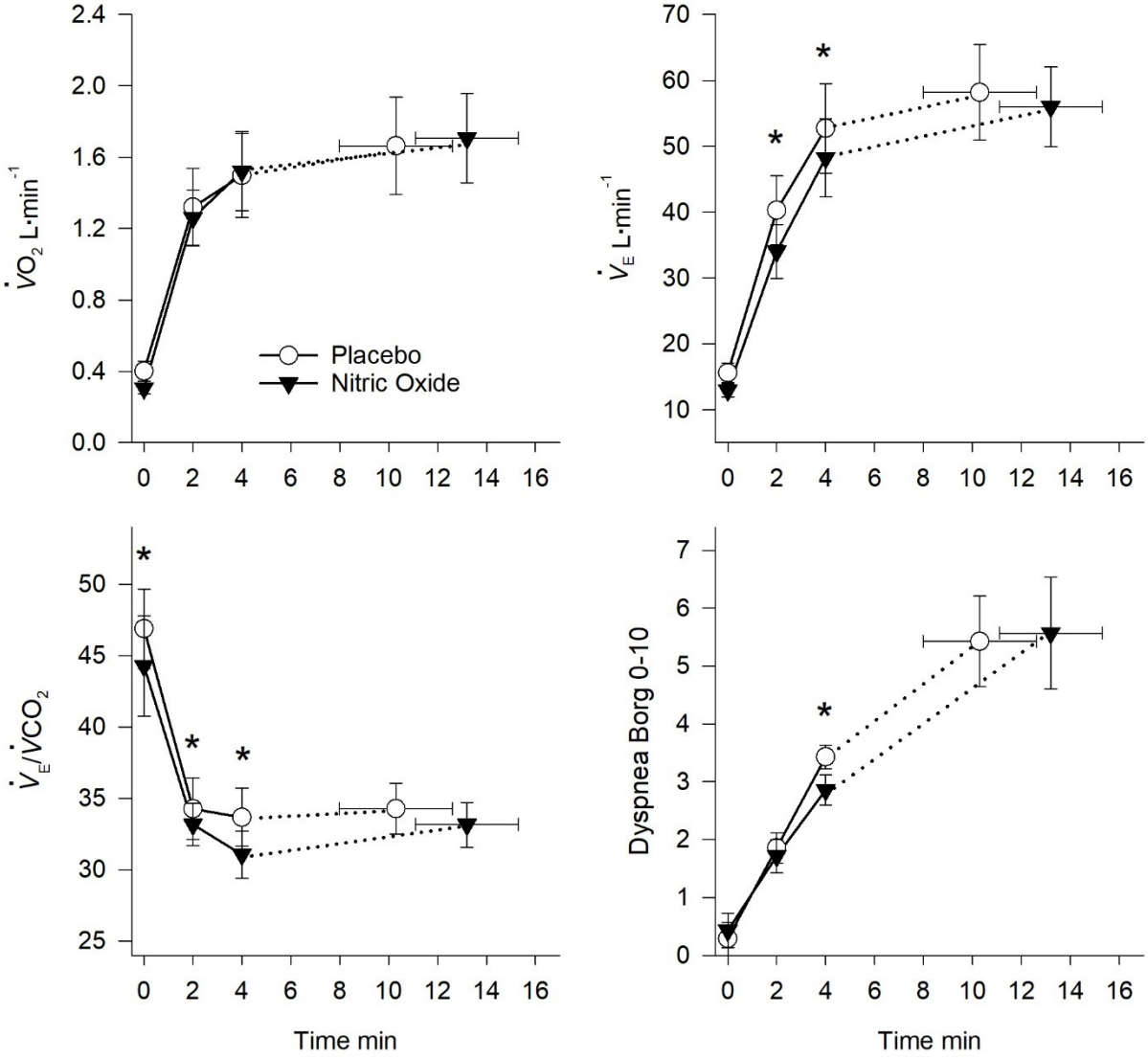


Figure 5.10. Mean ± SEM oxygen uptake ($\dot{V}O_{2peak}$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), ventilatory equivalent to carbon dioxide production ($\dot{V}_E/\dot{V}CO_2$), breathing frequency (f_B), tidal volume (V_T), partial pressure of end-tidal carbon dioxide (P_{ETCO2}) and operating lung volumes (OLV) at rest and during incremental exercise to symptom limitation in healthy controls. End-expiratory lung volume (EELV); end-inspiratory lung volume (EILV); total lung capacity (TLC).

Chapter Six

General Discussion

6.1 Dissertation overview

This dissertation was undertaken to better understand how autonomic and pulmonary vascular dysfunction contribute to reduced exercise capacity and elevated cardiovascular risk in patients with mild to moderate COPD. As illustrated in Figure 6.1, the mechanism(s) of reduced exercise capacity and cardiovascular risk are multifactorial and interrelated. The first study examined the effect of carotid chemoreceptor inhibition (CC) on resting cardiovascular function and ventilatory regulation in patients with mild to moderate COPD, and age- and risk-matched controls. Inhibiting the CC with low-dose dopamine infusion decreased arterial stiffness and ventilation, as indicated by significant reductions in pulse-wave velocity and expired minute ventilation, while no effect was observed in controls. Additionally, CC inhibition reduced muscle sympathetic nervous activity (MSNA) in COPD, while no effect was observed in controls. COPD is characterized by an elevated ventilatory response to exercise (high $\dot{V}_E/\dot{V}CO_2$) which is a key contributor to heightened dyspnea and exercise intolerance, however, the reason for the elevated $\dot{V}_E/\dot{V}CO_2$ is not well understood. Based on the results from Study One, the second experiment was completed as a follow up to determine if CC inhibition translated to a reduction in dyspnea and increased exercise tolerance, secondary to reduced $\dot{V}_E/\dot{V}CO_2$. Although significant increases in vascular conductance were observed, exercise ventilation, dyspnea and exercise tolerance were unaffected by CC inhibition in patients with mild to moderate COPD.

The purpose of the third study was to determine if acute improvements in pulmonary vascular function translated to increased exercise capacity, secondary to lower $\dot{V}_E/\dot{V}CO_2$ and reduced dyspnea, in patients with mild COPD. For this study, we hypothesized that improvements in pulmonary vascular function, using inhaled nitric oxide, would increase pulmonary microvascular perfusion, leading to better ventilation-perfusion $(\dot{V}_A/\dot{Q})$ matching, reduced

deadspace ventilation and therefore a reduced $\dot{V}_E/\dot{V}CO_2$. Further, the reduction in ventilation would decrease dyspnea and increase exercise capacity. Consistent with our hypothesis, iNO increased exercise capacity in mild COPD, secondary to reduced ventilation (lower $\dot{V}_E/\dot{V}CO_2$) and dyspnea.

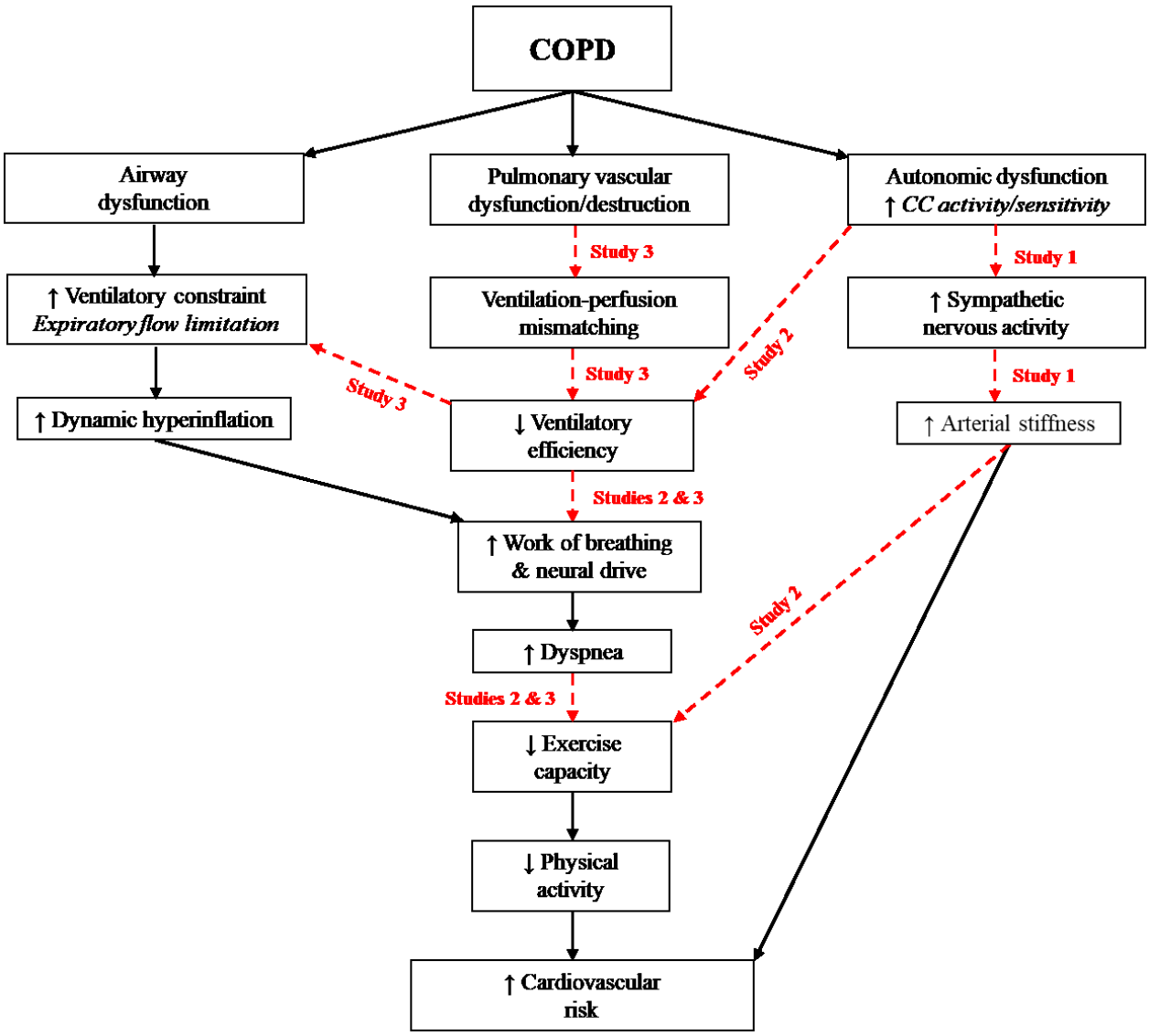


Figure 6.1. Flowchart of COPD-cardiovascular risk disease pathways relevant to the current dissertation. Red dashed lines were the central focus of this dissertation. COPD: chronic obstructive pulmonary disease; CC: carotid chemoreceptor.

6.2 Major findings

When the results of the three studies are combined, the major findings are threefold. First, CC inhibition decreased MSNA and pulse wave velocity in COPD, suggesting that tonic CC activity is elevated at rest and contributes to the elevated arterial stiffness in COPD. Second, the CCs appears to be a modulator of vasoconstrictor outflow during exercise in COPD patients, however, the exaggerated ventilatory response typically observed in COPD is not explained by heightened activity and sensitivity of the CC (*i.e*. autonomic dysfunction). Further, our data suggests that the CCs are not a key contributor to exertional dyspnea and exercise intolerance in COPD. Last, iNO-mediated improvements in ventilatory efficiency, dyspnea and exercise capacity suggest that mild COPD patients demonstrate pulmonary vascular dysfunction that contributes to exercise intolerance, secondary to inefficiencies in gas exchange.

6.3 Analyzing responders and non-responders to carotid chemoreceptor inhibition

6.3.1 Individual responses

In Chapter Four (study two), the primary outcome was the effect of CC inhibition on exercise tolerance in mild to moderate COPD and age- and risk- matched controls. In this study, there was no significant effect of CC inhibition on exercise tolerance in either COPD patients or controls. Despite no mean change in exercise endurance time with dopamine, large variability was observed in the change in endurance time from saline to dopamine in COPD patients. Correlations between the change in exercise endurance time (saline vs. dopamine) and the change in key variables of interest are displayed in Table 6.1. Within the COPD group, there was a strong negative correlation (r=-0.69, p=0.02) between the change in dyspnea from saline to dopamine at 4-minute isotime, and the change in exercise endurance time from saline to dopamine. There was also a strong negative correlation (r=-0.67, p=0.02) between the change in $\dot{V}_E/\dot{V}CO_2$ from saline to

dopamine at 4-minute isotime, and the change in exercise endurance time from saline to dopamine in the COPD group. Additionally, a strong positive correlation (r= 0.67, p=0.02) was observed between the change in vascular conductance from saline to dopamine at TLIM and the change in exercise endurance time from saline to dopamine. There was no significant correlation between the change in exercise endurance time and basal CC activity or sensitivity (*i.e.* hypoxic ventilatory response) in COPD. There were no significant correlations between the change in key variables of interest (dyspnea, $\dot{V}_E/\dot{V}CO_2$, vascular conductance or basal CC activity and sensitivity) and the change in exercise endurance time (saline to dopamine) in controls.

Figure 6.2 displays the change in exercise endurance time, from saline to dopamine, in responders and non-responders in both COPD patients and controls. COPD patients who showed improved $\dot{V}_E/\dot{V}CO_2$ and dyspnea at 4-minute isotime with dopamine (responders) had significantly greater improvements in exercise endurance time when compared to those who demonstrated no change or worsened $\dot{V}_E/\dot{V}CO_2$ and dyspnea (non-responders). In the control group, there was no significant difference in the change in exercise endurance time between responders and non-responders.

Although our sample size was small, analysis showed no relationship between basal chemosensitivity and the change in exercise endurance time from saline to dopamine. None of the patients had significant changes in pulmonary function or medication between trials and all patients were free from exacerbation within 6 months of study enrollment. In the control group, individual changes in exercise $\dot{V}_E/\dot{V}CO_2$ and dyspnea, between conditions, did not translate to large changes in exercise endurance time. However, in the COPD group, individual changes in exercise $\dot{V}_E/\dot{V}CO_2$ and dyspnea, between conditions, did translate to large increases or decreases in exercise endurance time. Further, COPD patients who had reduced $\dot{V}_E/\dot{V}CO_2$ and dyspnea (both >1 unit

reduction) on one test compared to the other, independent of condition, exercised longer in that respective condition. COPD patients who had increased $\dot{V}_E/\dot{V}CO_2$ and dyspnea within a trial, exercised for a shorter duration when compared to their other test, independent of condition. Although we did not observe a mean improvement in exercise tolerance with dopamine, the observed individual responses in $\dot{V}_E/\dot{V}CO_2$ demonstrate that COPD patients are sensitive to either improved or worsened ventilatory efficiency, and is consistent with previous work [17, 49, 50, 138]. The data in the current study provide further evidence that interventions to improve ventilatory efficiency (*i.e.* lower $\dot{V}_E/\dot{V}CO_2$) may be helpful to reduce dyspnea and increase tolerance in patients with COPD.

6.3.2 Is the variability in responses explained by a learning effect?

Equal numbers of participants completed the saline or the dopamine condition first. To check for the possibility of a learning effect during the constant load exercise test, Test 1 completion times were compared with Test 2, regardless of condition. In the control group, there was no significance difference between Test 1 (10.9 ± 1.5 min) and Test 2 (11.5 ± 01.5 min, p=0.54) in exercise endurance time. In the COPD group, there was also no significant difference between Test 1 (9.5 ± 1.5 min) and Test 2 (10.5 ± 1.6 min, p=0.53) in exercise endurance time. These data suggest that constant load exercise test endurance time was not influenced by a systematic learning effect.

6.4 Autonomic and pulmonary vascular dysfunction in COPD

The physiological underpinnings of elevated carotid chemoreceptor activity and sympathetic nerve activity, and systemic and pulmonary vascular dysfunction in COPD are poorly understood. Although speculative, it is likely that the autonomic dysfunction (Studies one and two) and pulmonary vascular dysfunction (Study three) observed in this dissertation may be linked by

an upstream mechanism. The carotid chemoreceptors are located within the carotid body at the level of the carotid sinus and are sensitive to stimuli including O_2, CO_2, inflammation, reactive oxygen species (ROS), NO and angiotensin II. Importantly, this dissertation examined non-hypoxemic, non-hypercapnic patients, indicating that another mechanism besides abnormal PO_2 and PCO_2 in arterial blood is responsible for the heightened CC activity and sensitivity.

Previous research in patients with either obstructive sleep apnea (OSA) or COPD has shown elevated levels of inflammation and oxidative stress and an associated reduction in NO bioavailability [61, 96, 219]. It has been well demonstrated that increased systemic inflammation directly elevates oxidative stress within the circulation [30, 155, 163]. Further, elevated oxidative stress contributes to greater levels of free radicals, which can; 1) oxidize endothelial NO synthase (eNOS) co-factors (*i.e.* tetrahydrobiopterin), leading to eNOS uncoupling, reduced NO bioavailability and vascular dysfunction and 2), stimulate the chemoreceptors, which increases sympathetic outflow (*i.e.* autonomic dysfunction). Additionally, decreased NO bioavailability has been shown to exacerbate the angiotensin II mediated increased in carotid body activity and sympathetic outflow [115]. Following treatment of OSA with continuous positive airway pressure, inflammation, oxidative stress and carotid chemosensitivity (*i.e.* ventilatory response to hypoxia) were reduced while NO-synthase expression and vascular function were improved [96]. Ives *et al.* demonstrated a reduction in oxidative stress and improvement in systemic vascular function in COPD following administration of an antioxidant cocktail (Vitamin-C and E, α-lipoic acid), while no effect was observed in healthy age- and sex-matched controls. When combined, these studies provide further experimental evidence that oxidative stress and inflammation (and associated reduction in NO bioavailability) may be the underlying mechanism that explains both the elevated carotid chemoreceptor activity/sensitivity and pulmonary vascular dysfunction observed in our

cohort of non-hypoxemic, non-hypercapnic COPD patients. However, further studies are required to better link oxidative stress, inflammation and NO bioavailability and its effect on autonomic and pulmonary vascular function in COPD.

6.5 Pulmonary diffusing capacity during exercise in mild COPD: identifying pulmonary vascular dysfunction and/or destruction

During exercise, diffusing capacity increases in order to facilitate efficient pulmonary gas-exchange and maintain arterial blood gas homeostasis. Two important determinants of diffusing capacity are pulmonary capillary blood volume (Vc) and diffusing membrane capacity (Dm). As pulmonary arterial pressure increases with exercise, Vc increases secondary to capillary recruitment and distension. With greater capillary recruitment, Dm is increased as more capillaries are perfused [193, 231]. The recruitment and distension of pulmonary capillaries also aids to reduce pulmonary vascular resistance and mitigate the increases in pulmonary artery pressure typically observed during high-intensity exercise [105, 199]. Our recent research demonstrated a blunted diffusing capacity for carbon monoxide (DLCO) at rest and during exercise in patients with mild COPD, secondary to a reduced Vc [206]. Low resting and exercise DLCO has been associated with elevated exercise $\dot{V}_E/\dot{V}CO_2$, exertional dyspnea, and exercise intolerance in COPD [49-51, 206]. It is likely that the reduced Vc is secondary to impaired pulmonary blood flow, leading to inadequate perfusion and elevated physiological deadspace in COPD. To overcome the elevated deadspace, COPD patients are required to increase total ventilation (i.e. $\dot{V}_E/\dot{V}CO_2$) in an attempt to maintain arterial blood gas homeostasis. It is not well understood how much of the capillary hypoperfusion, and increased deadspace and, subsequently, $\dot{V}_E/\dot{V}CO_2$ at rest and during exercise is due to pulmonary vascular dysfunction or destruction, or a combination of both. Pulmonary vascular dysfunction, secondary to impaired NO-bioavailability (reduced endothelial nitric oxide synthase; eNOS) could be reversed using an intervention to increase NO within the

endothelium, which has been previously demonstrated in patients with CHF and PAH [81, 94, 123, 156] and in Chapter Five (study three) of the current dissertation. However, pulmonary capillary destruction, secondary to emphysema, would likely be unresponsive to improvements in vascular function, as the alveolar-capillary connections are permanently impaired. Nambu *et al.* and Grydeland *et al.* examined relationships between DLCO and structural emphysema, quantified by computational tomography (CT) and found only modest associations between low DLCO and extent of emphysema in patients with COPD [69, 130]. These data suggest that the reduced DLCO in COPD patients is only partially explained by the emphysema severity. Hueper *et al.* demonstrated reduced pulmonary blood flow in non-emphysematous lung regions in patients with mild COPD and reduced DLCO, which would suggest a lower capillary perfusion second to impaired blood flow within an intact vascular bed [92]. Recent research by Elbehairy *et al.* compared the ventilatory and dyspnea response to exercise in mild-moderate COPD patients [51], stratified into normal DLCO and low DLCO groups using the Global Lung Initiative (GLI) 5th percentile as the lower limit normal (LLN) cutoff [187]. When matched for resting spirometric values (*i.e*. FEV_1), the authors demonstrated that the low DLCO group had 1) increased emphysema severity, elevated exercise $\dot{V}_E/\dot{V}CO_2$, heightened exertional dyspnea and reduced exercise tolerance, when compared to the normal DLCO group. However, only modest correlations between resting DLCO and emphysema severity were observed, which was consistent with the findings of Numba and Grydeland [69, 130]. Although these studies provide critical insight into the pulmonary vascular influences on lung structure and function, it is currently unclear whether the increase deadspace and elevated $\dot{V}_E/\dot{V}CO_2$ typically observed in mild COPD is a result of pulmonary vascular dysfunction or destruction.

In Chapter Five (study three), fifteen patients with mild COPD and fifteen healthy controls completed incremental CPETs with and without iNO. Inhaled NO reduced $\dot{V}_E/\dot{V}CO_2$ and increased peak oxygen uptake ($\dot{V}O_{2peak}$) in patients with mild COPD, while no effect was observed in controls. Further, we concluded that the elevated $\dot{V}_E/\dot{V}CO_2$ response to exercise in mild COPD was secondary to pulmonary vascular dysfunction. Although we observed consistent reductions in $\dot{V}_E/\dot{V}CO_2$ with iNO, there was large variability in the magnitude of responses to iNO within our mild COPD group. Additionally, there was a large range in resting DLCO (41–109% predicted) within mild COPD patients. As previously mentioned, low resting DLCO has been associated with elevated exercise $\dot{V}_E/\dot{V}CO_2$ and reduced $\dot{V}O_{2peak}$ in COPD [49-51, 206]. It is therefore plausible that the variable resting DLCO within mild COPD patients may help to partition between pulmonary vascular dysfunction and destruction, and their effects on $\dot{V}_E/\dot{V}CO_2$ in patients with mild COPD.

We completed an exploratory post-hoc analysis to investigate the potential effects of resting DLCO on the response to iNO. COPD patients were stratified into either normal DLCO (n=6, z-score > -1.67) or low DLCO (n=9, z-score < -1.67) based on the GLI 5th percentile as the LLN cutoff [51, 187]. We then compared the change in $\dot{V}O_{2peak}$ and Nadir $\dot{V}_E/\dot{V}CO_2$ (global index of ventilatory efficiency) from placebo to iNO in both COPD groups (normal and low DLCO) and healthy controls. With the exception of DLCO, there was no difference in lung function between the normal and low DLCO COPD groups (Table 6.2) The normal DLCO COPD group had a higher $\dot{V}O_{2peak}$ and lower nadir $\dot{V}_E/\dot{V}CO_2$ compared to the low DLCO group (p=0.01 and 0.03, respectively). In both COPD groups, iNO significantly improved $\dot{V}O_{2peak}$ and reduced nadir $\dot{V}_E/\dot{V}CO_2$ to the same magnitude, while as stated previously, no effect was observed in controls (Fig 6.3 & 6.4). In the placebo condition, the normal DLCO group trended towards a lower $\dot{V}O_{2peak}$ and elevated nadir $\dot{V}_E/\dot{V}CO_2$ when compared to healthy controls (p=0.08 and 0.06, respectively),

which is consistent with recent findings [50, 51]. Interestingly, the $\dot{V}O_{2peak}$ and the nadir $\dot{V}_E/\dot{V}CO_2$ in the iNO condition within the COPD group with normal DLCO were not different from the same data obtained in healthy controls during the placebo trial (p=0.448 and 0.756, respectively). In the low DLCO group, $\dot{V}O_{2peak}$ remained lower while the nadir $\dot{V}_E/\dot{V}CO_2$ was higher than controls, independent of condition. These data would suggest that acute improvements in pulmonary vascular function in patients with mild COPD and preserved DLCO may actually *normalize* exercise ventilatory efficiency and restore exercise capacity. It is possible that mild COPD patients with normal DLCO may present with reversible pulmonary vascular dysfunction, while mild COPD patients with low DLCO may have a combination of partially reversible pulmonary vascular dysfunction and irreversible pulmonary vascular destruction, secondary to more severe emphysema. This is illustrated by a significant reduction and "normalizing" of $\dot{V}O_{2peak}$ and nadir $\dot{V}_E/\dot{V}CO_2$ in the normal DLCO group with iNO, while, these parameters remained "abnormal" in the low DLCO group with iNO, despite a reduction of similar magnitude to the normal DLCO group. It must be noted that this is an exploratory analysis (n=6 normal DLCO group, n=9 low DLCO group), and as such, we are underpowered to detect within (placebo vs iNO) and between group (normal vs low DLCO) differences in $\dot{V}O_{2peak}$ and nadir $\dot{V}_E/\dot{V}CO_2$. Further, without CT analysis, we do not have a determination of emphysema severity and our conclusion that normal DLCO patients have reversible pulmonary vascular dysfunction and low DLCO patients have partially reversible pulmonary vascular dysfunction is speculative. Future research is required to compare normal versus low DLCO COPD patients, when accounting for emphysema severity.

6.6 The effects of healthy aging on pulmonary vascular function and exercise ventilation

Age-related reductions in pulmonary function are associated with ventilatory mechanical constraint, and worsened ventilatory efficiency [38, 55, 128, 147]. Further, the worsening of

ventilatory efficiency, secondary to ventilation-perfusion $\dot{V}_A/\dot{Q}$ mismatching, is evident by an elevated $\dot{V}_E/\dot{V}CO_2$ during exercise in elderly, when compared to younger individuals [55]. One proposed mechanism of elevated $\dot{V}_E/\dot{V}CO_2$ in elderly is increased deadspace, secondary to age-related impairments in pulmonary vascular function [52, 55, 108]. Previously, it has been shown that pulmonary vascular resistance and pressure are increased in older individuals compared to young individuals, secondary to vascular remodeling and stiffening [52, 108] and these age-related differences in pulmonary vascular function are exacerbated during exercise [218]. Additionally, previous research has demonstrated an age-related reduction in the alveolar-capillary surface area [207, 209]. Further, the alveolar septa diminish and the alveolar duct diameter increases with age, resulting in a decreased surface area for diffusion into the pulmonary capillaries [133, 209]. When the reduced surface area for gas-exchange is combined with the stiffening of the pulmonary arteries and capillaries, the net effect is an age-related decrease in pulmonary capillary blood volume [31, 64]. It could be hypothesized that increased pulmonary vascular resistance and reduced capillary blood volume lead to a reduction in perfusion in some areas of the lung, leading to areas of high $\dot{V}_A/\dot{Q}$ and elevated physiological deadspace, which would explain the elevated $\dot{V}_E/\dot{V}CO_2$ typically observed during exercise in healthy elderly people.

In Chapter Five (study three), patients with mild COPD and age-matched controls completed cardiopulmonary exercise tests (CPET) with and without iNO, which is a known selective pulmonary vasodilator. In the healthy controls (Age 61 ± 13 years, range 40-80), iNO decreased right ventricular systolic pressure (RVSP) at rest, however, $\dot{V}_E/\dot{V}CO_2$ was unaffected during exercise. It must be noted that these controls were age-matched to COPD patients, and as such, some individuals were not considered elderly (n=5, age 40-55 years). To further examine the effect of aging on exercise $\dot{V}_E/\dot{V}CO_2$, we completed a sub-analysis with ten healthy controls (≥ 65

years, 68 ± 7 years) and compared to ten young healthy individuals (24 ± 5 years) who recently completed a similar study in our laboratory. Participants were pairwise matched for sex (each group n=5 male, n=5 female), lung function and aerobic fitness (% predicted). As expected, the older group had an elevated resting RVSP, when compared to young individuals (17.0 ± 1.3 vs 7.7 ± 0.9 mmHg, $p<0.001$). With iNO, both groups observed a small but significant reduction in RVSP. During exercise, $\dot{V}_E/\dot{V}CO_2$ was significantly elevated in the older group when compared to the young individuals, however, iNO had no effect on $\dot{V}_E/\dot{V}CO_2$ in either group ($p=0.50$). These data suggest that acute reductions in pulmonary vascular resistance do not translate to 1) improved distribution of perfusion throughout the lung, and, 2) reduced deadspace and $\dot{V}_E/\dot{V}CO_2$ in healthy individuals, independent of age. When our current findings are combined with previous work, it is likely that the increase in $\dot{V}_A/\dot{Q}$ mismatching, deadspace and $\dot{V}_E/\dot{V}CO_2$ in older individuals, is secondary to age-related structural changes within the lung such as non-reversible vascular stiffening and changes in alveolar diameter. Future research in this area is required to better understand the relationship between aging and pulmonary gas-exchange during exercise.

6.7 Future research in COPD

Chapters Three and Four demonstrated that acute reductions in CC activity resulted in reductions in MSNA and arterial stiffness, and increased vascular conductance. Although these study results help us to better understand the pathophysiology of COPD, our findings are based on cross-sectional data. Future research is needed to evaluate the long-term effects of CC inhibition on cardiovascular function in patients with COPD. It would be of great interest to complete a prospective randomized-control trial investigating the effect of chronic CC inhibition on cardiovascular function in patients with COPD. Should chronic CC inhibition consistently reduce MSNA and arterial stiffness, this would clearly establish that heightened activity of the CC

contributes to cardiovascular dysfunction. Further, this finding would help identify a potential therapy to reduce cardiovascular risk in patients with COPD.

Chapter Five demonstrated that acute improvements in pulmonary vascular function with iNO translated to improved ventilatory efficiency (*i.e.* lower $\dot{V}_E/\dot{V}CO_2$), reduced dyspnea and increased exercise capacity in mild COPD. It is unclear whether iNO would lower $\dot{V}_E/\dot{V}CO_2$ and dyspnea, and in increase exercise capacity in more severe COPD. With advancing COPD, there would be capillary destruction, pulmonary arterial hypertension, and hypoxemia. In the presence of microvascular destruction, iNO is likely ineffective at improving pulmonary capillary perfusion and thus $\dot{V}_A/\dot{Q}$ matching. With advanced disease, hypoxic pulmonary vasoconstriction occurs in an attempt to optimize $\dot{V}_A/\dot{Q}$ matching [174], and therefore any release of hypoxic vasoconstriction may actually worsen $\dot{V}_A/\dot{Q}$ matching, resulting in arterial hypoxemia, greater ventilation and dyspnea, and impaired exercise capacity. This likely explains why interventions that target pulmonary vascular dysfunction in more advanced COPD patients without severe pulmonary arterial hypertension generally appear to be ineffective at improving exercise capacity, dyspnea and quality of life [26, 152, 175]. A small study by Ashutosh *et al.* observed that 24 hours of iNO in hypoxemic patients with severe COPD reduced pulmonary vascular resistance, but did not affect pulmonary gas-exchange or exercise capacity [5]. However, the authors noted that 6 of the 11 patients reported significant reductions in perceived dyspnea with iNO. It is unclear why dyspnea was reduced in these patients and highlights the need for additional research to better understand the impact of the pulmonary vasculature on pulmonary gas-exchange, dyspnea and exercise capacity across the continuum of COPD (*i.e.* mild to severe). Future randomized-control trials targeting the pulmonary vasculature (*i.e.* iNO) should be completed in patients with mild to severe COPD, incorporating structural (*i.e.* CT-derived emphysema severity) and functional (*i.e.* FEV_1,

DLCO) parameters to; 1) better understand and characterize responders and non-responders, and, 2) determine target treatment groups that would benefit from pulmonary vascular interventions.

The inclusion-exclusion criteria for study participants with COPD was rigorous and the findings from this dissertation are restricted to a small relatively homogenous group of patients with stable COPD and minimal co-morbidities. Future research should incorporate a more pragmatic approach to select study participants whom are more representative of the disease population, such as patients with more severe COPD or common co-morbidities (*i.e.* obstructive sleep apnea, heart failure, pulmonary hypertension). This approach could aid to improve relevance of study findings to inform clinical and policy decisions, and lead to widespread changes in health care [208, 215].

6.8 Summary and conclusion

In this dissertation, two fundamental important areas in the pathophysiology of COPD were examined: autonomic dysfunction and pulmonary gas-exchange abnormalities.

Stable non-hypoxemic and non-hypercapneic patients with COPD have increased central arterial stiffness and MSNA, both of which contribute to cardiovascular dysfunction and increased cardiovascular risk [3, 178, 221]. In Chapter Five (study one), we provided experimental evidence that the increased arterial stiffness and MSNA are secondary to enhanced activity of the carotid chemoreceptor, which suggest that reduced cardiovascular function is secondary to autonomic dysfunction.

Patients with COPD experience significant dyspnea and reduced exercise capacity, both of which profoundly reduce physical activity and increase risk of mortality [4, 93, 183]. In Chapter Four (study two), we showed evidence of improved cardiovascular function during whole-body

exercise following inhibition of the carotid chemoreceptor, which builds on our findings from Chapter Three. Despite improved cardiovascular function, exercise ventilation and exercise tolerance were unaffected by CC inhibition which suggests that altered CC activity and sensitivity is not the primary reason for the exaggerated ventilatory response to exercise in COPD.

Emerging evidence suggests that COPD is associated with pulmonary vascular dysfunction, and may be a key pathological mechanism that helps to explain the increased deadspace and exaggerated ventilation response to exercise, even in mild COPD. In Chapter Five (study three), we showed evidence that acutely improving pulmonary vascular function with iNO resulted in clinically meaningful reductions in exercise ventilation and dyspnea, which translated to improved exercise capacity in patients with mild COPD.

The findings from this dissertation help to explain why patients with COPD have 1) increased cardiovascular risk, and 2) reduced exercise capacity. The results of this dissertation may assist with identifying potential therapeutic targets to lower cardiovascular risk, improve dyspnea and exercise capacity, and by extension quality of life in patients with COPD.

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Table 6.1 Correlations for selected parameters at isotime and T_{LIM} with the change in exercise endurance time from saline to dopamine in COPD patients

Variable	r-value	p-value
COPD		
Δ $\dot{V}_E/\dot{V}CO_2$ at isotime	-0.67	0.03
Δ Dyspnea (Borg) at isotime	-0.69	0.02
Δ Vascular Conductance (ml/min/mmHg) at isotime	0.39	0.30
Δ $\dot{V}_E/\dot{V}CO_2$ at T_{LIM}	0.06	0.88
Δ Dyspnea (Borg) at T_{LIM}	0.47	0.17
Δ Vascular Conductance (ml/min/mmHg) at T_{LIM}	0.72	0.02
Hypoxic $\dot{V}_E$ response ($\Delta\dot{V}_E/\Delta SpO_2$ slope ($L \cdot min^{-1} \cdot \%^{-1}$)	0.39	0.39

$\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent to carbon dioxide production; T_{LIM}, time to symptom limitation; $\dot{V}_E$, minute ventilation; SpO_2, oxygen saturation measured by pulse oximetry.

Table 6.2 Participant demographics and resting lung function. In a post-hoc exploratory analysis, 15 patients with mild chronic obstructive pulmonary disease (COPD) were stratified into two groups to examine the impact of resting diffusing capacity on the response to inhaled nitric oxide during exercise.

	Normal DLCO	**Low DLCO**
Subjects (n)	6	9
Males/females (n)	6/0	5/4
Age (years)	63 ± 8	69 ± 10
Height (cm)	174 ± 8	170 ± 8
Mass kg	76 ± 15	77 ± 15
Body mass index ($kg \cdot m^{-2}$)	28 ± 4	26 ± 4
Smoking history (pack-years)	29 ± 15	35 ± 12
Pulmonary function		
Post-bronchodilator		
FEV_1 (% pred)	92 ± 15	83 ± 6
FEV_1/FVC (% pred)	82 ± 6	85 ± 9
Pre-bronchodilator		
FEV_1 (% pred)	92 ± 15	93 ± 11
FEV_1/FVC (% pred)	82 ± 6	85 ± 6
SVC (% pred)	106 ± 21	104 ± 10
IC (% pred)	104 ± 16	112 ± 15
FRC (% pred)	121± 24	115 ± 23
RV (% pred)	121 ± 18	122 ± 4
TLC (% pred)	110 ± 18	113 ± 27
D_{LCO} ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	25 ± 5	13 ± 3***
D_{LCO} (% pred)	90 ± 13	53 ± 8***
D_{LCO} (z-score)	-0.23 ± 0.61	-3.27 ± 0.95***
V_A (% pred)	83 ± 15	88 ± 14

Values are mean ± SD. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; SVC: slow vital capacity; IC: inspiratory capacity; FRC: functional residual capacity; RV; residual volume; TLC: total lung capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; V_A: alveolar volume.
***P<0.001

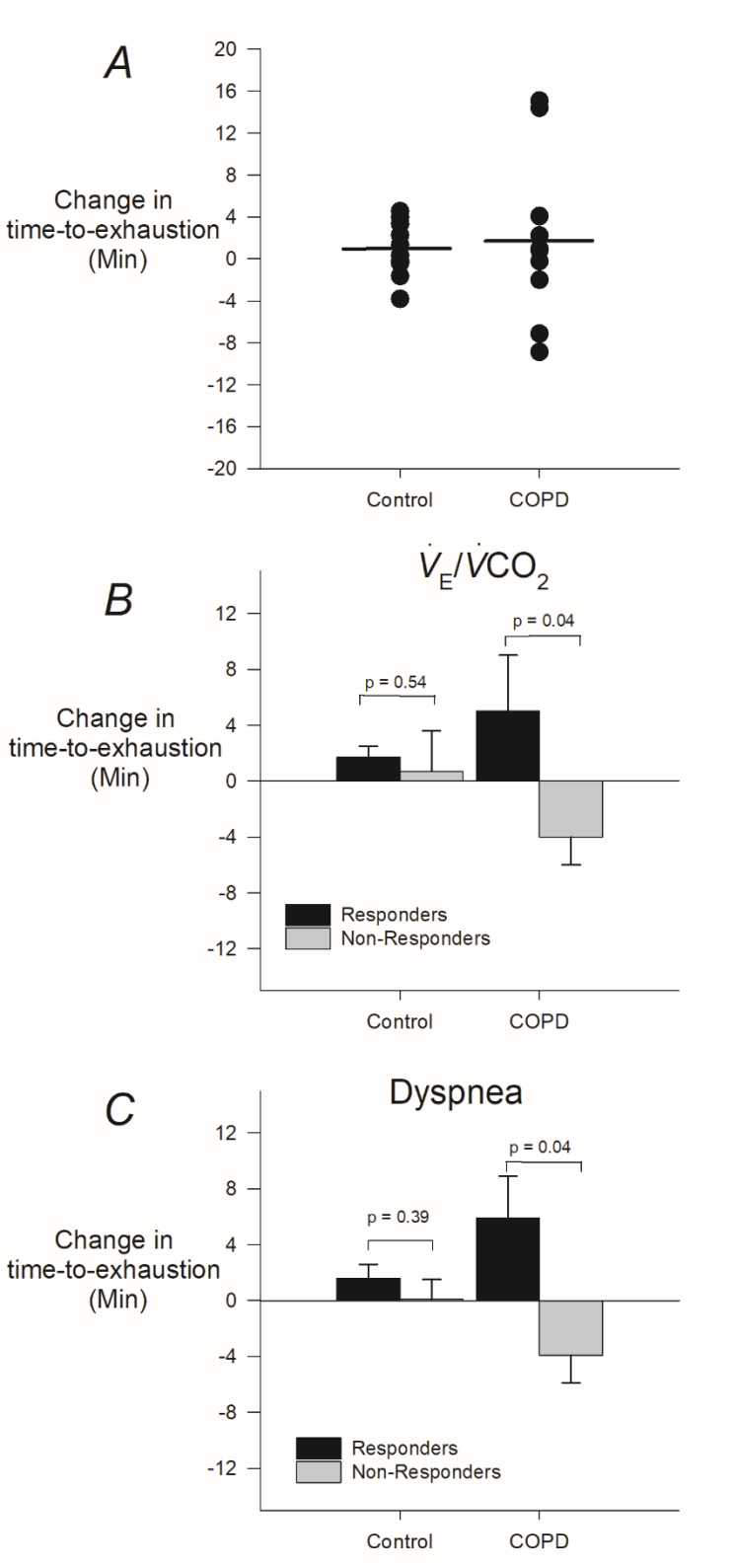

Figure 6.2. Fig. 6.2A displays individual responses in the change in exercise endurance time, from saline to dopamine, in controls and COPD. Fig. 6.2*B* illustrates the change in exercise endurance, from saline to dopamine, in individuals who had improved $\dot{V}_E/\dot{V}CO_2$ (responders) and individuals who showed no change or worsened $\dot{V}_E/\dot{V}CO_2$ (non-responders) at 4-minute isotime, with dopamine. Fig. 6.2*C* illustrates the change in exercise endurance, from saline to dopamine, in individuals who had reduced dyspnea (responders) and individuals who showed no change or worsened dyspnea (non-responders) at 4-minute isotime, with dopamine. In fig 6.2*B* and 6.2*C*, values are mean ± SD.

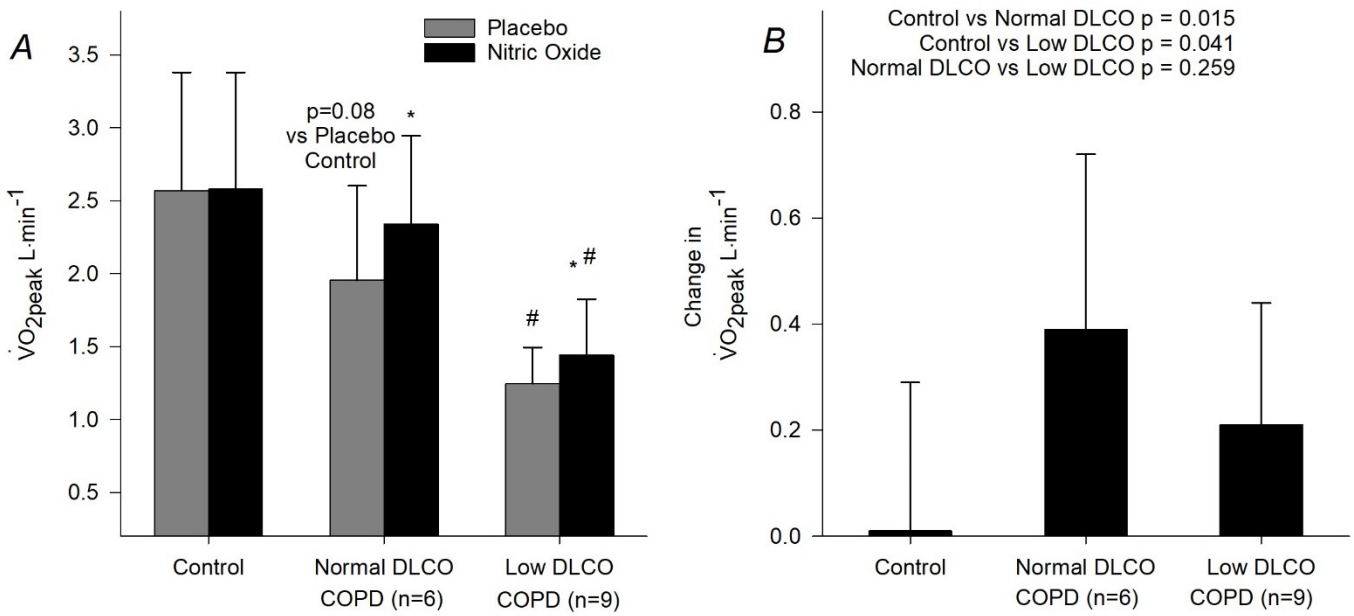

Figure 6.3. Figure 6.3.A displays mean ± SD oxygen uptake ($\dot{V}O_{2peak}$) in healthy controls, mild COPD patients with normal diffusing capacity for carbon monoxide (DLCO) and mild COPD patients with low DLCO. Figure 6.3.B displays the mean ± SD change in $\dot{V}O_{2peak}$ from placebo to inhaled nitric oxide.

* $p<0.05$ between condition within group
# $p<0.05$ compared to control placebo

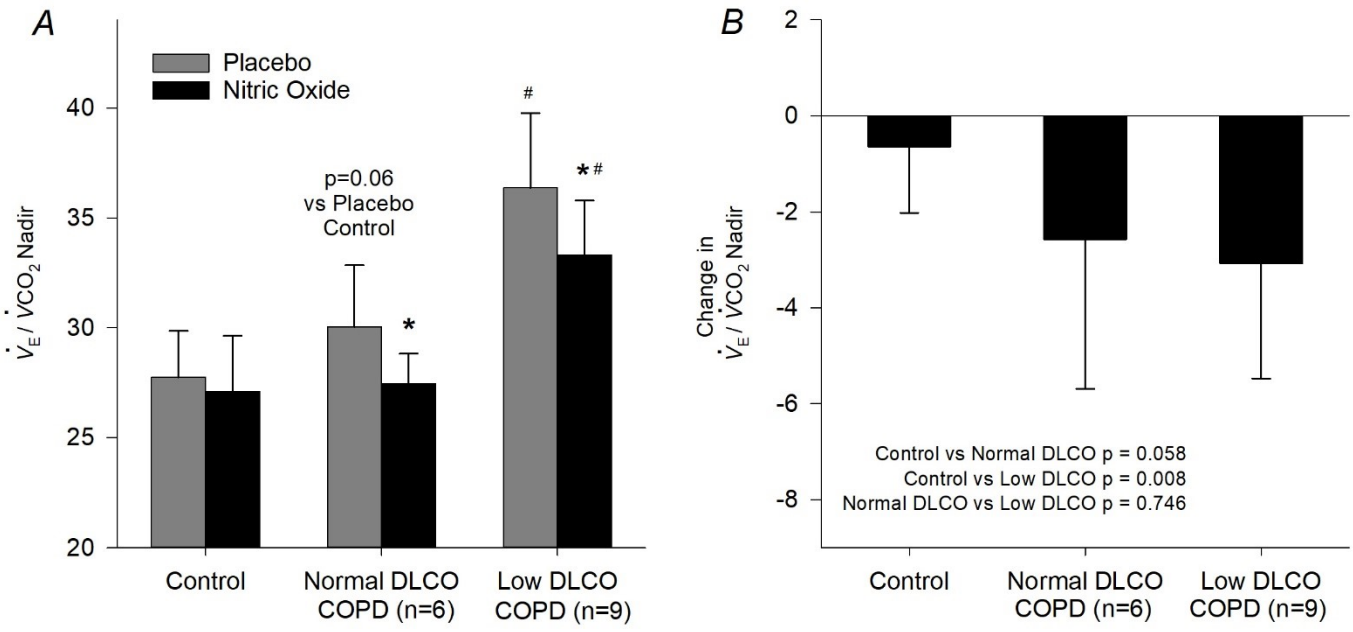

Figure 6.4. Figure 6.4.A displays mean ± SD nadir ventilatory equivalent to carbon dioxide production ($\dot{V}_E/\dot{V}CO_2$) in healthy controls, mild COPD patients with normal diffusing capacity for carbon monoxide (DLCO) and mild COPD patients with low DLCO. Figure 6.4.B displays the mean ± SD change in $\dot{V}_E/\dot{V}CO_2$ from placebo to inhaled nitric oxide.

* $p<0.05$ between condition within group
# $p<0.05$ compared to control placebo

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