# Understanding the impact of pulmonary vascular structure and function on exercise tolerance and dyspnea in health and chronic obstructive pulmonary disease

by

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## ABSTRACT

The purpose of this dissertation was to examine pulmonary vascular structure and function and its relationship with exercise capacity in individuals with and without chronic obstructive pulmonary disease (COPD). We hypothesized that pulmonary vascular structure and function play a key role in ventilation, dyspnea and exercise tolerance both in health and in COPD. This doctoral dissertation consists of three distinct studies. In the first and second study, we used multimodal research methods to evaluate the relationship between pulmonary vascular structure and function with exercise capacity and physical activity. These analyses used cross-sectional and longitudinal data from the Canadian Cohort Obstructive Lung Disease (CanCOLD) study. For the final study, we took an experimental approach to evaluate the therapeutic potential of pulmonary vascular targets in a prospective cohort of patients with COPD using a placebo controlled, double-blind, randomized control cross-over design.

The first study aimed to test if baseline physical activity and $\dot{V}O_{2peak}$ are related to baseline pulmonary diffusing capacity and quantitative computed tomography (CT) pulmonary vascular measurements. Participants from the CanCOLD study were categorized into three groups: 1) NS: never-smokers with normal spirometry; 2) ES: ever-smokers with normal spirometry; and 3) COPD: smokers with spirometric airflow obstruction. Total airway count (TAC), total vessel volume (TBV), the volume for all vessels with a cross-sectional area $\leq$5 $mm^2$ (BV5), and between 5-10 $mm^2$ (BV5-10) were calculated from CT scans, and pulmonary diffusing capacity for carbon monoxide ($D_LCO$) was evaluated via the single breath $D_LCO$ technique. $\dot{V}O_{2peak}$ was evaluated by symptom-limited incremental cycle cardiopulmonary exercise tests (CPET). A total of 1,004 participants (NS: 263; ES: 407; COPD: 334) met inclusion criteria. General linear regression models revealed that even after controlling for $FEV_1$, emphysema severity and body morphology,

$\dot{V}O_{2peak}$ was independently associated with $D_LCO$, TBV, BV5, BV5-10 but not TAC. Our results suggest that exercise capacity may be predictive of structural and functional differences in the pulmonary vasculature; and that these relationships are not limited to COPD.

The second project evaluated if higher baseline $\dot{V}O_{2peak}$ is protective of pulmonary vascular decline over three years and if higher baseline pulmonary vascular measures are protective of $\dot{V}O_{2peak}$ decline over the same timeline. A tertiary aim was to determine whether potential associations between pulmonary vascular structure/function and $\dot{V}O_{2peak}$ decline are mediated by exercise ventilatory inefficiency ($\dot{V}_E/\dot{V}CO_2$ nadir). Participants with baseline (visit 1) and 3 year follow-up data (visit 3) from the CanCOLD study were categorized into 2 groups: 1) ES; and 2) COPD. CT scan measurements at visits 1 and 3 were generated with identical methods as study #1. Separate random slope and intercept linear mixed effect models were built to evaluate the relationship between baseline $\dot{V}O_{2peak}$, longitudinal change in $D_LCO$ and CT-pulmonary vascular measures, while adjusting for baseline group and participant characteristics (e.g., height, $FEV_1$, etc.,), and vice-versa (relationship between baseline $D_LCO$ and CT-pulmonary vascular measures and longitudinal change in $\dot{V}O_{2peak}$). For the tertiary aim, a mediation model was built to evaluate the relationship between baseline $D_LCO$, LnLAA-950, and BV5/TVV (predictors), with change in $\dot{V}O_{2peak}$ (outcome) through baseline $\dot{V}_E/\dot{V}CO_2$ nadir (mediator). Our findings were threefold: 1) Independent of emphysema and degree of airflow obstruction, higher baseline $\dot{V}O_{2peak}$ was protective of small vessel volume decline in ES without COPD and in individuals with COPD; 2) Pulmonary vascular structure/function measures were not associated with longitudinal decline in $\dot{V}O_{2peak}$ in ES or in individuals with COPD; 3) however, lower baseline BV5/TVV and higher $D_LCO$ was associated with lower $\dot{V}_E/\dot{V}CO_2$ nadir, which in turn was associated with $\dot{V}O_{2peak}$ decline at 3-years follow-up, suggesting a mechanistic role of baseline pulmonary vascular structure and

function in determining decline in exercise capacity through exercise ventilatory efficiency in people with and without COPD.

In the third and final project of this dissertation, we conducted a randomized double-blind placebo-controlled cross-over trial to examine the effects of an acute inhaled selective pulmonary vasodilator (inhaled nitric oxide [iNO]) on $\dot{V}_E/\dot{V}CO_2$, dyspnea and $\dot{V}O_{2peak}$ in patients with mild to severe COPD without pulmonary hypertension (NCT03679312). Fifty-two patients with mild to severe COPD were recruited. The first visit comprised of participant enrollment, pulmonary function test, and a symptom limited incremental CPET to determine $\dot{V}O_{2peak}$. At visit 2, participants completed a cardiac ultrasound to estimate right ventricular systolic pressure with and without iNO. At the next two visits, participants completed an incremental CPET breathing either room air (placebo) or iNO (room air with 40ppm iNO; order of intervention randomized). Finally, at the last visit, participants underwent a chest CT scan to quantify emphysema severity, total vessel count (TVC; a measure of vascular pruning), TBV, and BV5. Twenty nine of 52 patients demonstrated an increase in $\dot{V}O_{2peak}$ with iNO, but in fully adjusted linear mixed models, iNO did not significantly improve $\dot{V}O_{2peak}$ (fixed effect of condition: β= -7.93, 95%CIs= -18.41 to 2.56) or $\dot{V}_E/\dot{V}CO_{2nadir}$ (fixed effect of condition: β=0.59, 95%CIs= -2.19 to 3.38); however, there was a significant effect of condition on dyspnea (β=-3.41, 95%CIs= -5.25 to -1.57). In secondary analyses, we found that there was no correlation between the change in $\dot{V}O_{2peak}$ (%pred) with iNO and $FEV_1$ (%pred) (condition by $FEV_1$ interaction: p=0.97), $D_LCO$ (condition by $D_LCO$ interaction: p=0.73) or emphysema (condition by LAA-950% interaction: p=0.20). Exploratory analyses revealed that among CT variables, the measure most closely associated with change in $\dot{V}O_{2peak}$ with iNO was TVC (Condition by TVC interaction: β= 0.001, 95%CIs= -4.74E-5 to 0.001), even after accounting for severity of airflow obstruction and emphysema.

The results within this dissertation highlight the key role of pulmonary vascular structure and function in health and disease and demonstrates that pulmonary vascular function may be a potential therapeutic target to improve ventilation and exercise capacity in those with COPD.

## PREFACE

This thesis is an original work by Sophie Collins. The research projects, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, "The effect of inhaled nitric oxide on dyspnea and exercise tolerance in COPD", Pro00078715, 3/13/2018; and "Physical activity and exercise capacity, and their relation to airway- and vascular disease in COPD", Pro00108856, 03/17/2021.

Some of the results reported in this thesis are a sub analysis of data stemming from a national research collaboration, the Canadian Cohort Obstructive Lung Disease (CanCOLD) (**Studies 1&2**). A version of the research project in **Chapter 3** of this thesis is currently under review: S. Collins., et al., "Exercise capacity is related to pulmonary vascular structure and function in health and COPD".

I was responsible for all parts of the three studies, including study #3 design, and CanCOLD secondary analysis design, data analysis, and manuscript preparation. The co-authors assisted with data collection (Study #3), interpretation of results and manuscript preparation (Studies #1-3). Stickland MK was the principal investigator and oversaw all aspects of the studies presented in this dissertation.

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## LIST OF ABBREVIATIONS

AWT: airway wall thickness

BHT: breath-hold time

BV5: volume for vessels <5 $mm^2$ in cross-sectional area

BV5-10: volume for vessels between 5-10 $mm^2$ in cross-sectional area

BV10: volume for vessels >10 $mm^2$ in cross-sectional area

CanCOLD: Canadian Cohort Obstructive Lung Disease

CAT: COPD assessment test

COPD: chronic obstructive pulmonary disease

CPET: cardiopulmonary exercise test

CT: computed tomography

$D_LCO$: capacity of the lungs for carbon monoxide

Dm: diffusing membrane capacity

EDV: end-diastolic volume

EELV: end-expiratory lung volume

EF: ejection fraction

EILV: end-inspiratory lung volume

ES: ever-smoker

ESV: end-systolic volume

$FEV_1$: forced expiratory volume in one second

$F_IO_2$: fraction of inspired oxygen

FVC: forced vital capacity

GLI: global lung initiative

GOLD: global obstructive lung disease

HPV: hypoxic pulmonary vasoconstriction

HU: Hounsfield units

IC: inspiratory capacity

IRV: inspiratory reserve volume

LAA: low attenuation areas

LAA-856: low-attenuation areas of the lung below -856 Hounsfield units

LAA-950: low-attenuation areas of the lung below -950 Hounsfield units

iNO: inhaled nitric oxide

KCO: carbon monoxide transfer coefficient

LVOT: left-ventricular outflow tract

LVSV: left-ventricular stroke volume

MRC: medical research council

MRI: magnetic resonance imaging

MVPA: moderate to vigorous physical activity

NO: nitric oxide

NS: never-smoker

$O_2$: oxygen

PA: pulmonary artery

PA: physical activity

PASP: pulmonary artery systolic pressure

Pi10: internal airway perimeter of 10 mm

PV1: percent of vessels with a radius < one voxel

$P_AO_2$: alveolar partial pressure of oxygen

RCT: randomized controlled trial

RA: right atrium

RV: right ventricle

RV: residual volume

RVEF: right-ventricular ejection fraction

RVSP: right-ventricular systolic pressure

SV: stroke volume

TAC: total airway count

TBV: total blood vessel volume

TLC: total lung capacity

TLV: total lung volume

TR: tricuspid regurgitation

TVC: total vessel count

TVV: total blood vessel volume

V1: visit 1

V3: visit 3

$V_A$: alveolar volume

Vc: capillary blood volume

# CHAPTER ONE: INTRODUCTION

### *1.1 General introduction*

Despite many decades of research and pharmacological treatment of airway pathophysiology in chronic obstructive pulmonary disease (COPD), there has been limited progress in slowing disease progression or mortality, due to the aging population, increasing smoking rates, and "scarcity of effective disease modifying therapies" worldwide (1, 2). Therefore, the latest Global Strategy for prevention, diagnosis and management of COPD emphasize the need to consider distinct endotypes of disease pathophysiology (3). In other words, there has been an appeal for scientists and clinicians to "go back to the drawing board", and identify novel potential therapeutic targets.

Research study designs can be either observational (i.e., descriptive or exploratory) or experimental, each with different goals, participant sampling techniques, and methodologies. As its name implies, the goal of observational research is to study a group or population as they naturally exist, without manipulation of variables via experiments, with the aim of finding relationships (4). As such, exploratory studies are well suited to identifying distinct characterizations of COPD pathophysiology. One such endotype that has been identified is pulmonary vascular dysfunction: Hueper and colleagues recently demonstrated that pulmonary microvascular blood flow is reduced across the continuum of COPD, even in those with mild disease (5). Similarly, others have demonstrated that reduced pulmonary diffusion capacity in COPD is secondary to lower pulmonary capillary blood volume (6), which appears to contribute to worse exercise ventilatory efficiency, dyspnea, and exercise capacity. Therefore, impairments in pulmonary vascular function may have important functional consequences in individuals with COPD, representing a promising therapeutic target.

Experimental research studies evaluate cause and effect. The experiment is the most rigorous form of scientific investigation, with the randomized control trial (RCT) being the gold standard of experimental study designs. An RCT is defined as an experimental study where a clinical treatment is compared to a control condition, and participants are randomly assigned to groups (4); providing the opportunity to test novel therapies. Using a placebo controlled randomized crossover design, our laboratory recently found that acute inhaled selective pulmonary vasodilation (via inhaled nitric oxide [iNO]) significantly improved ventilatory efficiency, dyspnea, and exercise capacity in individuals with mild COPD (7). However, it was noted that responses were largely heterogeneous, suggesting that there are responders and non-responders to the drug. Furthermore, since this study only included patients with mild COPD, it remains unclear whether iNO has similar effects in those with more severe airflow obstruction (without pulmonary hypertension or hypoxemia). Together, exploratory and experimental research methods have the potential to aid us in better understanding the effects of COPD on pulmonary vascular structure/function and exercise, and in filling the vast treatment gap currently observed in the clinical management of COPD.

### *1.2 Purpose of dissertation*

The overall purpose of this dissertation was to examine pulmonary vascular structure and function and its relationship with exercise capacity in individuals with and without COPD, using exploratory and experimental research methods. We hypothesized that pulmonary vascular structure and function play a key role in ventilation, dyspnea and exercise tolerance both in health and in COPD.

**Study #1** and **Study #2** utilized exploratory research methods to evaluate the relationship between pulmonary vascular structure and function with exercise capacity and physical activity. These secondary analyses used cross-sectional and longitudinal data from the Canadian Cohort Obstructive Lung Disease (CanCOLD) study. The first study aimed to test if baseline physical activity and $\dot{V}O_{2peak}$ are related to pulmonary diffusing capacity and CT-pulmonary vascular measures in never-smokers, smokers, and patients with COPD. The second project evaluated if higher baseline $\dot{V}O_{2peak}$ is protective of pulmonary vascular decline, or if higher baseline pulmonary vascular measures are protective of $\dot{V}O_{2peak}$ decline. It was hypothesized that lower $\dot{V}O_{2peak,}$ and physical activity would be associated with pulmonary vascular measures both cross-sectionally and longitudinally, even after controlling for severity of airflow limitation.

The final study of this thesis (**Study #3**) took an experimental approach to evaluate the therapeutic potential of pulmonary vascular targets in patients with COPD. We tested the effects of an acute inhaled selective pulmonary vasodilator (inhaled nitric oxide [iNO]) on $\dot{V}_E/\dot{V}CO_2$, dyspnea and $\dot{V}O_{2peak}$ in patients with mild to severe COPD. We aimed to identify characteristics beyond airflow obstruction (such as emphysema, $D_LCO$) associated with response to iNO. We hypothesized that iNO would improve $\dot{V}O_{2peak}$ specifically in individuals with a more intact pulmonary vasculature, as evaluated via advanced pulmonary function testing and pulmonary imaging techniques.

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# CHAPTER TWO: REVIEW OF THE LITERATURE

### *2.1 Diagnosis and classification of COPD severity*

According to the American Thoracic Society, a diagnosis of COPD should be considered in patients presenting with ongoing symptoms of cough, sputum production, or dyspnea, and/or a history of exposure to risk factors for the disease (i.e. tobacco smoke; occupational dusts and chemicals (vapors, irritants, and fumes); and indoor/outdoor air pollution) (8). To confirm a diagnosis, spirometry must be completed (9), and a postbronchodilator forced expiratory volume in 1 s ($FEV_1$)/forced vital capacity (FVC) ratio less than 0.70 (or <LLN) is required to confirm the presence of persistent airflow limitation (8). Patients are further stratified into 4 stages of disease severity based on spirometry: GOLD 1 (mild COPD) post bronchodilator $FEV_1 > 80\%$ predicted; GOLD 2 (moderate COPD) post bronchodilator $50\% \leq FEV_1 < 80\%$ predicted; GOLD 3 (severe COPD) post bronchodilator $FEV_1$ $30\% \leq FEV_1 < 50\%$ predicted; and GOLD 4 (very severe COPD) post bronchodilator $FEV_1 < 30\%$ predicted (10). As disease severity worsens, the frequency of COPD exacerbations increases (11).

### *2.2 The progression of COPD*

A combination of small airway disease due to obstructive inflammation (e.g., obstructive bronchiolitis) and parenchymal destruction (emphysema) cause the chronic airflow limitation observed in COPD (8). In short, emphysema is “a condition of the lung characterized by abnormal permanent enlargement of the airspaces distal to the terminal bronchiole, accompanied by destruction of their walls, and without obvious fibrosis"(12). The degree of emphysema has been shown to correlate well with the extent of impairment of diffusing capacity for carbon monoxide per litre of alveolar volume ($D_LCO/V_A$), even after accounting for $FEV_1$%predicted (13). Furthermore, COPD severity can be characterized by pathological changes at the level of the

airways, parenchyma and pulmonary vasculature (14). These pathological changes include chronic inflammation as well as structural changes related to recurring injury and repair.

### *2.3 Exercise intolerance in COPD*

Exertional dyspnea is a common symptom of COPD and is the primary reason for exercise intolerance in patients with even mild airway obstruction (15-17). Ratings of perceived dyspnea are elevated at any given rate of oxygen consumption ($\dot{V}O_2$), work-load or minute ventilation in patients with COPD relative to healthy controls (18, 19). Furthermore, common qualitative descriptor choices at end exercise suggest increased perceived "work/effort" and "unsatisfied inspiration" (19). Previous work in COPD has shown that dyspnea is the result of increased work of breathing during exercise (16). This increased work of breathing may arise from 1) airflow limitation (*i.e.,* expiratory flow limitation and dynamic hyperinflation), and/or 2) an exaggerated ventilatory response to exercise (*i.e.,* increased minute ventilation relative to carbon dioxide production, $\dot{V}_E/\dot{V}CO_2$)(17, 20-23).

With disease progression there is a gradual reduction in inspiratory capacity ([IC], the sum of inspiratory reserve volume and tidal volume). Consequently, patients prematurely arrive at their physiological limits during exercise (24). As reviewed in detail elsewhere (25), a low resting IC will limit the degree of tidal volume expansion available during exercise whereby increased metabolic demands lead to greater minute ventilation requirements. This is important in patients with COPD, as expiratory flow limitation and lung hyperinflation are common. Therefore, if a patient has a significantly low IC at rest, tidal volume expansion will be limited, and minute ventilation at peak exercise will be lower than in those with a greater resting IC (24). Tachypnea can also contribute to further worsening inspiratory muscle function as well as pulmonary gas

exchange (25). Specifically, patients reach a point whereby tidal volume no longer increases; this plateau represents a discrepancy between increasing inspiratory neural drive and the mechanical/muscular response of the respiratory system (26). This phenomenon is referred to as neuromechanical dissociation (or uncoupling or demand-capacity imbalance), and it has been shown to occur at lower exercise workloads as COPD severity worsens (26).

*2.3.1 Ventilatory inefficiency in COPD*

Patients with COPD have an elevated ventilation to carbon dioxide production relationship [$\dot{V}_E/\dot{V}CO_2$; a surrogate marker of ventilatory (in)efficiency] during exercise (27). An elevated $\dot{V}_E/\dot{V}CO_2$, which reflects greater ventilatory demand for a given metabolic load, has been associated with reduced pulmonary diffusing capacity for carbon monoxide ($D_LCO$), and worse emphysema (28-32). Elbehairy and colleagues found that the increased exercise $\dot{V}_E/\dot{V}CO_2$ observed in patients with mild COPD was secondary to increased deadspace ventilation, which resulted in a compensatory increase in total minute ventilation (*i.e.,* greater $\dot{V}_E/\dot{V}CO_2$) to maintain effective alveolar ventilation ($\dot{V}_A$) (17). Both ventilatory mechanical constraint as well as airflow limitation can negatively affect ventilation during exercise in these patients, and in the presence of ventilatory inefficiency, patients may experience earlier attainment of dynamic mechanical constraint (critical decrease in inspiratory reserve volume); these changes in ventilatory mechanics lead to elevated dyspnea and exercise intolerance (15, 27, 31, 33, 34). The elevated $\dot{V}_E/\dot{V}CO_2$ response to exercise in COPD is clinically important because it independently predicts mortality, and indicates that physiological abnormalities beyond airflow obstruction are important in determining exercise tolerance and dyspnea (20, 35).

The $\dot{V}_E/\dot{V}CO_2$ response to exercise during a CPET can be evaluated in the following three ways: 1) nadir $\dot{V}_E/\dot{V}CO_2$, 2) $\dot{V}_E/\dot{V}CO_2$ slope, and 3) $\dot{V}_E/\dot{V}CO_2$ y-intercept. As discussed in a recent review by Phillips et al., (36), the $\dot{V}_E/\dot{V}CO_2$ response is hyperbolic during light exercise and decreases progressively to its lowest point (nadir) before increasing at heavy exercise intensities (37). In short, there are two ventilatory threshold points during incremental exercise, with the first one occurring when anaerobic metabolism is activated (where the slope of $\dot{V}CO_2/\dot{V}O_2$ becomes greater than 1) (38-40). Of interest here, the second ventilatory threshold (termed the respiratory compensation point), occurs when metabolic acidosis exceeds bicarbonate buffering capacities, at which point hyperventilation develops ($\dot{V}_E$ increases relative to $\dot{V}CO_2$), leading to the observed increase in $\dot{V}_E/\dot{V}CO_2$ with heavier exercise intensities (38). The $\dot{V}_E/\dot{V}CO_2$ slope is determined by analyzing the slope of the regression line relating $\dot{V}_E$ and $\dot{V}CO_2$ during exercise (41, 42). Lastly, as the name suggests, the y-intercept is the point at which the regression line relating $\dot{V}_E$ (y-axis) and $\dot{V}CO_2$ (x-axis) crosses the y-intercept (x=0). Thus, both $\dot{V}_E/\dot{V}CO_2$ slope and y-intercept can be obtained through the linear regression equation ($y = mx + b$).

Neder and colleagues evaluated the above three measures of exercise ventilatory inefficiency (y-intercept, nadir and slope) in patients with varying degrees of COPD severity from mild to end-stage disease (GOLD stage 1-4) (31). The authors found that exercise ventilatory inefficiency is an important physiological marker that is related to clinically relevant end-points across all stages of COPD. All COPD patients in the study had greater $\dot{V}_E/\dot{V}CO_2$ y-intercept (GOLD stages 1-4) than controls. Those with mild-moderate COPD (GOLD stage 1 and 2) had higher slopes, resulting in higher nadir. In those with more severe COPD (GOLD stages 3 and 4) Y-intercept further worsened, but $\dot{V}_E/\dot{V}CO_2$ slope went down, with no difference in nadir $\dot{V}_E/\dot{V}CO_2$ between patient groups (31). This divergent response in slope and nadir is secondary to worsening mechanical

constraints (43, 44), shorter exercise test duration, and increases in carbon dioxide set-point (45). Therefore, the $\dot{V}_E/\dot{V}CO_2$ y-intercept better expresses the worsening of exercise ventilatory inefficiency across severities of COPD (31).

### *2.4 Pulmonary vascular dysfunction and destruction in COPD*

Microvascular abnormalities within the lungs may contribute to the increased deadspace ventilation and inappropriate $\dot{V}_E/\dot{V}CO_2$ response that occurs during exercise in COPD. An early study found morphological abnormalities of the endothelium of the pulmonary artery in patients with even *mild* COPD (46), suggestive of pulmonary vascular dysfunction. More recently, Hueper et al. found that pulmonary microvascular blood flow is reduced across the continuum of disease severity (reduced by 30% in mild COPD, 29% in moderate COPD, and 52% in severe COPD) on gadolinium enhanced magnetic resonance imaging (MRI) (5). The authors concluded that the reduced pulmonary perfusion in a nonemphysematous intact pulmonary vascular bed was likely the result of pulmonary vascular dysfunction (5). Together these studies provide compelling evidence that COPD is not just a disease of the airways, but has important effects on the pulmonary microvasculature.

In line with the hypothesis that pulmonary vascular dysfunction and hypoperfusion contribute to an exaggerated ventilatory response to exercise, our laboratory recently demonstrated a blunted pulmonary capillary blood volume (Vc) response to exercise in mild COPD compared to healthy age- and height-matched non-smoking controls (47). Importantly, the low Vc was associated with increased $\dot{V}_E/\dot{V}CO_2$ (i.e., worse ventilatory efficiency) during exercise, suggesting that low pulmonary perfusion leads to elevated deadspace and/or regions of high alveolar ventilation relative to perfusion ($\dot{V}_A/\dot{Q}$). Further, in patients with moderate COPD, Vc did not rise

with increasing exercise intensity. Similarly, we recently found that $D_LCO$ and Vc were persistently reduced in the supine position, which suggests that pulmonary vascular destruction is a contributing factor to the blunted $D_LCO$ and Vc response to exercise in patients with mild COPD (48). Together, these findings suggest that pulmonary vascular function worsens with disease severity, and that vascular destruction (i.e., emphysema) may occur even in mild COPD.

### *2.5 Radiological Findings in COPD*

A recent study by Kirby and colleagues, sought to determine whether total airway count [TAC, quantified *in vivo* using quantitative computed tomography (CT)] reflects early airway-related disease changes and is associated with lung function decline independent of emphysema in COPD (49). The authors evaluated patients enrolled in the multicenter, population-based, longitudinal CanCOLD (Canadian Cohort Obstructive Lung Disease) study who underwent spirometry testing at four visits over a 6-year period. They found that TAC was 19% lower in GOLD I and II compared to controls, that parent airways with missing daughter branches had smaller lumens and thinner walls, and finally, that a lower TAC was independently related to lung function decline over time. Importantly, among all CT measures, TAC had the greatest influence on $FEV_1$, $FEV_1$/FVC, and bronchodilator responsiveness. These findings suggest that TAC is a valuable clinical tool in addition to quantifying emphysema severity in predicting future disease progression.

In addition to evaluating anatomical changes to the airways, advances in CT technology also allow evaluation of pulmonary vascular changes in COPD. Alford and colleagues found that pulmonary blood flow heterogeneity (quantified by multidetector row CT perfusion contrast imaging) in emphysema-susceptible smokers with normal lung function, was greater than in never

smokers (50). The authors concluded that the increased blood flow heterogeneity observed in smokers susceptible to emphysema is likely not entirely attributable to parenchymal destruction and is suggestive of pulmonary vascular *dysfunction* (50). In a study by Estépar and colleagues (51) using volumetric CT scans of the lungs, it was found that the extent of distal pruning of pulmonary vasculature is predictive of COPD severity. The authors found that a lower total vascular volume was associated with a diminished $D_LCO$; however, the multiple fraction of inspired oxygen ($FiO_2$) method to estimate capillary blood volume (Vc) was not completed in this study. To our knowledge, no study to date has compared Vc as estimated by the multiple $FiO_2$ technique to CT-quantified total vascular volume (TBV, the volume of all vessels [arteries and veins combined] (43, 44)) or the small pulmonary vessel volume (BV5, the aggregate vessel volume for vessels less than 5 $mm^2$). However, Morrison and colleagues (52) compared emphysema severity as quantified by CT imaging with Vc, and found that Vc did not improve the discrimination of cases with and without emphysema. As stated earlier, this may be explained by the finding that vascular dysfunction may occur even in mild disease (without emphysema) (6). Although BV5 has been shown to be related to emphysema in patients with COPD (29), the rate of decline in BV5 and emphysema score are not linearly related over time (47). Work by Barker and colleagues (3) suggests that longitudinal changes in CT pulmonary vascular structure and MRI derived ventilation may be asynchronous both in ex-smokers with normal spirometry and patients with COPD. The authors showed a significant reduction in the percent of vessels with a radius < one voxel ($PV_1$), but not TBV ($p=0.06$), over 2.5 years in ex-smokers with and without COPD (3), which suggests pulmonary vascular pruning over time.

Together, these imaging studies provide evidence that tissue (emphysema), airway (TAC) and vascular (TBV and BV5) measures on CT imaging are likely important predictors of decline

in COPD. Interestingly, this recent work provides evidence that vascular, tissue and airway changes do not necessarily occur in parallel, and these different measures may help identify airway vs vascular predominant phenotypes of COPD, which may allow us to better identify future therapeutic targets.

### *2.6 Ventilation-perfusion mismatch in COPD*

Importantly, hypoxic pulmonary vasoconstriction (HPV) may occur as COPD severity progresses, in an attempt to improve $\dot{V}_A/\dot{Q}$ matching. This may lead to further lung structure changes such as intimal hyperplasia (thickening of the tunica intima; the innermost layer of an artery or vein, comprised of endothelium and elastic lamina) and smooth muscle hypertrophy and hyperplasia (53). The resulting pulmonary arterial hypertension may be secondary to a combination of airflow limitation as well as emphysematous destruction of the pulmonary capillary bed (54).

Importantly, $\dot{V}_A/\dot{Q}$ mismatch is the main cause of arterial hypoxemia both in stable COPD and during COPD exacerbations (55-58). The $\dot{V}_A/\dot{Q}$ ratio represents the relative matching of alveolar ventilation to perfusion, and thus $\dot{V}_A/\dot{Q}$ mismatch or inequality may be due to inadequate alveolar ventilation relative to perfusion, as occurs with obstructive lung disease, and/or by inadequate perfusion relative to alveolar ventilation of the respective alveolar unit (with shunt and deadspace at either extremes of the continuum). In their pioneering work, Wagner and colleagues evaluated the mechanism of gas exchange impairment using the multiple inert gas elimination technique in patients with severe to advanced stable COPD (59). Patients were stratified into 3 groups: 1) Type A COPD: with evidence of anatomical emphysema, minimal cough/sputum production, marked lung overdistension (hyperinflation), no history of peripheral edema,

attenuated vessels on chest X-ray, reduced elastic lung recoil, relatively mild hypoxemia, and no $CO_2$ retention; 2) Type B COPD: with chronic bronchitis, *cor pulmonale*, normal lung elastic recoil, airway resistance, severe hypoxemia, and $CO_2$ retention, and lastly 3) a combination of Type A and B features. The authors found that the majority of Type A COPD patients had a pattern of regions of high $\dot{V}_A/\dot{Q}$ ratios, no areas of low $\dot{V}_A/\dot{Q}$, and no areas of intrapulmonary shunt. These findings were suggested to be due to loss of blood flow related to alveolar wall destruction. Areas of low $\dot{V}_A/\dot{Q}$ ratios and no shunt were primarily observed in patients with Type B COPD (chronic bronchitis), with almost no high $\dot{V}_A/\dot{Q}$ areas. This pattern was attributed to low ventilation secondary to mechanical airway obstruction/distortion (59). In the three patients with a combination of Type A and B COPD, 2 had both high and low $\dot{V}_A/\dot{Q}$ areas, and one had a high $\dot{V}_A/\dot{Q}$ pattern. In a more recent study, Rodríguez-Roisin and colleagues studied the relationship between pulmonary gas exchange and airflow limitation in patients with COPD across the severity spectrum (60). The authors found that the progression of $\dot{V}_A/\dot{Q}$ inequality with spirometric severity ($FEV_1$) is modest. They suggested that this modest relationship may be related to reductions in local ventilation and perfusion in the same regions through airway and alveolar disease as well as capillary involvement (60). Both of these studies provide convincing evidence that not only is COPD a largely heterogeneous disease, but that traditional COPD staging (based on spirometric values) may not be useful in terms of identifying COPD phenotypes.

Transitioning from the upright to supine position has been shown to translocate blood from the lower limbs to the chest, leading to increased pulmonary perfusion pressure (61), and improved $D_LCO$ (62). Through this central shift in blood, and increased pulmonary perfusion pressure, it has been suggested that supine positioning may overcome pulmonary microvascular dysfunction in an otherwise intact alveolar-capillary interface (i.e., in non-emphysematous lung regions). With this

in mind, Ross and colleagues recently examined whether the supine position improves $D_LCO$ and Vc responses to exercise in mild COPD (63). In order to differentiate pulmonary vascular dysfunction from destruction, the authors used the multiple-$FiO_2$ technique to evaluate $D_LCO$, Dm and Vc in both the seated and supine position during exercise. The authors found that Vc was blunted in COPD in both conditions, but that the supine position increased exercise Vc (adjusted for alveolar volume) in COPD in a similar degree to healthy controls, which would be attributable to the recruitment and distension of intact capillary beds. The authors thus concluded that patients with mild COPD have partially reversible pulmonary vascular dysfunction, but that pulmonary vascular destruction is a contributing factor to the persistently blunted $D_LCO$ and Vc response to exercise in patients with COPD (63). In other words, this work supports the concept that patients with mild COPD have some potential for pulmonary vascular recruitment.

In summary, there appears to be variability in terms of pulmonary vascular pathophysiology along the continuum of COPD severity, with some describing a vascular phenotype of emphysema (5, 50, 63). Early on in the development of COPD, there appears to be pulmonary vascular dysfunction (5) which may occur before the detection of airflow obstruction (50). In these patients, pharmacotherapies targeting pulmonary vascular function may hold therapeutic benefit. Conversely, in the presence of advanced emphysema and diffuse pulmonary vascular destruction, drugs aiming at improving pulmonary vascular function may not have any beneficial effects.

### *2.7 Interventions targeting the pulmonary vasculature*

#### *2.7.1 Nitric oxide: a pharmacological intervention targeting the pulmonary vasculature*

Airspace enlargement (emphysema) and airway damage have been shown to be preceded by pulmonary vascular remodeling/dysfunction in animal models of tobacco smoke-induced COPD (64, 65). Patients with COPD have been shown to have airway and lung inflammation, as well as systemic inflammation. The downregulation of nitric oxide (NO) bioavailability, secondary to persistently reduced endothelial NO synthase (eNOS), contributes to pulmonary vascular endothelial dysfunction and the development of both emphysema and pulmonary arterial hypertension (PAH) in COPD (66). Interestingly, interventions aiming to increase NO bioavailability and improve pulmonary vascular function actually appear to prevent smoking-induced emphysema in animals (67, 68). Therefore, increasing pulmonary vascular NO bioavailability could hold therapeutic potential to reduce deadspace and dyspnea, and maybe even slow the progression of COPD. Exogenous inhaled nitric oxide (iNO) is known to have selective pulmonary vasodilatory effects, and has been shown to effectively treat pulmonary arterial hypertension (69, 70). The route by which iNO reaches pulmonary artery smooth muscle cells is unclear. However, NO is a lipophilic gaseous molecule, and is therefore readily able to diffuse across the lipid bilayer of cell membranes without requiring active transport. It is therefore likely that pre-capillary gas exchange occurs, whereby iNO diffuses across the airway into the pulmonary artery and/or arteriole smooth muscle (71, 72). In short, the mechanism of action of NO is as follows: 1) once arrived in the vascular smooth muscle cell, NO activates soluble guanylate cyclase; 2) guanylate cyclase catalyzes the conversion of guanosine-5'-triphosphate (GTP) to guanosine 3',5'-cyclic monophosphate (cGMP); and 3) the increased intracellular concentration of cGMP leads to smooth muscle relaxation. It is important to note that the vasodilatory actions of

NO are limited to either its area of synthesis (endogenous) or diffusion into smooth muscle cells by 2 mechanisms: 1) cGMP is hydrolyzed to GMP by cyclic nucleotide phosphodiesterases (PDE), subsequently inhibiting further vasodilation; and 2) once diffused into the blood, NO is scavenged by hemoglobin and is quickly inactivated (73). In other words, NO inhalation does not result in peripheral vasodilation.

Previous studies on the effects of iNO on PAH have used doses ranging from 5ppm to 80pm, but it has been established that doses greater than 20ppm have limited additional hemodynamic effects (73). As described by Ichinose et al. in their review, iNO has been shown to increase blood flow (via vasodilation) to well-ventilated regions in the lung (i.e. improving $\dot{V}_A/\dot{Q}$ matching) that may have elevated vasomotor tone, an effect that is unique from that of intravenous vasodilators which would produce vasodilation throughout the lungs (73).

Despite emerging evidence that COPD is associated with pulmonary vascular dysfunction, there is limited research evaluating the therapeutic potential of iNO along the continuum of COPD severity. However, our group recently completed a randomized double-blind controlled trial examining the effectiveness of 40ppm iNO on $\dot{V}O_{2peak}$ in patients with mild COPD. iNO effectively improved $\dot{V}O_{2peak}$ and dyspnea in patients with mild COPD secondary to a reduction in ventilatory inefficiency ($\dot{V}_E/\dot{V}CO_2$) when compared to placebo (inhaled room air). These findings suggest that vascular dysfunction is an important contributor to ventilation, dyspnea and exercise intolerance in *mild* COPD (7). In addition, these findings would suggest that iNO increases pulmonary microvascular perfusion during exercise, resulting in better $\dot{V}_A/\dot{Q}$ matching, reduced deadspace ventilation and finally reduced ventilation for a given metabolic demand. It is important to note, however, that spirometric values may not predict which patients will respond to iNO versus those who may not. Furthermore, the aforementioned study did not evaluate the following: emphysema

severity, anatomical airway and pulmonary vascular structure, or capillary blood volume. In more advanced COPD with significant emphysema, improving NO bioavailability may not provide clinically meaningful therapeutic benefit.

What remains to be evaluated is whether these effects can be translated to patients with more advanced stages of COPD. Since there is pulmonary vascular *destruction* in patients with severe COPD, iNO may not improve pulmonary microvascular perfusion and thus $\dot{V}_A/\dot{Q}$ matching. With more advanced disease, HPV occurs in an attempt to optimize $\dot{V}_A/\dot{Q}$ matching. In a group of hypoxemic patients with severe COPD and PH, iNO worsened $\dot{V}_A/\dot{Q}$ matching and hypoxemia (74), which would likely result in worse ventilatory efficiency, dyspnea and impaired exercise tolerance. Conversely, in non-hypoxemic patients with severe COPD *without* PH, Roger et al., (75) showed that iNO improved $\dot{V}_A/\dot{Q}$ matching. It is now clear that COPD severity may be defined by much more than the traditional GOLD criteria based on spirometry (8). Therefore, it is important to identify the specific phenotype along the continuum of COPD severity that responds positively to iNO, and that which responds negatively to the drug.

*2.7.2 Physical activity and exercise training and its relation to the pulmonary vasculature*

Exercise training has been shown to increase NO bioavailability in patients with endothelial dysfunction (76), while improving pulmonary diffusing capacity for carbon monoxide ($D_LCO$) (77) and arterial stiffness in patients with COPD (78). Interestingly, aerobic exercise training reduced airway inflammation and emphysema in an animal model of COPD (40). Therefore, increased physical activity may contribute to reducing airway/systemic inflammation, in turn slowing or even preventing further pulmonary vascular and airway changes related to COPD in humans. Individuals with a greater $\dot{V}O_{2peak}$ likely have higher physical activity levels

while showing lower levels of pulmonary and systemic inflammation. Consequently, lower levels of pulmonary and systemic inflammation may protect from pulmonary vascular and airway changes in smokers and patients with COPD; however, this remains unclear.

Population based prospective studies provide a unique opportunity to evaluate pulmonary vascular, tissue and airway changes in health and COPD, while being powered to control for important factors such as age, body morphology, and lung function. The CanCOLD study is built on the Canadian COPD prevalence study (COLD), and aims to better understand the heterogeneity of COPD and disease progression (79). In brief, participants were randomly identified using Statistics Canada census data and were recruited using random digit dialing. Participants recruited included patients with COPD and a balanced subset of non-COPD control participants (never and ever smokers) at nine sites. Participants completed assessments at baseline, 18 months, 3 years and beyond. Baseline visit 1 and visit 3 (V1 and V3, respectively) both included pulmonary function tests, incremental cardiopulmonary exercise tests, questionnaires and quantitative chest computed tomography (CT) scans (79). As such, the CanCOLD study may allow for the evaluation of cross-sectional and longitudinal pulmonary vascular and airway changes (from CT scan measures) and its association with lung function, physical activity, and exercise capacity in smokers and patients with COPD.

### *2.8 Summary*

While there has been much focus on treating airflow limitation in patients with COPD, there have been little improvements in patient outcomes, including survival (80). Indeed, COPD is one of the leading causes of morbidity and mortality worldwide, with disease burden projected to rise by 2030 (2). Ventilatory inefficiency contributes to worsening dyspnea, leading to reduced

exercise capacity in patients with COPD. Both exercise intolerance and ventilatory inefficiency are predictors of mortality in patients with COPD. Recent studies provide convincing evidence that pulmonary vascular function is a modifiable therapeutic target. Furthermore, pulmonary rehabilitation may improve pulmonary function. Therefore, greater exercise capacity and physical activity may be associated with better pulmonary structure/function in people with and without COPD. Furthermore, greater exercise capacity and physical activity may be protective of pulmonary structure/function decline in smokers and people with COPD. And lastly, there may be a pulmonary vascular phenotype most responsive to acute, selective pulmonary vasodilation; however, to our knowledge, no studies have evaluated these questions in COPD.

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# CHAPTER THREE: STUDY #1

## Exercise capacity is related to pulmonary vascular structure and function in health and COPD

A version of this chapter is currently under review - November 2023

### *3.1 Introduction*

The pulmonary circulation plays a key role in appropriately matching ventilation and perfusion, while maintaining a low pulmonary artery pressure, which becomes increasingly important as cardiac output increases with exercise (81). Pulmonary diffusing capacity ($D_LCO$) is associated with the peak rate of $O_2$ consumption ($\dot{V}O_{2peak}$, an index of exercise capacity) in ostensibly healthy adults (82), smokers with normal spirometry (83), and people with chronic obstructive pulmonary disease (COPD) (6, 84). In addition, a higher $\dot{V}O_{2peak}$ is associated with greater pulmonary arteriolar distensibility and capillary blood volume during exercise in health (85, 86), suggesting that individuals with superior pulmonary vascular structure and/or function have a greater $\dot{V}O_{2peak}$. Chronic cigarette smoke exposure contributes to reducing computed tomography (CT)-derived total and small pulmonary vessel volume (TBV and BV5, respectively), and impairing pulmonary microvascular blood flow (5, 87-91). Higher $\dot{V}O_{2peak}$ and moderate-to-vigorous levels of regular physical activity have been associated with attenuated lung function decline and reduced COPD risk among smokers (92, 93). Therefore, greater levels of moderate-to-vigorous physical activity (MVPA), above and beyond the effect of $\dot{V}O_{2peak}$, may be associated with enhanced pulmonary vascular structure and/or function.

Pulmonary vascular pruning (i.e., remodelling and loss of the small pulmonary vessels) on CT has been associated with reduced 6-minute walk distance and mortality in COPD (94). Recent work (95) has also shown that CT-derived pulmonary vascular volumes are positively associated with $\dot{V}O_{2peak}$ in apparently healthy adults without COPD. However, no study to date has evaluated how $\dot{V}O_{2peak}$ and MVPA are related to $D_LCO$ and CT pulmonary vascular measures in individuals with or without COPD. Therefore, the primary purpose of this study was to evaluate the cross-sectional relationship between $\dot{V}O_{2peak}$ and CT-derived pulmonary vascular measures and $D_LCO$

in never-smokers (NS) and ever-smokers (ES) without COPD, and people with COPD using cross-sectional data originating from the Canadian Cohort Obstructive Lung Disease (CanCOLD) study (79). The secondary purpose of this study was to evaluate the separate and combined relationship between $\dot{V}O_{2peak}$ and MVPA with CT-derived pulmonary vascular measures and $D_LCO$. We hypothesized that people with higher $\dot{V}O_{2peak}$ would have greater TBV, BV5, and $D_LCO$ independent of emphysema severity and airflow limitation. Some of the results of this study have been previously reported in the form of an abstract (96).

## *3.2 Methods*

### *3.2.1 Study Participants*

CanCOLD is a prospective, multicentre (nine sites across Canada) longitudinal study evaluating 1,532 adults > 40 years old which were originally recruited from the general population via random digit dialling (79). NS and ES with normal spirometry, and people with COPD from CanCOLD (79) studied at the baseline visit (study visit 1) were included in this cross-sectional analysis. Participants with a full pulmonary function test, quantitative CT scan (of adequate quality for analysis) with images taken at full suspended inspiration, and symptom limited incremental cardiopulmonary cycle exercise tests (CPET) were selected for analyses. The CanCOLD study was approved by the institutional human research ethics review boards of all participating centres and all participants provided written informed consent. Secondary analyses presented in this manuscript were approved by the University of Alberta Health Research Ethics Board (Health Panel Pro00108856).

Participants were categorized into the following groups: 1) Controls with normal spirometry (a post-bronchodilator forced expiratory volume in 1-sec to forced vital capacity

[$FEV_1$/FVC] ratio ≥5th percentile [LLN; from the Global Lung Function Initiative (GLI)], who were either NS or ES; and 2) individuals with evidence of spirometric airflow obstruction (post-bronchodilator $FEV_1$/FVC ratio <LLN) (97). $D_LCO$ reference values were based on Stanojevic et al. (98). Static lung volume reference equations established by Garcia-Rio et al. (99) and Hall et al. (100) were used for people aged >80 years and ≤80 years, respectively.

*3.2.2 Pulmonary function and cardiopulmonary exercise tests*

Pulmonary function and incremental CPET were completed following recommended guidelines (101-105), as previously described (79, 106). Peak data were taken as the average of the last 30s of loaded pedalling. $\dot{V}O_{2peak}$ data are presented as absolute (L/min) and relative to predicted normal reference values (107).

*3.2.3 CT image acquisition and analysis*

CT images were evaluated using Apollo 2.0 software (VIDA Diagnostics, Inc., Coralville, USA) and VIDA Diagnostics, Inc., clinical image analysis service, as previously described (106). Emphysema was quantified on full-inspiration CT images using the low-attenuation areas of the lung below -950 Hounsfield units (HU) (LAA-950%) (108-110). Total airway count (TAC) was quantified via summation of all airway segments from the segmented airway tree, as previously described by our group (106). Pulmonary vessel measurements were completed with 3-dimensional vascular reconstruction using a scale-space particle method of all vessels, which allowed for the automated evaluation of vessel volumes. This method uses the geometry of the blood vessels to identify vasculature in relationship to the parenchyma and estimates vessel size. The volume of all pulmonary vessels (TBV), the volume of pulmonary vessels <5 $mm^2$ in cross-sectional area (BV5), and the volume of pulmonary vessels between 5-10 $mm^2$ in cross-sectional area (BV5-10) were calculated for each participant. BV5 defines the transition between the distal

and proximal pulmonary vasculature (111), and previous work suggests that low BV5 represents vascular pruning, whereas larger cut-offs in cross-sectional area (i.e., BV>5mm$^2$) tend to represent more proximal vascular morphology (89, 90, 112). BV5-10 coincides with a vessel radius of approximately 1.25-2mm, and therefore may capture the smaller pulmonary arteries and transition toward arterioles.

*3.2.4 Questionnaires*

Participants completed questionnaires including sociodemographic information, smoking history, and co-morbid conditions during the study visit. Participants also completed the Medical Research Council (MRC) dyspnoea scale (113, 114) and the Community Health Activities Model Program for Seniors (CHAMPS) physical activity self-report questionnaire (115, 116); and disease specific questionnaires including the COPD Assessment Test (CAT) (117) and the St. George's Respiratory Questionnaire (SGRQ) (118), all of which have been previously validated.

*3.2.5 Statistical analyses*

Data are presented as mean and standard deviation for continuous variables, and as percentages for categorical variables within each study group (NS, ES, and COPD). One-way ANOVA with a Tukey post-hoc test for multiple comparison correction was performed for analyses between NS, ES, and COPD groups for continuous variables. For categorical variables, a Chi-square test was performed. Pearson correlation was utilized to evaluate the relationship between MVPA and $\dot{V}O_{2peak}$.

General linear regression modelling was used to determine the associations between $\dot{V}O_{2peak}$ (L/min) and MVPA (square root transformed Cal/week in MVPA) with each dependent variable (TBV, BV5, and BV5-10 in mL; and TAC in counts) and $D_LCO$ (in mL/min/mmHg). First, a null model (Model 0) was built including group (NS, ES, COPD) and the dependent

variable. Subsequent unadjusted models evaluated the separate relationships between $\dot{V}O_{2peak}$ and MVPA with each dependent variable (Model 0 + $\dot{V}O_{2peak}$; and Model 0 + MVPA). An intermediate model (Model 1) was built to adjust for potential confounders including race/ethnicity, sex, age (years), height (cm), body mass (kg), CT scanner model (for models evaluating CT dependent variables), $FEV_1$ (L), emphysema (LnLAA-950%), total lung volume on CT ($TLV_{CT}$), depth of inspiration on CT [$TLV_{CT}$/plethsymographic total lung capacity ratio ($TLC_{pleth}$)], and alveolar volume ($V_A$; only included in the $D_LCO$ model). Subsequent intermediate models were derived evaluating the separate relationships between $\dot{V}O_{2peak}$ and MVPA with the dependent variables (Model 1 + $\dot{V}O_{2peak}$; and Model 1 + MVPA). Model 2 included the same covariates as Model 1 plus $\dot{V}O_{2peak}$, MVPA, $FEV_1$ and LnLAA950%. Interaction terms between $\dot{V}O_{2peak}$ and group (NS, ES, COPD) were included in the final model.

$\dot{V}O_{2peak}$ in L/min (not mL/kg/min or % predicted) was utilized in all statistical analyses to avoid multicollinearity between independent variables, since CT outcomes require adjustment for body size (e.g., height). Independent variables did not show evidence of multicollinearity, with bivariate correlation coefficients <0.80. Calories expended per week in ≥MVPA was square root transformed for all regression analyses to obtain normally distributed residuals. Percent emphysema (LAA-950%) was transformed via natural logarithm to obtain a normal distribution (LnLAA-950%). All statistical analyses were completed using IBM SPSS Statistics 26 (IBM Corporation, Armonk, NY). For all inferential analyses, the probability of Type I error was two-sided, and set at <0.05.

### *3.3 Results*

#### *3.3.1 Participant characteristics, pulmonary function, and peak cardiopulmonary exercise responses*

A flow diagram on CanCOLD participant inclusion (n=1,004) is presented in **Figure 3.1**. Individuals with COPD were younger than NS and ES (both $p<0.001$). The proportion of participants self-reporting participating in MVPA was 88%, 87% and 85% for NS, ES and COPD, respectively. Participants with COPD had worse pulmonary function, greater respiratory symptom burden, greater % emphysema, and lower TAC and TBV/TLV than NS and ES (all $p<0.001$) (**Tables 3.1 and 3.2**). There was a moderate correlation between $\dot{V}O_{2peak}$ (L/min) and MVPA (Pearson $R^2=0.14$, $p<0.001$, n=1,004).

#### *3.3.2 The relationship between $\dot{V}O_{2peak}$ and MVPA with CT-airways and the pulmonary vasculature*

The 'final' adjusted general linear regression models for each dependent variable (TAC, TBV, BV5, BV5-10 and $D_LCO$) are reported in **Table 3.3**; while the 'null', intermediate, and 'final' models are included in the data supplement (Appendix A). **Figure 3.2** depicts the predicted individual CT-derived measurements (TVV, BV5 and BV5-10) and $D_LCO$ in the entire cohort relative to $\dot{V}O_{2peak}$ generated by general linear models adjusting for group, sex, race/ethnicity, age, height, body mass, $FEV_1$ and LnLAA-950%, and where appropriate, CT scanner model (or site ID), and $TLV_{CT}$, $TLV_{CT}/TLC_{pleth}$ (or $V_A$ for models evaluating $D_LCO$).

#### *3.3.3 CT measures*

$\dot{V}O_{2peak}$ (L/min) and MVPA were significantly associated with TBV in unadjusted models; but only $\dot{V}O_{2peak}$ remained significantly associated with TBV when both $\dot{V}O_{2peak}$ ($p<0.001$) and

MVPA (p=0.23) were included in the final model adjusting for anthropometrics and indices of disease severity. When $\dot{V}O_{2peak}$ by group interaction was examined in the final model, a significant interaction effect was only observed between COPD and $\dot{V}O_{2peak}$; where for every 1 L/min increase in $\dot{V}O_{2peak}$, TBV increased by 3.02 mL in COPD, and by 8.61 mL in ES and NS.

Both $\dot{V}O_{2peak}$ and MVPA were significantly associated with BV5 in unadjusted models. After adjusting for participant anthropometrics and indices of disease severity (model 2), the association between $\dot{V}O_{2peak}$ and BV5 remained significant (p=0.01), whereas MVPA was no longer significant (p=0.44). Model 2 determined there was no significant group by $\dot{V}O_{2peak}$ interaction.

$\dot{V}O_{2peak}$ (p<0.001) and MVPA (p<0.001) were significantly associated with BV5-10 in unadjusted models; but only $\dot{V}O_{2peak}$ remained significantly associated with BV5-10 in adjusted models (model 2 and final model). The final model revealed a significant interaction effect between group and $\dot{V}O_{2peak}$; where for every 1 L/min increase in $\dot{V}O_{2peak}$, BV5-10 rose by 0.35 mL and 1.76 mL in COPD and ES, respectively.

Neither $\dot{V}O_{2peak}$ nor MVPA were consistently associated with TAC in the unadjusted or in the final adjusted model (**Table 3.3**).

### *3.3.4 Pulmonary diffusing capacity for carbon monoxide*

Both $\dot{V}O_{2peak}$ (L/min) and MVPA were significant significantly associated with $D_LCO$ in unadjusted models. $\dot{V}O_{2peak}$ and MVPA remained significantly associated with $D_LCO$ when separately added to model 1. When included in the same model (model 2), $\dot{V}O_{2peak}$ (p<0.001) and MVPA (p=0.03) remained significantly associated with $D_LCO$. There was no interaction effect

between group and $\dot{V}O_{2peak}$ (**Table 3.3**). In summary, after adjusting for potential confounders, for every 1 L/min increase in $\dot{V}O_{2peak}$, $D_LCO$ increased by 2.95 mL/min/mmHg across all groups.

### *3.4 Discussion*

This is the first study to evaluate the relationship between MVPA and $\dot{V}O_{2peak}$ and pulmonary vascular structure and function measures in NS and ES without airflow obstruction, and people with COPD. Our key findings were: 1) $\dot{V}O_{2peak}$ was associated with pulmonary vascular volumes and $D_LCO$ in NS and ES without COPD and in individuals with COPD, independent of emphysema and degree of airflow obstruction; 2) COPD by $\dot{V}O_{2peak}$ interaction effects were observed for both TBV and BV5-10; and 3) self-reported MVPA was significantly associated with $D_LCO$ but not any CT-derived airway or pulmonary vascular measurements. Our findings suggest that $\dot{V}O_{2peak}$ may influence resting pulmonary vascular structure and function and that the relationship between $\dot{V}O_{2peak}$, pulmonary vascular structure and $D_LCO$ and are not limited to COPD.

#### *3.4.1 Pulmonary diffusing capacity and CT-vascular correlates*

$\dot{V}O_{2peak}$ was independently associated with TBV, BV5 and BV5-10 when controlling for degree of airflow obstruction, emphysema, and lung size. These findings indicate that $\dot{V}O_{2peak}$ is consistently associated with CT-derived pulmonary vascular measures in individuals with or without COPD, and are in line with previous work in healthy adults showing that lower $\dot{V}O_{2peak}$ is associated with lower $D_LCO$ (82), resting pulmonary capillary blood volume (85, 86), and TBV and BV5 (95). To our knowledge, our study is the first to evaluate the relationship between $\dot{V}O_{2peak}$ and CT pulmonary vascular measures in individuals with or without COPD, and to show there

exists a significant COPD by $\dot{V}O_{2peak}$ interaction effect on TBV and BV5-10. These findings extend previous work showing that pulmonary vascular pruning is associated with reduced 6-minute walk distance and increased risk of mortality in COPD (94). Combined, these findings highlight the importance of exercise capacity, and may help explain why a high $\dot{V}O_{2peak}$ is associated with lower mortality in health and COPD (119, 120); however, cause and effect cannot be ascertained from our cross-sectional results.

As previous work has shown that $\dot{V}O_{2peak}$ and MVPA are highly correlated (121), we hypothesized that both $\dot{V}O_{2peak}$ and MVPA would be associated with greater pulmonary vascular measures. We found that MVPA was moderately associated with $\dot{V}O_{2peak}$; however, contrary to our hypothesis, MVPA was not associated with any CT-derived pulmonary vascular measures in fully adjusted models. Although animal studies have shown that the pulmonary circulation may adapt to exercise (122, 123), work in healthy humans has reported mixed results (124, 125), making it unclear whether physical activity/exercise training modulates pulmonary vascular structure/function. MVPA was evaluated using a validated self-report questionnaire in the current study, which lacks accuracy as compared to other techniques such as activity monitors (116, 126). While regular MVPA is protective of lung function decline in physically active smokers at-risk of developing COPD (93), it remains to be determined if higher levels of directly measured MVPA would, above and beyond its effect on $\dot{V}O_{2peak,}$ be protective of further lung function decline in people with COPD and/or at-risk of developing COPD.

With correlative work, it is difficult to ascertain exposure and outcome. The pulmonary circulation is closely linked to right ventricular function and thus $\dot{V}O_{2peak}$ (127); thus, we subsequently examined the inverse relationship (see data supplement in Appendix A): the association between pulmonary vascular structure and $D_LCO$ with $\dot{V}O_{2peak}$ (dependent variable).

We found that $D_LCO$ and all CT-derived pulmonary vascular measurements were significantly associated with $\dot{V}O_{2peak}$. These associations, even after controlling for MVPA, would indicate that the pulmonary vasculature may be an important determinant of $\dot{V}O_{2peak}$ in adults with or without COPD. However, the directional nature of the relationship between pulmonary vascular structure/function with $\dot{V}O_{2peak}$ remains unclear. Future studies should utilize longitudinal data to evaluate whether greater baseline exercise capacity is protective of pulmonary vascular decline, or whether greater baseline pulmonary vascular structure and/or function is protective of longitudinal decline in exercise capacity.

### *3.4.2 Strengths and Limitations*

The CanCOLD study has many strengths; notably, it is the first prospective cohort study for COPD with random sampling from the general population (79), which allows for the phenotyping of COPD across a wide range of individuals aged ≥40 years. Most participants with COPD included in the CanCOLD study did not have a prior diagnosis of COPD (and were therefore untreated) and had relatively asymptomatic mild-to-moderate disease. As stated previously (128), the relative proportions of NS, ES and individuals with COPD are not reflective of the general population; therefore, our findings may not be generalized. The CanCOLD study objectively assessed $\dot{V}O_{2peak}$ on CPET alongside CT and post-bronchodilator spirometry and $D_LCO$ measurements at the same study visit in all participants, as compared to recent work (95) reporting CT and exercise tests completed approximately 8 years apart in apparently healthy participants without COPD. However, the analyses reported in our study are cross-sectional in nature, and therefore cause-effect cannot be determined.

Although quantitative CT is a useful tool to characterize the structure of the pulmonary vasculature, it is not without its limitations. First, the scans collected in the present study did not involve the use of a contrast agent, and the software utilized does not allow for the segmentation of pulmonary arteries and veins. Further, it is unclear whether the vascular volumes quantified include blood, vessel wall, or a combination of the two. As the pulmonary vascular tree prunes in COPD, there is potential for progressive narrowing of the larger proximal vessels, reduction in the relative volume of pulmonary veins, and loss of the smaller distal vessels (129), which may affect a calibre-based measurement such as BV5. BV5/TBV can be reduced by the loss or narrowing of distal vessels (BV5) or by a relative increase in the volume of proximal vasculature, contributing to increased TBV (130). Previous work in severe emphysema (131) has reported high BV5 relative to the overall pulmonary vasculature (i.e., high BV5/TBV), which was related to a smaller vascular tree on CT secondary to emphysema. Consistent with this, Schuhmann et al., (131) and our current data report that the small vessel volume relative to total vessel volume was higher in COPD, suggesting that the greater BV5/TBV in COPD compared to NS and ES may not be indicative of higher distal vessel volumes *per se* (less vascular pruning and/or vasoconstriction), but rather an increase in the contribution of the smallest vessels relative to a reduced TBV. Indeed, vascular density (TBV/TLV) was lower in people with COPD when compared with NS and ES in the present study. Therefore, it is unlikely that the presence of mild emphysema observed in the present study increased the ratio of BV5/TBV in COPD.

### *3.4.3 Conclusion*

In conclusion, $\dot{V}O_{2peak}$ was associated with resting pulmonary vascular structure and function (and vice-versa), in NS, ES and COPD, independent of emphysema and airflow obstruction severity. These results suggest that the relationship between pulmonary vascular

structure/function and $\dot{V}O_{2peak}$ are not specific to COPD. Self-reported MVPA was not consistently associated with any CT-derived airway and pulmonary vascular measurements, suggesting that other variables, such as body morphology, emphysema, severity of airflow obstruction and $\dot{V}O_{2peak}$ are more important determinants of airway count and pulmonary vascular volumes. Although cross-sectional, the results of our study underline the important relationship between the pulmonary circulation and exercise capacity among people with and without COPD. These novel findings provide a physiological rationale for a longitudinal examination of the cause-effect relationship between $\dot{V}O_{2peak}$ and the pulmonary circulation in individuals with and without COPD, and further underscore the need for targeted therapeutic interventions in people with COPD.

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**Table 3.1:** Baseline anthropometrics, demographics, pulmonary function in never-smokers, ever-smokers without airflow obstruction, and individuals with COPD (n = 1,004).

| Parameter | Never-Smokers n=263 | Ever-smokers n=407 | COPD n=334 |
|---|---|---|---|
| **Participant Demographics** | | | |
| Female, % | 48 | 35† | 43 |
| Caucasian, % | 94 | 96 | 96 |
| Age, years | 67.3 ± 9.9 | 67.2 ± 9.4 | 64.6 ± 10.0*† |
| Height, cm | 166.8 ± 9.8 | 169.5 ± 9.2† | 169.5 ± 9.6† |
| BMI, kg/m$^2$ | 26.9 ± 4.8 | 27.7 ± 4.7 | 27.0 ± 4.8 |
| Current smoker, % | 0 | 19 | 22 |
| Pack-years | 0 ± 0 | 21 ± 21† | 25 ± 25†* |
| HDHTDM, % | 49 | 55 | 48 |
| **Pulmonary Function and Exercise** | | | |
| $FEV_1$, %pred | 101.3 ± 16.3 | 98.7 ± 14.7 | 78.5 ± 17.3†* |
| FVC, %pred | 103.7 ± 16.5 | 102.8 ± 15.0 | 103.3 ± 18.2 |
| $FEV_1$/FVC, %pred | 97.3 ± 6.9 | 95.7 ± 7.1† | 75.4 ± 9.8†* |
| RV, %pred | 109.8 ± 28.0 | 112.6 ± 32.0 | 140.4 ± 41.9†* |
| TLC, %pred | 103.7 ± 16.5 | 104.1 ± 14.2 | 109.8 ± 15.3†* |
| RV/TLC, %pred | 106.2 ± 21.2 | 108.3 ± 25.8 | 127.8 ± 29.5†* |
| DLCO, %pred | 97.5 ± 20.6 | 95.7 ± 20.6 | 89.0 ± 23.7†* |
| $V_A$, %pred | 100.3 ± 17.4 | 100.0 ± 14.2 | 98.2 ± 17.2 |
| $D_LCO/V_A$, %pred | 97.9 ± 18.6 | 95.5 ± 16.4 | 90.7 ± 21.8†* |
| $\dot{V}O_{2peak}$, %pred | 93.4 ± 25.0 | 89.8 ± 24.2 | 84.2 ± 23.2†* |
| Peak work rate, %pred | 95.5 ± 25.4 | 90.5 ± 24.8† | 83.0 ± 24.3†* |
| Calories/week in MVPA | 2,128 ± 2,079 | 1,915 ± 1,919 | 2,118 ± 2,066 |
| **Symptoms** | | | |
| CAT total | 5.1 ± 4.3 | 5.7 ± 4.5 | 8.3 ± 6.5*† |
| SGRQ total | 8.6 ± 11.2 | 9.9 ± 10.8 | 16.4 ± 14.8*† |
| MRC dyspnoea | 1.3 ± 0.5 | 1.4 ± 0.6 | 1.6 ± 0.7*† |
| **Imaging** | | | |
| TLV, L | 4.39 ± 1.23 | 4.68 ± 1.16† | 5.02 ± 1.24*† |
| LAA-950, % | 3.22 ± 3.29 | 3.54 ± 3.45 | 6.23 ± 6.26*† |
| TAC, n | 215 ± 74 | 210 ± 68 | 161 ± 60*† |

Data are mean ± SD for continuous variables, or % for categorical variables. BMI: body mass index; HDHTDM: heart disease, hypertension and/or diabetes mellitus; $FEV_1$: forced expiratory volume in 1 sec; FVC: forced vital capacity; RV: residual volume; TLC: total lung capacity; $D_LCO$: diffusing capacity for carbon monoxide; $V_A$: alveolar volume; CAT: COPD Assessment Test; SGRQ: Saint-Georges Respiratory Questionnaire; MRC: Medical Research Council dyspnoea scale; TLV: CT-derived total lung volume; LAA-950%: low attenuation area of the lung with attenuation values below −950 HU on full inspiration CT.

*Significantly different from ever-smokers ($p<0.05$). †Significantly different from never-smoker ($p<0.05$).

**Table 3.2:** Distribution of Blood Vessel Volume Measures (n = 1,004).

| Parameter | Never-Smokers n=263 | Ever-smokers n=407 | COPD n=334 | p-value |
|---|---|---|---|---|
| **Whole lung** | | | | |
| TBV/TLV | 3.14 ± 0.42 | 3.21 ± 0.39 | 3.07 ± 0.40* | <0.001 |
| BV5/TBV, % | 53.13 ± 8.98 | 51.88 ± 7.91† | 54.54 ± 8.09* | <0.001 |
| BV5-10/TBV, % | 31.77 ± 5.52 | 33.09 ± 4.74† | 31.29 ± 4.61* | <0.001 |

Data are mean ± SD. TLV: CT-derived total lung volume; TAC: total airway count; TBV: total vessel volume; BV5: volume for vessels <5 $mm^2$ in cross-section. BV5-10: volume for vessels between 5-10 $mm^2$ in cross-section. *Significantly different from ever-smokers ($p<0.05$). †Significantly different from never-smoker ($p<0.05$).

**Table 3.3:** Separate General Linear Regression Models evaluating associations with CT-derived airway and vascular measurements.

| Parameter | Unstandardized estimate | 95% CI | P-value |
|---|---|---|---|
| ***TAC, n*** | | | |
| $\dot{V}O_{2peak}$ (L/min) | -8.90 | -21.48 to 3.68 | 0.17 |
| sqrtCal/week | 0.01 | -0.15 to 0.18 | 0.89 |
| $\dot{V}O_{2peak}$ x ES | -3.50 | -17.10 to 10.11 | 0.61 |
| $\dot{V}O_{2peak}$ x COPD | -2.99 | -17.22 to 11.25 | 0.68 |
| ***TBV, mL*** | | | |
| $\dot{V}O_{2peak}$ (L/min) | 8.61 | 5.36 to 11.86 | <0.001 |
| sqrtCal/week | 0.03 | -0.02 to 0.07 | 0.23 |
| $\dot{V}O_{2peak}$ x ES | -2.19 | -5.71 to 1.33 | 0.22 |
| $\dot{V}O_{2peak}$ x COPD | -5.59 | -9.27 to -1.91 | 0.003 |
| ***BV5, mL*** | | | |
| $\dot{V}O_{2peak}$ (L/min) | 1.00 | -1.02 to 3.02 | 0.33 |
| sqrtCal/week | 0.01 | -0.02 to 0.04 | 0.49 |
| $\dot{V}O_{2peak}$ x ES | 1.47 | -0.72 to 3.66 | 0.19 |
| $\dot{V}O_{2peak}$ x COPD | 1.19 | -1.10 to 3.48 | 0.31 |
| ***BV5-10, mL*** | | | |
| $\dot{V}O_{2peak}$ (L/min) | 3.64 | 2.00 to 5.28 | <0.001 |
| sqrtCal/week | 0.01 | -0.01 to 0.04 | 0.22 |
| $\dot{V}O_{2peak}$ x ES | -1.88 | -3.66 to -0.10 | 0.04 |
| $\dot{V}O_{2peak}$ x COPD | -3.29 | -5.15 to -1.43 | <0.001 |
| ***DLCO*** | | | |
| $\dot{V}O_{2peak}$ (L/min) | 2.95 | 2.19 to 3.71 | <0.001 |
| sqrtCal/week | 0.01 | 0.0005 to 0.02 | 0.04 |
| $\dot{V}O_{2peak}$ x ES | -0.33 | -1.15 to 0.49 | 0.43 |
| $\dot{V}O_{2peak}$ x COPD | 0.75 | -0.11 to 1.61 | 0.09 |

Data are unstandardized estimate (95%CI). Models are adjusted for CT scanner model, sex, race, age (years), height (cm); body mass (kg), $TLV_{CT}$: total lung volume on inspiratory CT; $TLV_{CT}/TLC_{pleth}$: depth of inspiration at CT; $FEV_1$: post-bronchodilator forced expiratory volume in 1 sec, and LnLAA-950%: natural logarithm of low-attenuating areas below -950 Hounsfield units. DLCO was adjusted by sex, race, age (years), height (cm); body mass (kg), $V_A$: alveolar volume (L); $FEV_1$, and LnLAA-950%.

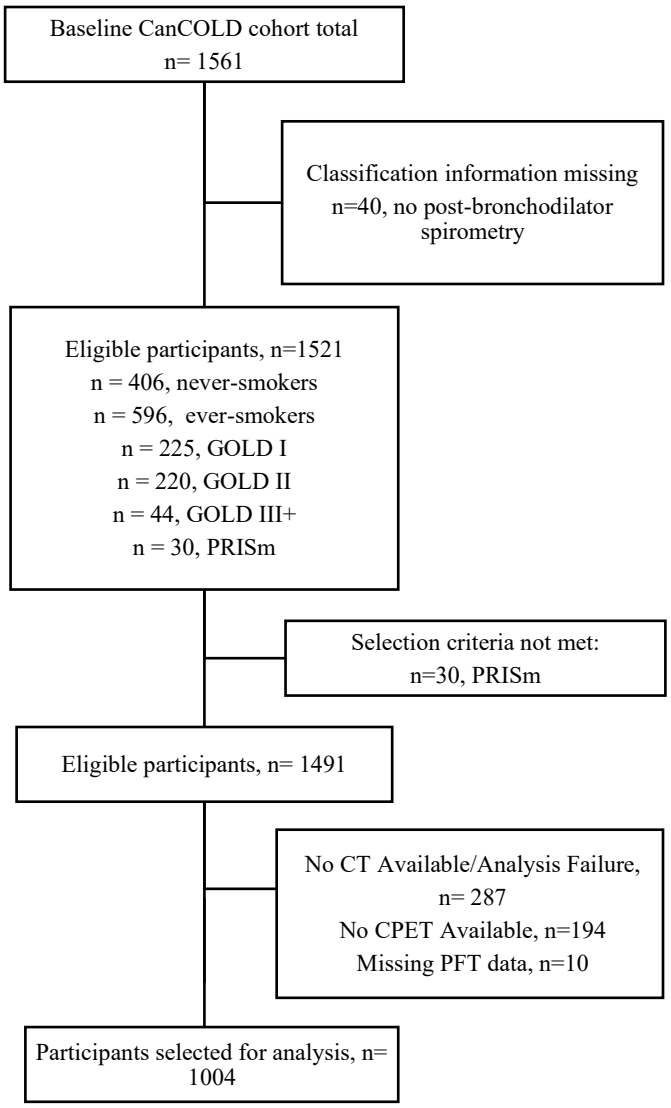


**Figure 3.1:** Participant selection. GOLD: Global initiative for Chronic Obstructive Pulmonary Disease; PRISm: preserved $FEV_1$/FVC ratio impaired spirometry; CPET: cardiopulmonary exercise test; PFT: pulmonary function test.

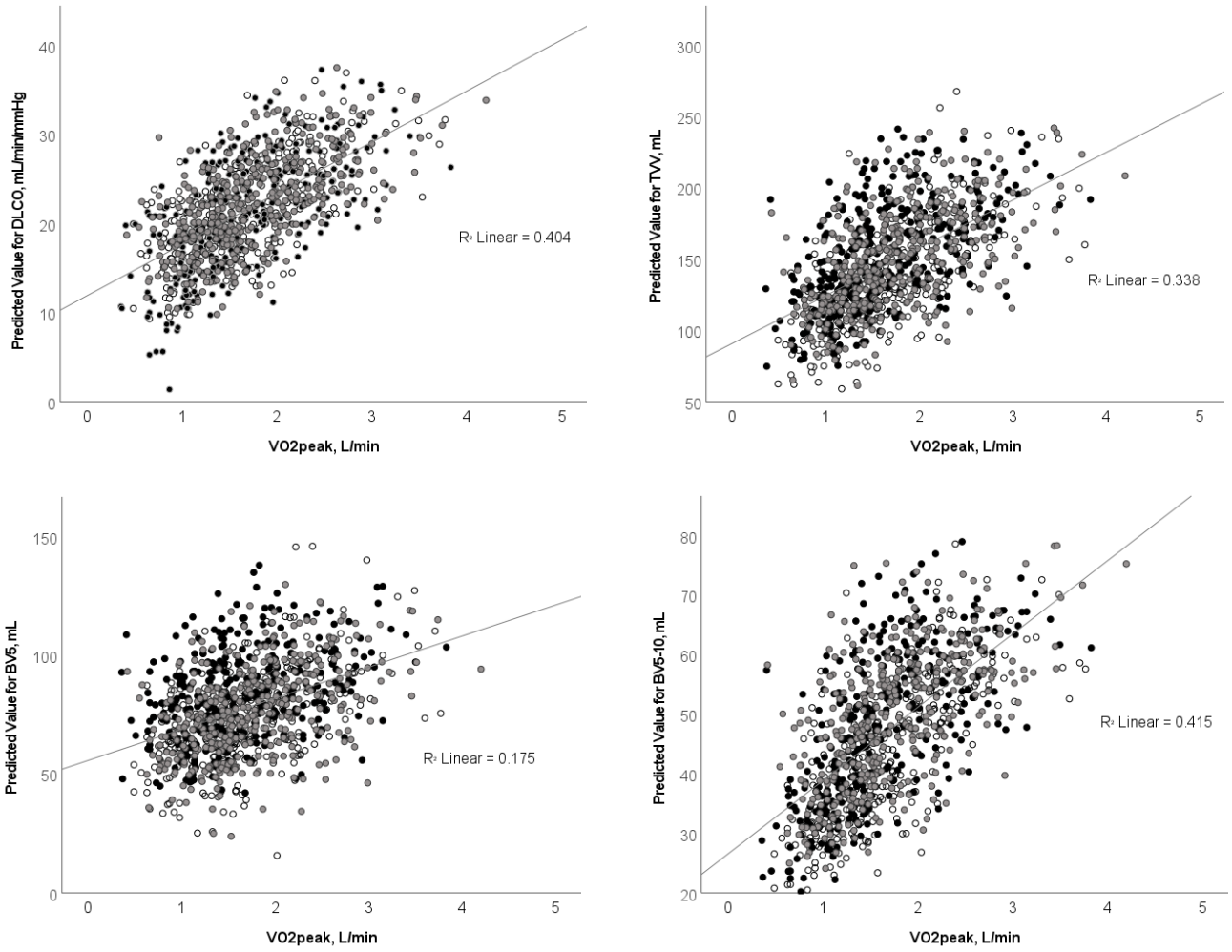


**Figure 3.2:** Regression slopes with 95% confidence intervals relating individual $\dot{V}O_{2peak}$ (L/min) to predicted outcome measures generated by the general linear models. Models adjusted by: group, sex, CT scanner model (for CT outcomes) age, height, body mass, $FEV_1$, LnLAA-950, as well as TLV and TLV/TLC (for CT outcomes), and $V_A$ (for $D_LCO$). White circles: never smokers; Grey circles: ever-smokers; Black circles: COPD.

# CHAPTER FOUR: STUDY #2

## Is higher exercise capacity protective of pulmonary vascular decline?

### *4.1 Introduction*

Chronic obstructive pulmonary disease (COPD) is progressive, and characterized by airway inflammation and persistent airflow obstruction (3). Individuals with COPD experience exertional dyspnea, which contributes to exercise intolerance (i.e., a low peak rate of $O_2$ consumption [$\dot{V}O_{2peak}$]). Importantly, a low $\dot{V}O_{2peak}$ is associated with an increased risk of mortality, reduced participation in activities of daily living, and lower quality of life (119, 120, 132, 133).

Pulmonary microvascular blood flow (5) and pulmonary vascular volumes (89, 90) are reduced across the continuum of COPD, and pulmonary vascular pruning is predictive of mortality in individuals with COPD (89, 94, 134). We have recently shown that $\dot{V}O_{2peak}$ was correlated with CT-derived pulmonary vascular volumes and $D_LCO$ in adults with and without airflow obstruction, independent of age, emphysema or spirometric airflow obstruction (Collins et al., under review). In an animal model of COPD, aerobic training was found to reduce the development of emphysema (135); while exercise training has been shown to increase pulmonary diffusing capacity for carbon monoxide ($D_LCO$) (77) in patients with COPD. Thus, aerobic exercise training may slow or even prevent pulmonary vascular decline. Both the progression of emphysema and estimated physiological deadspace seem to be proportional to $\dot{V}O_{2peak}$ decline in COPD (136), suggesting that pulmonary vascular remodelling or destruction may contribute to elevating deadspace, worsening ventilatory efficiency ($\dot{V}_E/\dot{V}CO_2$), further lowering $\dot{V}O_{2peak}$ (84, 137).

Therefore, the primary purpose of this study was to determine whether higher baseline $\dot{V}O_{2peak}$ is protective against pulmonary vascular decline in ever-smokers (ES) with normal spirometry and patients with COPD (aim 1). We hypothesized that those with higher baseline $\dot{V}O_{2peak}$ would demonstrate preserved $D_LCO$, TVV, BV5, and BV5-10 over three years, independent of severity of airflow limitation. As pathological changes in pulmonary vascular

structure (e.g., vascular pruning) and dysfunction (e.g., reduced $D_LCO$) may drive exercise intolerance, aim 2 was to evaluate the relationship between baseline pulmonary diffusing capacity ($D_LCO$), as well as computed tomography (CT) imaging measures of emphysema and pulmonary small vessel volume, with longitudinal $\dot{V}O_{2peak}$ decline. As exercise ventilatory inefficiency ($\dot{V}_E/\dot{V}CO_2$ nadir) has been shown to be a powerful determinant of $\dot{V}O_{2peak}$, a tertiary aim was to determine whether potential associations between pulmonary vascular structure/function and $\dot{V}O_{2peak}$ decline are mediated by exercise ventilatory inefficiency ($\dot{V}_E/\dot{V}CO_2$ nadir) in ES and COPD. We hypothesized that the relationship between pulmonary vascular function and structure with change in $\dot{V}O_{2peak}$ would be partly mediated by $\dot{V}_E/\dot{V}CO_2$ nadir.

## *4.2 Methods*

### *4.2.1 Study Design and Participants*

A summary of study design is shown in **Figure 4.1A**. ES with normal spirometry and patients with COPD were included in the study. Participants without classifying information (e.g., spirometry and smoking history) were excluded. Participants with a full pulmonary function test, quantitative CT scan (of adequate quality for analysis) with images taken at full suspended inspiration and expiration, and incremental cardiopulmonary exercise tests from the CanCOLD study were selected for analyses.

The CanCOLD study was approved by the institutional human research ethics review boards of all participating centers and all participants provided written informed consent, while the secondary analyses presented in this manuscript were approved by the University of Alberta Health Research Ethics Board (Health Panel Pro00108856).

*4.2.2 Group stratification*

Participants were categorized into the following groups at visits 1 and 3: **1)** ES with normal spirometry (i.e., a post-bronchodilator $FEV_1$/FVC ratio ≥5th percentile [LLN; from the Global Lung Function Initiative (GLI) or **2)** Individuals with evidence of spirometric airflow obstruction (i.e., a post-bronchodilator $FEV_1$/FVC ratio < LLN). $D_LCO$ reference values were based on Stanojevic et al. (98). Static lung volume reference equations established by Garcia-Rio et al. (99) and Hall et al. (100) were used for people aged >80 years and ≤80 years, respectively.

*4.2.3 Pulmonary function and cardiopulmonary exercise tests*

Pulmonary function and incremental CPET were completed following recommended guidelines (101-105), as previously described (79, 106). Peak data were taken as the average of the last 30s of loaded pedaling. $\dot{V}O_{2peak}$ data are presented as absolute (L/min) and relative to normative values (107).

*4.2.4 CT image acquisition and analysis*

CT images were evaluated using Apollo 2.0 software (VIDA Diagnostics, Inc.) and VIDA Diagnostics, Inc., clinical image analysis service, as previously described (106). Emphysema was quantified on full-inspiration CT images using the low-attenuation areas of the lung below -950 Hounsfield units (HU) (LAA-950%) (108-110). TAC was quantified via summation of all airway segments from the segmented airway tree, as previously described by our group (106). Vessel measurements were completed with 3-dimensional vascular reconstruction using a scale-space particle method of all vessels, which allowed for the automated evaluation of vessel volumes. This method uses the geometry of the blood vessels to identify vasculature in relationship to the

parenchyma and estimates vessel size. The volume of all pulmonary vessels (TVV), the volume of pulmonary vessels <5 $mm^2$ in cross-sectional area (BV5), and the volume of pulmonary vessels between 5-10 $mm^2$ in cross-sectional area (BV5-10) were calculated for each participant. BV5 defines the transition between the distal and proximal vasculature (111), and previous work suggests that low BV5 represents vascular pruning, while larger cut-offs in CSA (i.e., BV>5$mm^2$) tend to represent more proximal vascular morphology (89, 90, 112). BV5-10 coincides with a vessel radius of approximately 1.25-2mm, and therefore may capture the smaller arteries and transition toward arterioles.

*4.2.5 Questionnaires*

Participants completed questionnaires including sociodemographic information, smoking history, and co-morbid conditions during the study visit. Participants also completed the Medical Research Council (MRC) dyspnoea scale (113, 114) and disease specific questionnaires including the COPD Assessment Test (CAT) (117) and the St. George's Respiratory Questionnaire (SGRQ) (118), all of which have been previously validated.

*4.2.6 Statistical analyses*

Data are presented as mean and standard deviation for continuous variables, and as percentages for categorical variables within each study group (ever smokers without COPD, and patients with COPD) and study visit. One-way ANOVA with a Bonferroni post-hoc test for multiple comparison correction was performed for statistical comparison between ES, and COPD for continuous variables of participant characteristic, pulmonary function, and CT measurements. For categorical variables, a Chi-square test was used.

$\dot{V}O_{2peak}$ in L/min (not mL/kg/min or % predicted) was utilized in all mixed model analyses to avoid multicollinearity between independent variables, since CT outcomes require adjustment for body size (e.g., height). Independent variables did not show evidence of multicollinearity, with bivariate correlation coefficients <0.80. Percent emphysema (LAA-950%), was transformed via natural logarithm to obtain a normal distribution (LnLAA-950%). All statistical analyses were completed using IBM SPSS Statistics 26 (IBM Corporation, Armonk, NY). For all inferential analyses, the probability of Type I error was two-sided, and set at <0.05.

For aim 1, random intercept and random slope mixed effects linear regression models were used to investigate whether baseline $\dot{V}O_{2peak}$ is associated with the rate of change in pulmonary vasculature (i.e., TVV, BV5/TVV, TVC and $D_LCO$) and airway count (primary outcomes). Fixed effects were time (in years, between V1 and V3), baseline study group (AR and COPD), $FEV_1$ (L), sex, race, age (years), height (cm), weight (kg), LnLAA-950, and $FEV_1$ (L), and time-varying CT scanner model (or study site at V1 and V3), $TLV_{CT}/TLC_{pleth}$, or alveolar volume ($V_A$; only included in the $D_LCO$ model). Participant ID was used as the random intercept, and time (in years, between V1 and V3, with V1 fixed at 0) was used to define the random slope. For each model, the covariance structure for the random intercept and slope model was heterogenous first-order autoregressive. Separate models were built for each dependent variable ($D_LCO$, TVV/TLV, BV5/TVV, BV5-10/TVV, TAC), and were fitted using the restricted maximum likelihood. For aim 2, similar mixed models were built to evaluate the predictive value of baseline TVV, BV5/TVV, TVC and $D_LCO$ (independent variables) on the rate of change in $\dot{V}O_{2peak}$ (dependent variable), while adjusting for the same variables as described above.

For the tertiary aim, a mediation model was built to evaluate the relationship between baseline $D_LCO$, LnLAA-950, and BV5/TVV (predictors), with change in $\dot{V}O_{2peak}$ (outcome)

through baseline $\dot{V}_E/\dot{V}CO_2$ nadir (mediator) (**Figure 4.2**). We used the contemporary mediation analysis approach as described by Hayes (138), using the Process Macro for SPSS (139), rather than the "causal steps" approach popularized by Baron and Kenny (140), with focus on the indirect effect of the independent variable (X) on the dependent variable (Y) through the mediator (M) to establish mediation. Briefly, the indirect effect of X on Y through M estimates the change in Y for every one unit change in X. This is done through a series of steps whereby X affects M, which then affects Y (138). Due to the known effect of ageing and body size on $D_LCO$, and $\dot{V}O_{2peak}$, mediation analyses were run on % predicted values, while BV5 was normalized to TVV; allowing the authors to limit the number of covariates included in the mediation model.

### *4.3 Results*

Inclusion of participants in the visit 1 baseline analysis is demonstrated in **Figure 4.1B**, while inclusion of participants in the longitudinal analyses is described in **Figure 4.1C**. Participants having completed PFT, CPET, and CT testing at both visits were selected for the longitudinal analyses.

#### *4.3.1 Participant demographics, pulmonary function, and peak cardiopulmonary exercise responses*

A total of 299 participants completed the baseline assessments required for visit 1 and returned for visit 3. Baseline participant anthropometrics, demographics, pulmonary function, physical activity and peak exercise responses are presented in **Table 4.1**. In short, individuals with COPD had significantly worse pulmonary function, symptom scores, and emphysema severity, lower TAC and TVV/TLV, and greater BV5/TVV and BV5-10/TVV (all $p<0.001$), as shown in

**Table 4.1**. The mean change in $\dot{V}O_{2peak}$ from visit 1 to visit 3 was -2.09 ± 17.49% (calculated from % predicted, p=0.04) in ES and COPD (-2.00 ± 22.44% when calculated from mL/kg/min), and the mean change in $\dot{V}O_{2peak}$ % predicted from visit 1 to visit 3 in COPD was 0.70 ± 16.68% (p=0.04) (or – 1.57 ± 21.60% when calculated from mL/kg/min).

*4.3.2 Linear mixed effects models (Aim 1): Does elevated exercise capacity protect against pulmonary vascular decline?*

**Table 4.2** reports the results of the 'final' fitted multivariable linear mixed effects regression models for longitudinal $D_LCO$, TVV/TLV, BV5/TVV, BV5-10/TVV, TAC decline while adjusting for the aforementioned anthropometric, and disease covariates. Baseline $\dot{V}O_{2peak}$ was not significantly associated with greater decline in TAC (p= 0.86) or TVV/TLV (p=0.44). However, greater baseline $\dot{V}O_{2peak}$ was associated with an accelerated longitudinal decline in $D_LCO$ (β= -0.24 CI=-0.41 to -0.07), and BV5-10/TVV (β= -0.003, CI= -0.005 to -0.001), while greater baseline $\dot{V}O_{2peak}$ was associated with reduced decline in BV5/TVV (β=0.004, CI=0.001 to 0.008). Study group was significantly associated with $D_LCO$, TVV/TLV, BV5/TVV, BV5-10 and TAC (all $p<0.05$); with ES having a lower $D_LCO$ and BV5/TVV, and a higher TVV/TLV, BV510/TVV and TAC than COPD. **Figure 4.3** displays the fully adjusted fixed predicted outcome variables.

Due to the significant independent effect of group, the final mixed models were repeated with only the COPD group, and are reported in **Table 4.3**. Baseline $\dot{V}O_{2peak}$ was not significantly associated with decline in $D_LCO$ (p=0.06), TVV/TLV (p=0.55) or TAC (p=0.48). However, greater baseline $\dot{V}O_{2peak}$ was associated with a reduced decline in BV5/TVV (β= 0.005, CI= 0.001 to 0.01). In other words, for every 1L/min increase in baseline $\dot{V}O_{2peak}$, BV5/TVV increased by

1.5% at 3-year follow-up. Conversely, greater baseline $\dot{V}O_{2peak}$ was associated with accelerated decline in BV5-10/TVV (β= -0.004, CI= -0.007 to -0.001), where for every 1 L/min increase in baseline $\dot{V}O_{2peak}$, BV5-10/TVV was reduced by 1.2% at 3 years follow-up.

*4.3.3 Linear mixed effects models (Aim 2): Is baseline pulmonary vascular structure/function associated with change in exercise capacity over time?*

The multivariable linear mixed effects regression models for longitudinal $\dot{V}O_{2peak}$ decline in ES and COPD and COPD only are shown in **Table 4.4**. Models were adjusted for the aforementioned anthropometric, and disease covariates. After adjusting for potential covariates, neither baseline $D_LCO$, BV5/TVV, BV5-10/TVV nor TAC was significantly associated with longitudinal decline in $\dot{V}O_{2peak}$ in models including both ES and COPD, as well as in those limited to COPD (p>0.05). However, higher TVV/TLV was significantly associated with accelerated decline in $\dot{V}O_{2peak}$ in models including both ES and COPD (β= -0.04, CI= -0.07 to -0.004), but not in models limited to only individuals with COPD (p=0.84). Finally, higher baseline $\dot{V}_E/\dot{V}CO_2$ nadir was associated with less decline in $\dot{V}O_{2peak}$ in models including both ES and COPD (β= 0.004, CI= 0.0002 to 0.008), although this relationship disappeared in models limited to only COPD (p=0.17).

*4.3.4 Mediation model (Aim 3): Is the relationship between pulmonary vascular structure/function with change in $\dot{V}O_{2peak}$ over time mediated by ventilatory efficiency?*

Mediation model analysis results are presented in **Table 4.5**, demonstrating the relationship between X (independent variables: BV5/TVV, $D_LCO$ and LnLAA-950) and M (mediator: $\dot{V}_E/\dot{V}CO_2$ nadir), the total effect of X on Y (i.e., the sum of the indirect and direct effect of X on Y, with Y being the dependent variable $\dot{V}O_{2peak}$), the indirect effect (the product of the relationship

between X and M, as well as M and Y), and the mediation ratio (indirect effect/total effect). In the mediation model including both ES and people with COPD, there was no significant total effect of BV5/TVV, $D_LCO$ or LnLAA-950 on change in $\dot{V}O_{2peak}$ (all p>0.05). However, there was a significant relationship between baseline $\dot{V}_E/\dot{V}CO_2$ nadir and change in $\dot{V}O_{2peak}$ (% predicted, β= 1.11, CI= 0.67 to 1.55). There was no significant direct relationship between baseline BV5/TVV (p=0.62) or LnLAA-950 (p= 0.10) and change in $\dot{V}O_{2peak}$, while $D_LCO$ adjusted for $V_A$ was significantly associated with change in $\dot{V}O_{2peak}$ (%predicted, β= 0.18, CI= 0.09 to 0.28).

Baseline BV5/TVV, $D_LCO$, and LnLAA-950 were all significantly related with $\dot{V}_E/\dot{V}CO_2$ nadir (BV5/TVV: β= 11.75, CI= 5.22 to 18.29; $D_LCO$: β= -0.09, CI= -0.11 to -0.07; LnLAA-950: β= 0.83, CI= 0.29 to 1.37). Analyses of indirect effects revealed that BV5/TVV, $D_LCO$ and LnLAA-950 were all significantly associated with the change in $\dot{V}O_{2peak}$ through the mediator $\dot{V}_E/\dot{V}CO_2$ nadir (BV5/TVV: β= 13.06, CI= 4.68 to 23.25; $D_LCO$: β= -0.10, CI= -0.15 to -0.05; and LnLAA-950: β= 0.92, CI= 0.27 to 1.69). As such, the relationships between BV5/TVV and LnLAA-950 and the longitudinal change in $\dot{V}O_{2peak}$ were entirely mediated by $\dot{V}_E/\dot{V}CO_2$ nadir in ES and COPD (given the significant indirect effects and non-significant direct effects), while $D_LCO$ was partially mediated by $\dot{V}_E/\dot{V}CO_2$ nadir.

In the mediation model limited to people with COPD, there was no significant total effect of BV5/TVV, $D_LCO$ or LnLAA-950 on change in $\dot{V}O_{2peak}$ (all p>0.05). However, there was a significant relationship between baseline $\dot{V}_E/\dot{V}CO_2$ nadir and change in $\dot{V}O_{2peak}$ (% predicted) (β= 0.78, CI= 0.22 to 1.33). There was no significant direct relationship between baseline BV5/TVV and change in $\dot{V}O_{2peak}$ (p=0.47); however, $D_LCO$ adjusted for $V_A$ (%predicted, β=0.17, CI= 0.05 to 0.29) and LnLAA-950% (β= -3.88, CI= -6.78 to -0.98) were significantly associated with the change in $\dot{V}O_{2peak}$. BV5/TVV, $D_LCO$, and LnLAA-950 were all significantly related with $\dot{V}_E/\dot{V}CO_2$

nadir (BV5/TVV: β= 15.44, CI= 5.28 to 25.60; $D_LCO$: β= -0.09, CI= -0.13 to -0.06; LnLAA-950: β= 1.16, CI= 0.28 to 2.04). Analysis of indirect effects revealed that BV5/TVV, $D_LCO$ and LnLAA-950 were all was significantly associated with change in $\dot{V}O_{2peak}$ through the mediator $\dot{V}_E/\dot{V}CO_2$ nadir (BV5/TVV: β= 15.64, CI= 2.76 to 31.39; $D_LCO$: β= -0.10, CI= -0.19 to -0.03; and LnLAA-950: β= 1.18, CI= 0.25 to 2.37). Therefore, the relationship between baseline BV5/TVV and change in $\dot{V}O_{2peak}$ was entirely mediated by baseline $\dot{V}_E/\dot{V}CO_2$ nadir (given the significant indirect effect but non-significant direct effect), while the effect of baseline $D_LCO$ and LnLAA-950% on change in $\dot{V}O_{2peak}$ was partly mediated by $\dot{V}_E/\dot{V}CO_2$ nadir in individuals with COPD. Results are represented in **Figure 4.4.**

### *4.4 Discussion*

To our knowledge, this is the first study to focus on the longitudinal relationship between $\dot{V}O_{2peak}$ and pulmonary vascular structure and function estimates in ES without airflow obstruction, and people with COPD. Our key findings were twofold: 1) Higher baseline $\dot{V}O_{2peak}$ was protective of peripheral pulmonary vascular decline (BV5/TVV), in ES without COPD and in individuals with COPD, independent of emphysema and degree of airflow obstruction; 2) Baseline pulmonary vascular structure and function measures were not associated with longitudinal decline in $\dot{V}O_{2peak}$ in linear mixed models analyses, but rather the relationship between baseline pulmonary vascular structure and function with longitudinal change in $\dot{V}O_{2peak}$ was mediated through $\dot{V}_E/\dot{V}CO_2$ nadir. Combined, these data indicate that higher exercise capacity may be protective of structural decline in the distal pulmonary vasculature; but contrary to our initial hypothesis, no direct relationship was observed between baseline pulmonary vascular structure and function and decline in $\dot{V}O_{2peak}$. While these findings indicate that greater pulmonary vascular structure/function does not *directly*

affect longitudinal decline in exercise capacity over 3 years, our mediation analysis results suggest that baseline pulmonary vascular structure and function impacts ventilatory efficiency, which in turn is associated with longitudinal change in $\dot{V}O_{2peak}$. These data shed light on an important connection: exercise ventilatory inefficiency emerges as a significant factor linking pulmonary vascular structure/function and decline in exercise capacity. Combined, our findings provide further evidence regarding the complex interplay between pulmonary vascular structure/function and exercise, and its functional consequences in those with and without COPD.

*4.4.1 Does impaired exercise capacity impact longitudinal changes in pulmonary vascular structure and function?*

In the entire cohort, we found that greater baseline $\dot{V}O_{2peak}$ was protective of peripheral pulmonary vascular decline (BV5/TVV), even after controlling for participant body morphology, emphysema and degree of airflow obstruction. When analyses were limited to only COPD, $\dot{V}O_{2peak}$ became an even more important contributor to longitudinal change in BV5/TVV, with the association between baseline $\dot{V}O_{2peak}$ and change in BV5/TVV increasing 1.25 fold. For every 1L/min increase in baseline $\dot{V}O_{2peak}$, BV5/TVV increases by 1.8% in COPD, and by 1.2% in ES and COPD at follow-up. Our results extend recent work showing that greater physical activity and $\dot{V}O_{2peak}$ is protective of lung function decline (141), and reduces COPD risk in smokers (92, 93), and suggest that parameters beyond airflow obstruction may be susceptible to modification. Exercise capacity ($\dot{V}O_{2max}$) and physical activity have been shown to be inversely related to inflammation and immunity (142-148), with chronic inflammation suggested to be linked to emphysema and pulmonary vascular alterations including intima media thickening, thrombosis, and vascular pruning (149-152).

Surprisingly, we found that greater baseline $\dot{V}O_{2peak}$ was associated with *accelerated* decline in BV5-10/TVV and $D_LCO$ in ES and COPD (with the former effect disappearing in models isolated to COPD only), while there was no association between $\dot{V}O_{2peak}$ with longitudinal change in TAC or TVV/TLV. For every 1L/min increase in baseline $\dot{V}O_{2peak}$, BV5-10/TVV decreased by 1.4% in COPD, and by 1.2% in ES and COPD at follow-up. While the strength of the relationships between baseline $\dot{V}O_{2peak}$ with BV5-10/TVV and BV5/TVV were similar (Tables 2&3), the relative contribution of small vessel volume (BV5, a surrogate marker of distal vascular pruning) to total blood vessel volume is almost double that of intermediate blood vessel volume (BV5-10) (**Table 4.1**). In other words, the total volume of small vessels is larger than that of intermediate sized vessels, and in order to interpret our findings, it is necessary to consider the relative contributions of BV5-10 to the entire pulmonary vascular bed. These reductions in BV5-10/TVV with greater exercise capacity may be secondary to reversal of pathological remodelling of muscular arteries and arterioles such as thinning of the intimal layer. Histologic investigations have shown that pathologic vascular remodelling (e.g., increased relative area comprising the intima and media in arteries <1mm in diameter) is associated with pulmonary vascular pruning on CT (153). As such, the relationships between $\dot{V}O_{2peak}$ and small and intermediate sized vessels that are in opposite directions may reflect redistribution of blood away from the larger vessels, towards the smallest peripheral vessels detectable by CT. As for $D_LCO$, for every 1L/min increase in $\dot{V}O_{2peak}$, $D_LCO$ declined by 2 mL/min/mmHg 3 years later in ES and COPD. This decline in $D_LCO$ is similar to aging related decline previously reported in health (98, 154). Therefore, while statistically significant, these results suggest that the impacts of baseline $\dot{V}O_{2peak}$ on pulmonary diffusing capacity are minimal after accounting for participant characteristics (including age), and are therefore unlikely to be of clinical significance. Further research is needed to better understand

the potential implications of reduced in BV5-10/TVV. Moreover, there is a need to investigate the impacts of exercise capacity and perhaps exercise training on the relative distributions on small vs larger pulmonary vessel volumes.

*4.4.2 Are there consequences to impaired pulmonary vascular structure and function on changes in exercise capacity?*

Given that both exercise capacity and pulmonary vascular structure/function vary over time, the longitudinal relationship between changes in exercise capacity and decline in pulmonary vascular structure and function may be due to reverse causation. Therefore, a secondary aim of this study was to evaluate whether greater baseline pulmonary vascular structure and function is protective of $\dot{V}O_{2peak}$ decline. We found that neither baseline $D_LCO$, BV5/TVV, BV5-10/TVV, TVV/TLV nor TAC was consistently associated with longitudinal decline in $\dot{V}O_{2peak}$ in mixed models including both ES and COPD, as well as in those limited to COPD. These results were surprising, given previous reports showing that lower baseline $D_LCO$ is an important predictor in functional decline over time (e.g., 12-minute walk distance (155)), and that individuals with lower baseline $D_LCO$ have less improvements in 6-minute walk following pulmonary rehabilitation (77).

The non-significant total effects demonstrated in our mediation analyses confirmed the results of our linear mixed models; again, showing that neither baseline $D_LCO$, emphysema nor pulmonary vascular pruning on CT at baseline are associated with accelerated $\dot{V}O_{2peak}$ decline in ES and COPD at follow-up. However, our mediation analyses provide evidence that the relationship between baseline $D_LCO$, emphysema and BV5/TVV and change in $\dot{V}O_{2peak}$ was more complex as the effects of baseline $D_LCO$, emphysema and BV5/TVV on change in $\dot{V}O_{2peak}$ were found to be mediated by baseline $\dot{V}_E/\dot{V}CO_2$ nadir. Specifically, the effects of baseline BV5/TVV

and LnLAA-950 on change in $\dot{V}O_{2peak}$ were entirely mediated by $\dot{V}_E/\dot{V}CO_2$ nadir, while $D_LCO$ was partially mediated by $\dot{V}_E/\dot{V}CO_2$ nadir in ES and COPD. When analyses were conducted in COPD only, the relationship between baseline BV5/TVV and change in $\dot{V}O_{2peak}$ was entirely mediated by baseline $\dot{V}_E/\dot{V}CO_2$ nadir, while the effect of baseline $D_LCO$ and LnLAA-950% on change in $\dot{V}O_{2peak}$ were partly mediated by $\dot{V}_E/\dot{V}CO_2$ nadir. Collectively, these data indicate that while baseline BV5/TVV does not directly impact longitudinal changes in $\dot{V}O_{2peak}$, it exerts an influence on ventilatory efficiency, which in turn affects change in $\dot{V}O_{2peak}$ (see **Table 4.5** and **Figure 4.4**). Thus, our mediation analyses provide evidence beyond that which can be obtained from linear mixed model analyses as we demonstrate a mechanistic role of baseline pulmonary vascular structure and function in determining change in exercise capacity through baseline exercise ventilatory (in)efficiency in ES at risk of developing COPD and people with COPD. These results extend previous cross-sectional work in smokers and patients with COPD, whereby emphysema severity, $D_LCO$, $\dot{V}O_{2peak}$ and $\dot{V}_E/\dot{V}CO_2$ were shown to be interrelated (83, 84), and further support the notion that interventions aimed at modifying pulmonary vascular function may increase exercise capacity secondary to improved ventilatory efficiency (7, 48).

Recent cross-sectional work originating from the CanCOLD cohort has shown that $\dot{V}_E/\dot{V}CO_2$ nadir above the upper limit of normal (i.e., representing exercise ventilatory inefficiency) is associated with greater dyspnea and low $\dot{V}O_{2peak}$, independent of severity of airflow obstruction (137). Phillips and colleagues showed that dyspnea/$\dot{V}_E$ ratios and operating lung volumes were not different between individuals with normal vs elevated $\dot{V}_E/\dot{V}CO_2$ nadir, suggesting that increased ventilatory requirements, rather than respiratory mechanics contribute to the elevated dyspnea observed in those with ventilatory inefficiency (137). Our mediation analyses completed in CanCOLD participants with complete follow-up data revealed an unexpected finding, however:

that *higher* baseline $\dot{V}_E/\dot{V}CO_2$ nadir (i.e., worse ventilatory efficiency) was positively associated with an *increase* in $\dot{V}O_{2peak}$ 3 years later. The physiological underpinnings of this unexpected finding are difficult to ascertain from the current pre-specified analyses; however, they warrant discussion. During exercise, $\dot{V}_E/\dot{V}CO_2$ can be elevated secondary to alveolar hyperventilation, and/or high dead space (156), with increased dead space being the most consistent gas exchange abnormality in mild COPD (27). However, the interpretation of $\dot{V}_E/\dot{V}CO_2$ responses to exercise in moderate-severe COPD can become complex: with some patients demonstrating a *blunted* $\dot{V}_E/\dot{V}CO_2$ response to exercise due to severe ventilatory mechanical constraints (36). Severe exercise intolerance and short exercise test duration might overestimate $\dot{V}_E/\dot{V}CO_2$ nadir in those with more severe COPD due to the excessive ventilation observed during the initial stages of a CPET (157). However, this is unlikely within the current study given that most participants with COPD enrolled had mild-moderate disease. Notably, most participants in the CanCOLD study were not aware of their COPD diagnosis at time of enrolment (158), as participants were recruited from the general population rather than from clinical settings. As such, COPD pharmacotherapy may have been initiated (e.g., inhaled short- and long-acting bronchodilators, and corticosteroids) following the baseline assessment, and subsequent symptom improvement may be driving the observed improvement in $\dot{V}O_{2peak}$ in those with worse baseline ventilatory efficiency in the current study.

Finally, it is generally accepted that the rate of decline in relative $\dot{V}O_{2max}$ is approximately 1-1.5% per year in healthy adults (159-161). COPD has been referred to as a disease of senescence (162) (or accelerated ageing), which can lower the threshold for symptom limitation, therefore accelerating functional decline (163). We observed a mean reduction of 0.67% per year in $\dot{V}O_{2peak}$ (mL/kg/min for ES and COPD combined), which is slightly lower than the expected rate of decline

in exercise capacity in health. This discrepancy may be due to survivor bias: with those returning for the follow-up visit having a higher baseline exercise capacity and less $\dot{V}O_{2peak}$ decline at follow-up than those who did not. Indeed, percent predicted $\dot{V}O_{2peak}$ was about 3-5% higher at baseline in those who returned for the follow-up visit, as compared to the entire cohort of ES and COPD assessed at the baseline study visit. Therefore, since ventilatory inefficiency has been shown to be associated with lower exercise capacity in the same cohort (137), it is likely that individuals with better baseline ventilatory efficiency (i.e., lower $\dot{V}_E/\dot{V}CO_2$) had higher baseline $\dot{V}O_{2peak}$, which in turn would have contributed to slower $\dot{V}O_{2peak}$ decline at follow-up.

*4.4.3 Strengths and Limitations*

This study has many strengths. Firstly, this sub-analysis involves use of both visit 1 and visit 3 data collected through the national CanCOLD study (79), where participants were recruited via random digit dialling. As such, results from this study are more likely to be generalizable to ES and individuals with COPD within the Canadian population. However, never-smokers which were assessed at visit 1 did not return for visit 3 and were therefore not included in our analyses. While this reduces the heterogeneity of our sub-analyses, this prevented the comparison with a "healthier" sample of participants. Further, the authors recognize that a 3-year follow-up visit may not be sufficient to detect accelerated functional or structural changes beyond those associated with ageing. However, this is unlikely, given that $\dot{V}O_{2peak}$ was a significant predictor of longitudinal decline in distal pulmonary vascular structure, even after accounting for age at visit 1. Finally, mechanisms contributing to exercise limitation in COPD are multifactorial and complex and involve multiple factors beyond pulmonary vascular structure/function such as dynamic operating lung volumes, cardiac function, and peripheral oxygen delivery and utilization (22, 164-

167). Nonetheless, the purpose of this investigation was to further probe the specific impacts of exercise capacity on pulmonary vascular structure and function decline, which has not been previously investigated in a large, population-based sample of both ES and COPD.

### *4.4.4 Conclusion*

In conclusion, this is the first examination of the potential protective effects of baseline exercise capacity on longitudinal changes in pulmonary vascular structure/function in a cohort of ES at risk of developing COPD, and individuals with COPD. Combined, our results suggest that greater baseline exercise capacity may serve as a protective factor against longitudinal decline in distal pulmonary vascular volumes. Interestingly, neither $D_LCO$ nor pulmonary vascular volumes on CT at baseline were associated with accelerated $\dot{V}O_{2peak}$ decline in ES or COPD. However, we did observe a significant mediation effect of baseline exercise ventilatory (in)efficiency on $\dot{V}O_{2peak}$, supporting a mechanistic role of pulmonary vascular structure and function in determining decline in exercise capacity through $\dot{V}_E/\dot{V}CO_2$ nadir in ES and people with COPD.

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**Table 4.1:** Baseline anthropometrics, demographics, pulmonary function in ever-smokers without airflow obstruction, and individuals with COPD (n = 299).

| Parameter | Ever-smokers n=148 | | | COPD n=151 | | |
|---|---|---|---|---|---|---|
| **Participant Demographics** | | | | | | |
| Female, % | 36 | | | 45 | | |
| Caucasian, % | 98 | | | 97 | | |
| Age, years | 65.73 | ± | 9.36 | 63.4 | ± | 10.29 |
| Height, cm | 169.64 | ± | 8.57 | 169.8 | ± | 9.76 |
| BMI, $kg/m^2$ | 27.24 | ± | 3.74 | 26.48 | ± | 4.68 |
| Current smoker, % | 16 | | | 21 | | |
| Pack-years | 20.76 | ± | 20.3 | 21.22 | ± | 23.66 |
| HDHTDM, % | 53 | | | 50 | | |
| **Pulmonary Function** | | | | | | |
| $FEV_1$, %pred | 100.89 | ± | 14.38 | 78.76 | ± | 17.35* |
| FVC, %pred | 104.87 | ± | 15.09 | 102.38 | ± | 18.04 |
| $FEV_1$/FVC, %pred | 95.95 | ± | 6.76 | 76.29 | ± | 8.53* |
| RV, %pred | 104.06 | ± | 12.47 | 109.55 | ± | 17.53* |
| TLC, %pred | 110.82 | ± | 31.98 | 143.41 | ± | 43.12* |
| RV/TLC, %pred | 107.08 | ± | 27.13 | 128.94 | ± | 31.45* |
| $D_LCO$, %pred | 95.25 | ± | 18.83 | 90.88 | ± | 25.41 |
| $V_{A,}$ %pred | 100.28 | ± | 11.65 | 98.61 | ± | 17.42 |
| $D_LCO/V_A$, %pred | 94.55 | ± | 15.49 | 92.25 | ± | 23.84 |
| **Peak cardiopulmonary exercise responses** | | | | | | |
| Work rate, W | 126 | ± | 43 | 114 | ± | 46* |
| Work rate, % pred | 97.02 | ± | 23.22 | 86.39 | ± | 24.39* |
| $\dot{V}O_{2peak}$, L/min | 1.85 | ± | 0.65 | 1.68 | ± | 0.66* |
| $\dot{V}O_{2peak}$, mL/kg/min | 23.49 | ± | 7.20 | 21.93 | ± | 7.43 |
| $\dot{V}O_{2peak}$, % predicted | 94.91 | ± | 25.26 | 86.42 | ± | 23.65* |
| $\dot{V}_E$, L/min | 67.22 | ± | 23.47 | 60.21 | ± | 24.31* |
| $\dot{V}_E/\dot{V}CO_{2\ nadir}$ | 29.75 | ± | 4.32 | 30.84 | ± | 6.12 |
| $SpO_2$, % | 97 | ± | 2 | 96 | ± | 3* |
| Dyspnea, 0-10 Borg units | 6 | ± | 3 | 5 | ± | 3 |
| Leg discomfort, 0-10 Borg units | 7 | ± | 3 | 7 | ± | 2 |
| Symptoms | | | | | | |
| CAT | 4.8 | ± | 3.76 | 8.3 | ± | 6.57* |
| SGRQ total | 8.05 | ± | 7.62 | 15.48 | ± | 14.45* |
| MRC | 1.32 | ± | 0.51 | 1.55 | ± | 0.73* |
| Imaging | | | | | | |
| TLV, L | 4.83 | ± | 1.15 | 5.05 | ± | 1.28 |
| LAA950, % | 3.53 | ± | 3.31 | 5.68 | ± | 5.92* |
| LAA856, % | 59.81 | ± | 14.34 | 63.68 | ± | 13.53 |
| TAC, n | 212.44 | ± | 59.52 | 163.72 | ± | 65.87* |
| TVV/TLV, mL | 3.22 | ± | 0.37 | 3.05 | ± | 0.39* |
| BV5/TVV | 0.53 | ± | 0.08 | 0.56 | ± | 0.08* |
| BV5-10/TVV | 0.32 | ± | 0.04 | 0.31 | ± | 0.05* |

Data are mean ± SD for continuous variables, or % for categorical variables. BMI: body mass index; HDHTDM: heart disease, hypertension and/or diabetes mellitus; $FEV_{1:}$ forced expiratory volume in 1sec; FVC: forced vital

capacity; RV: residual volume; TLC: total lung capacity; $D_LCO$: diffusing capacity for carbon monoxide; $V_A$: alveolar volume; $\dot{V}O_{2peak}$: peak rate of oxygen consumption; $\dot{V}_E$: minute ventilation; $\dot{V}_E/\dot{V}CO_2$:minute ventilation to carbon dioxide output; $SpO_2$: peripheral oxygen saturation; CAT: COPD Assessment Test; SGRQ: Saint-Georges Respiratory Questionnaire; MRC: Medical Research Council dyspnea scale; TLV: CT-derived total lung volume; LAA950: low attenuation area of the lung with attenuation values below −950 HU on full inspiration CT; LAA856: low attenuation areas of the lung with attenuation values below −856 HU on full expiration CT; TAC: total airway count; TVV: total blood vessel volume; TLV: total lung volume; BV5: volume of blood vessels with a cross-sectional area <5mm$^2$; BV5-10/TVV: volume of blood vessels with a cross-sectional area between 5-10mm$^2$.

*Significantly different from ever-smokers ($p<0.05$).

**Table 4.2:** Is baseline $\dot{V}O_{2peak}$ associated with longitudinal airway and pulmonary vascular decline in ES and COPD?

| **Model 1: *$D_LCO$, n=290*** | **Estimate (95% CI)** | **p-value** |
|---|---|---|
| Intercept | -4.44 (-18.33 9.45) | 0.53 |
| Time (years) | 0.20 (-0.12 to 0.53) | 0.22 |
| $VO_2$ (L/min) | 2.72 (1.73 to 3.72) | <0.001 |
| Ever-smokers | -1.32 (-2.36 to -0.28) | 0.01 |
| COPD | | |
| $VO_2$ x time interaction | -0.24 (-.41 to -0.07) | 0.01 |
| **Model 2: *TVV/TLV n=282*** | | |
| Intercept | 3.68 (2.50 to 4.87) | <0.001 |
| Time (years) | 0.03 (0.006 to 0.06) | 0.02 |
| $VO_2$ (L/min) | 0.09 (0.002 to 0.17) | 0.046 |
| Ever-smokers | 0.11 (0.02 to 0.20) | 0.01 |
| COPD | | |
| $VO_2$ x time interaction | -0.006 (-0.02 to 0.01) | 0.44 |
| **Model 3: *BV5/TVV (n=279)*** | | |
| Intercept | 0.6 (0.39 to 0.81) | <0.001 |
| Time (years) | -0.01 (-0.02 to 0.00) | 0.005 |
| $VO_2$ (L/min) | -4.87E-6 (-0.02 to 0.02) | 1.00 |
| Ever-smokers | -0.02(-0.03 to 0.00) | 0.04 |
| COPD | | |
| $VO_2$ x time interaction | 0.004 (0.001 to 0.008) | 0.008 |
| **Model 4: *BV5-10 n=279*** | | |
| Intercept | 0.40 (0.27 to 0.52) | <0.001 |
| Time (years) | 0.006 (0.002 to 0.01) | 0.003 |
| $VO_2$ (L/min) | 0.003 (-0.006 to 0.012) | 0.50 |
| Ever-smokers | 0.01 (0.004 to 0.02) | 0.007 |
| COPD | | |
| $VO_2$ x time interaction | -0.003 (-0.005 to -0.001) | 0.005 |
| **Model 5: *TAC n=282*** | | |
| Intercept | -7.34 (-178.34 to 163.66) | 0.93 |
| Time (years) | -19.27 (-24.35 to -14.19) | <0.001 |
| $VO_2$ (L/min) | -5.75 (-18.41 to 6.91) | 0.37 |
| Ever-smokers | 23.82 (10.97 36.66) | <0.001 |
| COPD | | |
| $VO_2$ x time interaction | -0.27 (-2.96 to 2.42) | 0.84 |

$\dot{V}O_{2peak}$: peak rate of oxygen consumption; $D_LCO$: diffusing capacity for carbon monoxide; TVV: total blood vessel volume; TLV: total lung volume; BV5: volume of blood vessels with a cross-sectional area <5mm$^2$; BV5-10/TVV: volume of blood vessels with a cross-sectional area between 5-10mm$^2$; TAC: total airway count.
**Models are adjusted for:** Fixed effects are time (in years, between V1 and V3), baseline group (AR and COPD), $FEV_1$ (L), sex, race, and age (years), height (cm), and weight (kg), and time-varying, CT scanner model (or study site at V1 and V3), and CT measurements at V1 and V3 (LnLAA-950), $TLV_{CT}/TLC_{pleth}$, or alveolar volume ($V_A$; only included in the $D_LCO$ model). Participant ID was used as the random intercept, and time (in years, between V1 and V3, with V1 fixed at 0) was used to define the random slope. For each model, the covariance structure for the random intercept and slope model was heterogenous first-order autoregressive, AR(1).

| **Table 4.3:** Is baseline $VO_2$ associated with longitudinal airway and pulmonary vascular decline in COPD? | | |
|---|---|---|
| **Model 1: *$D_LCO$*** | **Estimate (95% CI)** | **p-value** |
| Intercept | -12.76 (-35.26 to 9.73) | 0.26 |
| Time (years) | 0.23 (-0.25 to 0.71) | 0.35 |
| $VO_2$ (L/min) | 3.13 (1.34 to 4.92) | <0.001 |
| $VO_2$ x time interaction | -0.26 (-0.53 to 0.01) | 0.057 |
| **Model 2: *TVV/TLV*** | | |
| Intercept | 4.38 (2.64 to 6.12) | <0.001 |
| Time (years) | 0.04 (-3.96E-5 to 0.08) | 0.05 |
| $VO_2$ (L/min) | 0.05 (-0.09 to 0.18) | 0.50 |
| $VO_2$ x time interaction | -0.007 (-0.03 to 0.02) | 0.55 |
| **Model 3: *BV5/TVV*** | | |
| Intercept | 0.48 (0.19 to 0.78) | 0.001 |
| Time (years) | -0.01 (-0.02 to 0.00) | 0.01 |
| $VO_2$ (L/min) | 0.005 (-0.03 to 0.02) | 0.68 |
| $VO_2$ x time interaction | 0.005 (0.001 to 0.01) | **0.03** |
| **Model 4: *BV5-10*** | | |
| Intercept | 0.48 (0.29 to 0.66) | <0.001 |
| Time (years) | 0.008 (0.002 to 0.01) | 0.009 |
| $VO_2$ (L/min) | 0.007 (-0.007 to 0.02) | 0.33 |
| $VO_2$ x time interaction | -0.004 (-0.007 to -0.001) | **0.02** |
| **Model 5: *TAC*** | | |
| Intercept | -8.68 (-273.13 to 257.77) | 0.95 |
| Time (years) | -12.98 (-19.69 to -6.27) | <0.001 |
| $VO_2$ (L/min) | -1.21 (-21.07 to 18.65) | 0.90 |
| $VO_2$ x time interaction | -1.32 (-4.99 to 2.34) | 0.48 |

$\dot{V}O_{2peak}$: peak rate of oxygen consumption; $D_LCO$: diffusing capacity for carbon monoxide; TVV: total blood vessel volume; TLV: total lung volume; BV5: volume of blood vessels with a cross-sectional area <5mm$^2$; BV5-10/TVV: volume of blood vessels with a cross-sectional area between 5-10mm$^2$; TAC: total airway count. **Models are adjusted for:** Fixed effects are time (in years, between V1 and V3), baseline group (AR and COPD), $FEV_1$ (L), sex, race, and age (years), height (cm), and weight (kg), and time-varying, CT scanner model (or study site at V1 and V3), and CT measurements at V1 and V3 (LnLAA-950), $TLV_{CT}/TLC_{pleth}$, or alveolar volume ($V_A$; only included in the $D_LCO$ model). Participant ID was used as the random intercept, and time (in years, between V1 and V3, with V1 fixed at 0) was used to define the random slope. For each model, the covariance structure for the random intercept and slope model was heterogenous first-order autoregressive, AR(1).

**Table 4.4:** Are baseline pulmonary vascular and airway structure and function predictive of change in $\dot{V}O_{2peak}$ in ES and COPD?

| **Predictors** | **ES and COPD** | | **COPD only** | |
|---|---|---|---|---|
| | **Estimate (95% CI)** | **p-value** | **Estimate (95% CI)** | **p-value** |
| BL $D_LCO$ x time | 0.001 (-0.001 to 0.003) | 0.31 | 0.002 (-0.001 to 0.004) | 0.15 |
| BL TVV/TLV x time | -0.04 (-0.07 to -0.004) | **0.03** | 0.005 (-0.04 to 0.05) | 0.84 |
| BL BV5/TVV x time | 0.09 (-0.06 to 0.26) | 0.24 | -0.005 (-0.22 to 0.21) | 0.96 |
| BL BV5-10/TVV x time | -0.19 (-0.47 to 0.09) | 0.18 | 0.05 (-0.31 to 0.41) | 0.79 |
| BL TAC x time | -6.43E-5 (-0.0002 to 0.0001) | 0.45 | -7.80E-5 (-0.0003 to 0.0001) | 0.50 |
| BL $\dot{V}_E/\dot{V}CO_{2nadir}$ | 0.004 (0.0002 to 0.008) | **0.04** | 0.002 (-0.001 to 0.005) | 0.17 |

$\dot{V}O_{2peak}$: peak rate of oxygen consumption; $D_LCO$: diffusing capacity for carbon monoxide; TVV: total blood vessel volume; TLV: total lung volume; BV5: volume of blood vessels with a cross-sectional area <5mm$^2$; BV5-10/TVV: volume of blood vessels with a cross-sectional area between 5-10mm$^2$; TAC: total airway count; $\dot{V}_E/\dot{V}CO_2$:minute ventilation to carbon dioxide output. Fixed effects are time (in years, between V1 and V3), baseline $FEV_1$ (L), LnLAA-950, sex, race, age (years), height (cm), and weight (kg), and time-varying pack-years, CT scanner model (or site ID for $D_LCO$ and $\dot{V}_E/\dot{V}CO_2$ models), $TLV_{CT}/TLC_{pleth}$ (only included in CT measure models) or alveolar volume ($V_A$; only included in the $D_LCO$ model). Participant ID was used as the random intercept, and time (in years, between V1 and V3, with V1 fixed at 0) was used to define the random slope. The covariance structure for each random intercept and slope model was heterogenous first-order autoregressive, AR(1).

**Table 4.5:** Mediation analysis evaluating the relationship between baseline BV5/TVV, $D_LCO$, and LnLAA-950 (predictors) with $\dot{V}O_{2peak}$ through $\dot{V}_E/\dot{V}CO_2$ nadir (mediator).

| **Predictors** | Direct effect of X on M | Total effect | Indirect effect | Mediation ratio |
|---|---|---|---|---|
| **ES and COPD** | | | | |
| BV5/TVV, mL | 11.75 (5.22 to 18.29) | 6.66 (-18.91 to 32.22) | 13.06 (4.68 to 23.25) | 1.96 |
| $D_LCO$ adj for $V_A$, %pred | -0.09 (-0.11 to -0.07) | 0.08 (-0.001 to 0.18) | -0.10 (-0.15 to -0.05) | -1.18 |
| LnLAA-950, % | 0.83 (0.29 to 1.37) | -0.82 (-2.94 to 1.30) | 0.92 (0.27 to 1.69) | -1.12 |
| **COPD only** | | | | |
| BV5/TVV, mL | 15.44 (5.28 to 25.60) | 2.71 (-31.52 to 36.94) | 15.64 (2.76 to 31.39) | 5.78 |
| $D_LCO$ adj for $V_A$, %pred | -0.09 (-0.13 to -0.06) | 0.07 (-0.04 to 0.19) | -0.10 (-0.19 to -0.03) | -1.32 |
| LnLAA-950, % | 1.16 (0.28 to 2.04) | -2.71 (-5.67 to 0.25) | 1.18 (0.25 to 2.37) | -0.43 |

Data are unstandardized estimates and 95% confidence intervals. $\dot{V}O_{2peak}$: peak rate of oxygen consumption; $D_LCO$: diffusing capacity for carbon monoxide; $V_A$: alveolar volume; TVV: total blood vessel volume; BV5: volume of blood vessels with a cross-sectional area <5mm$^2$; $\dot{V}_E/\dot{V}CO_2$:minute ventilation to carbon dioxide output. M = mediator ($\dot{V}_E/\dot{V}CO_2$ nadir); X = independent variables BV5/TVV, DLCO adj for $V_A$, LnLAA-950; Y= change in $\dot{V}O_{2peak}$. BV5/TVV: , DLCO adj for $V_A$, LnLAA-950. Indirect effect of X on Y = a*b; total effect = sum of direct and indirect effect = (a*b)+c'.

## A. Study Design

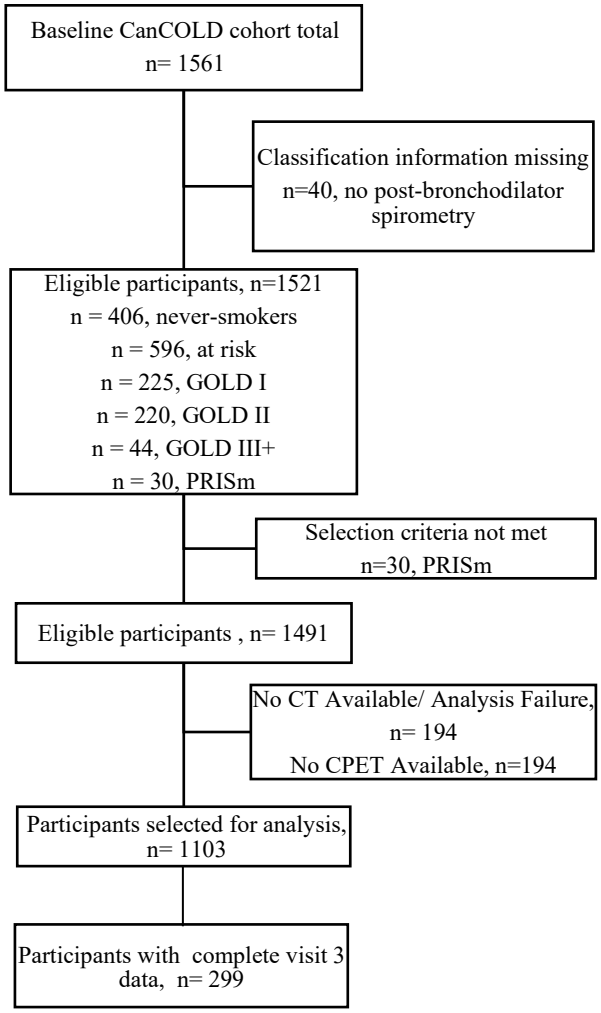
| CanCOLD longitudinal Cohort | |
|---|---|
| **Visit 1** | **Visit 3** |
| Demographics | Demographics |
| Smoking History | Smoking History |
| Spirometry | Spirometry |
| Plethysmography | Plethysmography |
| Single breath $D_LCO$ | Single breath $D_LCO$ |
| CPET | CPET |
| Questionnaires | Questionnaires |
| CT Imaging | CT Imaging |

## B. Longitudinal Data

| Visit 1 | Visit 3 |
|---|---|
| n=1561 | n= 299 |

**Figure 4.1:** Study Design and participant selection flowchart. (A) Participants included in the COLD (Chronic Obstructive Lung Disease) prevalence study were selected to participate in the longitudinal CanCOLD (Canadian Chronic Obstructive Lung Disease) study as previously described (4). (B) CPET = cardiopulmonary exercise test; CT = computed tomography; GOLD = Global Initiative for Chronic Obstructive Lung Disease; PRISm = preserved ratio but impaired spirometry.

## C. Participant Selection – Baseline



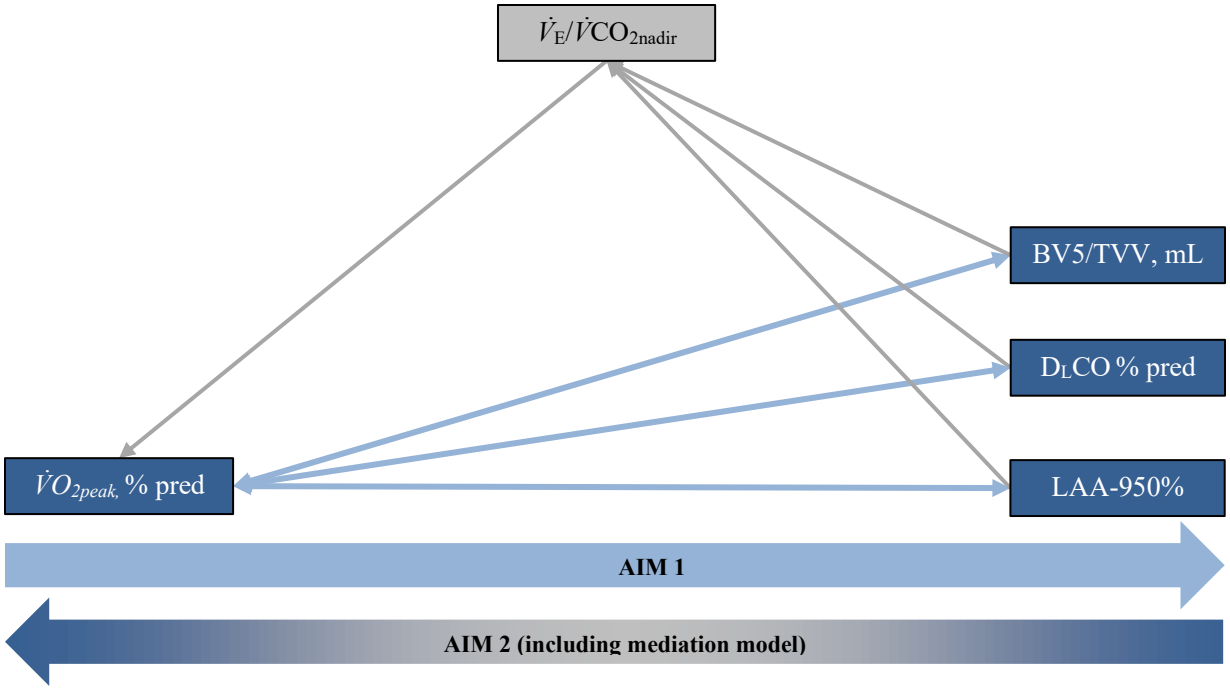



**Figure 4.2:** Theoretical model of the study. $\dot{V}O_{2peak}$: peak rate of oxygen consumption; $D_LCO$: diffusing capacity for carbon monoxide; $V_A$: alveolar volume; TVV: total blood vessel volume; BV5: volume of blood vessels with a cross-sectional area <5mm$^2$; $\dot{V}_E/\dot{V}CO_2$:minute ventilation to carbon dioxide output**.**

Arrow from left to right (Aim 1): the association between baseline $\dot{V}O_{2peak}$ (predictor) with longitudinal change in $D_LCO$, LAA-950, and BV5/TVV (outcomes). Arrow from right to left (Aim 2): the association between baseline $D_LCO$, LAA-950, and BV5/TVV (predictors) with longitudinal change in $\dot{V}O_{2peak}$ (outcome), and through baseline $\dot{V}_E/\dot{V}CO_{2nadir}$ (mediation analysis).

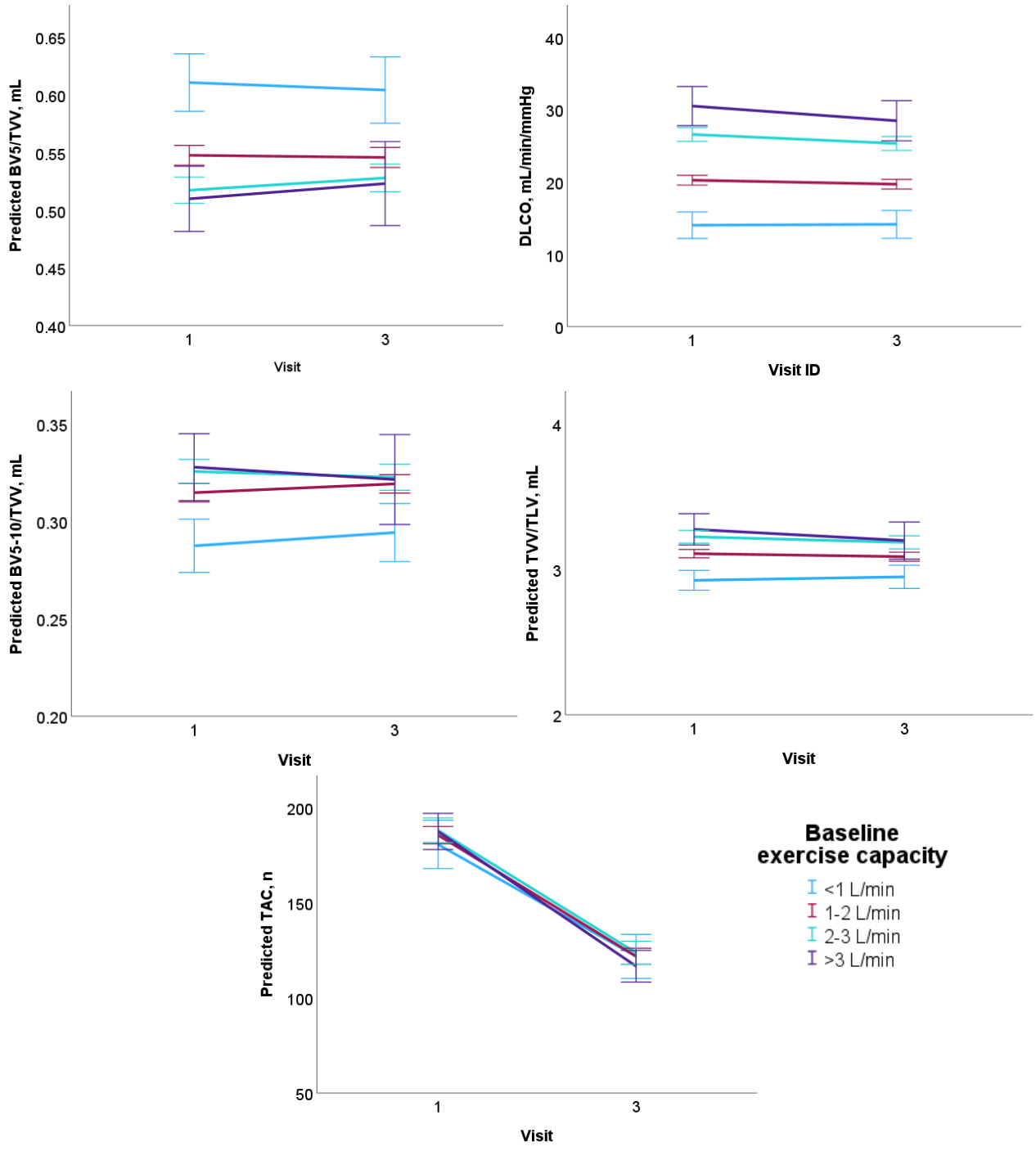



**Figure 4.3:** Longitudinal change in the fully adjusted fixed predicted outcome variables in ES and COPD by exercise capacity (L/min).

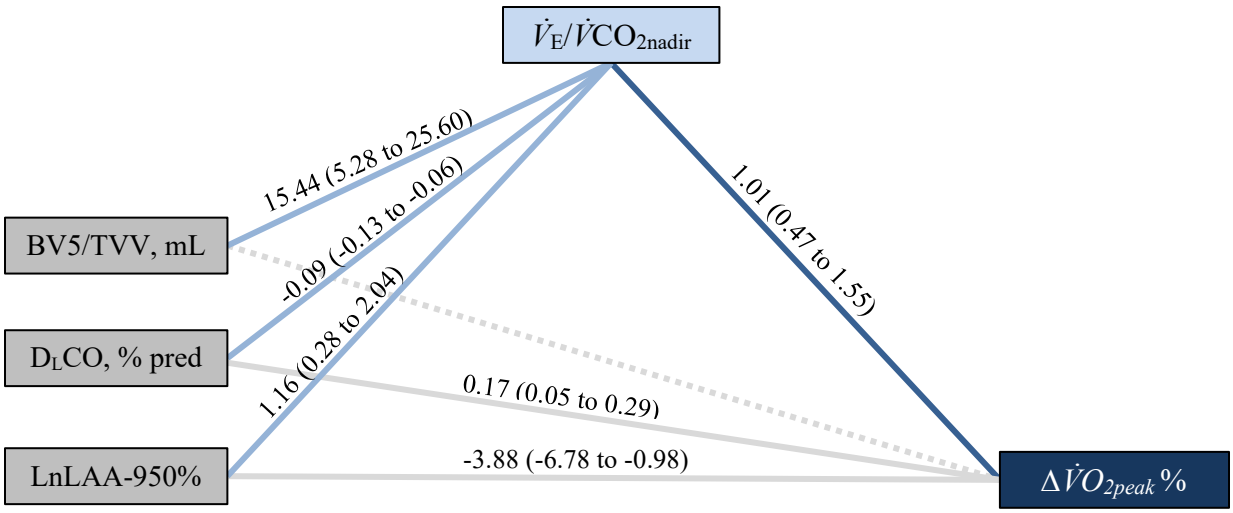



**Figure 4.4:** Model for the association between baseline $D_LCO$, LAA-950, and BV5/TVV (predictors), with change in $\dot{V}O_{2peak}$ (outcome) through baseline $\dot{V}_E/\dot{V}CO_{2nadir}$ (mediator) in COPD. Data are reported as: unstandardized β estimates (95% upper and lower limit confidence intervals). $\dot{V}O_{2peak}$: peak rate of oxygen consumption; $D_LCO$: diffusing capacity for carbon monoxide; $V_A$: alveolar volume; TVV: total blood vessel volume; BV5: volume of blood vessels with a cross-sectional area <5mm$^2$; $\dot{V}_E/\dot{V}CO_2$:minute ventilation to carbon dioxide output. Grey lines: relationship between independent variables (X) and dependent variable (Y; path c': the direct effect). Light blue lines: relationship between X and mediator (M; path a). Navy blue line: relationship between M and Y (path b). Indirect effect of X on Y = a·b. Total effect (c path, not shown here) = c' + a·b. Mediation occurs when the indirect effect is significant. Full mediation occurs when the indirect effect is significant, and the direct effect is non-significant. Dotted grey

## CHAPTER FIVE: STUDY #3

### The effect of iNO on $\dot{V}O_{2peak}$, ventilatory efficiency and dyspnea across the continuum of COPD: a randomized-controlled cross-over trial

*Results presented in this Chapter are preliminary data from a larger prospective trial. Additional data and participants will be included in the final manuscript.

### *5.1 Introduction*

Chronic Obstructive Pulmonary Disease (COPD) is characterized by dyspnea and exercise intolerance. Dyspnea in patients with COPD is partly explained by an exaggerated ventilatory response to exercise [determined by the ventilatory equivalent for carbon dioxide production ($\dot{V}_E/\dot{V}CO_2$)](137). The elevated $\dot{V}_E/\dot{V}CO_2$ response to exercise in COPD is clinically important because it independently predicts mortality, and indicates that physiological abnormalities beyond airflow obstruction are important in determining exercise tolerance and dyspnea (20, 35, 137). The increased $\dot{V}_E/\dot{V}CO_2$ in COPD may be explained in part by pulmonary vascular dysfunction resulting in ventilation-perfusion abnormalities; specifically, areas of normal ventilation but low relative perfusion (i.e., deadspace ventilation). Indeed, others have demonstrated that reduced pulmonary vascular volumes are related with low pulmonary diffusing capacity ($D_LCO$), increased $\dot{V}_E/\dot{V}CO_2$, and exertional dyspnea in symptomatic smokers with minor emphysema (6, 168). Recently, we have documented that targeting pulmonary vascular dysfunction using inhaled nitric oxide (iNO) in mild COPD reduces $\dot{V}_E/\dot{V}CO_2$ and dyspnea, resulting in an improved peak rate of $O_2$ consumption ($\dot{V}O_{2peak}$, an index of cardiopulmonary fitness) (7). iNO is a selective pulmonary vasodilator that has been shown to improve pulmonary perfusion in conditions with elevated vascular tone (70, 73, 75), and has a rapid onset and short washout period (half-life < 15 seconds (169, 170)), with minimal effects on the systemic circulation (171).

Pulmonary vasodilators have been shown to have a variable effect in advanced COPD (74, 75, 172), where a progressive deterioration of pulmonary microvascular function is expected along with distal vessel pruning, independent of emphysema severity (5). iNO appears to worsen ventilation-perfusion matching in hypoxemic COPD patients with coexisting pulmonary hypertension (74); likely due to reversing hypoxic pulmonary vasoconstriction. Comparatively, in

severe and very severe COPD patients without pulmonary hypertension (75, 172), iNO does not appear to worsen gas-exchange efficiency, with one study finding over 50% of patients reported reductions in exertional dyspnea with iNO (172). Together, these studies suggest that iNO may be beneficial in individuals with mild (7) to severe COPD without pulmonary hypertension (75, 172); however, the response appears heterogeneous, and anatomic and physiologic predictors of response have not been identified.

While iNO improves $\dot{V}_E/\dot{V}CO_2$, dyspnea, and $\dot{V}O_{2peak}$ in mild COPD (7), it remains unclear whether iNO has similar effects in more severe, non-hypoxemic COPD patients without pulmonary hypertension. Therefore, the purpose of this study was to determine the effect of the selective local pulmonary vasodilator, iNO, on $\dot{V}_E/\dot{V}CO_2$, dyspnea and $\dot{V}O_{2peak}$ (primary outcome: $\dot{V}O_{2peak}$), in patients with mild-severe COPD without pulmonary hypertension. It is important to note that we opted to use a selective, inhaled pulmonary vasodilator rather than an intravenous vasodilator (e.g., dopamine or prostacyclin) for the following reasons. First, the inhaled gas would be preferentially delivered to well-ventilated lung regions, therefore potentially reducing physiological deadspace ventilation. Second, using an inhaled pulmonary vasodilator avoids the confounding effect of systemic vasodilation, severe arterial hypotension and syncope (70, 73). It is now clear that COPD severity may be defined by much more than traditional spirometry (8). As such, we aimed to identify characteristics beyond airflow obstruction (such as emphysema, $D_LCO$) associated with response to iNO. We hypothesized that iNO would improve $\dot{V}O_{2peak}$, $\dot{V}_E/\dot{V}CO_2$ and dyspnea in patients with COPD with a more preserved pulmonary vasculature (e.g., as demonstrated by a greater $D_LCO$ or pulmonary vascular measures as quantified by CT), while patients with more severe COPD and/or worse emphysema on CT would not experience

improvements in $\dot{V}O_{2peak}$ with iNO. Some of the preliminary results of this study have been previously reported in the form of abstracts (173-175).

## *5.2 Methods*

### *5.2.1 Ethical Approval and Participant Selection*

This study was a single-site, randomized, placebo-controlled double-blind crossover trial (ClinicalTrials.gov Identifier: NCT03679312). A cross-over design was selected due to both the short washout period of iNO and the short duration of treatment (approximately 10-20 minutes), and therefore we did not anticipate any carry-over effects. The study was approved by the University of Alberta Health Research Ethics Board (Pro00078715), and all participants provided written, informed consent. In total, 52 participants with clinically stable COPD and a smoking history (10 ≥ pack-years) were recruited. Participants with significant cardiovascular or metabolic disease, pulmonary arterial hypertension, taking medications affecting NO bioavailability (e.g., phosphodiesterase-5 inhibitors), with diseases affecting pulmonary vascular function, or those with musculoskeletal injuries preventing them from being able to exercise on a cycle ergometer were excluded. Participants were recruited from the G.F. MacDonald Centre for Lung Health and our community COPD clinics.

Previous work in our laboratory has shown that iNO increases $\dot{V}O_{2peak}$ by 3.4±3.5ml/kg/min (pre/post correlation: 0.92) in mild COPD (7). Assuming that participants with more severe COPD would show increases in $\dot{V}O_{2peak}$ of roughly 50% of that previously observed in mild COPD, we expected to need 15 COPD participants to detect a significant change in $\dot{V}O_{2peak}$ ($\alpha$=0.05, power=80%). We recruited an additional 35 COPD participants to allow for additional exploratory

analyses examining clinically relevant predictors of response to iNO (including $D_LCO$, $FEV_1$, emphysema severity, and pulmonary vascular volume or count).

*5.2.2 Experimental design*

Five sessions were completed over a 4-week period in this single-site, randomized, placebo-controlled double-blind crossover study, and are described in Figure 5.1.

The screening visit included participant enrollment, medical history and pre- and post-bronchodilator (400ug salbutamol) pulmonary function test (PFT) including the multiple fraction of inspired $O_2$ ($F_IO_2$) diffusing capacity for carbon monoxide ($D_LCO$) technique to estimate capillary blood volume and diffusing membrane capacity. Participants then completed a symptom-limited incremental cardiopulmonary exercise test (CPET) to determine $\dot{V}O_{2peak}$. Eligible participants were then randomized in a 1:1 allocation (computer generated) to one of 2 intervention sequences for the upcoming visits 3 and 4: placebo – iNO or iNO – placebo. On visit 2, participants underwent a resting cardiac ultrasound while breathing either iNO (room air with 40 ppm iNO) or room air (placebo: 21% $O_2$, 79% $N_2$) in the resting supine position to estimate right ventricular systolic pressure (RVSP; repeated measures, randomized order). On visits 3 & 4, participants completed an incremental CPET breathing either room air or iNO (order randomized). Participants breathed through the identical apparatus during both trials. Finally, on the 5$^{th}$ visit participants were sent for a prospective quantitative CT scan of the lungs. Participants were asked to refrain from short-acting bronchodilators, smoking, caffeine, vigorous exercise and alcohol for a minimum of 6 hrs prior to testing, and to avoid long-acting bronchodilators for at least 24 hrs prior to testing.

*5.2.3 Intervention*

We selected a dose of 40 ppm iNO because it has previously been shown to reduce pulmonary vascular resistance in more severe COPD (176), and is effective in mild COPD (7). With VitalAire™, we have developed a system to effectively deliver iNO during exercise using the SoKINOX™ device (VitalAire™) which is Health Canada approved. We have used this system in several studies to date in both health (177, 178) and COPD (7). To maintain double blinding, participants breathed through the identical apparatus on the resting cardiac ultrasound day as well as the placebo exercise days, as previously described (7).

*5.2.4 Pulmonary function test*

Plethysmography, single-breath $D_LCO$, and pre- and post-bronchodilator (200ug salbutamol) spirometry were completed based on standardized techniques (102-105), and measurements were compared to normative values (97, 98, 100, 179).

As previously described in detail by our group (6, 48, 180-182), the multiple $F_IO_2$ $D_LCO$ technique was utilized to assess pulmonary capillary blood volume (Vc) and membrane diffusing capacity (Dm). Importantly, participants were coached to avoid doing Müller or Valsalva maneuvers during the breath holds. Furthermore, participants were given five breaths from a Douglas bag (Hans Rudolph, Shawnee, KS, USA) at the respective $F_IO_2$ (0.21, 0.40 or 0.60) for each $D_LCO$ test gas to maintain consistent alveolar $PO_2$ values. Hemoglobin (183) concentrations were estimated (HemoCue 201+, HemoCue AB, Angelholm, Sweden), and $D_LCO$ was adjusted for [Hb] as recommended (184), using different equations for males and females. The order of $F_IO_2$ was randomized for each participant. The inspired carbon monoxide and methane concentrations (0.3 and 0.3%, respectively) were held constant between test gasses. Methane (tracer gas) dilution was used to estimate alveolar volume ($V_A$). If $V_A$ estimates varied by >10%

between tests, breath-holds were repeated. $D_LCO$ can be broken down into its 2 components: 1) membrane conductivity (Dm) signifying the diffusion properties of the alveolar capillary membrane; and 2) the product of carbon monoxide reaction rate ($\theta; 1/\theta = 0.0058 \cdot P_AO_2 + 0.73$ (185)) and capillary blood volume (186):

$$\frac{1}{DLCO} = \frac{1}{D_M} + \frac{1}{\theta V_C}$$

Finally, $1/D_LCO$ versus $1/\theta_{CO}$ was plotted in a graph, and a regression line was generated, whereby 1/Vc is the slope of the regression line, and 1/Dm is the y-intercept (185). The minimum acceptable coefficient of determination ($r^2$) value of the regression line was set to 0.95, as previously described (86, 181, 182).

*5.2.5 Incremental cardiopulmonary exercise tests*

All exercise tests were completed on an electronically braked cycle ergometer (Ergoselect II 1200; Ergoline, Blitz, Germany), and cardiorespiratory data were recorded using a metabolic measurement system (Encore229 Vmax, SensorMedics, Yorba Linda, CA, USA). Participants were instrumented with finger pulse oximetry (N-595; Nellcor Oximax, Boulder, CO, USA) to estimate arterial $O_2$ saturation, and a 12-lead ECG (CardioSoft, GG Medical Systems, Milwaukee, WI, USA) to record heart rate. Cardiac output was estimated using the Physioflow® Impedance Cardiography device (Manatec® Biomedical), and pre- and post-exercise hemoglobin was measured to estimate oxygen delivery. Arterial blood pressure was obtained via manual auscultation.

All incremental exercise tests were preceded by a steady state resting period followed by a 2-minute unloaded cycling period. Participants then followed a step-wise cycling exercise

protocol, wherein the work rate was increased by 10 or 20 watt increments every 2-minutes (in severe, and mild-moderate COPD, respectively). Consistent with our previous work (47, 187, 188), metabolic data (ventilation, $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E/\dot{V}CO_2$ etc.) were collected continuously. Ratings of perceived exertion and dyspnea were obtained using the modified Borg scale (0-10 points) during each exercise test. These data were collected during the second minute of each workload, before inspiratory capacity (IC) maneuvers and at test termination (189). Operating lung volumes were determined from measurements of IC collected at steady state rest, during the last 30s of every 2-min stage of the CPET, and at the end of exercise (187). It is important to note that during every exercise test, all continuous ventilatory and cardiovascular measurements were collected during the first 30 seconds of every second minute of each stage and subsequently linked to the corresponding perceptual ratings and IC maneuvers to avoid contamination of the expired gas data from the IC maneuvers (42).

*5.2.6 CT image acquisition and analysis*

CT images were evaluated using Apollo 2.0 software (VIDA Diagnostics, Inc.) and VIDA Diagnostics, Inc., clinical image analysis service, as previously described (106). Emphysema was quantified on full-inspiration CT images using the low-attenuation areas of the lung below -950 Hounsfield units (HU) (LAA-950%) (108-110). TAC was quantified via summation of all airway segments from the segmented airway tree, as previously described by our group (106). Vessel measurements were completed with 3-dimensional vascular reconstruction using a scale-space particle method of all vessels, which allowed for the automated evaluation of vessel volumes. This method uses the geometry of the blood vessels to identify vasculature in relationship to the parenchyma and estimates vessel size. The volume of all pulmonary vessels (TBV), the volume of

pulmonary vessels <5 $mm^2$ in cross-sectional area (BV5), and the volume of pulmonary vessels between 5-10 $mm^2$ in cross-sectional area (BV5-10) were calculated for each participant. BV5 defines the transition between the distal and proximal vasculature (111), and previous work suggests that low BV5 represents vascular pruning, while larger cut-offs in CSA (i.e., BV>5$mm^2$) tend to represent more proximal vascular morphology (89, 90, 112). BV5-10 coincides with a vessel radius of approximately 1.25-2mm, and therefore may capture the smaller arteries and transition toward arterioles.

*5.2.7 Resting echocardiography*

Following a 10 minute baseline resting period, a resting cardiac ultrasound with the participant breathing either iNO (room air with 40 ppm iNO) or room air (placebo: 21% $O_2$, 79% $N_2$) in the supine position was completed to characterize cardiac function and RVSP (repeated measures, randomized order) using a 2D ultrasound (Vivid Q, GE Healthcare, Fairfield, CT USA), following recommended guidelines (190, 191). Each condition was separated by a minimum (placebo or iNO) washout time of 5 minutes as previously described by our group (7), and return to baseline was confirmed before continuing to the next condition (e.g., HR, $SpO_2$). Images were collected by an experienced sonographer over five cardiac cycles, and data were analyzed in triplicate, with the analyst blinded to the experimental condition (EchoPAC PC software, GE Healthcare, Horten, Norway).

Briefly, RVSP was be determined from peak tricuspid regurgitant jet velocity ($V_{TR}$, in meters per second), calculating the product of the simplified Bernoulli equation and an estimate of right atrial (RA) pressure:

$$RVSP = 4(V_{TR})^2 + RA\ pressure$$

RA pressure was estimated via imaging of the inferior vena cava from a subcostal view using inferior vena cava diameter and collapse during an inspiratory sniff, as previously described (192).

Left ventricular stroke volume (LVSV) was calculated as the product of the cross-sectional area of the LV outflow tract (LVOT, estimated from 2D ultrasound in the parasternal short-axis view) and the velocity-time integral of the aortic outflow, estimated from continuous wave doppler ultrasound from a five-chamber view (193). Finally, cardiac output was calculated as the product of SV and HR measured on ECG at the time of the velocity time integral measurement.

Left (Simpson's biplane) ventricular volumes were estimated by calculating end-diastolic volume (EDV) and end-systolic volume (ESV) with two-dimensional echocardiography of apical 4- and 2-chamber images (193). EDV and ESV allowed for the calculation of SV, and ejection fraction (EF) of the left ventricle:

$$SV = EDV\text{-}ESV$$

$$EF = (SV/EDV) \times 100$$

Finally, global right ventricular function was estimated using the tricuspid annular plane systolic excursion (TAPSE) from the apical four chamber view using an M-mode cursor. The cursor was placed through the tricuspid lateral annulus, and the amount of longitudinal motion of the annulus was measured at peak systole.

### *5.2.8 Statistical analyses*

Data are presented as mean and standard deviation for continuous variables, and as percentages for categorical variables. Paired t-tests were used to compare within participant physiological and perceptual data with placebo vs. iNO at rest and peak exercise. For categorical

variables, a Chi-square test was performed. Pearson correlation was utilized to explore bivariate relationships between variables.

A mixed effects linear regression model was used to explore predictors of response to iNO on exercise capacity ($\dot{V}O_{2peak}$, primary outcome), while accounting for within-subject correlations expected from cross-over designs. The fixed effect was the intervention (placebo vs. iNO) and random effects was participants as well as the order of intervention. The repeated covariance structure was unstructured. To further examine the influence of COPD severity, $FEV_1$% and emphysema score were entered as fixed covariates in additional models (secondary aim). Additional clinically relevant covariates ($D_LCO$ %, %emphysema, Vc) were also entered in subsequent models. Similar models were developed for each secondary outcome ($\dot{V}_E/\dot{V}CO_2$, dyspnea).

To further understand characteristics of responders and non-responders to iNO, participants were divided into tertiles of absolute change in $\dot{V}O_{2peak}$ in % predicted, and were compared using one-way ANOVA with a Bonferroni post-hoc test for multiple comparison correction in exploratory analyses.

$\dot{V}O_{2peak}$ in % predicted was utilized in all statistical analyses to aid in limiting the number of covariates required in the mixed models. Percent emphysema (LAA-950%), was transformed via natural logarithm to obtain a normal distribution (LnLAA-950%). All statistical analyses were completed using IBM SPSS Statistics 26 (IBM Corporation, Armonk, NY). For all inferential analyses, the probability of Type I error was two-sided, and set at $<0.05$. Data were analyzed per protocol (i.e., in those who completed both experimental CPETs).

## *5.3 Results*

### *5.3.1 Participants*

**Figure 5.2** displays the CONSORT participant flow diagram. Of the 172 patients who were assessed for eligibility, 52 were randomized to an intervention sequence for the experimental CPET visits (25 received placebo followed by iNO, and 27 received iNO followed by placebo). Participant recruitment began in October 2019 and ended in November 2023. Participant characteristics are provided in **Table 5.1**. Participants were below the lower limit of normal for $FEV_1/FVC$, and ranged from mild-severe airflow limitation (mean $FEV_1$: 63.95 ± 18.05% predicted). Participants demonstrated impaired pulmonary function (hyperinflation, impaired pulmonary diffusing capacity) and exercise intolerance (low peak power output and $\dot{V}O_{2peak}$ %predicted) relative to normative values. Quantitative CT measurements revealed that participants exhibited severe emphysema (LAA-950%: 11.07 ± 8.93%), while demonstrating no evidence of resting pulmonary artery hypertension (pulmonary artery-to-aorta diameter ratio <1.0, RVSP = 25.00 ± 7.10 mmHg).

**Table 5.2** reports unadjusted resting supine echocardiographic and cardiorespiratory data with placebo or iNO. Paired t-tests revealed that iNO had no effect on TAPSE, SV or Q ($p>0.05$). There was a significant reduction in RVSP with iNO in the supine position (normoxia: 28.37 ± 7.73 mmHg vs iNO: 24.54 ± 5.97 mmHg, $p<0.001$, n=17).

### *5.3.2 Change in exercise capacity with iNO, and predicting response to iNO*

**Figure 5.3** displays the mean $\dot{V}O_{2peak}$ % pred with placebo vs iNO (paired t-test between conditions: $p>0.05$), as well as the range of responses across participants (both figures A and B are unadjusted for covariates). Twenty nine of 52 patients demonstrated an increase in $\dot{V}O_{2peak}$ with

iNO. **Table 5.3** reports the mixed effects linear regression model predicting change in $\dot{V}O_{2peak}$ % pred with iNO. The unadjusted linear mixed model (null) shows that there was no effect of iNO on $\dot{V}O_{2peak}$ (p=0.10). Model 1 revealed a significant TVC by condition interaction effect, where TVC was associated with greater change in $\dot{V}O_{2peak}$ % pred with iNO (β=0.001, CI=0.0001 to 0.001, p=0.019). However, Models 2-4 revealed that neither $FEV_1$ by condition (p=0.97), LnLAA-950% by condition (p=0.20) or $D_LCO$ by condition (p=0.73) interactions were associated with change in $\dot{V}O_{2peak}$ % pred. In the final model, the TVC by condition interaction effect remained significantly associated with change in $\dot{V}O_{2peak}$ % pred after adjusting for $FEV_1$, LnLAA-950% and DLCO, whereby for every additional 1000 pulmonary blood vessels, $\dot{V}O_{2peak}$ % pred increased by 1 % with iNO (p=0.036). **Figure 5.4** displays the unadjusted relationships between $FEV_1$, $D_LCO$, LnLAA-950% and TVC with change in $\dot{V}O_{2peak}$ % pred.

*5.3.3 Change in exercise ventilatory efficiency and dyspnea with iNO, and predicting response to iNO*

**Table 5.4** reports the mixed effects linear regression models predicting change in $\dot{V}_E/\dot{V}CO_2$ nadir with iNO, and shows that $\dot{V}_E/\dot{V}CO_2$ nadir was significantly higher with iNO (β=0.67, CI= 0.02 to 1.31, p=0.04) (Null model); however, this relationship disappeared in subsequent models. Model 1 revealed no significant TVC by condition interaction effect, showing that greater TVC was not associated with greater change in $\dot{V}_E/\dot{V}CO_2$ nadir with iNO (p=0.88). Results from models 2-5 revealed that neither $FEV_1$ by condition (p=0.23), LnLAA-950% by condition (p=0.63) or $D_LCO$ by condition (p=0.21) interactions were associated with change in $\dot{V}_E/\dot{V}CO_2$ nadir with iNO. Even after adjusting for $FEV_1$, LnLAA-950% and $D_LCO$, there was no significant relationship between the TVC by condition interaction and change in $\dot{V}_E/\dot{V}CO_2$ nadir (p=0.92). Furthermore,

there was no significant relationship between change in $\dot{V}_E/\dot{V}CO_2$ nadir and the change in $\dot{V}O_{2peak}$ % pred with iNO; however, there appeared to be a trend whereby individuals showing a greater reduction in $\dot{V}_E/\dot{V}CO_2$ nadir had the greatest improvement in $\dot{V}O_{2peak}$ % pred with iNO (**Figure 5.5,** Pearson $R^2$= 0.07, p=0.08, values unadjusted for covariates).

**Figure 5.6** reports the change in dyspnea against power output (unadjusted for covariates). **Table 5.5** reports the mixed effects linear regression models predicting change in dyspnea at peak exercise with iNO, and shows that there was no significant effect of iNO on dyspnea at peak exercise (p=0.27) (Null model). Model 1 revealed a significant TVC by condition interaction effect on change in dyspnea, suggesting that higher TVC was significantly associated with a greater increase in dyspnea (β=0.0002, CI=6.091E-5 to 0.000, p=0.003). The TVC by condition interaction remained significantly associated with change in dyspnea in the final model (β= 0.0002, CI= 7.82E-5 to 0.0003). However, the final model shows that dyspnea was significantly reduced with iNO at peak exercise (β= -3.41, CI= -5.25 to -1.57), and that individuals with a greater TVC report less dyspnea during exercise (β=-8.46E-5, CI= -0.0003 to 0.0001).

**Table 5.6** reports the effects of iNO on physiological and perceptual responses at rest, and peak exercise (results are unadjusted). At peak exercise, there were differences in $\dot{V}_E/\dot{V}CO_2$ with iNO (placebo: 33.83 ± 5.21 vs iNO: 34.92 ± 5.32, p<0.005), minute ventilation (placebo: 47.5 ± 15.3 vs. iNO: 50.6 ± 17.3 L/min, p<0.001), tidal volume (placebo: 1.52 ± 0.47 vs. iNO: 1.59 ± 0.5 L, p<0.005), but not breathing frequency (p>0.05). Neither EELV% or EILV% were affected by iNO; however, there was a trend towards increased IRV% with iNO (p=0.07). The end-tidal partial pressure of $CO_2$ ($P_{ET}CO_2$) was slightly reduced with iNO both at rest (placebo: 32.42 ± 3.19 vs. iNO: 31.05 ± 3.26 mmHg, p<0.001) and at peak exercise (placebo: 37.1 ± 5.29 vs. iNO: 35.93 ± 5.22 mmHg, p<0.005) with iNO. Peripheral oxygen saturation was also slightly reduced at peak

exercise with iNO (placebo: 93 ± 3 vs iNO: 92 ± 3 %, $p<0.05$), while heart rate, stroke volume, cardiac output and vascular conductance remained unchanged (all $p>0.05$). There was no effect of iNO on any other variable either at rest or at peak exercise (all $p>0.05$).

*5.3.4 Exploratory analysis: Comparison of responders vs. non-responders*

**Table 5.7** reports participant characteristics, resting pulmonary function, quantitative CT, echocardiographic and baseline cardiorespiratory data across tertiles of response to iNO (unadjusted). Tertiles of absolute change in $\dot{V}O_{2peak}$ (%pred) were divided as follows: lower tertile: <-1.45%; middle tertile: ≥-1.45 to <5.06%; upper tertile: ≥5.06%. One way ANOVA revealed a significant effect of tertile on FVC ($p=0.04$), where individuals in the lower tertile of response to iNO show a trend towards a lower FVC compared to the middle tertile ($p=0.07$). We also found a significant effect of tertile on exercise capacity (as evaluated by $\dot{V}O_{2peak}$ at the screening visit), where individuals in the upper tertile of response showed a trend toward a higher $\dot{V}O_{2peak}$ % predicted than the middle tertile ($p=0.054$) and lower tertile ($p=0.08$). There were no other differences in lung function, CT-derived variables or hemodynamics between tertiles.

## *5.4 Discussion*

This is the first study to evaluate the predictors of response to iNO in non-hypoxemic patients across the continuum of COPD severity *without* evidence of pulmonary arterial hypertension. This randomized, double-blind, placebo controlled cross-over study has two important findings. First, iNO significantly reduced dyspnea at peak exercise, while there was no significant effect on $\dot{V}_E/\dot{V}CO_2$ nadir or $\dot{V}O_{2peak}$ in patients with mild-severe COPD after adjusting for emphysema, $FEV_1$, $D_LCO$ and TVC. Second, there was a significant positive interaction effect

between TVC and condition with $\dot{V}O_2$ at peak exercise, suggesting that individuals with a preserved pulmonary vasculature show the greatest improvement with iNO, independent of emphysema, $FEV_1$ and $D_LCO$. Combined, these findings suggest that response to iNO is independent of severity of airflow obstruction, emphysema, or pulmonary diffusing capacity, and that responders tend to have a greater number of intraparenchymal blood vessels. This investigation demonstrates, for the first time, a pulmonary vascular endotype that is responsive to acute and selective pulmonary vasodilation, across the continuum of COPD severity.

*5.4.1 Predicting the response to selective pulmonary vasodilation*

Previous work from our laboratory (7) has shown that iNO improves exercise capacity in non-hypoxemic patients with mild COPD and no evidence of pulmonary hypertension secondary to reduced $\dot{V}_E/\dot{V}CO_2$ and dyspnea (at an equivalent work rate of 60 watts). This previously reported reduction in $\dot{V}_E/\dot{V}CO_2$ during submaximal exercise without changes in airway mechanics or peripheral oxygen saturation supports a reduction in physiological deadspace ventilation with iNO. Few studies have evaluated the effects of selective inhaled pulmonary vasodilators on exercise capacity, ventilation and dyspnea in patients with mild-severe COPD *without* hypoxemia or pulmonary hypertension. Previous work in hypoxemic patients with severe COPD and pulmonary hypertension has shown that inhaled vasodilators reduce pulmonary artery pressure at rest but do not improve gas-exchange efficiency (74). In severe hypoxemic COPD patients *without* pulmonary hypertension, 24hrs of iNO reduced pulmonary vascular resistance but did not affect exercise tolerance (172). However, roughly 50% of patients in the study by Ashutosh et al. (172) reported reductions in dyspnea with iNO, suggesting that there may be responders and non-responders to iNO. Finally, in *non-hypoxemic* patients with severe COPD and no pulmonary hypertension, iNO

decreased pulmonary artery pressure both at rest and during exercise and improved exercising ventilation-perfusion matching (75). Specifically, the authors reported increased perfusion to lung units with normal ventilation-perfusion ratios, and reduced perfusion to lung units with low ventilation-perfusion ratios, although peak aerobic exercise capacity was not evaluated (75). Together, these studies suggest that response to iNO may be independent of severity of airflow obstruction, and that other factors may determine response to selective inhaled pulmonary vasodilators.

Our results extend the above findings, showing that severity of airflow obstruction and emphysema are not important predictors of response to iNO. We found that dyspnea was significantly reduced at peak exercise with iNO after accounting for $FEV_1$, emphysema, $D_LCO$ and TVC. Most importantly, our linear mixed models support a pulmonary vascular endotype: whereby individuals with a more intact pulmonary vasculature on quantitative CT are most likely to exhibit improvements in exercise capacity with iNO, independent of $FEV_1$ %predicted, LnLAA-950%, or $D_LCO$ adj %predicted. Further, our bivariate correlation results suggest a trend (Pearson $R^2$= 0.07, p= 0.08) whereby those who improved their ventilatory efficiency (i.e., reduced $\dot{V}_E/\dot{V}CO_2$ nadir, suggesting reduced deadspace ventilation) with iNO also demonstrated increased exercise capacity. Together, our novel findings expand our knowledge on the therapeutic potential of selective pulmonary vasodilators across the continuum of COPD.

*5.4.2 Dyspnea, ventilation, and cardiocirculatory responses at rest and peak exercise*

We found that dyspnea at peak exercise was reduced by more that 3 Borg units with iNO in the fully adjusted linear mixed regression models. Our results show that TVC (included in an intermediate model and the final model) explains some of the variability in dyspnea with iNO

which was not accounted for in the initial null model; with individuals with higher TVC reporting lower ratings of perceived dyspnea at peak exercise. This observed improvement in ratings of perceived dyspnea far exceeded the minimally clinically important difference of 1 Borg unit (194). Phillips et al., did not find a significant effect of iNO on dyspnea at peak exercise in repeated measures ANOVA; however, they showed a 1.1 unit reduction at 60watts in mild COPD (while no change was observed in controls) (7). Comparatively, Guenette et al., found no change in dyspnea at an isolated time point or at peak exercise (constant-load exercise) with the bronchodilator fluticasone propionate in COPD (195). Similarly, a systematic review describing the effects of bronchodilators on exercise tolerance and dyspnea in patients with COPD showed heterogenous results, with some studies reporting improvements in dyspnea, others showing worsening, and others showing no effect (196). The combined results in mild and mild to severe COPD suggest that targeting the pulmonary vasculature may have a greater impact on dyspnea than traditional bronchodilator therapy. Although TVC was a significant predictor of reduced dyspnea at peak exercise in the current study, the mechanisms explaining this change are unclear. Specifically, $\dot{V}_E/\dot{V}CO_2$ was not included as a covariate in the linear mixed regression modelling change in dyspnea, and therefore it is unknown whether improvements in ventilatory efficiency during exercise directly contributed to reduced dyspnea.

Despite a reduction in dyspnea, there was no change in $\dot{V}_E/\dot{V}CO_2$ nadir in linear mixed model regression, while unadjusted pairwise comparisons actually revealed a *significant* increase in mean $\dot{V}_E/\dot{V}CO_2$ ratio with iNO at peak exercise (**Table 5.6**). The increased $\dot{V}_E/\dot{V}CO_2$ ratio at peak exercise was secondary to increased minute ventilation (via augmented tidal volume, but not breathing frequency), while $P_{ET}CO_2$ was lower with iNO both at rest and peak exercise. This finding is somewhat consistent with our previous work, where minute ventilation at peak exercise

was increased with iNO; however, this change was driven by greater breathing frequency in mild COPD (7). Neither end-expiratory or end-inspiratory lung volumes (%TLC) were altered with iNO at rest or during exercise in the current investigation. As such, our current results suggest that iNO did not alter lung mechanics in individuals with mild-severe COPD at peak exercise. Peripheral oxygen saturation was also slightly reduced at peak exercise with iNO, while cardiac output and vascular conductance remained unchanged. Combined, these results suggest that breathing iNO may have resulted in increased alveolar ventilation, contributing to reducing $P_{ET}CO_2$ at peak exercise.

iNO reduced RVSP at rest in the supine posture, similar to previous work (7, 70, 172, 197). Secondary analyses revealed no effect of iNO on cardiac output (or HR and SV), or vascular conductance at rest or at peak exercise. This finding is consistent with previous work in COPD, whereby iNO did not significantly affect Q or conductance at rest or during exercise (7, 198). Combined, these findings suggest that reductions in RVSP (and likely a reduction in RV afterload) did not translate to an improvement in cardiac output at rest or during exercise.

### *5.4.3 Limitations*

We observed a small effect of iNO on $\dot{V}O_{2peak}$ % predicted in COPD (effect size=0.16, pre-/post-correlation=0.90, calculated via repeated measures ANOVA). The observed power was calculated as 0.21; this finding was not surprising, given that we recruited a heterogeneous sample of individuals with mild-severe COPD. It must be noted, however, that the primary aim of our study was not to evaluate the mean change in $\dot{V}O_{2peak}$ with iNO, but rather to evaluate predictors of response to iNO. While increasing the generalizability of our results due to the variability across

participants, our findings likely underestimate the effectiveness of iNO in individuals with a more intact pulmonary vascular bed.

While 49 of 52 participants completed cardiac ultrasound assessments with and without iNO, secondary analyses of cardiac ultrasound data were limited to those with images of adequate quality. Estimation of RVSP requires the assessment of the peak tricuspid regurgitant jet velocity, an outcome measure which is present in 65-85% of the population (199, 200). Only 17 participants in the current investigation showed evidence of measurable tricuspid regurgitant jet velocity during both placebo and iNO conditions in the supine posture. Tricuspid regurgitation becomes even more difficult to measure in the setting of lung hyperinflation and rightward rotation of the heart (201), and it must be acknowledged that estimates of RVSP have been shown to be poorly correlated with PASP derived from invasive right heart catheterization in patients with severe emphysema (202). Nonetheless, participants showed reductions in resting supine RVSP with iNO. Although RVSP data were collected on a separate day and we could not obtain RVSP data during upright exercise, we assume a similar response to iNO during upright exercise as observed in the resting supine position.

### *5.4.4 Conclusion*

In conclusion, acute, selective pulmonary vasodilation via inhaled nitric oxide improved dyspnea but not exercise capacity or ventilatory efficiency in a heterogenous sample of participants with mild to severe COPD. Our study is the first to demonstrate a pulmonary vascular endotype of response to iNO, whereby individuals with a more intact pulmonary vasculature (i.e., a larger number of intraparenchymal blood vessels) are more likely to respond favourably to iNO. Furthermore, these findings increase our understanding of the complexities of COPD, shifting the

focus beyond airway dysfunction. Future studies should further probe the physiological impacts of impaired pulmonary vascular structure and function, and explore practical pulmonary vascular therapies to reduce dyspnea and increase exercise tolerance in COPD.

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**Table 5.1:** Participant characteristics (n=52)

| Parameter | | | |
|---|---|---|---|
| Female, % | 46 | | |
| Age, years | 68 | ± | 7 |
| Height, cm | 168.08 | ± | 8.60 |
| BMI, kg/m$^2$ | 27.58 | ± | 5.05 |
| Pack-years | 41.10 | ± | 21.10 |
| SABA, n | 30 | | |
| LABA, n | 2 | | |
| ICS (incl. combination inhalers), n | 31 | | |
| Pulmonary Function and Exercise | | | |
| $FEV_1$, %pred | 63.95 | ± | 18.05 |
| FVC, %pred | 101.30 | ± | 18.66 |
| $FEV_1$/FVC, %pred | 62.51 | ± | 12.72 |
| RV, %pred | 153.15 | ± | 52.89 |
| TLC, %pred | 114.71 | ± | 16.75 |
| RV/TLC, %pred | 133.12 | ± | 36.56 |
| $D_LCO$, %pred | 86.70 | ± | 20.53 |
| Vc adj, mL | 63.82 | ± | 27.87 |
| Dm adj, mL/min/mmHg | 38.68 | ± | 24.50 |
| $V_A$, %pred | 65.43 | ± | 19.13 |
| $\dot{V}O_{2peak}$, %pred | 74.3 | ± | 19.35 |
| Peak work rate, %pred | 81.15 | ± | 33.99 |
| Imaging | | | |
| $TLV_{CT}$, mL | 6577 | ± | 1441 |
| LAA-950, % | 11.07 | ± | 8.93 |
| Pi10 Leq | 3.88 | ± | 0.06 |
| BV5/TVV, mL | 0.70 | ± | 0.05 |
| BV5-10/TVV, mL | 0.22 | ± | 0.03 |
| BV10/TVV, mL | 0.08 | ± | 0.02 |
| TVC, n | 15763 | ± | 4022 |
| TVV/TLV, mL | 2.34 | ± | 0.21 |
| Pa/Ao | 0.74 | ± | 0.10 |
| Cardiac function in the supine position | | | |
| RVSP, mmHg | 25.00 | ± | 7.10 |
| LV-EDV, mL | 84.96 | ± | 25.85 |
| LV-ESV, mL | 40.32 | ± | 15.96 |
| LV-EF, % | 53.68 | ± | 6.85 |

Data are mean ± SD for continuous variables, or % for categorical variables. BMI: body mass index; SABA: short-acting beta agonists; LABA: long-acting beta agonists; ICS: inhaled corticosteroids; $FEV_1$: forced expiratory volume in 1 sec; FVC: forced vital capacity; RV: residual volume; TLC: total lung capacity; $D_LCO$: diffusing capacity for carbon monoxide; Vc adj: capillary blood volume adjusted for hemoglobin and alveolar volume; Dm adj: diffusing membrane capacity adjusted for hemoglobin and alveolar volume; $V_A$: alveolar volume; $\dot{V}O_{2peak}$: peak rate of $O_2$ consumption; TLV: CT-derived total lung volume; LAA-950%: low attenuation area of the lung with attenuation values below −950 HU on full inspiration CT; Pi10: average airway wall thickness normalized to a 'theoretical' airway lumen of 10-mm inner perimeter; TVV: total vessel volume; BV5: volume of blood vessels with a cross-sectional area <5mm$^2$; BV5-10: volume of blood vessels with a cross-sectional area between 5-10mm$^2$; BV10: volume of blood vessels with a cross-sectional area >10mm$^2$; TVC: total vessel count; Pa/Ao: pulmonary artery-to-aorta diameter ratio; RVSP: right ventricular systolic pressure; LV: left ventricle; EDV: end-diastolic volume; ESV: end-systolic volume; EF: ejection fraction.

**Table 5.2:** Resting supine echocardiographic and cardiorespiratory data with placebo or iNO (unadjusted).

| Parameter | Placebo | iNO | p-value | n |
|---|---|---|---|---|
| RVSP, mmHg | 28.37 ± 7.73 | 24.54 ± 5.97 | <0.001 | 17 |
| TAPSE, cm | 2.37 ± 0.38 | 2.27 ± 0.33 | 0.07 | 35 |
| $HR_{LVOT}$, bpm | 67 ± 11 | 68 ± 11 | 0.98 | 38 |
| $SV_{LVOT}$, mL | 60.83 ± 16.41 | 60.23 ± 15.48 | 0.65 | 38 |
| $Q_{LVOT}$, L/min | 4.04 ± 1.02 | 4.02 ± 1.02 | 0.75 | 38 |

Data are presented as the mean ± SD. iNO, inhaled nitric oxide; RVSP: right ventricular systolic pressure; TAPSE: tricuspid annular plane systolic excursion; HR: heart rate; SV: stroke volume; Q: cardiac output; LVOT: left-ventricular outflow tract; Data analyzed via paired t-test.

**Table 5.3:** Linear mixed effects models evaluating predictors of change in $\dot{V}O_{2peak}$ with iNO.

| | **Null** | **Model 1:** null + TVC | **Model 2:** null + $FEV_1$ | **Model 3:** null + LAA-950 | **Model 4:** null+ $FEV_1$ & LAA-950 + $D_LCO$ | **Final** |
|---|---|---|---|---|---|---|
| **Intercept** | 74.34 (68.79 to 79.89), p<0.001 | 74.88 (51.19 to 98.58) p<0.001 | 37.04 (19.60 to 54.48) p<0.001 | 86.78 (76.06 to 97.49) p<0.001 | 34.33 (8.04 to 60.62) p=0.01 | 42.72 (15.61 to 69.83) p=0.003 |
| **Condition: iNO** | 2.18 (-0.39 to 4.74), p=0.10 | -9.19 (-19.32 to 0.95) p=0.07 | 2.07 (-7.55 to 11.68) p=0.67 | 0.10 (-4.86 to 5.06) p=0.97 | 1.32 (-8.44 to 11.07) p=0.79 | -7.93 (-18.41 to 2.56) p=0.14 |
| **$FEV_1$ % pred** | | | 0.59 (0.33 to 0.85) p<0.001 | | 0.30 (-0.01 to 0.61) p=0.06 | 0.31 (-0.01 to 0.64), p=0.06 |
| **LnLAA-950%** | | | | -6.48 (-11.23 to -1.72) p=0.01 | -1.52 (-5.89 to 2.85) p=0.49 | -1.50 (-6.77 to 3.76) p=0.57 |
| **$D_LCO$ adj** | | | | | 0.35 (0.05 to 0.66) p=0.03 | 0.36 (0.03 to 0.68) p=0.03 |
| **TVC** | | -6.561E-6 (-0.001 to 0.001) p=0.99 | | | | -0.001 (-0.002 to 0.001) p=0.43 |
| **iNO*$D_LCO$ adj % pred** | | | | | 0.02 (-0.12 to 0.16) p=0.73 | |
| **iNO *$FEV_1$** | | | 0.003 (-0.14 to 0.15), p=0.97 | | | |
| **iNO* LnLAA-950%** | | | | 1.42 (-0.80 to 3.63) p=0.20 | | |
| **iNO*TVC** | | 0.001 (0.0001 to 0.001) p=0.02 | | | | 0.001 (4.74E-5 to 0.001), p=0.04 |
| **AIC** | 836.52 | 784.25 | 808.21 | 739.34 | 739.34 | 738.43 |

Data are presented as estimate (95% CI), p-value. iNO: inhaled nitric oxide; $FEV_1$: forced expiratory volume in 1second; LnLAA-950%: natural logarithm of the % low attenuation <-950 Hounsfield units; $D_LCO$: pulmonary diffusing capacity; TVC: total vessel count.

**Table 5.4:** Linear mixed effects models evaluating predictors of change in $\dot{V}_E/\dot{V}CO_2$ nadir with iNO.

| | Null | Model 1: null + TVC | Model 2: null + $FEV_1$ | Model 3: null + LAA-950 | Model 4: null+ $D_LCO$ | Model 4: null+ $FEV_1$ & LAA-950 + $D_LCO$ | Final |
|---|---|---|---|---|---|---|---|
| **Intercept** | 34.33 (32.85 to 35.81), p<0.001 | 34.34 (28.10 to 40.58) p<0.001 | 38.01 (32.49 to 43.53), p<0.001 | 29.43 (26.91 to 31.95), p<0.001 | 46.43 (42.29 to 50.56), p<0.001 | 37.84 (31.67 to 44.01), p<0.001 | 36.99 (30.81 to 43.18), p<0.001 |
| **Condition: iNO** | 0.67 (0.02 to 1.31) p=0.04 | 0.48 (-2.20 to 3.15) p=0.72 | -0.74 (-3.17 to 1.70) p=0.55 | 0.97 (-0.31 to 2.25) p=0.13 | -0.72 (-3.03 to 1.60), p=0.54 | -1.32 (-5.13 to 2.50), p=0.49 | 0.59 (-2.19 to 3.38), p=0.67 |
| **$FEV_1$ (% pred)** | | | -0.06 (-0.14 to 0.03), p=0.17 | | | 0.08 (0.003 to 0.15), p=0.04 | 0.08 (0.01 to 0.16), p=0.03 |
| **LnLAA-950%** | | | | 2.43 (1.31 to 3.56) p<0.001 | | 1.59 (0.55 to 2.64), p=0.004 | 1.69 (0.47 to 2.90), p=0.008 |
| **$D_LCO$ adj Hb (%pred)** | | | | | -0.19 (-0.25 to -0.13) p<0.001 | -0.18 (-0.28 to -0.11), p<0.001 | -0.16 (-0.24 to -0.09), p<0.001 |
| **TVC (n)** | | -1.21E-5 (0.00 to 0.00) p=0.95 | | | | | -3.34E-5 (0.00 to 0.00), p=0.85 |
| **iNO*$D_LCO$** | | | | | 0.02 (-0.01 to 0.06) p=0.21 | 0.02 (-0.03 to 0.06), p=0.40 | |
| **iNO*$FEV_1$** | | | 0.02 (-0.01 to 0.06) p=0.23 | | | 0.01 (-0.04 to 0.06), p=0.65 | |
| **iNO* LnLAA-950%** | | | | -0.14 (-0.71 to 0.43), p=0.63 | | 0.06 (-0.59 to 0.71), p=0.86 | |
| **iNO*TVC** | | 1.29E-5 (0.00 to 0.00) p=0.88 | | | | | 8.42E-6 (0.000 to 0.000) p=0.92 |
| **AIC** | 557.96 | 542.08 | 556.97 | 484.47 | 540.55 | 475.62 | 497.42 |

Data are presented as estimate (95% CI), p-value. iNO: inhaled nitric oxide; $FEV_1$: forced expiratory volume in 1second; LnLAA-950%: natural logarithm of the % low attenuation <-950 Hounsfield units; $D_LCO$: pulmonary diffusing capacity; TVC: total vessel count.

| **Table 5.5:** Linear mixed effects models evaluating predictors of change in dyspnea with iNO. | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Null | Model 1: null + TVC | Model 2: null + $FEV_1$ | Model 3: null + LAA-950 | Model 4: null+ $D_LCO$ | Model 4: null+ $FEV_1$ & LAA-950 + $D_LCO$ | Final |
| **Intercept** | 7.15 (6.55 to 7.76) p<0.001 | 7.44 (4.89 to 9.99), p<0.001 | 8.15 (5.83 to 10.47) p<0.002 | 7.03 (5.81 to 8.25), p<0.001 | 6.76 (4.58 to 8.94), p<0.001 | 7.27 (3.61 to 10.93), p<0.001 | 6.91 (3.30 to 10.51), p<0.001 |
| **Condition: iNO** | -0.28 (-0.78 to 0.22), p=0.27 | -3.12 (-4.98 to -1.27), p=0.001 | -1.66 (-3.51 to 0.19), p=0.08 | -0.66 (-1.62 to 0.29), p=0.17 | -.53 (-2.34 to 1.28), p=0.56 | -3.72 (-6.39 to -1.04), p=0.008 | -3.41 (-5.25 to -1.57), p<0.001 |
| **$FEV_1$ % pred** | | | -0.02 (-0.05 to 0.02), p=0.38 | | | -0.02 (-0.06 to 0.03), p=0.38 | -0.01 (-0.05 to 0.04), p=0.80 |
| **LnLAA-950%** | | | | 0.13 (-0.42 to 0.68), p=0.63 | | 0.16 (-0.46 to 0.79), p=0.60 | 0.35 (-0.37 to 1.06), p=0.33 |
| **$D_LCO$ adj** | | | | | 0.006 (-0.03 to 0.04), p=0.70 | 0.02 (-0.03 to 0.06), p=0.49 | 0.02 (-0.02 to 0.07), p=0.33 |
| **TVC** | | -1.28E-5 (0.000 to 0.000), p=0.87 | | | | | -8.46E-5 (-0.0003 to 0.0001), p=0.04 |
| **iNO*$D_LCO$ adj % pred** | | | | | 0.004 (-0.02 to 0.03), p=0.77 | 0.01 (-0.02 to 0.04), p=0.44 | |
| **iNO*$FEV_1$** | | | 0.02 (-0.01 to 0.05), p=0.12 | | | 0.03 (-0.004 to 0.06), p=0.08 | |
| **iNO* LnLAA-950%** | | | | 0.13 (-0.30 to 0.55), p=0.55 | | 0.36 (-0.09 to 0.82), p=0.12 | |
| **iNO*TVC** | | .0002 (6.09E-5 to 0.00), p=0.003 | | | | | 0.0002 (7.82E-5 to 0.0003), p=0.001 |
| **AIC** | 435.54 | 424.24 | 438.87 | 391.41 | 448.60 | 402.11 | 418.09 |

Data are presented as estimate (95% CI), p-value. iNO: inhaled nitric oxide; $FEV_1$: forced expiratory volume in 1second; LnLAA-950%: natural logarithm of the % low attenuation <-950 Hounsfield units; $D_LCO$: pulmonary diffusing capacity; TVC: total vessel count.

**Table 5.6:** Effect of placebo vs. iNO on unadjusted physiological and perceptual responses at rest and peak exercise (n=52).

| Parameter | Rest | | Peak | |
|---|---|---|---|---|
| | Placebo | iNO | Placebo | iNO |
| Work rate | - | - | 76 ± 33 | 77 ± 34 |
| Dyspnea | 0.5 ± 0.7 | 0.4 ± 0.8 | 7.3 ± 2.1 | 7.0 ± 2.3 |
| Leg discomfort | 0.5 ± 0.7 | 0.4 ± 0.7 | 7.4 ± 2.5 | 7.3 ± 2.3 |
| $\dot{V}O_2$ (L/min) | 0.33 ± 0.09 | 0.31 ± 0.07$^{*}$ | 1.36 ± 0.44 | 1.39 ± 0.47 |
| $\dot{V}CO_2$ (L/min) | 0.29 ± 0.08 | 0.30 ± 0.06 | 1.44 ± 0.53 | 1.48 ± 0.55 |
| $\dot{V}_E$ (L/min) | 14.5 ± 3.4 | 14.6 ± 2.9 | 47.5 ± 15.3 | 50.6 ± 17.3$^{***}$ |
| $\dot{V}_E/\dot{V}CO_2$ | 50.6 ± 9.7 | 49.7 ± 8.7 | 33.83 ± 5.21 | 34.92 ± 5.32$^{**}$ |
| $V_T$ (L) | 0.81 ± 0.19 | 0.89 ± 0.27$^{*}$ | 1.52 ± 0.47 | 1.59 ± 0.5$^{**}$ |
| $f_B$ (breaths/min) | 18.6 ± 5.49 | 17.5 ± 5.41 | 31.6 ± 6.73 | 32.31 ± 7.1 |
| EELV (% TLC) | 62.51 ± 11.41 | 62.25 ± 7.87 | 67.28 ± 15.87 | 68.52 ± 7.49 |
| EILV (% TLC) | 74.91 ± 11.03 | 75.66 ± 7.44 | 90.24 ± 11.71 | 88.85 ± 16.20 |
| IRV (%TLC) | 23.98 ± 7.33 | 24.31 ± 7.52 | 7.22 ± 3.88 | 8.28 ± 3.53 |
| $SpO_2$ (%) | 95 ± 2 | 95 ± 2 | 93 ± 3 | 92 ± 3$^{*}$ |
| $P_{ET}CO_2$ (mmHg) | 32.42 ± 3.19 | 31.05 ± 3.26$^{***}$ | 37.1 ± 5.29 | 35.93 ± 5.22$^{**}$ |
| HR (bpm) | 84 ± 14 | 81 ± 12 | 122 ± 19 | 122 ± 18 |
| SV (mL) | 62.17 ± 15.02 | 61.02 ± 15.36 | 79.36 ± 34.12 | 78.87 ± 22.69 |
| Q (L/min) | 5.14 ± 1.05 | 4.96 ± 1.2 | 9.68 ± 3.61 | 10.05 ± 3.37 |
| Q/MAP (mL/min·mmHg)$^{\#}$ | 56.4 ± 12.9 | 54.37± 13.8 | 89.00 ± 5.23 | 91.01 ± 4.64 |

Data are presented as the mean ± SD. iNO, inhaled nitric oxide; $\dot{V}O_2$, 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; $P_{ET}CO_2$,partial pressure of end-tidal $CO_2$; Q, cardiac output; MAP, mean arterial pressure; Q/MAP, vascular conductance. Within workload data were analysed using a paired samples t-test.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ between conditions.

**Table 5.7:** Comparison of responders vs. non-responders to iNO (tertiles)

| Parameter | Lower tertile n=17 | Middle tertile n=18 | Upper tertile n=17 | p-value |
|---|---|---|---|---|
| Female, % | 53 | 39 | 47 | 0.70 |
| Age, years | 69.2 ± 9.24 | 68.94 ± 6.49 | 65.94 ± 7.38 | 0.41 |
| Height, cm | 168.15 ± 9.75 | 169.42 ± 7.11 | 166.59 ± 9.10 | 0.63 |
| BMI, $kg/m^2$ | 29.46 ± 5.48 | 25.94 ± 4.68 | 27.44 ± 4.61 | 0.12 |
| Pack-years | 38.36 ± 17.05 | 45.30 ± 28.88 | 39.41 ± 14.56 | 0.58 |
| **Pulmonary Function and Exercise** | | | | |
| $FEV_1$, %pred | 62.55 ± 17.95 | 62.27 ± 17.90 | 67.04 ± 19.25 | 0.70 |
| FVC, %pred | 92.13 ± 16.90 | 106.66 ± 19.99 | 105.09 ± 16.70 | **0.04** |
| $FEV_1$/FVC, %pred | 67.26 ± 13.01 | 57.61 ± 11.78 | 62.67 ± 12.39 | 0.09 |
| RV, %pred | 147.99 ± 43.14 | 150.04 ± 39.57 | 161.82 ± 74.00 | 0.17 |
| TLC, %pred | 108.15 ± 15.67 | 117.58 ± 15.31 | 118.04 ± 18.63 | 0.73 |
| RV/TLC, %pred | 137.50 ± 35.51 | 128.00 ± 28.29 | 134.51 ± 46.80 | 0.75 |
| $D_LCO$, %pred | 64.45 ± 21.65 | 63.56 ± 17.52 | 68.40 ± 19.20 | 0.74 |
| $V_A$, %pred | 82.68 ± 15.49 | 90.34 ± 17.62 | 86.87 ± 27.48 | 0.56 |
| $\dot{V}O_{2peak}$, %pred | 71.64 ± 25.31 | 70.91 ± 17.66 | 87.63 ± 16.65 | **0.03** |
| Peak work rate, %pred | 64.00 ± 30.79 | 59.35 ± 18.86 | 71.90 ± 25.70 | 0.35 |
| $\dot{V}CO_2$ (L/min) | 1.39 ± 0.53 | 1.38 ± 0.42 | 1.69 ± 0.66 | 0.18 |
| $\dot{V}_E$ (L/min) | 46.91 ± 17.21 | 48.09± 14.77 | 56.90 ± 19.06 | 0.18 |
| $\dot{V}_E/\dot{V}CO_2$ nadir | 35.03 ± 5.88 | 35.31 ± 3.77 | 34.42 ± 6.29 | 0.89 |
| **Imaging** | | | | |
| $TLV_{CT}$, L | 6.20 ± 1.29 | 6.95 ± 1.42 | 6.57 ± 1.6 | 0.36 |
| LAA-950, % | 11.33 ±13.11 | 13.99 ± 6.34 | 8.11 ± 6.04 | 0.17 |
| Pi10 Leq | 3.88 ± 0.07 | 3.87 ± 0.06 | 3.89 ± 0.05 | 0.68 |
| BV5/TVV, mL | 0.69 ± 0.06 | 0.70 ± 0.05 | 0.72 ± 0.04 | 0.35 |
| BV5-10/TVV, mL | 0.23 ± 0.04 | 0.22 ± 0.03 | 0.21 ± 0.03 | 0.47 |
| BV10/TVV, mL | 0.08 ± 0.03 | 0.08 ± 0.03 | 0.07 ± 0.02 | 0.28 |
| TVC, n | 14705 ± 4196 | 16536 ± 3996 | 15968 ± 3993 | 0.45 |
| TVV/TLV, mL | 2.37 ± 0.24 | 2.38 ± 0.21 | 2.29 ± 0.19 | 0.41 |
| Pa/Ao | 0.74 ± 0.12 | 0.72 ± 0.09 | 0.75 ± 0.09 | 0.66 |
| **Change in resting cardiac function with iNO in the supine position** | | | | |
| ΔLV-Q, L/min | 0.06 ± 0.26 | -0.15 ± 0.58 | -0.71 ± 0.61 | 0.63 |
| ΔTAPSE, mm | -0.06 ± 0.30 | -0.18 ± 0.21 | -0.03 ± 0.38 | 0.44 |
| ΔRVSP, mmHg | -7.38 ± 8.82 | 3.07 ± 3.75 | -4.32 ± 4.16 | 0.48 |

Data are mean ± SD for continuous variables, or % for categorical variables. BMI: body mass index; $FEV_1$: forced expiratory volume in 1 sec; FVC: forced vital capacity; RV: residual volume; TLC: total lung capacity; $D_LCO$: diffusing capacity for carbon monoxide; $V_A$: alveolar volume; $\dot{V}O_{2peak}$: peak rate of $O_2$ consumption; TLV: CT-derived total lung volume; LAA-950%: low attenuation area of the lung with attenuation values below −950 HU on full inspiration CT; Pi10: average airway wall thickness normalized to a 'theoretical' airway lumen of 10-mm inner perimeter; TVV: total vessel volume; BV5: volume of blood vessels with a cross-sectional area $<5mm^2$; BV5-10: volume of blood vessels with a cross-sectional area between 5-$10mm^2$; BV10: volume of blood vessels with a cross-sectional area $>10mm^2$; TVC: total vessel count; Pa/Ao: pulmonary artery-to-aorta diameter ratio; TAPSE: tricuspid annular plane systolic excursion; HR: heart rate; SV: stroke volume; Q: cardiac output; LVOT: left-ventricular outflow tract; RVSP: right ventricular systolic pressure.

Data analyzed via one-way ANOVA and Pearson $Chi^2$**.**

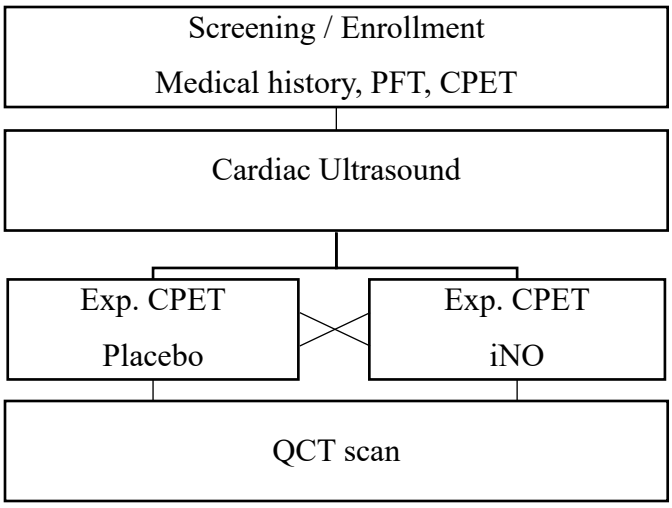


**Figure 5.1:** Study design. Visit 1: screening visit including participant enrollment, medical history and pre- and post-bronchodilator pulmonary function test (PFT) and symptom-limited incremental cardiopulmonary exercise test (CPET); Visit 2: resting cardiac ultrasound while breathing either iNO (room air with 40 ppm iNO) or room air (placebo: 21% $O_2$, 79% $N_2$; order randomized); Visits 3 & 4: incremental CPET breathing either room air or iNO (order randomized); Visit 5: prospective quantitative computed tomography (QCT) scan of the lungs.

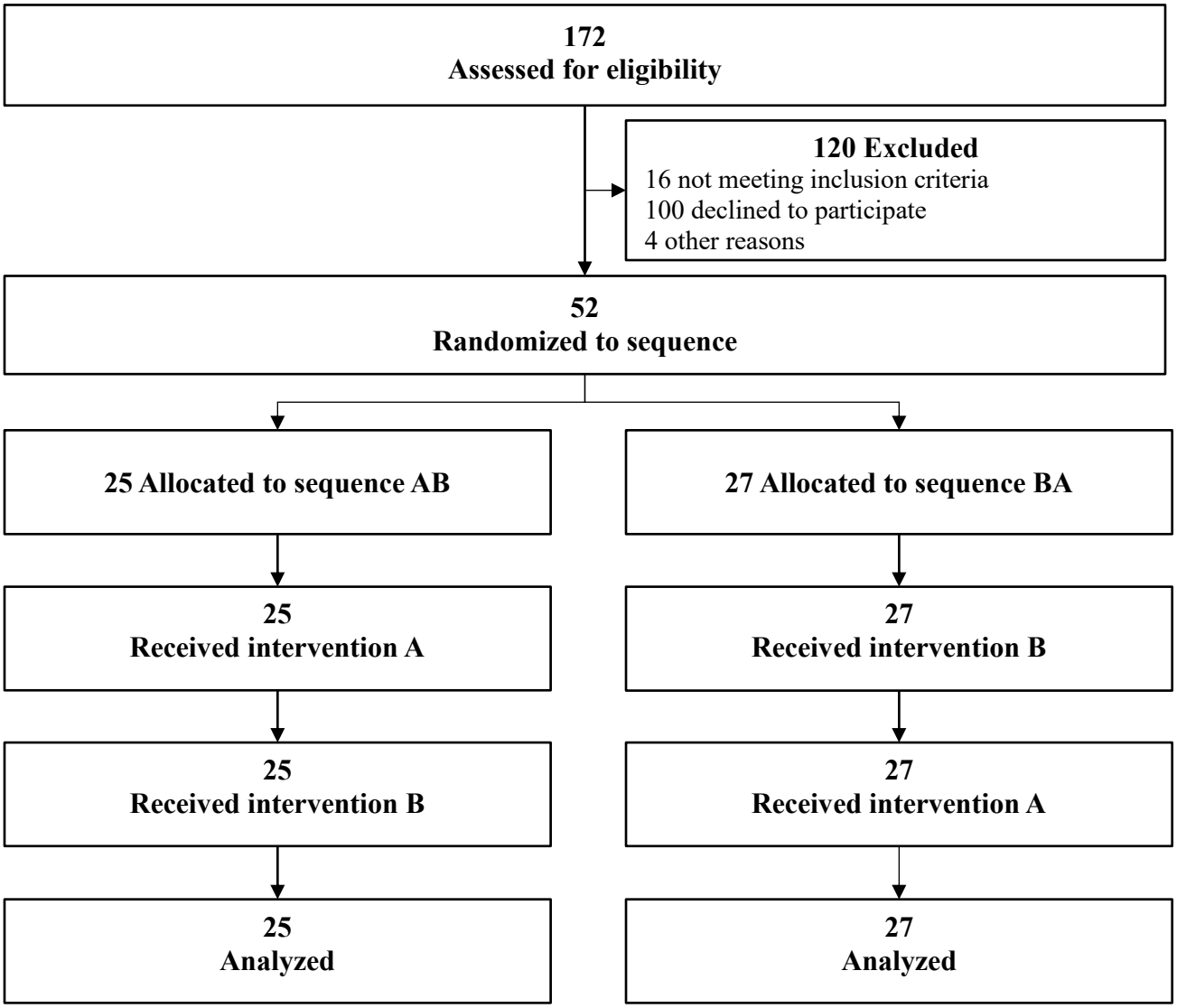


**Figure 5.2**: CONSORT participant flow diagram. A: placebo, B: iNO.

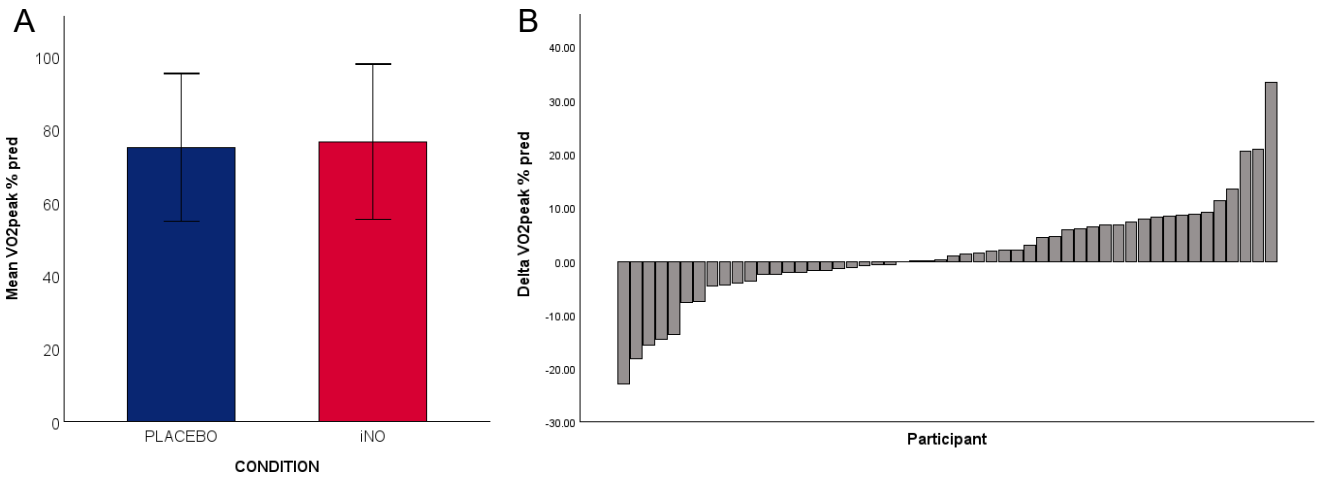


**Figure 5.3:** A) Mean (p>0.05) and B) individual change in $\dot{V}O_{2peak}$ (% predicted) with placebo vs iNO. $\dot{V}O_{2peak}$: peak rate of oxygen consumption.

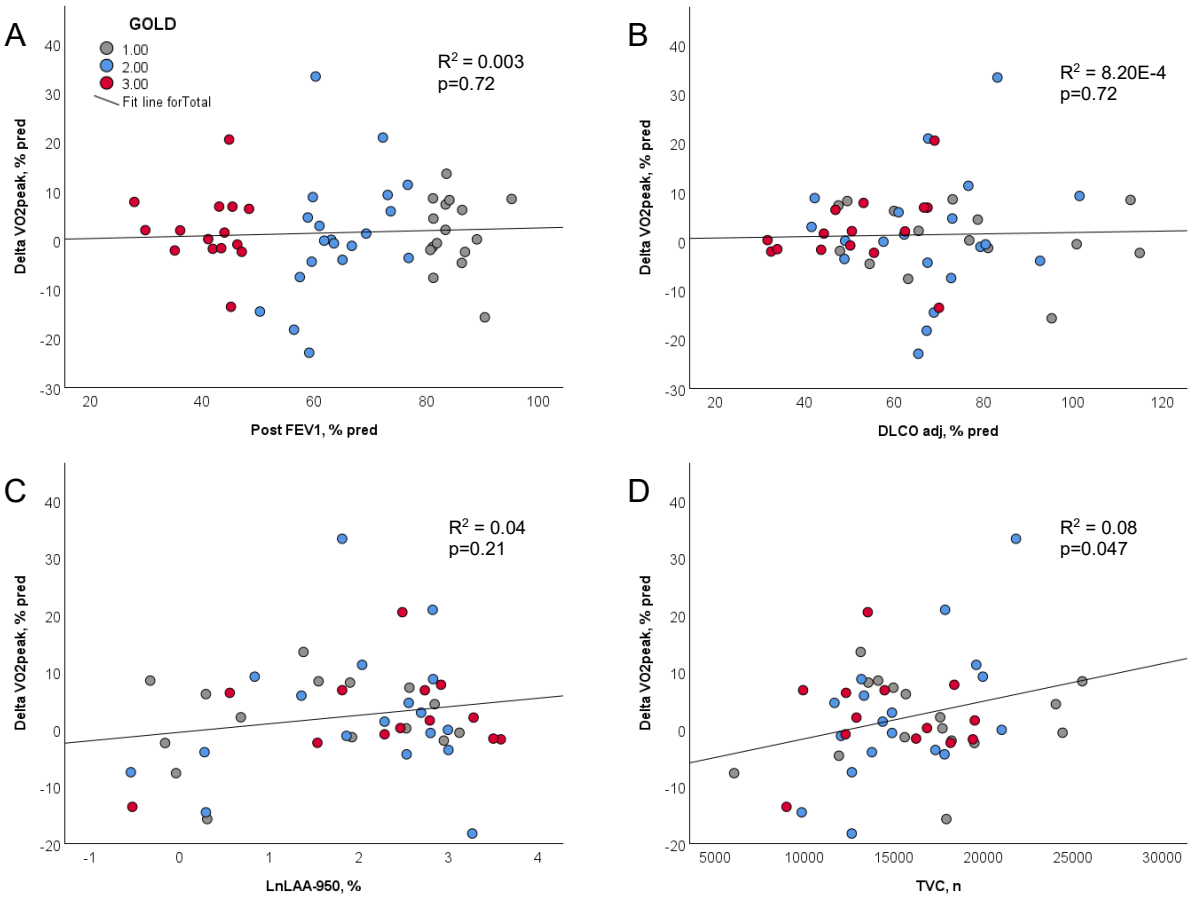


**Figure 5.4:** The unadjusted Pearson correlations between A) $FEV_1$, B) $D_LCO$, C) LnLAA-950% and D) TVC with change in $\dot{V}O_{2peak}$ % pred. $FEV_1$: forced expiratory volume in 1 second; $D_LCO$: diffusing capacity for carbon monoxide; LnLAA-950: natural logarithm of low attenuation areas of the lung <-950 Hounsfield units; TVC: total vessel count; $\dot{V}O_{2peak}$: peak rate of oxygen consumption.

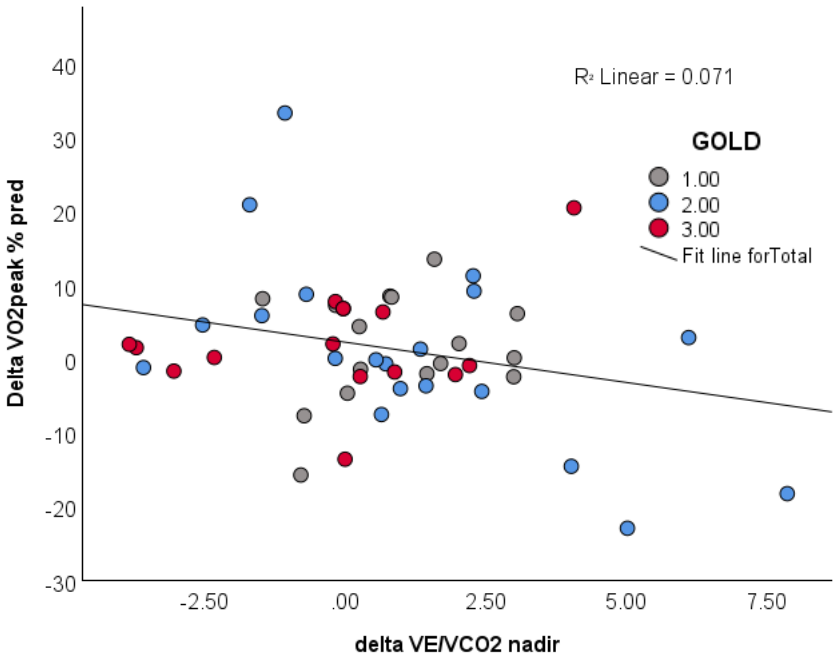


**Figure 5.5:** Represents the association between change in $\dot{V}_E/\dot{V}CO_2$ nadir and the change in $\dot{V}O_{2peak}$ % pred (Pearson $R^2$= 0.07, p= 0.08).

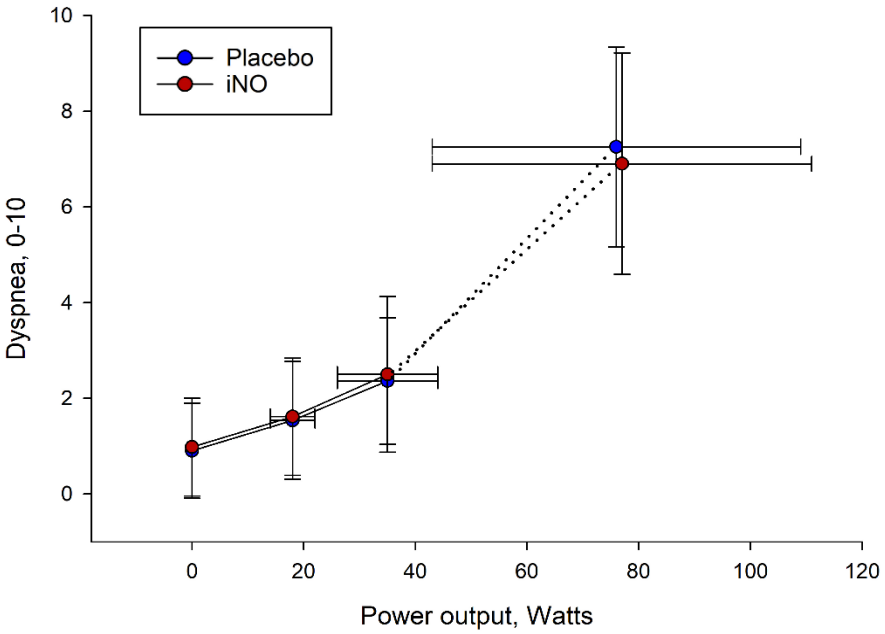


**Figure 5.6:** Dyspnea responses to incremental exercise with placebo vs. iNO. Data are mean ± SD.

# CHAPTER 6: GENERAL DISCUSSION

### *6.1 Dissertation overview*

The overall purpose of this doctoral dissertation was to evaluate the impacts of pulmonary vascular structure and function on ventilation and exercise capacity in health and chronic obstructive pulmonary disease (COPD). As summarized in **Figure 6.1,** which integrates the International Classification of Functioning framework, COPD is a complex, heterogenous disease, involving multiple factors (including personal, environmental, and pathophysiological processes), which together contribute to activity and participation limitations.

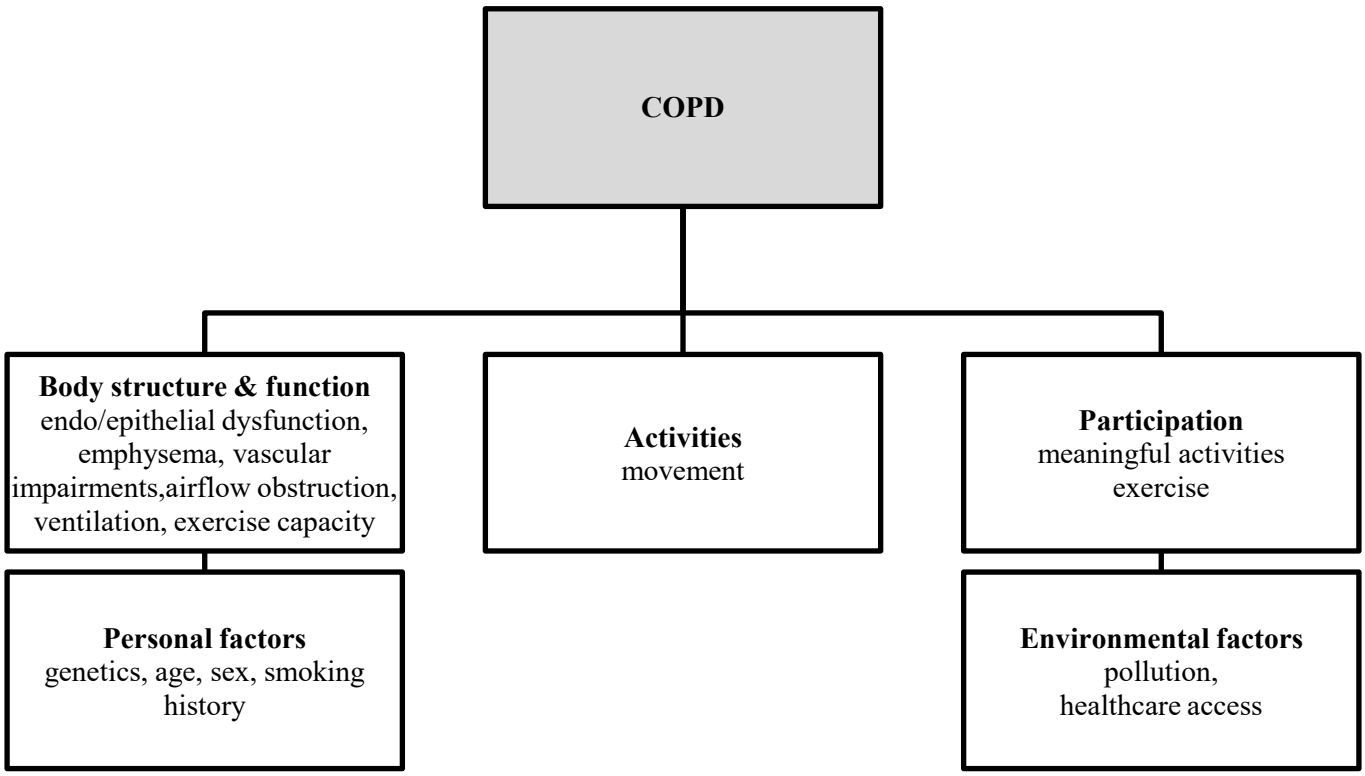


**Figure 6.1:** Chart of COPD disease pathway, integrated within the International Classification of Functioning (ICF) COPD framework.

In the first and second study of this dissertation, we used exploratory research methods to evaluate the relationship between pulmonary vascular structure and function with exercise capacity and physical activity. Data from both studies originated from the Canadian Cohort Obstructive Lung Disease (CanCOLD) study, which is a multi-site, longitudinal study. In **Study #1**, we evaluated if baseline physical activity and exercise capacity ($\dot{V}O_{2peak}$) are associated with pulmonary diffusing capacity ($D_LCO$) and quantitative computed tomography (CT) pulmonary vascular measurements. We found that in a cohort of 1,004 participants, $\dot{V}O_{2peak}$ was independently associated with $D_LCO$, total pulmonary blood vessel volume (TBV), volume of vessels < 5 $mm^2$ in cross-sectional area (BV5), and volume of vessels between 5-10 $mm^2$ in cross-sectional area (BV5-10) but not total airway count (TAC), even after controlling for severity of airflow obstruction (forced expiratory volume in 1 second [$FEV_1$]), emphysema severity and body morphology. Further, we observed COPD by $\dot{V}O_{2peak}$ interaction effects with TBV and BV5-10, suggesting that exercise capacity may be predictive of structural and functional differences in the pulmonary vasculature; and that these relationships are modified in COPD. Surprisingly, self-reported physical activity was not consistently associated with any CT-derived airway or pulmonary vascular measurements, suggesting that other variables, such as body morphology, emphysema, severity of airflow obstruction and $\dot{V}O_{2peak}$ are more important determinants of airway count and pulmonary vascular volumes. Our findings suggest that $\dot{V}O_{2peak}$ may be an important determinant of the pulmonary vasculature in adults with or without COPD. However, due to the cross-sectional design of the study, the directional relationship between pulmonary vascular structure/function with $\dot{V}O_{2peak}$ could not be determined.

The results from **Study #1** generated further questions about the longitudinal relationship between $\dot{V}O_{2peak}$ and pulmonary vascular structure and function. Therefore, **Study #2** was

completed to evaluate if higher baseline $\dot{V}O_{2peak}$ is protective of pulmonary vascular structure/function decline over three years, or if higher baseline pulmonary vascular measures are protective of $\dot{V}O_{2peak}$ decline over the same time period. A sub aim was to determine whether these associations are mediated by exercise ventilatory inefficiency ($\dot{V}_E/\dot{V}CO_2$ nadir). We found that higher baseline $\dot{V}O_{2peak}$ was protective of small vessel volume decline in ES without COPD and in individuals with COPD, even after controlling for emphysema and degree of airflow obstruction. The inverse was not observed; pulmonary vascular structure/function measures were not directly associated with longitudinal decline in $\dot{V}O_{2peak}$ in those at risk of developing COPD or in individuals with COPD. However, mediation analysis demonstrated that $\dot{V}_E/\dot{V}CO_2$ nadir mediates the relationship between baseline pulmonary vascular structure/function and $\dot{V}O_{2peak}$ decline at 3-years follow-up, suggesting a mechanistic role of baseline pulmonary vascular structure and function in determining decline in exercise capacity *through* exercise ventilatory efficiency in people with and without COPD. The latter finding provides further evidence suggesting that pulmonary vascular structure/function may be an appropriate therapeutic target to improve $\dot{V}O_{2peak}$ through optimizing ventilatory efficiency in COPD.

In **Study #3**, we completed a randomized double-blind placebo-controlled cross-over trial to examine the effects of an acute inhaled selective pulmonary vasodilator (inhaled nitric oxide [iNO]) on $\dot{V}_E/\dot{V}CO_2$, dyspnea and $\dot{V}O_{2peak}$ in patients with *mild to severe* COPD (NCT03679312). We aimed to identify characteristics (e.g., lung function or pulmonary vascular structure) of participants most likely to respond to iNO. Responses to the intervention were largely heterogeneous, with 29/52 patients demonstrating an increase in $\dot{V}O_{2peak}$ with iNO. We found that there was no correlation between the change in $\dot{V}O_{2peak}$ (%pred) with iNO and $FEV_1$, or emphysema. While iNO did not significantly improve $\dot{V}O_{2peak}$ at peak exercise; we found that TVC

was a significant predictor of improved $\dot{V}O_{2peak}$ with iNO, even after accounting for severity of airflow obstruction, $D_LCO$ and emphysema. **Study #3** is the first to show that COPD patients with an intact pulmonary vasculature are more likely to respond favourably to iNO. This study increases our understanding of the complexities of COPD, and offers evidence suggesting that response to pulmonary vasodilators is independent of the degree of airflow obstruction and emphysema – a concept that has persisted for decades in the scientific literature.

### *6.2 Major Findings*

Taken together, the studies presented in this dissertation show that 1) $\dot{V}O_{2peak}$ and pulmonary vascular structure/function are positively associated, independent of traditional markers of COPD severity; 2) Individuals with higher baseline $\dot{V}O_{2peak}$ will show less decline in distal pulmonary vascular volumes over time. While having greater pulmonary vascular structure/function is not directly protective of decline in $\dot{V}O_{2peak}$, the pulmonary vasculature exerts its effects on changes in $\dot{V}O_{2peak}$ *through* ventilatory efficiency; 3) Among all of the *a priori* selected markers of disease severity ($FEV_1$, $D_LCO$, emphysema, CT-measured pulmonary vascular structure), total vessel count was the only predictor of improvement in $\dot{V}O_{2peak}$ with acute selective pulmonary vasodilation. The entire body of this thesis substantiates the concept that COPD is much more than just airflow obstruction and emphysema. When considering COPD, as highlighted recently, we should use our senses, "*looking beyond the curtain of* $FEV_1$" (203), to unveil the "*pulmonary vasculature in COPD: the silent component*" (152).

### *6.3 Cardiovascular function in COPD*

While various tools were used to assess pulmonary vascular and cardiac structure/function in **Studies #1-3**, our results may not provide a full picture of the pathological changes associated

with COPD. Together, hypoxic pulmonary vasoconstriction, hypercapnia, acidosis, and pulmonary vascular remodelling can increase pulmonary vascular resistance leading to pulmonary hypertension (PH). However, the progressive increase in pulmonary artery pressure has been shown to be very slow in a large study evaluating COPD patients with no PH and mild-moderate hypoxemia at enrolment (204). Further, PH was shown to only occur in a minority of patients, with those with high exercising pulmonary pressures being more likely to develop resting PH over time (204). Chronic PH can lead to increased right ventricular (RV) afterload, leading to *cor pulmonale* (RV dilation and hypertrophy)(205). Further, distal pulmonary artery pruning (suggestive of capillary destruction) measured through quantitative CT in smokers and patients with COPD has been associated with *cor pulmonale* (94, 130, 206). Work from the MESA Study has described a new phenotype of RV dysfunction termed *cor pulmonale parvus* (parvus is from the Latin word parva meaning "small") (207). Briefly, increased intrathoracic pressure (as can occur with hyperinflation) impairs venous return and therefore reduces RV volume (with normal RV mass and ejection fraction). *Cor pulmonale parvus* is often found in emphysema (207). Segmentation of the vascular tree via quantitative CT imaging may provide insight into symmetric or asymmetric involvement of arterial and venous beds in COPD (130). For example, the pulmonary arterial-venous volume (A/V) ratio is a marker of dilation of the arteries relative to the veins (130). Rahaghi and colleagues (130) found that an increased A/V ratio in the *large vessels* (suggestive of arterial engorgement) was associated with reduced right ventricular ejection fraction (RVEF, indicating *cor pulmonale*), while *small vessel* A/V ratios were positively correlated with RVEF in smokers with moderate COPD (% emphysema was not reported). While the VIDA Diagnostics quantitative imaging software used in this thesis currently does not differentiate arteries from veins, this functionality may have provided stronger predictive power in identifying how higher exercise

capacity may be protective of pulmonary vascular decline, and may help us further identify characteristics of pulmonary vascular structure most responsive to pulmonary vasodilation.

Vasodilators can modulate cardiovascular function in several ways, including release of tonic vasoconstriction in the pulmonary, coronary and systemic circulations. iNO is an effective, selective pulmonary vasodilator which does not cause systemic hypotension (7, 178, 208). In **Study #3**, we enrolled non-hypoxemic patients with mild-moderate COPD with a normal ejection fraction, indicating the absence of heart failure and making it unlikely to observe changes in stroke volume or cardiac output (Q). Although we did not observe any changes in stroke volume or Q either at rest or at peak exercise with iNO, any potential effects of iNO on cardiac function would have been expected to occur secondary to pulmonary vasodilation, and not systemic vasodilation. Inhalation of NO would reduce pulmonary vascular resistance (PVR)(70), which is calculated as:

$$PVR = (mPAP - LAP) / Q$$

Where mPAP is mean pulmonary arterial pressure, LAP is left atrial pressure. As such, PVR represents the resistance to blood flow, and a reduction in PVR could thus decrease RV afterload and strain, therefore improving right heart function. However, Sitbon et al., showed that while iNO improved stroke volume in mildly hypoxemic patients with primary PH, it did *not* change Q in responders to iNO (209). This finding is surprising, as we would expect that improved pulmonary blood flow towards the left heart would increase diastolic filling (preload), thus improving stroke volume and subsequently Q. The reason for the absence of an effect on Q was due to a *decrease* in heart rate with iNO. Others have reported similar findings in COPD with and without PH, with no change in Q (7, 74, 75, 198). Conversely, Ashutosh et al., reported a rise in resting Q with iNO in hypoxemic patients with severe COPD (at risk of PH) (172). Given that "*Q is only as good as your worst ventricle"* (210), we would expect to observe improvements in Q with iNO in right

heart failure. Indeed, previous studies have reported that iNO increased Q in patients with acute right heart failure (211, 212), and that the etiology of right heart failure did not predict response to iNO (212). Finally, in patients with *left* heart failure, iNO caused a large increase in pulmonary artery wedge pressure (estimate of left ventricular filling pressure) secondary to decreased PVR, which contributed to a *reduction* in cardiac output, likely secondary to "excessive dilation" of the left ventricle (213). In conclusion, the effects of iNO on cardiovascular function appear to vary across studies, with the majority of findings suggesting that while iNO reduces PVR, it does not improve Q in COPD without overt right heart failure.

### *6.4 The pulmonary (micro)vascular phenotype: a previously identified therapeutic target?*

Numerous review articles and communications have been published over the last few years discussing the pulmonary vasculature in the setting of COPD (152, 214-216). Although previous work in quantitative CT imaging of the lung has demonstrated physiological COPD phenotypes as being either emphysema dominant, small airway dysfunction dominant or mixed, more recent work in COPD has shown that some patients may develop a distinct pulmonary vascular phenotype (94, 214, 215). The pulmonary vascular phenotype in COPD is characterized by *severe PH*, less severe airflow limitation (mainly moderate obstruction), hypoxemia, very low $D_LCO$, normo- or hypocapnia, and exercise intolerance caused by cardiovascular impairments (214, 217-219). PH has been found at various stages of COPD (220-222), with an estimated 30-70% of COPD patients having clinically significant pulmonary vascular disease (223-226). The primary cause of PH in COPD is pulmonary vascular remodeling, which affects small and precapillary arteries. Histologically, the most pronounced feature of pulmonary vascular remodeling in COPD is the thickening of the intimal layer (i.e., innermost layer) of muscular arteries, which is caused by

chronic smoke inhalation (152). Importantly, histologic remodelling has been shown to be associated with pulmonary vascular pruning on CT (153, 227). Current guidelines do not recommend targeted PAH therapy in individuals with PH caused by lung disease (214, 228), likely due to the adverse effects of pulmonary vasodilators on ventilation-perfusion matching and oxygen saturation in individuals with severe COPD (74, 75, 198, 229). Instead, treatment of the underlying lung condition is recommended, despite no evidence suggesting that treatment of the lung disease improves PH (151, 230).

However, we know that pulmonary vascular remodelling occurs overtime, with evidence suggesting that even individuals with *mild* COPD have evidence of impaired pulmonary vascular function (5). In their review article, Kovacs et al., propose that individuals with the "pulmonary vascular phenotype" characterized by severe PH, less severe airflow limitation and very low $D_LCO$, are appropriate candidates for pulmonary vascular therapies (214). Given recent evidence that iNO effectively improves exercise capacity secondary to improved ventilatory efficiency and dyspnea in individuals with *mild* COPD with no evidence of PH (7), we propose re-evaluating, or expanding the definition of the "pulmonary vascular phenotype".

When also considering the results of **Study #3**, we propose that the "pulmonary vascular phenotype" appears to be independent of COPD severity (i.e., post-bronchodilator $FEV_1$% predicted), emphysema severity, or $D_LCO$. We found that TVC was the strongest predictor of response to iNO. A higher TVC likely represents less distal pulmonary vascular pruning, and is likely associated with a greater number of capillaries available to participate in gas-exchange (i.e., the pulmonary *micro*vascular phenotype). Attempts have been made to identify the phenotype of participants with PH (without COPD) who are most responsive to iNO. Work by Krasuski and colleagues (231) showed that individuals with RV diastolic dysfunction and right-ventricular

failure (*cor pulmonale*) were less likely to respond to iNO. The authors suggested that these predictors of response were likely signs of long-standing pulmonary hypertension (irreversible pulmonary vascular dysfunction). In patients with PH and left heart failure, low pulmonary arterial compliance, high pulmonary vascular resistance, and low diastolic blood pressure predict the non-responsiveness to intravenous nitroprusside (a systemic vasodilator), while right-sided heart failure predicted poor prognosis (232). While these findings are in individuals without COPD, they may offer insight and further clues on predictors of response to iNO in non-hypoxemic patients with COPD and no evidence of PH.

As discussed earlier in this chapter, COPD may contribute to the development of *cor pulmonale* (RV dilation and hypertrophy and reduced RVEF), or *cor pulmonale parvus* (characterized by impaired venous return and therefore reduced RV output due to increased intrathoracic pressure) (207, 233, 234). Given that cardiac (dys)function occurs along a continuum, the pulmonary vascular alterations discussed earlier would not be limited to individuals with right-sided dysfunction. Indeed, there is strong evidence to support that pulmonary vascular dysfunction precedes emphysema development (5). Therefore, assessment of large and small arteries and veins via quantitative CT may offer valuable insight in our quest to identify patients who would respond to pulmonary vascular targets.

In summary, the key points of this section are: 1) Previous work suggests that patients with *cor pulmonale* are less likely to respond positively to pulmonary vasodilators (231); and 2) Reduced RVEF (i.e., *cor pulmonale*) is associated with an increased large vessel A/V ratio but a reduced small vessel A/V ratio in COPD (130). Within the context of the findings reported in **Study #3** whereby responders to iNO appeared to have a greater number of intraparenchymal vessels, it is plausible that responders may be further differentiated from non-responders by

separating arteries from veins on CT. It is likely that responders would have less distal *arterial* pruning (larger arterial BV5/TVV), a lower large vessel A/V ratio (representing less arterial engorgement), and a higher small vessel A/V ratio. In contrast to the review by Kovacs et al., (214) on the pulmonary vascular phenotype, we would suggest there may be a pulmonary *micro*-vascular phenotype characterized by a preserved distal pulmonary vasculature, without PH and less proximal artery engorgement on imaging.

### *6.5 The relationship between pulmonary vascular structure and pulmonary vascular function*

Previous work from our laboratory has shown that people with COPD have persistently reduced pulmonary diffusing capacity for carbon monoxide ($D_LCO$) compared to healthy controls, both at rest, and during exercise (6). $D_LCO$ can be broken down into its components: membrane diffusing capacity (Dm; reflecting the surface area available for gas exchange and alveolar membrane thickness), as well as pulmonary capillary blood volume (Vc; reflecting pulmonary capillary recruitment) (185). Therefore, pathological processes that decrease the surface area for gas-exchange (e.g., emphysema), increase membrane thickness (e.g., pulmonary edema or pulmonary hypertension), or reduce capillary blood volume can contribute to impairing $D_LCO$. Indeed, previous work (87) found that CT-derived pulmonary vascular volumes were associated with $D_LCO$ in individuals with and without COPD, suggesting a link between pulmonary vascular structure and function. However, no study to-date has evaluated CT- pulmonary vascular structure measures alongside pulmonary vascular function estimates derived from the multiple fraction of inspired oxygen $D_LCO$ technique (i.e., Vc and Dm). Therefore, we completed exploratory analyses of data from 35 people with COPD enrolled in **Study #3**, and found that $D_LCO$ adjusted for alveolar volume was moderately correlated with TVV, BV5 and BV5-10 (175) (**see Figure 6.2**

**below**). Exploratory analyses revealed that among CT variables, Dm was associated with BV5-10 and TVV, while Vc was not associated with any CT pulmonary vascular measurements. Our results support an association between pulmonary vascular function as estimated via $D_LCO$ and structure as measured through novel quantitative CT imaging.

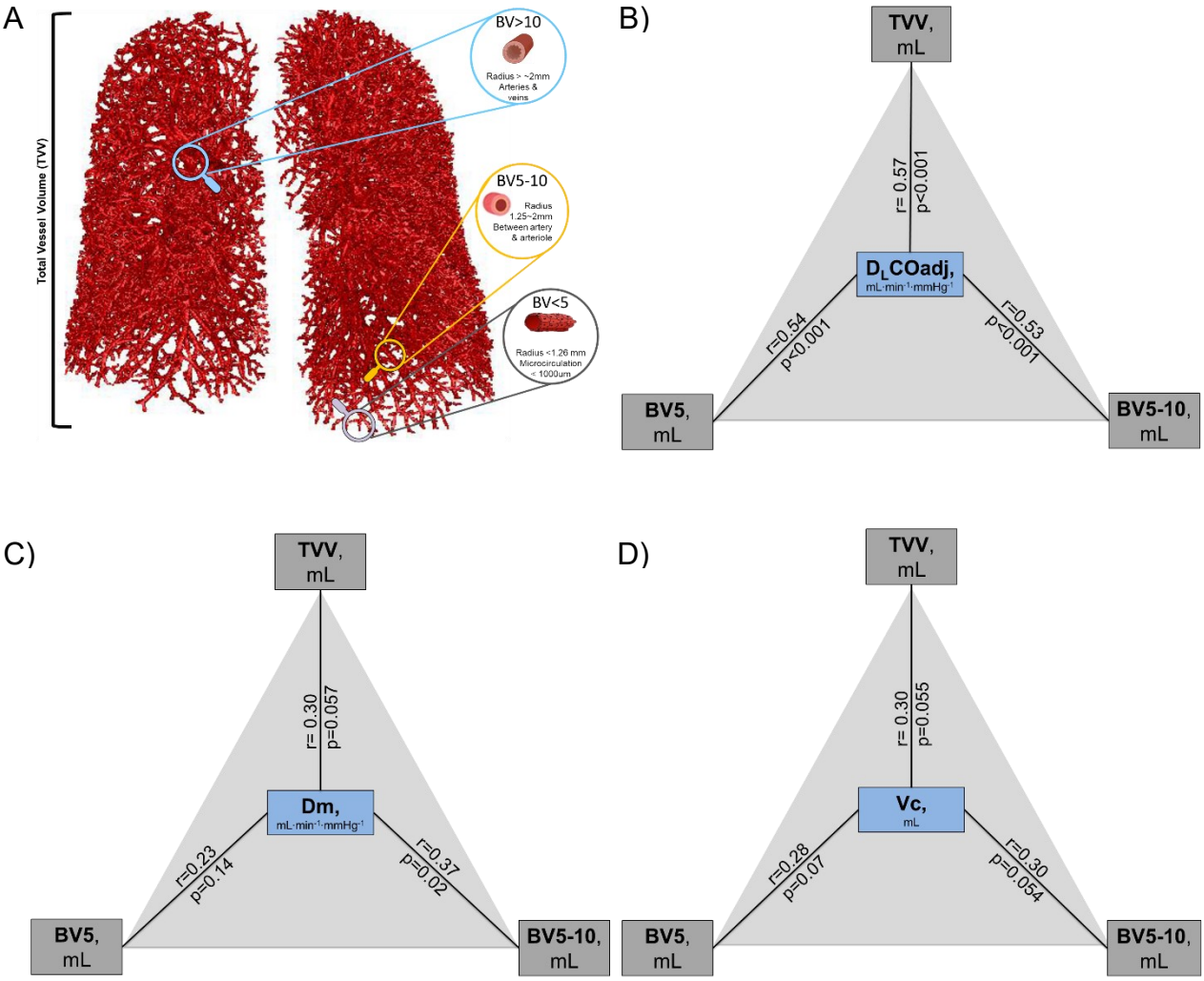


**Figure 6.2: A)** 3D reconstruction of intraparenchymal pulmonary vessels in a participant with COPD. **B-D)** Pearson correlations between CT measured pulmonary vascular volumes with $D_LCO$ adjusted for alveolar volume (B), Dm (C), and Vc (D). $D_LCO$: pulmonary diffusing capacity for carbon monoxide; Dm: diffusing membrane capacity; Vc: pulmonary capillary blood volume.

### *6.6 Future directions and implications for the discipline*

The previous studies discussed in this discussion section combined with the findings reported in **Studies 1-3** support further exploration into the complex interactions between pulmonary vascular structure/function and exercise in health and COPD. Findings discussed in **sections 6.3-6.5,** support the further use of quantitative imaging analysis alongside lung function testing to gain a better understanding of the underlying pathological processes affecting individuals with COPD, and perhaps may be utilized to better identify candidates for pulmonary vascular therapies. For example, researchers may use techniques such as those used by Iyer and colleagues (235) to evaluate changes in peripheral pulmonary vascular perfusion (using CT) before and after administering pulmonary vascular therapies, and to evaluate whether these acute changes in pulmonary perfusion translate to improved pulmonary gas-exchange and vascular function (via the single breath multiple $FiO_2$ $D_LCO$ technique). Future studies could further differentiate the artery vs. veinous adaptations occurring in patients with COPD using advanced image analysis techniques (130), as these parameters may help us more precisely predict response to selective pulmonary vasodilators, and perhaps better identify patients at risk of developing PH. These modalities may also help identify patients who might benefit the most from pulmonary rehabilitation, given that higher exercise capacity appears to protect from longitudinal decline in pulmonary vascular structure. Finally, **Study #3** provides a physiological rationale for future studies to probe the physiological impacts of impaired pulmonary vascular structure and function, and explore practical pulmonary vascular therapies (such as inhaled Treprostinil). Future studies could also explore airway vs vascular phenotypes of COPD, or systemic vascular measures (e.g., circulating nitrates/nitrites, inflammatory markers, peripheral vascular function) that might further help in identifying responders to iNO.

### *6.7 Summary and conclusion*

In this dissertation, we probed the relationship between the pulmonary vasculature, ventilation and exercise capacity in health and COPD utilizing data from a large, population-based cohort, and through experimental manipulation of the pulmonary vasculature.

Pulmonary vascular pruning (i.e., remodelling and loss of the small pulmonary vessels) measured on CT is related to reduced 6-minute walk distance and increased mortality in patients COPD (94). Similarly, others (95) have shown that healthy adults *without* COPD with higher CT-derived pulmonary vascular volumes have increased $\dot{V}O_{2peak}$; however, it was unclear whether these relationships existed in COPD, and whether these relationships differed across never-smokers, smokers and patients with COPD. In **Study #1**, we found a cross-sectional relationship between $\dot{V}O_{2peak}$ and resting pulmonary vascular structure and function (and vice-versa), in never-smokers, smokers and patients with COPD, independent of emphysema and airflow obstruction severity.

Since higher $\dot{V}O_{2peak}$ and moderate-to-vigorous levels of regular physical activity have been associated with attenuated lung function decline and reduced COPD risk among smokers (92, 93), we wanted to identify whether higher baseline $\dot{V}O_{2peak}$ was also protective of pulmonary vascular decline in smokers at risk of developing COPD, and in people with COPD. In **Study #2**, we found that 1) Independent of emphysema and degree of airflow obstruction, higher baseline $\dot{V}O_{2peak}$ was protective of small vessel volume decline in ES without COPD and in individuals with COPD; 2) Pulmonary vascular structure/function measures were not associated with longitudinal decline in $\dot{V}O_{2peak}$ in those at risk of developing COPD or in individuals with COPD; 3) However, baseline BV5/TVV and $D_LCO$ was associated with $\dot{V}_E/\dot{V}CO_2$ nadir, which in turn was associated with $\dot{V}O_{2peak}$ decline at 3-years follow-up, suggesting a mechanistic role of baseline pulmonary vascular

structure and function in determining decline in exercise capacity through exercise ventilatory efficiency in people with and without COPD.

Ventilatory inefficiency contributes to increasing dyspnea and exercise intolerance in people with COPD, and recent evidence shows that acute selective inhaled pulmonary vasodilation improves exercise capacity secondary to improved ventilatory efficiency and dyspnea in mild COPD (7). In **Study #3**, we demonstrated, for the first time in patients with mild-severe COPD, that responders to iNO have more intraparenchymal blood vessels, even after controlling for severity of airflow obstruction, $D_LCO$ and emphysema.

Together, findings from this dissertation highlight the key role of pulmonary vascular structure and function in health and disease and shows that pulmonary vascular structure and function may be a modifiable factor to improve ventilation, dyspnea, and exercise capacity in those with COPD.

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# APPENDICES

## *Appendix A: Supplementary materials for Study #1*

**Table E1:** General Linear Regression Models for TAC

| | Model 0 | Model 0 + $\dot{V}O_{2peak}$ | Model 0 + MVPA | Model 1 | Model 1 + $\dot{V}O_{2peak}$ | Model 1 + MVPA | Model 2 | Final |
|---|---|---|---|---|---|---|---|---|
| Intercept | 214.93 (206.78 to 223.08)* | 212.27 (198.55 to 225.98)* | 209.99 (199.22 to 220.76)* | -6.93 (-155.02 to 141.16) | -7.77 (-156.32 to 140.79) | -5.75 (-153.91 to 142.4) | -46.51 (-189.44 to 96.41) | -48.92 (-192.34 to 94.49) |
| ES | -4.57 (-15.03 to 5.89) | -4.66 (-15.13 to 5.81) | -4.28 (-14.74 to 6.18) | -6.52 (-15.78 to 2.73) | -6.46 (-15.75 to 2.84) | -6.24 (-15.52 to 3.05) | -5.23 (-14.17 to 3.71) | 0.74 (-24.21 to 25.69) |
| COPD | -54.36 (-65.26 to -43.46)* | -54.28 (-65.18 to -43.37)* | -54.28 (-65.17 to -43.38)* | -63.09 (-72.9 to -53.29)* | -62.91 (-73.01 to -52.8)* | -62.82 (-72.66 to -52.99)* | -34.3 (-45.7 to -22.89)* | -29.28 (-55.83 to -2.72)$ |
| Never-smoker | - | - | - | - | - | - | - | - |
| $\dot{V}O_{2peak}$ (L/min) | - | 1.58 (-4.96 to 8.12) | - | - | 0.67 (-8.02 to 9.36) | - | -11.27 (-20.17 to -2.37)$ | -8.9 (-21.48 to 3.68) |
| sqrtCal/week | - | - | 0.12 (-0.05 to 0.3) | - | - | 0.06 (-0.1 to 0.23) | 0.01 (-0.15 to 0.17) | 0.01 (-0.15 to 0.18) |
| Female | - | - | - | 5.44 (-6.02 to 16.91) | 5.64 (-6.11 to 17.39) | 5.54 (-5.94 to 17.01) | 14.17 (2.58 to 25.76)$ | 14.17 (2.57 to 25.78)$ |
| Caucasian | - | - | - | -13.13 (-45.52 to 19.25) | -13.26 (-45.7 to 19.19) | -13.79 (-46.24 to 18.65) | -8.41 (-39.57 to 22.74) | -8.26 (-39.5 to 22.97) |
| Age (years) | - | - | - | 1.11 (0.71 to 1.51)* | 1.13 (0.68 to 1.58)* | 1.13 (0.73 to 1.53)* | 2.07 (1.59 to 2.54)* | 2.07 (1.59 to 2.54)* |
| Height (cm) | - | - | - | 0.57 (-0.14 to 1.29) | 0.57 (-0.14 to 1.28) | 0.56 (-0.16 to 1.27) | -0.37 (-1.09 to 0.35) | -0.38 (-1.1 to 0.34) |
| Body mass (kg) | - | - | - | -0.97 (-1.26 to -0.69)* | -0.98 (-1.27 to -0.69)* | -0.98 (-1.26 to -0.69)* | -0.69 (-0.98 to -0.4)* | -0.69 (-0.97 to -0.4)* |
| TLV (L) | - | - | - | 16.05 (10.45 to 21.64)* | 15.96 (10.24 to 21.68)* | 15.88 (10.27 to 21.5)* | 6.78 (0.33 to 13.23)$ | 6.78 (0.33 to 13.24)$ |
| TLV/TLC | - | - | - | 64.32 (23.2 to 105.43)$ | 64.54 (23.3 to 105.77)$ | 65.12 (23.94 to 106.3)$ | 124.91 (83.18 to 166.64)* | 124.51 (82.67 to 166.35)* |
| $FEV_1$ (L) | - | - | - | - | - | - | 43.28 (34.12 to 52.45)* | 43.28 (34.1 to 52.45)* |
| LnLAA-950% | - | - | - | - | - | - | -1.99 (-7.11 to 3.13) | -1.99 (-7.14 to 3.17) |
| INTERACTIONS | - | - | - | - | - | - | - | - |
| $\dot{V}O_{2peak}$ x ES | - | - | - | - | - | - | - | -3.5 (-17.1 to 10.11) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $\dot{V}O_{2peak}$ x COPD | - | - | - | - | - | - | - | -2.99 (-17.22 to 11.25) |
| $R^2$ | 0.116 | 0.116 | 0.118 | 0.352 | 0.352 | 0.352 | 0.406 | 0.406 |
| Adjusted $R^2$ | 0.114 | 0.114 | 0.115 | 0.338 | 0.338 | 0.338 | 0.391 | 0.390 |

Data are unstandardized estimate (95%CI). Models 1 and after are adjusted for CT scanner model (not reported here). ES: ever smokers; COPD: chronic obstructive pulmonary disease; $\dot{V}O_{2peak}$: peak rate of oxygen consumption; sqrtCal/week: square-root of calories expended per week in moderate or greater intensity exercise; $TLV_{CT}$: total lung volume on inspiratory CT; $TLV_{CT}/TLC_{pleth}$: depth of inspiration at CT; $FEV_1$: post-bronchodilator forced expiratory volume in 1 sec, and LnLAA-950%: natural logarithm of low-attenuating areas below -950 Hounsfield units.
$^{*}p<0.001$, $^{\$}p<0.05$

| Table E2: General Linear Regression Models for TBV | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Model 0 | Model 0 + $\dot{V}O_{2peak}$ | Model 0 + MVPA | Model 1 | Model 1 + $\dot{V}O_{2peak}$ | Model 1 + MVPA | Model 2 | Final |
| Intercept | 137.68 (132.93 to 142.43)$^{\$}$ | 76.91 (70.43 to 83.38)* | 119.28 (113.25 to 125.32)* | -43.79 (-81.69 to -5.89)$^{\$}$ | -52.67 (-89.91 to -15.43)$^{\$}$ | -42.72 (-80.52 to -4.92)$^{\$}$ | -52.05 (-89.15 to -14.95)$^{\$}$ | -55.89 (-92.95 to -18.83)$^{\$}$ |
| ES | 12 (5.91 to 18.1)* | 9.82 (4.88 to 14.76)* | 13.08 (7.22 to 18.94)* | 1.25 (-1.12 to 3.62) | 1.98 (-0.36 to 4.31) | 1.51 (-0.86 to 3.88) | 1.99 (-0.33 to 4.31) | 5.65 (-0.79 to 12.1) |
| COPD | 15.16 (8.81 to 21.51)* | 17.07 (11.92 to 22.21)* | 15.47 (9.36 to 21.57)* | -3.53 (-6.04 to -1.01)$^{\$}$ | -1.54 (-4.07 to 0.99) | -3.28 (-5.79 to -0.77)$^{\$}$ | 0.85 (-2.11 to 3.81) | 10.28 (3.41 to 17.14)$^{\$}$ |
| Never-smoker | - | - | - | - | - | - | - | - |
| $\dot{V}O_{2peak}$ (L/min) | - | 36.03 (32.95 to 39.12)* | - | - | 7.17 (4.99 to 9.34)* | - | 6 (3.69 to 8.31)* | 8.61 (5.36 to 11.86)* |
| sqrtCal/week | - | - | 0.46 (0.36 to 0.56)* | - | - | 0.06 (0.01 to 0.1)$^{\$}$ | 0.02 (-0.02 to 0.06) | 0.03 (-0.02 to 0.07) |
| Female | - | - | - | -5.72 (-8.65 to -2.78)* | -3.65 (-6.59 to -0.7)$^{\$}$ | -5.63 (-8.56 to -2.71)* | -3.93 (-6.94 to -0.92) $^{\$}$ | -4.04 (-7.04 to -1.04)$^{\$}$ |
| Caucasian | - | - | - | -0.99 (-9.28 to 7.29) | -2.29 (-10.43 to 5.84) | -1.59 (-9.87 to 6.68) | -1.88 (-9.97 to 6.2) | -1.25 (-9.32 to 6.82) |
| Age (years) | - | - | - | -0.29 (-0.39 to -0.18)* | -0.12 (-0.23 to 0.00)$^{\$}$ | -0.27 (-0.37 to -0.17)* | -0.05 (-0.17 to 0.07) | -0.04 (-0.17 to 0.08) |
| Height (cm) | - | - | - | 0.4 (0.22 to 0.58)* | 0.35 (0.17 to 0.53)* | 0.38 (0.2 to 0.57)* | 0.24 (0.05 to 0.42)$^{\$}$ | 0.22 (0.04 to 0.41)$^{\$}$ |
| Body mass (kg) | - | - | - | 0.2 (0.13 to 0.27)* | 0.15 (0.07 to 0.22)* | 0.2 (0.13 to 0.27)* | 0.18 (0.11 to 0.26)* | 0.19 (0.12 to 0.27)* |
| TLV (L) | - | - | - | 24.42 (22.99 to 25.85)* | 23.46 (22.03 to 24.9)* | 24.27 (22.84 to 25.7)* | 23.97 (22.29 to 25.64)* | 23.98 (22.32 to 25.65)* |
| TLV/TLC | - | - | - | 0.19 (-10.33 to 10.71) | 2.56 (-7.78 to 12.89) | 0.92 (-9.58 to 11.43) | 8.82 (-2.01 to 19.66) | 9.06 (-1.75 to 19.87) |
| $FEV_1$ (L) | - | - | - | - | - | - | 2.32 (-0.06 to 4.7) | 2.36 (-0.01 to 4.73) |
| LnLAA-950% | - | - | - | - | - | - | -2.24 (-3.57 to -0.91)* | -2.41 (-3.74 to -1.08)* |
| INTERACTIONS | - | - | - | - | - | - | - | - |
| $\dot{V}O_{2peak}$ x ES | - | - | - | - | - | - | - | -2.19 (-5.71 to 1.33) |
| $\dot{V}O_{2peak}$ x COPD | - | - | - | - | - | - | - | -5.59 (-9.27 to -1.91)$^{\$}$ |
| $R^2$ | 0.023 | 0.360 | 0.099 | 0.862 | 0.868 | 0.863 | 0.870 | 0.871 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Adjusted $R^2$ | 0.021 | 0.358 | 0.096 | 0.859 | 0.865 | 0.860 | 0.866 | 0.867 |
| Data are unstandardized estimate (95%CI). Models 1 and after are adjusted for CT scanner model (not reported here). ES: ever smokers; COPD: chronic obstructive pulmonary disease; $\dot{V}O_{2peak}$: peak rate of oxygen consumption; sqrtCal/week: square-root of calories expended per week in moderate or greater intensity exercise; $TLV_{CT}$: total lung volume on inspiratory CT; $TLV_{CT}/TLC_{pleth}$: depth of inspiration at CT; $FEV_1$: post-bronchodilator forced expiratory volume in 1 sec, and LnLAA-950%: natural logarithm of low-attenuating areas below -950 Hounsfield units.<br>$^{*}p<0.001$, $^{\$}p<0.05$ | | | | | | | | |

| Table E3: General Linear Regression Models for BV5 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Model 0 | Model 0 + $\dot{V}O_{2peak}$ | Model 0 + MVPA | Model 1 | Model 1 + $\dot{V}O_{2peak}$ | Model 1 + MVPA | Model 2 | Final |
| Intercept | 73.12 (70.5 to 75.74)* | 49.23 (45.22 to 53.23)* | 65.55 (62.16 to 68.93)* | 31.03 (7.89 to 54.17)$ | 28.18 (5.1 to 51.27)$ | 31.44 (8.31 to 54.57)$ | 25.81 (2.8 to 48.81)$ | 26.78 (3.71 to 49.85)$ |
| ES | 3.9 (0.54 to 7.26)$ | 3.04 (-0.01 to 6.1) | 4.35 (1.06 to 7.64)$ | -0.13 (-1.58 to 1.31) | 0.1 (-1.35 to 1.54) | -0.03 (-1.48 to 1.41) | 0.26 (-1.18 to 1.7) | -2.24 (-6.26 to 1.77) |
| COPD | 9.68 (6.18 to 13.18)* | 10.43 (7.24 to 13.61)* | 9.8 (6.38 to 13.23)* | -1.02 (-2.55 to 0.51) | -0.38 (-1.95 to 1.19) | -0.92 (-2.46 to 0.61) | -0.08 (-1.92 to 1.75) | -2.09 (-6.36 to 2.18) |
| Never-smoker | - | - | - | - | - | - | - | - |
| $\dot{V}O_{2peak}$ (L/min) | - | 14.17 (12.26 to 16.07)* | - | - | 2.29 (0.94 to 3.65)* | - | 1.98 (0.54 to 3.41)$ | 1 (-1.02 to 3.02) |
| sqrtCal/week | - | - | 0.19 (0.13 to 0.25)* | - | - | 0.02 (0 to 0.05) | 0.01 (-0.02 to 0.04) | 0.01 (-0.02 to 0.04) |
| Female | - | - | - | 3.83 (2.04 to 5.62)* | 4.5 (2.67 to 6.32)* | 3.86 (2.07 to 5.66)* | 5.27 (3.41 to 7.14)* | 5.27 (3.4 to 7.14)* |
| Caucasian | - | - | - | 0.16 (-4.9 to 5.22) | -0.25 (-5.29 to 4.79) | -0.07 (-5.13 to 5) | -0.42 (-5.44 to 4.59) | -0.48 (-5.5 to 4.55) |
| Age (years) | - | - | - | -0.16 (-0.23 to -0.1)* | -0.11 (-0.18 to -0.04)* | -0.16 (-0.22 to -0.1)* | -0.09 (-0.17 to -0.02)$ | -0.09 (-0.17 to -0.02)$ |
| Height (cm) | - | - | - | -0.04 (-0.15 to 0.07) | -0.06 (-0.17 to 0.05) | -0.05 (-0.16 to 0.06) | -0.04 (-0.16 to 0.07) | -0.04 (-0.16 to 0.08) |
| Body mass (kg) | - | - | - | -0.23 (-0.28 to -0.19)* | -0.25 (-0.3 to -0.21)* | -0.23 (-0.28 to -0.19)* | -0.26 (-0.3 to -0.21)* | -0.26 (-0.31 to -0.21)* |
| TLV (L) | - | - | - | 16.64 (15.77 to 17.52)* | 16.33 (15.45 to 17.22)* | 16.58 (15.71 to 17.46)* | 15.27 (14.23 to 16.3)* | 15.26 (14.22 to 16.3)* |
| TLV/TLC | - | - | - | -0.06 (-6.48 to 6.37) | 0.7 (-5.71 to 7.11) | 0.22 (-6.2 to 6.65) | 0.59 (-6.12 to 7.31) | 0.77 (-5.96 to 7.5) |
| $FEV_1$ (L) | - | - | - | - | - | - | 1.4 (-0.08 to 2.87) | 1.4 (-0.08 to 2.87) |
| LnLAA-950% | - | - | - | - | - | - | 1.54 (0.72 to 2.36)* | 1.54 (0.71 to 2.37)* |
| INTERACTIONS | - | - | - | - | - | - | - | - |
| $\dot{V}O_{2peak}$ x ES | - | - | - | - | - | - | - | 1.47 (-0.72 to 3.66) |
| $\dot{V}O_{2peak}$ x COPD | - | - | - | - | - | - | - | 1.19 (-1.1 to 3.48) |
| $R^2$ | 0.030 | 0.200 | 0.071 | 0.832 | 0.834 | 0.832 | 0.836 | 0.837 |
| Adjusted $R^2$ | 0.028 | 0.197 | 0.069 | 0.828 | 0.830 | 0.828 | 0.832 | 0.832 |

Data are unstandardized estimate (95%CI). Models 1 and after are adjusted for CT scanner model (not reported here). ES: ever smokers; COPD: chronic obstructive pulmonary disease; $\dot{V}O_{2peak}$: peak rate of oxygen consumption; sqrtCal/week: square-root of calories expended per week in moderate or greater intensity exercise; $TLV_{CT}$: total lung volume on inspiratory CT; $TLV_{CT}/TLC_{pleth}$: depth of inspiration at CT; $FEV_1$: post-bronchodilator forced expiratory volume in 1 sec, and LnLAA-950%: natural logarithm of low-attenuating areas below -950 Hounsfield units.
$^{*}p<0.001$, $^{\$}p<0.05$

| Table E4: General Linear Regression Models for BV5-10 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Model 0 | Model 0 + $\dot{V}O_{2peak}$ | Model 0 + MVPA | Model 1 | Model 1 + $\dot{V}O_{2peak}$ | Model 1 + MVPA | Model 2 | Final |
| Intercept | 43.72 (42.02 to 45.42)* | 21.86 (19.54 to 24.17)* | 37.31 (35.14 to 39.48)* | -29.61 (-48.88 to -10.34)$ | -32.50 (-51.67 to -13.33)* | -29.20 (-48.45 to -9.96)$ | -30.08 (-48.85 to -11.3)$ | -32.43 (-45.84 to -12.449)* |
| ES | 5.69 (3.5 to 7.87)* | 4.9 (3.13 to 6.67)* | 6.06 (3.95 to 8.17)* | 1.55 (0.35 to 2.76)$ | 1.79 (0.59 to 2.99) $ | 1.65 (0.44 to 2.86)$ | 1.69 (0.52 to 2.87)$ | 4.87 (1.414 to 7.741)$ |
| COPD | 4.05 (1.77 to 6.32)* | 4.73 (2.89 to 6.57)* | 4.15 (1.96 to 6.35)* | -0.84 (-2.11 to 0.44) | -0.19 (-1.5 to 1.11) | -0.74 (-2.02 to 0.53) | 0.87 (-0.63 to 2.37) | 6.42 (3.317 to 10.101)* |
| Never-smoker | - | - | - | - | - | - | - | - |
| $\dot{V}O_{2peak}$ (L/min) | - | 12.96 (11.86 to 14.07)* | - | - | 2.33 (1.21 to 3.45)* | - | 1.86 (0.69 to 3.03)$ | 3.64 (2.295 to 5.464)* |
| sqrtCal/week | - | - | 0.16 (0.13 to 0.2)* | - | - | 0.02 (0.00 to 0.04) | 0.01 (-0.01 to 0.03) | 0.01 (-0.01 to 0.04) |
| Female | - | - | - | -5.97 (-7.46 to -4.47)* | -5.29 (-6.81 to -3.78)* | -5.93 (-7.42 to -4.44)* | -6.06 (-7.59 to -4.54)* | -6.11 (-7.63 to -4.6)* |
| Caucasian | - | - | - | 0.2 (-4.01 to 4.41) | -0.22 (-4.41 to 3.96) | -0.03 (-4.24 to 4.19) | 0.07 (-4.02 to 4.17) | 0.4 (-3.68 to 4.48) |
| Age (years) | - | - | - | -0.08 (-0.13 to -0.03) $ | -0.03 (-0.08 to 0.03) | -0.08 (-0.13 to -0.02)$ | 0 (-0.06 to 0.06) | 0 (-0.06 to 0.06) |
| Height (cm) | - | - | - | 0.23 (0.14 to 0.33)* | 0.22 (0.13 to 0.31)* | 0.23 (0.13 to 0.32)* | 0.14 (0.05 to 0.24)$ | 0.13 (0.04 to 0.23)$ |
| Body mass (kg) | - | - | - | 0.24 (0.21 to 0.28)* | 0.23 (0.19 to 0.26)* | 0.24 (0.21 to 0.28)* | 0.25 (0.21 to 0.29)* | 0.26 (0.22 to 0.3)* |
| TLV (L) | - | - | - | 4.76 (4.03 to 5.48)* | 4.44 (3.71 to 5.18)* | 4.7 (3.97 to 5.43)* | 5.54 (4.69 to 6.39)* | 5.55 (4.71 to 6.39)* |
| TLV/TLC | - | - | - | -3.71 (-9.06 to 1.64) | -2.94 (-8.26 to 2.38) | -3.43 (-8.78 to 1.92) | 0.6 (-4.89 to 6.08) | 0.6 (-4.86 to 6.07) |
| $FEV_1$ (L) | - | - | - | - | - | - | 0.17 (-1.03 to 1.38) | 0.19 (-1.01 to 1.39) |
| LnLAA-950% | - | - | - | - | - | - | -2.41 (-3.08 to -1.73)* | -2.48 (-3.16 to -1.81)* |
| INTERACTIONS | - | - | - | - | - | - | - | - |
| $\dot{V}O_{2peak}$ x ES | - | - | - | - | - | - | - | -1.88 (-3.66 to -0.1)$ |
| $\dot{V}O_{2peak}$ x COPD | - | - | - | - | - | - | - | -3.29 (-5.15 to -1.43)* |
| $R^2$ | 0.026 | 0.364 | 0.097 | 0.723 | 0.728 | 0.724 | 0.741 | 0.744 |
| Adjusted $R^2$ | 0.024 | 0.362 | 0.094 | 0.717 | 0.721 | 0.718 | 0.735 | 0.737 |

Data are unstandardized estimate (95%CI). Models 1 and after are adjusted for CT scanner model (not reported here). ES: ever smokers; COPD: chronic obstructive pulmonary disease; $\dot{V}O_{2peak}$: peak rate of oxygen consumption; sqrtCal/week: square-root of calories expended per week in moderate or greater intensity exercise; $TLV_{CT}$: total lung volume on inspiratory CT; $TLV_{CT}/TLC_{pleth}$: depth of inspiration at CT; $FEV_1$: post-bronchodilator forced expiratory volume in 1 sec, and LnLAA-950%: natural logarithm of low-attenuating areas below -950 Hounsfield units.
$^{*}p<0.001$, $^{\$}p<0.05$

| Table E5: General Linear Regression Models for DLCO | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Model 0 | Model 0 + $\dot{V}O_{2peak}$ | Model 0 + MVPA | Model 1 | Model 1 + $\dot{V}O_{2peak}$ | Model 1 + MVPA | Model 2 | Final |
| Intercept | 21.68 (20.86 to 22.5)* | 10.15 (9.09 to 11.2)* | 18.32 (17.28 to 19.36)* | 8.51 (0.98 to 16.04)$ | 6.1 (-0.96 to 13.16) | 8.99 (1.53 to 16.45)$ | 5.84 (-0.98 to 12.66) | 6.2 (-0.65 to 13.04) |
| ES | 0.54 (-0.51 to 1.59) | 0.12 (-0.68 to 0.93) | 0.73 (-0.27 to 1.74) | -0.63 (-1.22 to -0.03) | -0.34 (-0.9 to 0.22) | -0.52 (-1.11 to 0.07) | -0.31 (-0.85 to 0.23) | 0.28 (-1.22 to 1.78) |
| COPD | -0.85 (-1.94 to 0.25) | -0.49 (-1.33 to 0.35) | -0.79 (-1.84 to 0.25) | -2.07 (-2.69 to -1.44)* | -1.39 (-1.99 to -0.8)* | -1.99 (-2.61 to -1.37)* | -0.35 (-1.03 to 0.33) | -1.6 (-3.2 to -0.01)$ |
| Never-smoker | - | - | - | - | - | - | - | - |
| $\dot{V}O_{2peak}$ (L/min) | - | 6.84 (6.34 to 7.34)* | - | - | 3.23 (2.7 to 3.77)* | - | 3.04 (2.5 to 3.58)* | 2.95 (2.19 to 3.71)* |
| sqrtCal/week | - | - | 0.08 (0.07 to 0.1)* | - | - | 0.02 (0.01 to 0.04)* | 0.01 (0 to 0.02)$ | 0.01 (0 to 0.02)$ |
| Female | - | - | - | -0.91 (-1.66 to -0.16)$ | -0.11 (-0.82 to 0.6) | -0.91 (-1.66 to -0.17)$ | -0.53 (-1.23 to 0.18) | -0.51 (-1.21 to 0.2) |
| Caucasian | - | - | - | -0.25 (-2.33 to 1.82) | -0.75 (-2.7 to 1.19) | -0.47 (-2.53 to 1.59) | -0.47 (-2.35 to 1.41) | -0.6 (-2.48 to 1.28) |
| Age (years) | - | - | - | -0.18 (-0.21 to -0.15)* | -0.11 (-0.14 to -0.08)* | -0.17 (-0.2 to -0.15)* | -0.08 (-0.11 to -0.05)* | -0.08 (-0.11 to -0.05)* |
| Height (cm) | - | - | - | 0.04 (0 to 0.09) | 0.03 (-0.02 to 0.07) | 0.04 (-0.01 to 0.08) | 0.01 (-0.04 to 0.05) | 0.01 (-0.04 to 0.05) |
| Body mass (kg) | - | - | - | 0.07 (0.06 to 0.09)* | 0.05 (0.03 to 0.07)* | 0.07 (0.05 to 0.09)* | 0.06 (0.04 to 0.08)* | 0.06 (0.04 to 0.07)* |
| $V_A$ (L) | - | - | - | 2.56 (2.27 to 2.86)* | 2.08 (1.79 to 2.37)* | 2.47 (2.18 to 2.77)* | 2.02 (1.69 to 2.35)* | 2 (1.68 to 2.33)* |
| $FEV_1$ (L) | - | - | - | - | - | - | 0.69 (0.09 to 1.28)$ | 0.69 (0.09 to 1.29)$ |
| LnLAA-950% | - | - | - | - | - | - | -1.02 (-1.27 to -0.77)* | -0.99 (-1.24 to -0.73)* |
| INTERACTIONS | - | - | - | - | - | - | - | - |
| $\dot{V}O_{2peak}$ x ES | - | - | - | - | - | - | - | -0.33 (-1.15 to 0.49) |
| $\dot{V}O_{2peak}$ x COPD | - | - | - | - | - | - | - | 0.75 (-0.11 to 1.61) |
| $R^2$ | 0.008 | 0.421 | 0.094 | 0.702 | 0.739 | 0.708 | 0.758 | 0.760 |
| Adjusted $R^2$ | 0.006 | 0.419 | 0.091 | 0.696 | 0.734 | 0.702 | 0.752 | 0.754 |

Data are unstandardized estimate (95%CI). ES: ever smokers; COPD: chronic obstructive pulmonary disease; $\dot{V}O_{2peak}$: peak rate of oxygen consumption; sqrtCal/week: square-root of calories expended per week in moderate or greater intensity exercise; $V_A$: alveolar volume; $FEV_1$: post-bronchodilator forced expiratory volume in 1 sec, and LnLAA-950%: natural logarithm of low-attenuating areas below -950 Hounsfield units.
$^{*}p<0.001$, $^{\$}p<0.05$

***Secondary aim:***

General linear regression models were also built to evaluate the associations between MVPA (sqrt Cal/week in ≥moderate intensity PA), TBV, BV5, BV5-10, TAC and $D_LCO$ with $\dot{V}O_{2peak}$. The models were adjusted for group, sex, race/ethnicity, age, height, body mass CT scanner model, TLVCT, TLVCT/TLCpleth (or $V_A$), $FEV_1$, and LnLAA-950%, and MVPA. The final models further included an interaction term between the independent variable and group (NS, ES, COPD). Only the final models are reported in Table E6.

TAC was not significantly associated with $\dot{V}O_{2peak}$ in the fully adjusted model (p=0.65), and no significant group by TAC interaction was observed (p=0.37). We found significant associations between CT-derived pulmonary vascular measures (TBV, BV5, and BV5-10) and $\dot{V}O_{2peak}$ in fully adjusted models (all p<0.001). Further, significant group by CT-pulmonary vascular (TBV, BV5 and BV5-10) interactions were observed in COPD but not ES (COPD*TBV, p=0.003; ES*TBV, p=0.24; COPD*BV5, p=0.005; ES*BV5, p=0.61; COPD*BV5-10, p=0.01; ES*BV5-10, p=0.17). Finally, $D_LCO$ was associated with $\dot{V}O_{2peak}$ in adjusted models (p<0.001), but no significant group by $D_LCO$ interaction was observed (p=0.30).

When included in the same model, $D_LCO$ had the strongest association with $\dot{V}O_{2peak}$ (standardized β=0.26, p<0.001), followed by TBV (standardized β=0.25, p=0.01), whereas LnLAA950% was not associated with $\dot{V}O_{2peak}$ (standardized β =0.02, p= 0.44) (Table E7).

| **Table E6: Separate general linear regression models evaluating associations with $\dot{V}O_{2peak}$ (L/min)** | | | |
|---|---|---|---|
| Parameter | Unstandardized estimate | 95%CI | p-value |
| TAC | | | |
| Model 1 | -0.001 | -0.001 to -0.0001 | 0.013 |
| Final model | -0.0002 | -0.001 to 0.001 | 0.65 |
| TBV | | | |
| Model 1 | 0.004 | 0.003 to 0.006 | <0.001 |
| Final model | 0.005 | 0.003 to 0.007 | <0.001 |
| BV5 | | | |
| Model 1 | 0.004 | 0.001 to 0.006 | 0.007 |
| Final model | 0.006 | 0.003 to 0.010 | <0.001 |
| BV5-10 | | | |
| Model 1 | 0.005 | 0.002 to 0.009 | 0.002 |
| Final model | 0.008 | 0.004 to 0.012 | <0.001 |
| $D_LCO$ | | | |
| Model 1 | 0.037 | 0.030 to 0.043 | <0.001 |
| Final model | 0.042 | 0.033 to 0.051 | <0.001 |
| Data are unstandardized estimate (95%CI). Models 1 and after are adjusted for CT scanner model (not reported here). Model 1: adjusted for group, sex, race/ethnicity, age, height, body mass, $FEV_1$, LnLAA-950%, CT scanner model, $V_A$ (or TLV and TLV/TLC) and MVPA; Final model: Model 1+interaction term between the IV and group. $TLV_{CT}$: total lung volume on inspiratory CT; $TLV_{CT}/TLC_{pleth}$: depth of inspiration at CT; $FEV_1$: post-bronchodilator forced expiratory volume in 1 sec, and LnLAA-950%: natural logarithm of low-attenuating areas below -950 Hounsfield units. | | | |

| **Table E7: General linear regression model evaluating associations between DLCO, LnLAA-950%, and TBV with $\dot{V}O_{2peak}$ (L/min)** | | | |
|---|---|---|---|
| Parameter | Standardized estimate | SE | p-value |
| TBV | 0.248 | 0.001 | <0.001 |
| LnLAA-950% | 0.023 | 0.0179 | 0.44 |
| $D_LCO$ | 0.255 | 0.0028 | <0.001 |
| Data are standardized estimate (SE). Models adjusted for group, sex, race/ethnicity, age, height, body mass, MVPA, CT scanner model, $TLV_{CT}$: total lung volume on inspiratory CT; $TLV_{CT}/TLC_{pleth}$: depth of inspiration at CT. TBV: total vessel volume, $FEV_1$: post-bronchodilator forced expiratory volume in 1 sec, and LnLAA-950%: natural logarithm of low-attenuating areas below -950 Hounsfield units. | | | |