Abstract

Background: Individuals with chronic obstructive pulmonary disease (COPD) have higher than normal ventilatory equivalents for carbon dioxide ($V_E/\dot{V}CO_2$) during exercise. There is growing evidence that emphysema on thoracic computed tomography (CT) scans is associated with poor exercise capacity in COPD patients with only mild-to-moderate airflow obstruction. We hypothesized that emphysema is an underlying cause of microvascular dysfunction and ventilatory inefficiency, which in turn contributes to reduced exercise capacity. We expected ventilatory inefficiency to be associated with a) the extent of emphysema; b) lower diffusing capacity for carbon monoxide; c) a reduced pulmonary blood flow response to exercise; and d) reduced exercise capacity.

Methods: In a cross-sectional study, 19 subjects with mild-to-moderate COPD (mean ± SD FEV₁= 82 ± 13% predicted, 12 GOLD grade 1) and 26 age-, sex-, and activity-matched controls underwent a ramp-incremental symptom-limited exercise test on a cycle ergometer. Ventilatory inefficiency was assessed by the minimum $V_E/\dot{V}CO_2$ value (nadir). A subset of subjects also completed repeated constant work rate exercise bouts with non-invasive measurements of pulmonary blood flow. Emphysema was quantified as the percentage of attenuation areas below -950 Housefield Units on CT scans. An electronic scoresheet was used to keep track of emphysema sub-types.

Results: COPD subjects typically had centrilobular emphysema (76.8 ± 10.1% of total emphysema) in the upper lobes (upper/lower lobe ratio= 0.82 ± 0.04). They had lower peak oxygen uptake ($\dot{V}O_2$), higher $V_E/\dot{V}CO_2$ nadir and greater dyspnea scores than
controls (p<0.05). Lower peak \( \dot{V}O_2 \) and worse dyspnea were found in COPD subjects with \( \dot{V}_E/\dot{V}CO_2 \) nadirs \( \geq 30 \). COPD subjects had blunted increases in pulmonary blood flow from rest to iso-\( \dot{V}O_2 \) exercise (p<0.05). Higher \( \dot{V}_E/\dot{V}CO_2 \) nadir in COPD subjects correlated with emphysema severity (r= 0.63), which in turn correlated with reduced lung diffusing capacity (r= -0.72) and blunted changes in pulmonary blood flow from rest to exercise (r= -0.69) (p<0.01).

**Conclusions:** Ventilation “wasted” in emphysematous areas is associated with reduced exercise ventilatory efficiency in mild-to-moderate COPD. Exercise ventilatory inefficiency links structure (emphysema) and function (gas transfer) to a key clinical outcome (reduced exercise capacity) in COPD patients with modest spirometric abnormalities.
Acknowledgements

The original work presented in this document was made possible by the generous members of the public that decided to participate in the research study. Their time and effort were greatly appreciated by the entire staff at the Laboratory of Clinical Exercise Physiology (LACEP), and we recognize that several participants even commuted from outside the Kingston area for their visitations. I sincerely thank all the participants who completed the study, as well as those who were motivated to participate but were excluded for reasons outside their control.

The LACEP was established owing to the Canadian Foundation for Innovation’s Leader Operating Fund. Financial support was provided to me by the McLaughlin Fellowship.

I am indebted to Dr. J Alberto Neder and Dr. John Fisher for supervising me for the past two years. Dr. Fisher taught me respiratory physiology while I was an undergraduate student, and he was the first person who encouraged me to enroll in graduate studies. Dr. Neder gave me the opportunity to help establish the LACEP in Kingston General Hospital. It was an invigorating feeling to have been part of something new that will have a positive impact on the lives of persons with cardiorespiratory disease. Future staff and students should adopt Dr. Neder’s comprehensive “From Mouth to Mitochondria” philosophy to facilitate the accomplishment of his numerous goals, all of which are set to improve the quality of life of patients.
The LACEP is privileged to have the world-renowned Respiratory Investigations Unit (RIU) as its neighbor and collaborator. Dr. Denis O'Donnell was the co-investigator of the project described herein, and his publications with Kathy Webb, Dr. Amany Elbehairy, and other colleagues have had a large influence on this work. I would like to thank Kathy Webb, Kristin MacLeod, and Casey Ciavaglia for dedicating their valuable time to performing pulmonary function tests. A close relationship between the LACEP and the RIU is an exciting development, and I hope future graduate students in either laboratory recognize their fortunate position.

There are numerous colleagues and peers that deserve more praise than I can offer in this document. Briefly: Dr. Ian Spreadbury’s administrative prowess was essential for establishing the LACEP, and his technical skills were often needed to train staff members such as myself; Dr. Daniel Hirai provided me with instruction on numerous occasions, and together we met several of the lab’s pesky challenges; Joel Zelt and I complemented and exchanged each other’s knowledge of exercise physiology and the life sciences, respectively; Luiza Castanhas and Ingrid Rafferty skillfully managed the lab’s numerous projects, financial administration, and clinical operations. They also provided administrative and technical assistance for the project described herein. Luiza Castanhas’ persistence and natural creativity should benefit her in her future career. Ingrid Rafferty’s kindness and intelligence will make her a great mother to Jack Rafferty; Dr. Nicolle Domnik gave me valuable advice and helped me navigate the ‘unwritten’ aspects of a graduate program in the Department of Biomedical and Molecular Sciences; Guests and
visiting students that shared their knowledge and made the LACEP an international enterprise include Dr. Samuel Verges, Dr. Daniel Langer, Marianne Da Silva, Camilla Diniz, Aida Zaza, and Antenor Rodrigues.

My family has always provided me with unconditional support, and this thesis is dedicated to them. The type of polarity that my brother Caleb Jones and I have subconsciously achieved is common among brothers, and should be perceived positively in this case. I respect and admire Caleb and wish him the best in his future career. My father John Jones and I are more alike than he may recognize; overbearing parents often nurture resentment rather than admiration or agreement, and it is a reflection of my father’s great character that his subtle guidance encourages my imitation of him. My mother Shawn Jones displays adulation that is out of proportion to what my abilities deserve, but I appreciate it nonetheless. Lots of love go out to my brother, father, and mother.
Statement of Originality

I hereby certify that all of the work described within this thesis is the original work of the author. Any published (or unpublished) ideas and/or techniques from the work of others are fully acknowledged in accordance with the standard referencing practices.

Joshua Henry Jones

August, 2016
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List of Abbreviations

A bar above any symbol indicates a mixed or mean value (e.g. \( \bar{v} \) mixed venous blood, \( \bar{E} \) mixed expired gas)

A dot above any symbol indicates a time derivative (e.g. \( \dot{V} \) for ventilation, \( \dot{Q} \) for blood flow)

**Primary Symbols for Gas Phase (Large Capital Letters)**

D: Diffusing capacity

F: Fractional concentration in a dry gas phase

f: Respiratory frequency

K: Transfer coefficient

P: Gas pressure or partial pressure

RER: Respiratory exchange ratio

V: Gas volume

\( \dot{V} \): Gas volume per unit time

**Secondary Symbols for Gas Phase (Small Capital Letters)**

A: Alveolar

B: Barometric

D: Dead space

E: Expired

\( \bar{E} \): Mixed expired

ET: End tidal
I: Inspired
L: Lung
T: Tidal

BTPS: Body temperature and pressure, saturated with water vapor
STPD: Standard temperature and pressure (0°C, 760mmHg), dry

**Primary Symbols for Blood Phase**

C: Concentration of gas in blood phase

\( \dot{Q} \): Volume of blood per unit time

S: Percentage saturation of hemoglobin with oxygen

**Secondary Symbols for Blood Phase**

a: Arterial blood

c: Capillary blood

v: Venous blood

\( \bar{v} \): Mixed venous blood

**Tertiary Symbols for Particular Gases**

CO: Carbon Monoxide

CO\(_2\): Carbon Dioxide

He: Helium

N\(_2\): Nitrogen

N\(_2\)O: Nitrous Oxide

NO: Nitric Oxide
O₂: Oxygen
SF₆: Sulfur Hexafluoride

**Lung Volumes and Pulmonary Function**

EELV: End-expiratory lung volume

FEF₂₅-₇₅%: Forced expiratory flow between 25% and 75% of forced vital capacity

FEV₁: Forced expired volume in 1 second

FEV₁/FVC: Forced expiratory volume to forced vital capacity ratio

FRC: Functional residual capacity

FVC: Forced vital capacity with maximal expiratory effort

IC: Inspiratory capacity

IRV: Inspiratory reserve volume

MVV: Maximal voluntary ventilation

PCF: Poorly communicating fraction

RV: Residual volume

RV/TLC (%): Residual volume to total lung capacity ratio expressed as a percentage

sRaw: Specific airway resistance

TLC: Total lung capacity

V₆: Physiological dead space

V₆/V₁: Physiological dead space fraction of tidal volume

V₁: Tidal volume
Gas Exchange and Miscellaneous Physiological Variables

BMI: Body mass index

GET: Gas exchange threshold

HR: Heart rate

PEEPi: Intrinsic positive end-expiratory pressure

$P_{es}$: Esophageal pressure

$S_{p}O_{2}$: Arterial oxygen saturation estimated by pulse oximetry

$\dot{V}_{A}/\dot{Q}$: Alveolar ventilation/perfusion ratio

VCP: Ventilatory compensation point

$\dot{V}CO_{2}$: Carbon dioxide output

$\dot{V}E/\dot{V}CO_{2}$: Ventilatory equivalent for one unit of carbon dioxide output

$\dot{VO}_{2}$: Oxygen uptake

$\dot{VO}_{2}{\text{PEAK}}$: Maximal aerobic power

Technical

CPET: Cardiopulmonary exercise test

CT: Computed Tomography

ECG: Electrocardiogram

HRCT: High-resolution computed tomography

HU: Housefield Units

IGR: Inert gas rebreathing

LAA: Low attenuation areas
MIGET: Multiple inert gas elimination technique

MRI: Magnetic resonance imaging

Pi10: Airway wall thickness of airways standardized to hypothetical airways with an internal perimeter of 10mm

RPM: Rotations per minute

WR: Work rate

6MWD: 6-minute walk distance

**Other**

ADP: Adenosine diphosphate

ATS: American Thoracic Society

CAT: COPD Assessment Test

COPD: Chronic obstructive pulmonary disease

ERS: European Respiratory Society

GOLD: Global Initiative for Obstructive Lung Disease

ILD: Interstitial lung disease

LACEP: Laboratory of Clinical Exercise Physiology

LLN: Lower limit of normality

ln: Natural logarithm

mMRC: Modified Medical Research Council

RIU: Respiratory Investigations Unit
Chapter 1

Introduction

1.1 Chronic Obstructive Pulmonary Disease

The 2016 Global Initiative for Chronic Obstructive Lung Disease (GOLD) report defines Chronic Obstructive Pulmonary Disease (COPD) as “a common preventable and treatable disease characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung parenchyma” (Vogelmeier et al., 2016). It is a leading cause of death and disability worldwide (Murray & Lopez, 2013), and is projected to become the 3rd leading cause of death in the world by 2020 (Murray & Lopez, 1997). In adults ≥40 years of age, the global prevalence and Canadian prevalence of COPD are ~9-10% and 9.6%, respectively (Buist et al., 2007; Raghavan et al., 2012). These statistics may be underestimated (Evans et al., 2014), and will likely grow as the population ages (Feenstra et al., 2001), thereby increasing the already substantial socioeconomic burden of COPD (Wouters, 2003).

Tobacco smoke inhalation is the primary cause of COPD, and is responsible for the heterogeneity of the disease due to the variable degrees of damage it inflicts on multiple pulmonary system components. The most common and distressing symptom patients experience is dyspnea, which becomes intolerable at progressively lower exercise workloads as the disease progresses (O’Donnell et al.,...
Important pathophysiological features that become more severe as COPD progresses include abnormal pulmonary mechanics (O’Donnell et al., 2014a), ventilation-perfusion inequality (Rodríguez-Roisin et al., 2009), and ventilatory inefficiency in expiring metabolically-produced carbon dioxide (CO₂), particularly upon the stress of exercise (Neder et al., 2015a).

1.2 Diagnosis and Assessment of Disease Severity

The disease is defined and graded in severity by the functional result of decreased airway cross-sectional area, airway patency during expiration, and forced vital capacity as measured by pulmonary function tests before and after the administration of bronchodilators. Spirometry is used to define and stratify COPD because of its simplicity and reproducibility rather than its relevance to functional limitations, cardiovascular co-morbidities, and symptoms in a given patient (Vogelmeier et al., 2016). Clinical management of a patient is aided by further stratification of disease severity based on the patient’s symptoms and tendency to have exacerbations (Vogelmeier et al., 2016).

The GOLD criterion for airway obstruction is a forced expiratory volume in one second divided by forced vital capacity (FEV₁/FVC) quotient less than 0.7 (Vogelmeier et al., 2016). Alternatively, or as a complement, the criterion for obstruction can be an FEV₁/FVC less than the lower limit of normality (LLN). The LLN is the lower 5th percentile of the frequency distribution of reference population values (Pellegrino et al., 2005). A substantial fraction of smokers with
FEV₁/FVC < 0.7 have a FEV₁/FVC that is greater than the age-corrected LLN (Figure 1). There is ongoing controversy whether the increased rate of potential false-positives for COPD in older individuals according to the 0.7 criterion (Hardie et al., 2002; Hansen et al., 2007) is clinically-relevant (Miller et al., 2009; Hoesein et al., 2011). Nevertheless, the authoritative GOLD document still endorses this value as a compromise between a statistically sounder metric (the LLN) and the time-honored threshold of 0.7.

Figure 1. The potential over-diagnosis of obstruction in older smokers. If the FEV₁/FVC<0.7 criterion is used to determine the presence of obstruction, a substantial number of individuals >40 years old with normal lung function (FEV₁/FVC>LLN) will be diagnosed. Source: unpublished data from the Queen’s Pulmonary Function Database.

In order to grade the severity of airflow limitation, the post-bronchodilator FEV₁ is compared to its predicted value expressed as a function of sex, age, and
height (Table 1). For the sake of simplicity, the GOLD document suggests the 80% predicted value as the threshold to indicate abnormality.

Table 1. The GOLD stratification of COPD severity. From (Vogelmeier et al., 2016) with permission from the publisher.

| 2016 Global Initiative for Chronic Obstructive Lung Disease (GOLD) Classification of Chronic Obstructive Pulmonary Disease Severity |
|---|---|
| Presence of Obstruction | Post-bronchodilator FEV₁/FVC < 0.70 |
| GOLD Grade I | Mild | FEV₁ ≥ 80% predicted |
| GOLD Grade II | Moderate | 50% ≤ FEV₁ < 80% predicted |
| GOLD Grade III | Severe | 30% ≤ FEV₁ < 50% predicted |
| GOLD Grade IV | Very Severe | FEV₁ < 30% predicted |

There is well-established evidence that the large majority of patients with COPD have mild-to-moderate disease (Buist et al., 2007; Raghavan et al., 2012). However, there is growing evidence that the 80% predicted FEV₁ threshold does not coincide with the LLN in many patients. The use of a fixed value may lead to a diagnosis of COPD for individuals with statistically normal lung function (Hardie et al., 2002; Hansen et al., 2007; Hoesein et al., 2011). Recognizing the importance of symptoms to gauge treatment, patients are also categorized into group A or B depending on their modified Medical Research Council (mMRC) or COPD Assessment Test (CAT) scores (Fletcher et al., 1959; Brooks, 1982; Jones et al., 2009). CAT scores <10 or mMRC scores of 0-1 indicate less symptoms (group A) while CAT scores ≥10 or mMRC scores ≥2 indicate more symptoms (group B) (Vogelmeier et al., 2016) (Table 2).
Table 2. Modified Medical Research Council scores with associated GOLD symptom categorization (Brooks, 1982; Vogelmeier et al., 2016).

<table>
<thead>
<tr>
<th>mMRC Score</th>
<th>Description of Severity of Breathlessness</th>
<th>GOLD Symptom Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Patient experiences breathlessness during strenuous exercise</td>
<td>A</td>
</tr>
<tr>
<td>1</td>
<td>Patient experiences breathlessness when hurrying on the level or walking up a slight hill</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>The patient walks slower than people of the same age on the level because of breathlessness, or has to stop for breath when walking at their own pace on the level</td>
<td>B</td>
</tr>
<tr>
<td>3</td>
<td>Patient stops for breath after walking about 100 meters or after a few minutes on the level</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>Patient is too breathless to leave the house or is breathless when dressing or undressing</td>
<td>B</td>
</tr>
</tbody>
</table>

The American Thoracic Society (ATS) and European Respiratory Society (ERS) have published a grading scheme for the severity of any spirometric abnormality- excluding upper airway obstruction- based on post-bronchodilator FEV$_1$ as a percentage of its predicted value (Table 3) (Pellegrino et al., 2005).

There is a potential for disagreement between the severity of airflow limitation as defined by the GOLD document and the severity of spirometric abnormality in a subject with COPD defined by ATS/ERS criteria. For example, subjects with moderate COPD according to GOLD criteria may have mild spirometric abnormality according to ATS/ERS criteria (Table 1 and Table 3, respectively). As long as the airflow limitation is not reversible, chronic airway
obstruction is present according to both GOLD and ATS/ERS criteria. Both sets of
criteria are limited in that they use percent-predicted FEV$_1$ as a marker of
stratification, which oversimplifies the vast heterogeneity of airway resistance, gas
transfer, and lung volumes within grades of COPD (Deesomchok et al., 2010). It
also does not reveal the presence or extent of vascular dysfunction in any patient.

Table 3. ATS/ERS stratification of spirometric abnormalities with the exception of
upper airway obstruction. Reproduced with permission of the European Respiratory
Society ©. European Respiratory Journal Nov 2005, 26 (5) 948-968; DOI: 10.1183/09031936.05.00035205

<table>
<thead>
<tr>
<th>Severity</th>
<th>FEV$_1$ % predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Moderate</td>
<td>60-69</td>
</tr>
<tr>
<td>Moderately severe</td>
<td>50-59</td>
</tr>
<tr>
<td>Severe</td>
<td>35-49</td>
</tr>
<tr>
<td>Very severe</td>
<td>&lt;35</td>
</tr>
</tbody>
</table>

1.3 Exercise Ventilatory Inefficiency in Health and Disease

1.3.1 Basic Physiological Concepts

It has long been established that changes in exercise pulmonary ventilation
($\dot{V}_E$, liters per minute, L min$^{-1}$), at least before the development of metabolic
acidosis (or hypoxemia in disease), are closely commensurate to the rate at which
metabolically-produced CO$_2$ is expired from the lungs ($\dot{V}$CO$_2$, L min$^{-1}$) (Whipp et
al., 1984). Thus, the $\dot{V}_E$-$\dot{V}$CO$_2$ relationship has been termed “ventilatory efficiency”
(Forster & Pan, 1988) because the ‘goal’ of ventilation is to increase with metabolic
demand in order to maintain blood gas and pH homeostasis (Whipp et al., 1984). If \( \dot{V}_E \) did not increase in proportion to \( \dot{V}_{CO_2} \), metabolic acidosis would ensue, resulting in neural and cardiac cellular dysfunction. Conversely, if \( \dot{V}_E \) increased out of proportion to \( \dot{V}_{CO_2} \), metabolic alkalosis would develop and also lead to cellular dysfunction (Wasserman et al., 1967).

In this context, \( \dot{V}_E \) to washout a given amount of \( CO_2 \) is higher the lower the level at which arterial partial pressure for \( CO_2 \) (\( P_aCO_2 \), milliliters of mercury, mmHg) is regulated by the respiratory controller. More \( \dot{V}_E \) is needed to keep \( P_aCO_2 \) at a given level if a larger amount of \( \dot{V}_E \) is “wasted” in the dead space (\( V_D, L \)) (Equation 1) (Whipp et al., 1984):

\[
\frac{\dot{V}_E}{\dot{V}_{CO_2}} = \frac{k}{P_aCO_2 \left(1 - \frac{V_D}{V_T}\right)}
\]

where \( \dot{V}_E/\dot{V}_{CO_2} \) is the ventilatory equivalent for a unit of \( CO_2 \) output and \( V_D/V_T \) is the physiological (anatomic plus alveolar) dead space fraction of tidal volume (\( V_T, L \)). Of note, \( V_D/V_T \) decreases hyperbolically as exercise progresses, i.e., a larger fraction of \( V_T \) participates in gas exchange as \( \dot{V}_E \) increases (Figure 2A). This happens in part because the effect of increased \( V_D \) due to expansion of the large airways is diminished as better ventilation-perfusion matching in expanded alveoli occurs (Palange et al., 2007; Whipp, 2008). Moreover, \( V_T \) increases markedly owing to a large increase in end-inspiratory lung volume and a small, but
important, decrease in end-expiratory lung volume; thus, V_T remains on the most compliant portion of the pressure-volume relationship (Johnson et al., 1999).

Figure 2. Selected ventilatory and gas exchange responses to incremental exercise in a healthy subject. See text for details.

In healthy young male subjects, the V_D/V_T ratio decreases from 0.3-0.35 at rest to 0.20 during moderate intensity exercise on a cycle ergometer. The ratio declines to 0.15 during heavy constant power output exercise or as an incremental test reaches the heavy intensity (Figure 2A). During incremental exercise, there is a corresponding hyperbolic decrease in V_E/VCO_2 ratio (Figure 2B) so P_aCO_2 is maintained close to its resting value (⇌, Equation 2) during moderate exercise in
healthy humans (Whipp et al., 1984; Forster et al., 2012; Dempsey & Smith, 2014) (Figure 2C). If $\dot{V}_E/\dot{V}CO_2$ did not decrease in tandem with $V_D/V_T$ in moderate exercise, the resulting alveolar hyperventilation would lower $P_aCO_2$ and lead to progressive respiratory alkalosis (Wasserman et al., 1967). The mechanisms that cause the hyperbolic decreases in $\dot{V}_E/\dot{V}CO_2$ and $V_D/V_T$ are still unclear (see (Poon & Tin, 2013) and (Ward, 2013) for a debate on the topic). At the ventilatory compensation point (VCP), $\dot{V}_E$ increases out of proportion to $\dot{V}CO_2$, leading to respiratory alkalosis to compensate for lactacidemia (Figure 2B-D).

$$\Leftrightarrow P_aCO_2 = \frac{k}{\left(\downarrow \frac{\dot{V}_E}{\dot{V}CO_2}\right)\left(1 - \downarrow \frac{V_D}{V_T}\right)}$$ (2)

These considerations provide the physiological bases for the assertion that the $\dot{V}_E/\dot{V}CO_2$ profile provides useful information on the $V_D/V_T$ trajectory, particularly if $P_aCO_2$ is concurrently measured (Whipp et al., 1984; Sun et al., 2002). The major assumptions, however, are the absence of mechanical constraints to $\dot{V}_E$ increase (Ward, 2007), i.e. the “output” ($\dot{V}_E$) can appropriately adjust to its determinants ($\dot{V}CO_2$, $V_D/V_T$ and $P_aCO_2$), and there is neither exercise-induced hypercapnia nor increased chemostimulation by, for example, hypoxemia (Whipp et al., 1984; Palange et al., 2007; Forster et al., 2012; Dempsey & Smith, 2014).

In practice, the $\dot{V}_E-\dot{V}CO_2$ relationship is analyzed in the $\dot{V}_E/\dot{V}CO_2$ ratio versus $\dot{V}CO_2$ plot (Figure 2B) and in the $\dot{V}_E$ versus $\dot{V}CO_2$ plot (Figure 2D) (Palange et al., 2007). The minimum value for $\dot{V}_E/\dot{V}CO_2$ (nadir) is reached just before $\dot{V}_E$
compensates for lactacidosis at the VCP (Figure 2B-D). Thus, $\dot{V}_{E}/\dot{V}_{CO_2}$ nadir and $\dot{V}_{E}/\dot{V}_{CO_2}$ at the lactate threshold are almost indistinguishable in normal subjects (Sun et al., 2002), being independent of the rate of [H+] accumulation. The nadir is highly reproducible in normal subjects (Sun et al., 2002) and in subjects with COPD (Barron et al., 2014). As expected, $\dot{V}_{E}/\dot{V}_{CO_2}$ peak is higher than the nadir as the former incorporates the hyperventilatory response to acidosis. In other words, $\dot{V}_{E}/\dot{V}_{CO_2}$ peak, by definition, does not constitute an index of ventilatory inefficiency in those who are able to exercise beyond the VCP. Most subjects with moderate to severe COPD, however, either do not reach the VCP or are unable to further increase $\dot{V}_{E}$. Thus, $\dot{V}_{E}/\dot{V}_{CO_2}$ nadir and peak are equivalent in most subjects with COPD, with the exception of less impaired subjects (Neder et al., 2015a).

It is important to recognize that, mathematically, the $\dot{V}_{E}/\dot{V}_{CO_2}$ response contour depends on how $\dot{V}_{E}$ changes relative to $\dot{V}_{CO_2}$ taking into consideration its starting point (Whipp et al., 1984; Palange et al., 2007; Poon & Tin, 2013; Ward, 2013). The former is reflected by the slope of the regression line between $\dot{V}_{E}$ and $\dot{V}_{CO_2}$ and the latter by its intercept on the ordinate (i.e., $\dot{V}_{E}$ when $\dot{V}_{CO_2}=0$) (Figure 2D). The minimum value (nadir) of $\dot{V}_{E}/\dot{V}_{CO_2}$ approximates the sum of the slope and intercept (Figure 2B). Considering that most healthy subjects have a positive intercept (Sun et al., 2002), the nadir is slightly greater than the slope. It should be noted that considering data points after the VCP will necessarily increase the slope and reduce the intercept. However, a linear regression line up to peak exercise
generally fits the $\dot{V}_E$ response in most subjects with advanced COPD because of their reduced exercise capacity (Neder et al., 2015a). More detailed considerations on the impact of varying slope and intercept on the nadir are provided in Appendix A.

1.3.2 Exercise Ventilatory Inefficiency: A Key Physiological Abnormality in Mild-to-Moderate COPD

There is growing evidence that reduced ventilatory efficiency is a key physiological abnormality in symptomatic patients with largely preserved FEV$_1$ and smokers without obstruction (Ofir et al., 2008; Chin et al., 2013; O’Donnell et al., 2014b; Guenette et al., 2014; Neder et al., 2015a; Elbehairy et al., 2015b, 2015a) (Table 4). Subjects with mild-to-moderate COPD (GOLD grades I-II) frequently have higher intercepts and/or slopes leading to higher $\dot{V}_E$/\dot{V}CO$_2$ nadirs (Ofir et al., 2008; Chin et al., 2013; O’Donnell et al., 2014b; Guenette et al., 2014; Neder et al., 2015a; Elbehairy et al., 2015b, 2015a). As shown in Figure 3, worsening mechanical constraints on $\dot{V}_E$ as the disease progresses (O’Donnell et al., 2014a), and hypercapnia in end-stage disease (O’Donnell et al., 2002; Poon et al., 2015), lead to lower slopes but higher intercepts. In non-hypercapnic subjects with milder airflow obstruction, all ventilatory inefficiency parameters (nadir, slope and intercept) increase in response to increased “wasted” ventilation (Figure 3).
Table 4. Overview of key studies on ventilatory efficiency in mild-to-moderate COPD.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Disease Severity</th>
<th>Main Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ofir et al., 2008)</td>
<td>FEV$_1$=91 ± 8%</td>
<td>↑ $\dot{V}<em>E$/$\dot{V}</em>{CO_2}$ nadir in smokers with chronic dyspnea</td>
</tr>
<tr>
<td>(Guenette et al., 2011)</td>
<td>FEV$_1$= 86 ± 11%</td>
<td>No sex-related effect on $\dot{V}_E$/CO$_2$ nadir</td>
</tr>
<tr>
<td>(Chin et al., 2013)</td>
<td>FEV$_1$= 87 ± 11%</td>
<td>↑ $\dot{V}<em>E$/$\dot{V}</em>{CO_2}$ with added external dead space in mild COPD</td>
</tr>
</tbody>
</table>
| (Teopompi et al., 2014)        | FEV$_1$= 26-80%  | ↑ $\dot{V}_E$/$\dot{V}_{CO_2}$ intercept related to greater dynamic hyperinflation  
|                                |                  | ↑ $\dot{V}_E$/$\dot{V}_{CO_2}$ slope associated with lower maximal exercise capacity |
| (Guenette et al., 2014)        | FEV$_1$= 93 ± 9% | ↑ $\dot{V}_E$/$\dot{V}_{CO_2}$ throughout incremental exercise in mild COPD |
| (O’Donnell et al., 2014b)      | GOLD 1 and 2     | ↑ $\dot{V}_E$/$\dot{V}_{CO_2}$ throughout incremental treadmill tests in GOLD 1 and 2 |
| (Neder et al., 2015b)          | GOLD 1 to 4      | ↑ $\dot{V}_E$/$\dot{V}_{CO_2}$ slope associated with ventilation inhomogeneity in GOLD 1&2 |
| (Elbehairy et al., 2015b)      | FEV$_1$=91 ± 10% | ↑ $\dot{V}_E$/$\dot{V}_{CO_2}$ nadir in GOLD grade 1B                      |
| (Neder et al., 2015a)          | GOLD 1 to 4      | ↑ $\dot{V}_E$/$\dot{V}_{CO_2}$ intercept from GOLD 1 to 4 associated with exertional dyspnea  
|                                |                  | ↑ $\dot{V}_E$/$\dot{V}_{CO_2}$ slope in GOLD 1 and 2 but lower slopes in GOLD 3 and 4 |
| (Elbehairy et al., 2015a)      | FEV$_1$=94 ± 10% | ↑ $\dot{V}_E$/$\dot{V}_{CO_2}$ associated with greater V$_D$/V$_T$ in symptomatic GOLD 1 |

Abbreviations and symbols: ↑=increased; FEV$_1$= forced expiratory volume in one second; $\dot{V}_E$: ventilation; $\dot{V}_{CO_2}$: carbon dioxide output; GOLD: Global Initiative for Obstructive Lung Disease.
Figure 3. Effects of COPD severity on the parameters of ventilatory inefficiency. (a) Ventilation (\(\dot{V}_E\))–carbon dioxide output (\(\dot{V}CO_2\)) intercept increased and (b) \(\dot{V}_E\)-\(\dot{V}CO_2\) slope diminished as the disease progressed from GOLD stages (grades) 1 to 4. (c) The nadir remained elevated at each disease severity compared to controls (C). (d) Increasing nadir-slope differences from GOLD stages 1 to 4 reflects the impact of a progressively higher intercept. Reproduced with permission of the European Respiratory Society ©. European Respiratory Journal Feb 2015, 45 (2) 377-387; DOI: 10.1183/09031936.00135514

The physiological bases for these derangements seem to stem from an enlarged \(V_D\) per se rather than a small \(V_T\) or a low \(P_{a}CO_2\) (Figure 4) (Elbehairy et al., 2015a). In fact, external (series) \(V_D\) predictably increased \(\dot{V}_E/\dot{V}CO_2\) in subjects with mild COPD (Chin et al., 2013) (Figure 5). Regardless of the potential mechanism(s) (see Chapter 2), high \(\dot{V}_E/\dot{V}CO_2\) nadir due to increased \(\dot{V}_E\)-\(\dot{V}CO_2\) slope and/or intercept erodes the mechanical reserves throughout incremental exercise,
thereby contributing to exertional dyspnea and exercise intolerance in these subjects (Ofir et al., 2008; Chin et al., 2013; O'Donnell et al., 2014b; Guenette et al., 2014; Elbehairy et al., 2015b, 2015a). This pattern of abnormalities was also seen in most subjects with moderate airflow obstruction (GOLD grade II) (Neder et al., 2015a).

Figure 4. Dead space ventilation in mild COPD. (a) Increased ventilation ($\dot{V}_E$) was associated with (b) an upward displacement of the $\dot{V}_E / \dot{V}CO_2$ ratio, i.e. poor ventilatory efficiency in mild COPD. These abnormalities were secondary to (c) an increased dead space ($V_D$) / tidal volume ($V_T$) ratio due to (d) a high $V_D$. (e-f) Thus, greater $V_D/V_T$ explained the larger difference between $\dot{V}_E$ and alveolar ventilation ($\dot{V}_A$) in COPD subjects than controls. Reprinted with permission of the American Thoracic Society. Copyright © 2016 American Thoracic Society. From Elbehairy AF, et al. Pulmonary Gas Exchange Abnormalities in Mild Chronic Obstructive Pulmonary Disease. Implications for Dyspnea and Exercise Intolerance. Am. J. Respir. Crit. Care Med. 2015; 191: 1384–1394.
Little is known about the structural and functional correlates of $V_{E}-V_{CO_2}$ slope and intercept (and, therefore, the nadir) in COPD. Adding external (series) $V_D$ had a more discernible effect on the intercept than the slope in healthy subjects (Ward & Whipp, 1980; Gargiulo et al., 2014) and in subjects with mild COPD (Chin et al., 2013) (Figure 5). However, series $V_D$ may not perfectly mimic alveolar (parallel) $V_D$ found in diseased humans because the former is associated with a greater $CO_2$ airway load that might further challenge the ventilatory control mechanisms (Poon & Tin, 2013; Ward, 2013). In fact, interpretation of an increased physiological $V_D$ in diseased humans is fraught with complexities.
The crucial observation made by Bohr in 1891 (Bohr, 1891) (Equation 3) provided an ingenious way to measure the volume of gas within the conducting airways, i.e., the anatomic $V_D$. It assumes that the inhaled fractional concentration of $CO_2$ is 0 ($F_{iO_2}$=0):

$$\frac{V_D}{V_T} = \frac{F_{ACO_2} - F_{ECO_2}}{F_{ACO_2}}$$  \hspace{1cm} (3)

where $F_{ECO_2}$ is the fractional concentration of $CO_2$ in the mixed exhaled gas and $F_{ACO_2}$ is an estimate of mean alveolar $CO_2$ concentration based on a sample of gas collected late in the exhalation. Note that the term physiological $V_D$ has been the dominant term in this document.

Enghoff (and others) recognized that a gas sample captured within a breath would not necessarily reflect the true mean alveolar gas composition, particularly in subjects with cardiorespiratory disease (Enghoff, 1938; Nunn & Holmdahl, 1979). Even in modern breath-by-breath gas sampling systems, the estimation of $F_{ACO_2}$ by the end-tidal fractional concentration of $CO_2$ ($F_{ET}CO_2$) is prone to errors since $F_{ET}CO_2$ consistently exceeds the mean $F_{ACO_2}$ in normal subjects, thereby overestimating anatomical $V_D/V_T$. Conversely, $F_{ET}CO_2$ tends to underestimate mean $F_{ACO_2}$ in subjects with $\dot{V}_A/\dot{Q}$ abnormalities, thereby underestimating anatomical $V_D/V_T$. Enghoff proposed the following modification to the Bohr equation to calculate physiological $V_D/V_T$ (Enghoff, 1938):

$$\frac{V_D}{V_T} = \frac{P_{aCO_2} - P_{ECO_2}}{P_{aCO_2}}$$  \hspace{1cm} (4)
Equation 4 is based on the assumption that there is equilibration between alveolar PCO₂ (PₐCO₂) and end-capillary PCO₂. In subjects without large venous-arterial shunts, PₐCO₂ would therefore represent the mean PₐCO₂ and eliminate the need to estimate PₐCO₂ from FₑTCO₂ (Comroe, 1963). V_D in Equation 4 is physiological V_D, which is equal to the sum of anatomical V_D and alveolar V_D. Alveolar V_D is the volume of gas that enters under-perfused or un-perfused alveoli such that PₐCO₂ is not representative of the PₐCO₂ of those alveoli (Nunn & Hill, 1960).

Enghoff’s equation adds a new component to the dead space estimate that is relevant when the equation is utilised to describe gas exchange in disease. That is, the mean PₐCO₂ is always less than the PₐCO₂ in abnormal lungs. Any pathophysiological mechanism contributing to an increased arterial–alveolar CO₂ difference will increase the measured physiological V_D, including intrapulmonary shunt, diffusion impairment, and ventilation delivered to unperfused alveolar spaces [i.e. high alveolar ventilation/perfusion ratio (Vₐ/Q̇)]. In practice, therefore, the original concept by Bohr that the alveolar dead space only reflected the influence of regions of lung parenchyma receiving no pulmonary artery perfusion does not hold true. In fact, the extent of overall Vₐ/Q̇ heterogeneity is an important contributor to V_D/V_T in subjects with cardio-respiratory diseases. These considerations set the scene for an appreciation of the potential abnormalities leading to Vₐ/Q̇ heterogeneity (and poor ventilatory efficiency) in mild-to-
moderate COPD, e.g., ventilation distribution heterogeneity due to small airway disease, emphysema and pulmonary microvascular disease.
Chapter 2

Literature Review: Potential Determinants of Exercise Ventilatory Inefficiency in Mild-to-Moderate COPD

2.1 Background

The airflow limitation that defines COPD is often the result of heterogeneous structural abnormalities including mucous gland hypertrophy, small airway fibrosis, and parenchymal destruction that are caused by repeated cigarette smoke inhalation and disrupted repair and defense mechanisms (Hogg, 2004). Although the understanding of COPD pathogenesis is limited given the relative absence of longitudinal studies, the lung periphery is the site of early damage (Thomashow et al., 2013). Small peripheral airways are the initial and major site of airway resistance in obstructive disease (Hogg et al., 1968), and oxidative stress and inflammation facilitate destruction of the pulmonary parenchyma prior to clinically-definable obstruction (Santos et al., 2002). Other indications of pre-clinical abnormalities include reduced lung diffusing capacity for carbon monoxide (DL\textsubscript{CO}) (Kirby et al., 2013) and markers of pulmonary vascular endothelial destruction in smokers without COPD (Gordon et al., 2011). Subjects with mild COPD have ventilation-perfusion inequality (Barbera et al., 1990) and abnormal pulmonary mechanics (Guenette et al., 2014). The former increases the ventilatory requirements needed for a given amount of work (Neder et al., 2015a), while the latter increases the elastic load on inspiratory muscles for a
given ventilation (O’Donnell et al., 1997; Guenette et al., 2014; Faisal et al., 2016) (see sections 2.5 and 2.6, respectively). Therefore, the pathogenesis of COPD likely begins in smokers well before airway function declines enough to establish a clinical diagnosis by spirometry, and these early alterations contribute to subsequent abnormal gas exchange and reduced exercise capacity in subjects with COPD. The following sections will outline the potential mechanisms underlying exercise ventilatory inefficiency in subjects with mild-to-moderate COPD.

2.2 Small Airway Dysfunction

The small (2-3mm diameter) airways are the major site of airway resistance in obstructive disease (Hogg et al., 1968). In an inflammatory response to the deposition of tobacco pollutants, immune cells infiltrate the small airways and the production of mucous increases (Saetta et al., 1997). Small airway fibrosis is initiated when innate immune cells (neutrophils and macrophages) and lymphocytes deposit extracellular matrix below the airway wall epithelium (Hogg et al., 2004). It is notable that the inflammatory response and airway wall thickness is similar between mild COPD subjects and smokers without obstruction but with similar smoking histories (Hogg et al., 2004). McDonough et al. used multidetector computed tomography (CT) scanning on isolated lungs to quantify the number of airways 2.0-2.5mm in diameter in mild COPD subjects and controls with similar smoking history. Narrowing and/or airway destruction caused a 27% reduction in the number of small airways in mild COPD subjects (McDonough et al., 2011).
Moreover, microCT scanning was utilized to determine that the cross-sectional area of smaller terminal bronchioles may decrease prior to the appearance of centrilobular emphysema (McDonough et al., 2011).

Sensitive pulmonary function tests indicate that small airway dysfunction is likely more relevant than reductions in FEV\textsubscript{1} to the maldistribution of ventilation. This is evidenced by the significant correlation between abnormal distribution of ventilation and the accumulation of inflammatory cells in membranous bronchioles in mild COPD subjects (Barbera et al., 1990). The slope of nitrogen concentration \textit{versus} volume during phase III of a single-breath nitrogen washout test curve can indicate the maldistribution of ventilation if different time constants for emptying exist between lung regions (Lumb, 2012). Cosio et al. graded smokers from I-IV according to indices of small airway pathology, with higher grades indicating greater pathology. The slope of phase III was significantly higher in group II than group I smokers, even though both groups had normal FEV\textsubscript{1}/FVC ratios (Cosio et al., 1978). To build on the work of Cosio et al., Verbanck et al. measured peripheral airway dysfunction using more sophisticated multiple breath washout tests in non-smokers and smokers grouped according to smoking history (Verbanck et al., 2004). Smokers with 10-20 pack-years of smoking history, but without airway obstruction, had significantly greater nitrogen washout slopes and reduced percent-predicted D\textsubscript{L}CO compared to never-
smokers. Both findings were attributed to ventilation heterogeneity resulting from small airway dysfunction (Verbanck et al., 2004).

Small airway dysfunction in smokers without COPD can be considered a contributing factor to the mechanisms of reduced exercise capacity once spirometric abnormalities appear. Smokers with FEV$_1$/FVC $\geq$0.7 have lower increases in maximal expiratory flow in response to breathing helium than do non-smokers (Dosman et al., 1975). When maximal attainable flows are decreased, mild COPD subjects are predisposed to expiratory flow limitation that contributes to ventilatory limitations to exercise (section 2.6). The maldistribution of ventilation also contributes to impaired gas exchange, which increases ventilatory drive for a given amount of work, and reduces exercise capacity (section 2.5) (Neder et al., 2015b). The characterization of early pulmonary function decline in smokers may prove useful to advance the knowledge of mechanisms of reduced exercise capacity in COPD (Gagnon et al., 2014).

2.3 Emphysema

Emphysema is characterized by abnormal, permanent enlargement of airspaces distal to the terminal bronchioles accompanied by the destruction of their walls (Snider et al., 1985). It is often present in combination with chronic bronchitis, which is defined as a productive cough for at least three months in each of two consecutive years (Vogelmeier et al., 2016). Emphysema is facilitated by oxidative stress and a protease-anti-protease imbalance created by macrophages
recruited to the respiratory bronchioles in smokers (Abboud & Vimalanathan, 2008). The destruction of the pulmonary parenchyma may occur in conjunction with the narrowing and obliteration of the terminal bronchioles (McDonough et al., 2011; Mitzner, 2011). There is evidence of pulmonary vascular endothelial destruction, impaired gas exchange, and mechanical abnormalities in smokers with and without mild COPD, indicating that the pathogenesis of emphysema can occur well before airway obstruction.

As part of the inflammatory response to tobacco smoke, monocytes are recruited to the respiratory bronchioles and alveoli where they differentiate into macrophages. Respiratory bronchiolitis, defined by alveolar macrophages in the respiratory bronchioles, has been found in the lungs of smokers under 40 years old without emphysema. The level of macrophage secretions necessary for the destruction of alveolar walls may therefore be preceded by respiratory bronchiolitis (Niewoehner et al., 1974). After finding that elastolytic enzymes secreted by macrophages were more resistant to protease inhibition than neutrophil elastases, Chapman et al. suggested that alveolar macrophages may be the dominant cause of elastin degradation in emphysema (Chapman & Stone, 1984). In a review of mechanisms of protease-antiprotease imbalance, Abboud and Vimalanathan supported this conclusion and indicated that neutrophil elastase was likely only the dominant cause of protease-antiprotease imbalance in subjects deficient in alpha (α) 1 anti-trypsin (Abboud & Vimalanathan, 2008). Smokers with
emphysema as measured by CT scanning have greater numbers of alveolar macrophages in bronchoalveolar lavage than smokers without emphysema. The number of alveolar macrophages has also been negatively correlated to \( D_L \)CO in smokers without COPD (Abboud et al., 1998). This indicates that alveolar wall degradation and impairments in gas transfer occur prior to airway obstruction.

The degradation of alveolar walls in emphysema produces biomarkers of pulmonary vascular endothelial cell destruction. Endothelial microparticles are 0.1-1.5\( \mu \)m vesicles released into the systemic circulation from activated or apoptotic endothelial cells (Horstman et al., 2004). Microparticles from apoptotic endothelial cells can be identified and specified by the presence of platelet-endothelial cell adhesion marker CD31, and the absence of platelet-specific markers, respectively. A positive test result for angiotensin converting enzyme may also indicate a pulmonary vascular origin (Gordon et al., 2011; Thomashow et al., 2013). Endothelial microparticles with these characteristics have been found in greater quantities in the systemic circulation of smokers with isolated low \( D_L \)CO (<80% of the predicted value) compared to smokers and non-smokers with normal pulmonary function test results (Gordon et al., 2011). Correlations between emphysema and endothelial microparticles or emphysema and \( D_L \)CO did not appear between or within groups, suggesting that assessment of endothelial microparticle levels may indicate early lung destruction before abnormal results appear using conventional clinical tests (Gordon et al., 2011). Thomashow et al.
conducted a more recent study in COPD subjects and controls with similar smoking history (Thomashow et al., 2013). They found that CD31+ endothelial microparticles were elevated in participants with mild COPD, and levels remained relatively steady across the spectrum of disease severity. Moreover, CD31+ microparticle concentrations were positively correlated with the extent of emphysema quantified by CT scanning (Thomashow et al., 2013). Across the spectrum of COPD, CD31+ microparticle concentrations were inversely related to $D_{L,CO}$ and pulmonary microvascular blood flow measured by gadolinium-enhanced magnetic resonance imaging (MRI). Collectively, these studies suggest that pulmonary vascular endothelial cell apoptosis occurs early in the pathogenesis of emphysema and contributes to reductions in gas transfer. Pulmonary vascular remodeling also occurs in smokers and mild COPD subjects (section 2.4).

Destruction of the alveolar-capillary membrane decreases the conductance of gases into and out of the pulmonary capillary blood by reducing the surface area available for gas exchange. Computed tomography scanning and $D_{L,CO}$ tests have been well-suited to detect these abnormalities. Abnormal $D_{L,CO}$ (<80% predicted) can be present and inversely correlated with emphysema in smokers without airway obstruction (Klein et al., 1992; Nagelmann et al., 2011). New methods of imaging and gas transfer assessment may shed light on the pathogenesis of emphysema and its subtypes. Hyperpolarized Helium-3 magnetic
resonance imaging (He-3 MRI) has been used to compare the regional apparent
diffusion coefficients in ex-smokers with normal $D_L$CO, ex-smokers with abnormal
$D_L$CO, and ex-smokers with mild COPD (Kirby et al., 2013). Even though both non-
COPD groups had a similar extent of CT-quantified emphysema, the group with
abnormal $D_L$CO had lower regional apparent diffusion coefficients than subjects
with normal $D_L$CO. Furthermore, ex-smokers with low $D_L$CO had worse
symptoms and exercise capacity than ex-smokers with normal $D_L$CO, and
symptoms similar to mild COPD subjects. Mild COPD subjects had worse
apparent diffusion coefficients and exercise capacity than those with isolated
abnormal $D_L$CO (Kirby et al., 2013). Results from new imaging techniques,
therefore, support the notion that microvascular destruction is an important part
of the pathogenesis of emphysema (Santos et al., 2002; Gordon et al., 2011), and
begins to contribute to exercise limitation prior to airway obstruction.

Correlations between variables and $D_L$CO and FEV$_1$/FVC may be
misleading because of complex pathogenic processes of airway obstruction that
lead to distinct phenotypes of the disease. For example, Paoletti et al. found that
percent-predicted $D_L$CO only correlated well with CT attenuation in COPD
subjects when lung parenchymal destruction was extensive (Paoletti et al., 2015).
In a study of 1140 male smokers with and without COPD, Hoesein et al. found that
subjects with dominant emphysema had a lower percent-predicted KCO
compared to subjects predominantly characterized by thickening of airway walls
and a similar smoking history (Hoesein et al., 2014). Therefore, mild COPD subjects may have a phenotype that is confined primarily to the airways rather than the alveoli and blood vessels, which could influence associations between measurements of gas transfer and airway function.

The connective tissue architecture surrounding airways recoils during expiration and provides radial traction to help maintain airway patency. The loss of lung elastic recoil itself decreases the conductance of the airways for a given lung volume and transpulmonary pressure, while the enhanced airway collapsibility reduces the change in airway conductance for a given change in transpulmonary pressure (Leaver et al., 1973). The emphysema in COPD subjects therefore contributes to expiratory flow limitation beyond what can be attributed to small airway obstruction and chronic bronchitis (Leaver et al., 1973). Reduced elastic recoil and expiratory flow limitation lead to dynamic hyperinflation in subjects with mild COPD when ventilatory requirements are increased and expiratory time is decreased (Gagnon et al., 2014) (see section 2.6).

Resting pulmonary gas trapping (Deesomchok et al., 2010) and maldistribution of ventilation because of small airways disease (Hogg et al., 1968) results in affected regions of the lung having poor communication with the large bronchi and atmosphere. The poorly communicating fraction (PCF) is equal to one subtract the quotient of accessible volume (VA) divided by total lung capacity (TLC). VA is measured by insoluble gas dilution during a single-breath Dl,CO test.
and TLC is measured by body plethysmography (Neder et al., 2015b). In a retrospective study, 59 mild COPD subjects had mildly sized PCF (≤23%), and 19 had a moderately sized PCF (24-33%). Despite the variability in PCF within grades of COPD, mild-to-moderate subjects with PCF≥17% had significantly greater peak \( \dot{V}_E/\dot{V}CO_2 \) than controls (Neder et al., 2015b). Conversely, when mild-to-moderate subjects had normal peak \( \dot{V}_E/\dot{V}CO_2 (<34) \), they all had a low (<17%) PCF. Maldistribution of ventilation in combination with alveolar-capillary membrane destruction likely contributes to ventilatory inefficiency in mild-to-moderate COPD.

In summary, emphysema occurs early in the pathogenesis of COPD, possibly as a continuation of a chronic inflammatory response in respiratory bronchioles accompanied by the secretion of proteases. The destruction of the alveolar-capillary membrane and lung parenchyma causes abnormalities in gas transfer, lung structure, and lung mechanics during exercise in mild COPD. The abnormal distribution of ventilation and destruction of surface area for gas exchange are likely contributing factors to ventilatory inefficiency in mild-to-moderate COPD.

2.4 Pulmonary Vascular Remodeling

Structural changes in the pulmonary vasculature in smokers with and without mild COPD are evidenced by morphometric and histochemical analysis of pulmonary arteries excised from subjects undergoing lung transplantation.
(Peinado et al., 1998, 1999; Santos et al., 2002; Firă-Mladinescu et al., 2008). Potential mechanisms of pulmonary vascular remodeling and endothelial dysfunction include the direct effects of tobacco smoke, secretion of cytokines and growth factors from inflammatory cells recruited to the pulmonary vasculature, and regional hypoxia that prevents vasodilation and promotes remodeling. In mild COPD, the latter mechanism may be absent, but the vascular remodeling and endothelial dysfunction could have functional impairments in mild-to-moderate COPD subjects despite the large pulmonary vascular reserve (Hilde et al., 2013; Elbehairy et al., 2015a).

Studies on the morphometric properties of pulmonary arteries from mild-to-moderate COPD subjects, smokers without obstruction, and non-smoking controls have found consistent results. Pulmonary arteries 100-500µm in diameter from smokers and mild-to-moderate COPD subjects have greater intimal thickness as a percentage of total arterial cross sectional area compared to controls (Peinado et al., 1998; Santos et al., 2002; Firă-Mladinescu et al., 2008). Endothelial cell proliferation and thickening of the tunica media appear to be absent (Santos et al., 2002). Increased intimal thickness may not lead to statistically significant reductions in lumen area, but may be of physiological significance given that resistance is inversely proportional to the radius raised to the fourth power. The increase in intimal thickness is associated with the deposition of collagen into the extracellular matrix (Santos et al., 2002), and is correlated with pack-years of
smoking history (Firă-Mladinescu et al., 2008). Additionally, smooth muscle cell migration into the intima occurs, and these cells appear to have different staining characteristics than, and an orientation perpendicular to, their counterparts in the tunica media (Santos et al., 2002). Hypoxia is not likely a major mechanism driving remodeling, as most subjects with mild-to-moderate COPD and smokers without airway obstruction are not hypoxemic (Peinado et al., 1998, 1999; Santos et al., 2002).

The inflammatory response to tobacco smoke may be an important factor in pulmonary vascular remodeling and endothelial dysfunction preceding, or in conjunction with, regional hypoxia (Ferrer et al., 2009, 2011). An increased leukocyte concentration in the pulmonary adventitia has been found in smokers and mild-to-moderate COPD subjects compared to controls. The concentration was positively correlated with intimal thickness and endothelial dysfunction measured by in vivo endothelium-dependent vasodilation (Peinado et al., 1999). The pulmonary vascular remodeling may occur as a result of the secretion of cytokines and growth factors from inflammatory cells. However, it is unclear if endothelial cell dysfunction occurs because of regional hypoxia, inflammation, the direct effects of smoking, or a combination of the three (Dinh-Xuan et al., 1991; Peinado et al., 1999; Firă-Mladinescu et al., 2008). The majority of mild-to-moderate COPD subjects have normal P_aO_2 (>80mmHg), but this may still be significantly lower than nonsmoking controls and be accompanied by an increased alveolar-
arterial PO$_2$ difference (P$_{A-a}$O$_2$) indicative of regional hypoxia (Barbera et al., 1990; Peinado et al., 1999; Santos et al., 2002; Elbehairy et al., 2015a). In a widely-cited study by Peinado (Peinado et al., 1998), endothelial dysfunction as measured by ADP-induced endothelium-dependent vasodilation was reduced in pulmonary arteries excised from mild-to-moderate COPD subjects compared to smokers and non-smokers. It is notable that only one concentration (10$^{-5}$ M) of ADP produced relaxation significantly less than smokers and non-smoking controls, while lesser and greater concentrations did not yield significant differences. In the context of Peinado’s finding that endothelial dysfunction correlated with P$_{A-a}$O$_2$, endothelial dysfunction and regional hypoxia may be inter-related with inflammation in a complex fashion. If the pathological remodeling of pulmonary arterioles alters the distribution or amount of perfusion beyond that of hypoxic pulmonary vasoconstriction, perfusion could be decreased to ventilated lung regions and inefficient ventilation could occur in mild-to-moderate COPD subjects without hypoxemia (Hilde et al., 2013; Elbehairy et al., 2015a).

2.5 Ventilation-Perfusion Inequality

The primary function of the respiratory system is to maintain arterial blood gas homeostasis, and this is in large part achieved by the matching of ventilation and perfusion. Ventilation-perfusion inequality is present in mild COPD, and gradually worsens with increasing disease severity (Rodríguez-Roisin et al., 2009). Ventilatory inefficiency in COPD is, in part, a compensation for ventilation-
perfusion inequality that would otherwise lower \( P_aO_2 \) and increase \( P_aCO_2 \) for a
given oxygen uptake (\( \dot{V}O_2 \)) and \( \dot{V}CO_2 \) (Wagner, 2015). The locations and relative
magnitudes of small airway dysfunction, emphysema, and pulmonary vascular
remodeling (sections 2.2, 2.3, and 2.4, respectively) largely determine the
dispersion of ventilation and perfusion in COPD, but are not predictive of arterial
blood gas values (Wagner et al., 1977). The characteristics of exercise ventilatory
inefficiency in mild-to-moderate COPD likely depend on the pattern of
ventilation-perfusion inequality, compensatory increases in ventilation, and
constraints imposed by abnormal pulmonary mechanics.

The multiple inert gas elimination technique (MIGET) is used to quantify
the frequency distribution of ventilation and perfusion to different ventilation-
perfusion ratio (\( \dot{V}_A/\dot{Q} \)) in the lungs (Wagner et al., 1974b). Briefly, the technique
involves intravenous infusion of six inert gases with known solubility and steady-
state measurements of arterial, mixed venous (\( \overline{V} \)), and mixed expired
concentrations of those gases (Roca & Wagner, 1994). The solubility and relative
retention (\( P_a/P_v \)) of each gas can be used to calculate the \( \dot{V}_A/\dot{Q} \) of the lung regions
with which the gases equilibrate. Soluble gases will have higher retentions for a
given \( \dot{V}_A/\dot{Q} \) than less soluble gases. The amount of ventilation and perfusion
contributing to lung compartments with many different \( \dot{V}_A/\dot{Q} \) values can therefore
be calculated based on the relative solubility of these gases (Farhi, 1967; Chang,
1989). The resultant plot traditionally has fifty data points each for ventilation and
perfusion at different $V_A/\dot{Q}$ values, the sums of which are total ventilation and total perfusion, respectively. Ventilation-perfusion inequality may be manifested by large differences between total ventilation and total perfusion, or between regional ventilation and regional perfusion (mismatching) in areas of the lung with specific $V_A/\dot{Q}$ values. Dispersion is the state of total ventilation and/or total perfusion being distributed to lung areas with a wide range of $V_A/\dot{Q}$ values.

In healthy subjects, the $V_A/\dot{Q}$ values at the mean perfusion and mean ventilation are approximately 0.8 and 1, respectively, while the overall $V_A/\dot{Q}$ (total ventilation divided by total perfusion) is approximately 0.8. Very little ventilation and perfusion supplies areas with $V_A/\dot{Q}$ values less than 0.3 and greater than 2.1, although low $V_A/\dot{Q}$ (0.005-0.1) areas develop with healthy aging (Wagner et al., 1974a). The dispersion of the frequency distribution of $V_A/\dot{Q}$ ratios that receive ventilation (logSDV) and perfusion (logSDQ) is analogous to the standard deviation of a normal distribution, and larger dispersions are indexes of inequality. The upper limits of normality for logSDQ and logSDV are 0.6 and 0.65, respectively, in 20-40 year-old subjects, and 0.7 and 0.75, respectively, in 70 year-old subjects (Cardús et al., 1997). Shunt is defined as blood flow through regions with $V_A/\dot{Q}<0.005$, while wasted ventilation supplies regions with $V_A/\dot{Q}>100$. Shunt is not present to an appreciable extent in normal subjects (Wagner et al., 1974a; Cardús et al., 1997) or subjects with COPD (Wagner et al., 1977).
Ventilation-perfusion inequality contributes to abnormal $P_aO_2$, $P_aCO_2$, and $P_{A-a}O_2$. A high $\dot{V}_A/\dot{Q} (>10)$ region will substantially decrease $P_aCO_2$ but only mildly increase $P_aO_2$ because of the relative shapes of their dissociation curves. Furthermore, $P_aO_2$ and $P_aCO_2$ are dependent on gas content and blood flow from each lung area rather than the partial pressure of blood flowing from each area (West, 1971; Petersson & Glenny, 2014). The ventilatory compensation for low $\dot{V}_A/\dot{Q}$ regions that would otherwise substantially lower $P_aO_2$ and raise $P_aCO_2$ may be pronounced, leading to a high work of breathing and a low $P_aCO_2$. Therefore, subjects with a high logSDQ will have a lower $P_aO_2$ and a variable $P_aCO_2$ depending on the magnitude of compensatory ventilation (Wagner, 2015). Compensatory ventilation that helps maintain $P_aO_2$ also increases $P_{A}O_2$, allowing $P_{A-a}O_2$ to be used as an indicator of compensated $\dot{V}_A/\dot{Q}$ inequality.

Wasted ventilation quantified by the MIGET is the ventilation supplying areas with $\dot{V}_A/\dot{Q}>100$. However, expired air from regions with high $\dot{V}_A/\dot{Q} (>10)$ still dilutes air that is representative of arterial blood and therefore decreases $P_{\overline{E}}CO_2$. The large discrepancy between $P_aCO_2$ and $P_{\overline{E}}CO_2$ therefore increases the physiological dead space as calculated by the Enghoff-Bohr equation (Equation 4), even without completely unperfused alveoli (Robertson, 2015). Subjects with COPD who have compensatory ventilation and high $\dot{V}_A/\dot{Q}$ areas will therefore have a large physiological dead space. Ventilatory efficiency for expiring CO$_2$ is reduced (section 1.3.2) and the work of breathing increases. Abnormal pulmonary
mechanics may limit ventilatory compensation, thereby masking the ventilatory inefficiency that comes as a consequence of a large physiological dead space (section 2.6) (Neder et al., 2015a).

The aforementioned arterial blood gas characteristics (lowered but normal P₃O₂, high Pₐ₅O₂, and lowered P₃CO₂) are present in mild-to-moderate COPD subjects who are able to maintain alveolar ventilation (Barbera et al., 1990; Neder et al., 2015a; Elbehairy et al., 2015a). In addition, the overall $\dot{V}/\dot{Q}$ increase and improved distribution of ventilation in COPD during exercise increases Pₐ₅O₂ more in mild-to-moderate subjects than controls (Barbera et al., 1991; Wagner, 1992; Elbehairy et al., 2015a). However, arterial blood gas composition is determined by a variety of factors, including the ratio between cardiac output ($\dot{Q}$) and tissue oxygen uptake that determines mixed-venous PO₂ (PᵥO₂). For the purposes of this document, cardiac output will be assumed to equal pulmonary blood flow (PBF). A blunted cardiac output response to exercise (increased oxygen uptake, PBF/VO₂) will lower PᵥO₂ and therefore P₃O₂ to the extent that the aforementioned blood gas characteristics may not be exclusively attributed to $\dot{V}/\dot{Q}$ inequality in advanced COPD (Wagner, 1992). It does not appear that PBF is abnormal at any COPD severity at rest (Rodríguez-Roisin et al., 2009), although its dispersion is abnormal as a result of vascular abnormalities in mild-to-moderate COPD (Barbera et al., 1994).
Several works from Barbera et al. have found correlations between lung structure abnormalities and ventilation-perfusion inequality in mild-to-moderate COPD subjects (Barbera et al., 1990, 1991, 1994). The finding of increased $P_{A-a}O_2$ but normal $P_aO_2$ (>80mmHg) at rest (Barbera et al., 1990) has been confirmed in physiological studies with exclusively mild COPD subjects (Elbehairy et al., 2015a). The logSDV and logSDQ are above normal in mild-to-moderate COPD (Barbera et al., 1990). An index of general $V_A/Q$ heterogeneity (DISP R-E*) was also higher than normal in mild-to-moderate subjects and correlated with bronchiolar inflammation (Barbera et al., 1990). Morphometrically defined emphysema correlated positively with logSDQ and negatively with $P_aO_2$ (Barbera et al., 1990, 1991). Since FEV$_1$ was relatively preserved in this subject pool, it was concluded that small airway dysfunction and pulmonary microvascular remodeling contribute substantially to ventilation-perfusion inequality (Barbera et al., 1990). This was supported by a retrospective analysis indicating that ventilation-perfusion inequality increases more from health to mild COPD than from mild to severe COPD (Rodríguez-Roisin et al., 2009).

In a separate study from the same group, it was hypothesized that pulmonary vascular remodeling would interfere with the ability of hypoxic pulmonary vasoconstriction to maintain $V_A/Q$ matching (Barbera et al., 1994). The hypothesis was supported based on the finding that COPD subjects with more intimal thickening were less sensitive to oxygen breathing than COPD subjects.
with similar small airway pathology. The change in logSDQ of the group with greater remodeling was significantly less, indicating that hypoxic pulmonary vasoconstriction did not have as prominent a role in maintaining $\dot{V}_A/\dot{Q}$ relationships (Barbera et al., 1994). Increased blood flow to areas of low $\dot{V}_A/\dot{Q}$ would decrease $P_{aO_2}$ if it were not for compensatory ventilation.

The respiratory system is able to fulfill its primary function to maintain arterial blood gases as long as lung mechanics are able to increase alveolar ventilation (Wagner, 2015). This is true even in the context of $\dot{V}_A/\dot{Q}$ inequality. Ventilatory inefficiency does not in itself cause dyspnea, but represents a consequence of increased afferent feedback that stimulates ventilation and burdens abnormal pulmonary mechanics. In turn, this contributes to neuro-mechanical dissociation that causes dyspnea and exercise intolerance (Guenette et al., 2014).

### 2.6 Abnormal Pulmonary Mechanics

As COPD progresses, pulmonary mechanics worsen and exertional dyspnea intensity increases for a given ventilation and work rate. Structural abnormalities in the airways and lung parenchyma predispose the lungs to dynamic hyperinflation in the context of high ventilatory demand. Hallmarks of exercise ventilation in COPD include high ventilatory demand, dynamic hyperinflation, and rapid and shallow breathing patterns compared to healthy age-matched controls (Ofir et al., 2008). Even in mild COPD, dyspnea intensity
correlates with measurements of dynamic hyperinflation and the resultant neuro-mechanical dissociation of the inspiratory muscles (Guenette et al., 2014). Intolerable dyspnea and exercise cessation will occur when inspiratory reserve volume and expiratory time are decreased to the extent that subjects are unable to increase ventilation in response to increased ventilatory demand (O’Donnell et al., 2012; Faisal et al., 2016).

The structural abnormalities in the airways and lung parenchyma in smokers with and without mild COPD are described in sections 2.2 and 2.3. Briefly, airway remodeling and parenchymal destruction increase airway resistance and decrease airway patency and lung elastic recoil. The time constants for lung emptying are long and expiratory flow limitation can occur. The latter is present when the maximal attainable expiratory flow is reached during tidal breathing (Hyatt, 1983). Ventilatory demand increases and the time available for expiration decreases during incremental exercise. As a consequence, subjects with COPD are required to end expirations at progressively higher lung volumes (O’Donnell et al., 2016). A corollary of this is decreased inspiratory capacity and inspiratory reserve volume. In 90% of mild COPD subjects, inspiratory capacity declines from rest to peak exercise, and the average change is -0.54L. By comparison, 24% of age-matched controls have a decline in inspiratory capacity, and the average change is +0.06L (Ofir et al., 2008).
Intrinsic (alveolar) positive end-expiratory pressure (PEEP\textsubscript{i}) is associated with pulmonary gas trapping in subjects with COPD (Faisal \textit{et al.}, 2016). In health, the alveolar-atmospheric pressure gradient approximates zero when flow becomes zero at the end of a spontaneous expiration. When expiratory flow limitation is present, inspiratory muscles are activated when the alveolar-atmospheric pressure gradient is still positive. Under this circumstance, a PEEP\textsubscript{i} must be overcome before inspiratory flow is generated. The magnitude of the PEEP\textsubscript{i} can be calculated using simultaneously recorded flows at the mouth and esophageal pressure (P\textsubscript{es}), the latter of which approximates pleural pressure (Blanch \textit{et al.}, 2005). Pleural pressure must become negative enough to overcome PEEP\textsubscript{i} and create a negative alveolar-atmospheric pressure gradient to generate inspiratory flow. Therefore, the magnitude to which P\textsubscript{es} becomes negative before inspiratory flow is produced approximates the PEEP\textsubscript{i} (Blanch \textit{et al.}, 2005).

Faisal \textit{et al.} measured and calculated EELV and PEEP\textsubscript{i} during incremental exercise in subjects with moderate COPD, subjects with moderate interstitial lung disease (ILD), and healthy controls (Faisal \textit{et al.}, 2016). PEEP\textsubscript{i} in the large majority of healthy and ILD subjects with preserved expiratory function (FEV\textsubscript{1}/FVC≥0.7) remained below 4 cmH\textsubscript{2}O. Conversely, PEEP\textsubscript{i} increased to an average of 12.8 cmH\textsubscript{2}O in subjects with COPD at the end of exercise. Both patient groups had limited ventilation at the end of exercise due to reduced inspiratory reserve volume. However, subjects with moderate COPD had a greater increase in end-
expiratory lung volume (EELV) to account for this reduction, indicating an association between PEEP\textsubscript{i} and EELV that ultimately reduces ventilatory capacity (Faisal et al., 2016).

Dynamic hyperinflation simultaneously constrains the inspiratory muscles and increases the elastic load in addition to PEEP\textsubscript{i}. As the EELV increases, the diaphragm shortens and depresses to a sub-optimal position in which the length-tension relationship needed to produce effective contractions is disturbed (Laghi & Tobin, 2003). This is referred to as ‘functional weakening’ of the inspiratory muscles (O’Donnell et al., 2014a). Tidal breathing moves to a less compliant portion of the pulmonary pressure-volume relationship. Collectively, these abnormalities increase the elastic work of breathing in emphysema as measured by inspiratory muscle oxygen consumption for a given ventilation (Roussos & Macklem, 1982).

Dyspnea and exercise intolerance are multifactorial, but the aforementioned mechanical abnormalities that limit ventilation certainly contribute. Neuro-mechanical dissociation of the respiratory muscles occurs when the magnitude of the central neural drive begins to exceed the diaphragm’s capability to generate the corresponding inspiratory pressures necessary to distend the lung. Indirect measurements of neuro-mechanical dissociation have been associated with dyspnea during cardiopulmonary exercise testing in mild COPD (Guenette et al., 2014). As ventilation becomes frequency-dependent in order to minimize the work of breathing, the necessary velocity of inspiratory
muscle shortening increases. This causes larger pressure swings for a given ventilation as COPD severity increases (O’Donnell et al., 2014a). The expiratory time is further reduced by the dependence on breathing frequency, thereby aggravating hyperinflation.

Eventually dynamic hyperinflation proceeds to a point represented by diminished inspiratory reserve volume (IRV). This indicates that the ventilatory capacity has been utilized. Further loads that increase ventilatory drive are met by neuro-mechanical dissociation and dyspnea rather than commensurate increases in ventilation. The O’Donnell threshold is the IRV at which intolerable dyspnea limits exercise (Casaburi & Rennard, 2015). In healthy controls and subjects with mild COPD, this critical IRV is approximately 0.7L (Ofir et al., 2008; Chin et al., 2013; O’Donnell et al., 2014b; Guenette et al., 2014). Although there is a large coefficient of variation, there is a trend for this critical IRV to decline as COPD severity increases (O’Donnell et al., 2012, 2014b). Another indicator this threshold has been reached is when there is an inflection of dyspnea intensity when the tidal volume/inspiratory capacity quotient reaches approximately 75. This inflection point occurs at around 85% peak ventilation regardless of disease severity, and reflects the frequency-dependence of increases in ventilation (O’Donnell et al., 2012). The O’Donnell threshold is reached at lower absolute values of ventilation as COPD severity progresses or when mechanical dead space is added (O’Donnell et al., 2012; Chin et al., 2013).
2.7 Objective

This cross-sectional investigation aimed to determine the structural and functional correlates of impaired ventilatory efficiency during incremental exercise in subjects with COPD and mild-to-moderate airflow obstruction.

2.8 Hypotheses

We hypothesized that, compared to healthy controls, COPD subjects would have:

i) Reduced peak exercise capacity;

ii) Impaired ventilatory efficiency as indicated by a high $\dot{V}_E/\dot{V}_{CO_2}$ minimum (“nadir”);

iii) More extensive emphysema on high-resolution computed tomography (HRCT) scans of the chest;

iv) Lower resting lung diffusing capacity for carbon monoxide ($D_L CO$); and

v) Impaired exercise pulmonary blood flow

Moreover, we anticipated:

vi) A significant association between reduced exercise capacity and impaired ventilatory efficiency within the COPD group, and

vii) Significant correlations between impaired ventilatory efficiency and $D_L CO$ decrement, emphysema severity, and deficits in pulmonary blood flow

Confirmation of the study hypotheses would shed new light on the pathophysiological mechanisms underlying an augmented ventilatory response
to exertion in COPD patients with only modest spirometric abnormalities. Additionally, confirmation would lend novel support to the notion that detection of emphysema on HRCT and D_LCO impairment in these patients should be clinically valued, as they predict a relevant patient-centered outcome: reduced exercise capacity.
Chapter 3
Materials and Methods

3.1 Study Design

This was a cross-sectional, observational study contrasting selected resting and exercise characteristics between subjects with mild-to-moderate COPD and healthy controls matched for age, sex, and scores of physical activity in daily life. After screening, subjects underwent symptom-limited incremental cardiopulmonary exercise tests. On a different day (at least 48 hours later), a subgroup of subjects completed constant work rate exercise tests at 25W and 50W with pulmonary blood flow measurements.

3.2 Study Population

Participants were recruited using posted advertisements (Appendix C and Appendix D) with the exception of eight participants that permitted other investigators (Elbehairy et al., 2015a) to provide us with their contact information. All participants were screened using the inclusion and exclusion criteria below. Participants were instructed to maintain their regular medication regimen, and to refrain from smoking/consuming tobacco, caffeinated products, and heavy meals, and to avoid heavy exercise, for at least 12 hours before each visit.

Inclusion Criteria

a) 45 years old or greater
b) Preserved left ventricular ejection fraction (>40%) as assessed by echocardiography
c) Long-term physical inactivity according to Baecke’s questionnaire (Baecke et al., 1982; Florindo & Latorre, 2003)

d) Clinical stability for at least 3 months

e) Control participants were required to have normal spirometry \( \text{FEV}_1/\text{FVC} \geq 0.7 \) and \( \text{FEV}_1 \geq 80\% \) predicted

f) COPD participants had long-term previous \((\geq 10 \text{ pack-years})\) or current smoking history

**Exclusion Criteria**

a) A history of asthma

b) Pulmonary, cardiac, or metabolic conditions that could contribute to dyspnea or exercise limitation, or an inability to safely complete exercise tests according to American Heart Association guidelines (Balady et al., 2010)

c) The use of daytime oxygen therapy

d) Hospitalization or exacerbation within the last 6 weeks

Clinical and physiological data from 54 individuals with COPD were reviewed for potential inclusion in the study. Twenty individuals were excluded due to major co-morbidities that could interfere with their responses to exercise (neoplasms= 4, heart failure= 4, active coronary disease= 3, recent myocardial infarction= 3, advanced liver disease= 2, orthopedic limitation= 2, morbid obesity= 2). Fifteen individuals either excused themselves from the study or failed to attend the initial screening visit. Thus, 19 individuals with COPD completed the first visit. Twelve COPD subjects were receiving short-acting bronchodilators as needed, and 10 were under long-acting bronchodilator treatment (7 of them with associated inhaled steroids). Disease severity was classified according to the latest recommendations from the GOLD committee (Vogelmeier et al., 2016). Participants were graded for symptoms using the modified Medical Research
Council (mMRC) questionnaire (Fletcher et al., 1959; Brooks, 1982). Comorbidity burden was determined by the combined 19-disease Charlson Index (Charlson et al., 1987). Twenty-six sedentary controls were enrolled.

The study received ethics approval from the Queen’s University and Affiliated Teaching Hospitals Research Ethics Board (DMED-1701-14) (Appendix G and Appendix H). Informed and written consent according to institutional criteria was obtained from all participants at the beginning of their first visitation to the Laboratory of Clinical Exercise Physiology (LACEP) (Appendix E).

3.3 Physical Activity Questionnaire

The physical activity in the daily life of participants was determined by the Baecke’s questionnaire (Baecke et al., 1982) after informed consent was given during the first visit. The questionnaire includes 16 questions on the type, intensity, and frequency of occupational, leisure time, and sport-related physical activity (Appendix B).

3.4 Pulmonary Function Tests

3.4.1 Spirometry

Participants with COPD had a diagnosis established from pre- and post-bronchodilator pulmonary function tests conducted within the last year, or were graded according to pre-bronchodilator FEV\textsubscript{1} in accordance with ATS/ERS standards (Miller et al., 2005). A FVC maneuver is a forceful expiration to residual volume following a maximal inspiration from functional residual capacity. The
forceful expiration was completed in a seated position, lasted at least six seconds, and was followed by another rapid and complete inspiration. Three acceptable maneuvers were obtained, and the two largest FVC and FEV₁ values were within 150mL of each other. Even when the highest FVC and FEV₁ values came from different maneuvers, the FEV₁/FVC ratio was calculated using these values (Miller et al., 2005). Subjects were removed from the study if they reported a history of asthma, and were placed into the mild-to-moderate COPD group if they had a FEV₁/FVC<0.7 and post-bronchodilator FEV₁≥60% predicted (Pellegrino et al., 2005).

### 3.4.2 Body Plethysmography

Body plethysmography uses gas compression in a body box of known volume to measure thoracic gas volume, which is equal to functional residual capacity (FRC) if the maneuver is completed at the end of a normal expiration (DuBois et al., 1956). Subjects wearing nose clips rested with their hands on their cheeks and, at the end of a normal expiration, panted into a mouthpiece at a rate of approximately one breath each second. After an obstruction was introduced, the panting maneuver continued for 3-5 more breaths. The calculation of FRC is based on Boyle’s law and relies on the inverse relationship between the change in lung volume and change in body box pressure while panting. The maneuver was repeated until at least three FRC values agreed within 5%, while the reported FRC
was averaged from three acceptable and repeatable panting maneuvers (Wanger et al., 2005).

3.4.3 Diffusing Capacity for Carbon Monoxide (D\textsubscript{L}CO)

Subjects with COPD completed single-breath D\textsubscript{L}CO tests to determine the effect of emphysema and pulmonary microvasculature damage on gas transfer. The subjects exhaled to residual volume, at which point they were instructed to take a full breath of test gas. The inhalation of test gas proceeded to 85\% of the vital capacity in less than 4 seconds, after which the breath was held for 10 seconds to allow carbon monoxide to transfer into the blood. Subsequent exhalation was relatively rapid (less than four seconds), but not forced, to ensure proper dead space clearance and gas sampling. The reported D\textsubscript{L}CO value was the average of two or more acceptable tests within 3 mL min\textsuperscript{-1} mmHg\textsuperscript{-1} or 10\% of each other (Macintyre et al., 2005).

3.5 High Resolution Computed Tomography

Thoracic CT scans were acquired in the COPD group at suspended inspiration without intravenous contrast, and reconstructed using a spatial contrast algorithm with 1.25-mm slice thickness. Study scans were acquired on Siemens and GE 64-slice scanners (GE Healthcare, Waukesha, Wisconsin) using a single spiral acquisition from apex to base (64 × 0.625 mm collimation, 120 kVp, 100 mA). Quantitative measures of emphysema and airway wall thickness were generated with VIDA software (VIDA Diagnostics, Iowa City, Iowa). Threshold-
based measures of the percentage of low attenuation areas (% LAA)-950 were calculated for each lung CT scan by quantifying the percentage of the overall lung density histogram below the −950 Housefield unit threshold (emphysema index, %) (Kirby et al., 2015). The radiologist used an electronic score sheet to record the extent of each emphysema subtype assessed visually on CT according to the following definitions (Hansell et al., 2008): a) centrilobular emphysema: focal regions of low attenuation, surrounded by normal lung attenuation, located within the central portion of secondary pulmonary lobules; b) panlobular emphysema: diffuse regions of low attenuation involving entire secondary pulmonary lobules and c) paraseptal emphysema: regions of low attenuation adjacent to visceral pleura (including fissures). Upper lobe (including right middle)/lower lobe ratio was calculated for each lung and averaged for reporting. Airway wall thickness of airways standardized to hypothetical airways with an internal perimeter of 10 mm (Pi10) were also obtained (Kirby et al., 2015).

3.6 Exercise Tests

Cardiopulmonary exercise tests (CPETs) were performed on a stationary electrically braked upright cycle ergometer (VIAsprint 150P; Ergoline, Bitz, Germany) during two visitations to the LACEP on non-consecutive days. Cardiac rhythm and heart rate and oxygen saturation were continuously monitored using an electrocardiogram (ECG) and pulse oximeter (SpO2, %), respectively. Flow and expired gas fraction data were collected breath-by-breath then averaged every 20s.
using a Vmax229d system (Sensormedics, Yorba Linda, CA). The heated wire mass flow sensor and gas analyzers were calibrated prior to each test. The mechanical dead space of the breathing apparatus (110mL from pneumotachometer, saliva trap, and mouthpiece) was provided by the manufacturer. Variables obtained include minute ventilation ($\dot{V}_E$, L min$^{-1}$), oxygen uptake ($\dot{V}O_2$, L min$^{-1}$), carbon dioxide output ($\dot{V}CO_2$, L min$^{-1}$), tidal volume ($V_T$, L), breathing frequency ($f$, min$^{-1}$), and end-tidal partial pressures of oxygen and carbon dioxide ($P_{ET}O_2$ and $P_{ET}CO_2$ in mmHg, respectively). Subjects breathed through a mouthpiece and wore a nose-clip to avoid air leakage.

3.6.1 ‘Ramp’ Incremental Test (First Visit)

The first visitation included a symptom-limited ramp exercise protocol on a cycle ergometer. The subject breathed at rest for approximately three minutes or until the breathing pattern stabilized. The rest period was followed by two minutes of unloaded (0W) cycling during which the subjects pedaled at a frequency of 50 to 60 revolutions per minute (RPM). The load on the electrically braked cycle then increased at a linear rate until the subject reached their symptom-limited maximum or could not maintain a cadence $\geq$50RPM. All subjects were constantly encouraged to perform as much work as possible. Load increments between 5 W min$^{-1}$ and 20 W min$^{-1}$ were selected by a physician in order to obtain an exercise duration of 8-12 minutes (Wasserman et al., 2011). The physician took into account the age, pulmonary function, and medical history of
the subjects. Subjects were asked to rate the intensity of their dyspnea and leg discomfort using the modified 10-point category-ratio BORG scale (Noble et al., 1983) throughout and at the end of exercise.

The primary outcomes measured in the first visitation were the indexes of ventilatory inefficiency. The intercept and slope of the \( \dot{V}_E - \dot{V}CO_2 \) relationship was determined by performing a linear regression through data points below the ventilatory compensation point (VCP) (Neder et al., 2001). The minimum \( \dot{V}_E / \dot{V}CO_2 \) (nadir) throughout the course of exercise was taken as the lowest 20-second average, which corresponds to the VCP (Neder et al., 2001). The anaerobic threshold was approximated by the gas exchange threshold (GET) using the V-slope method (Beaver et al., 1986) and the ventilatory equivalent method (Reinhard et al., 1979). The average of the final 20 seconds for all measured variables was considered the peak exercise data. Peak \( \dot{V}_E \) was also expressed relative to the estimated maximal voluntary ventilation [MVV, (L min\(^{-1}\)) = FEV\(_1\) x 35).}

### 3.6.2 Constant Work Rate Tests (Second Visit)

An exercise protocol consisting of several intermittent constant work rate exercise bouts took place a minimum of 48 hours after the ramp exercise test. The protocol consisted of alternating 5-minute 25W and 50W exercise bouts separated by 5-minute resting periods until two bouts at each power output were performed (Figure 6). The final 20-second average of gas exchange data (\( \dot{VO}_2, \dot{V}_E / \dot{V}CO_2, \) and \( P_{ET}CO_2 \)) at each work rate was assumed to correspond to the pulmonary blood
flow measurement taken directly thereafter. Inert gas rebreathing measurements of PBF were performed between the 3rd and 5th minute of each constant work rate exercise. The bag volume and rebreathing frequency were adjusted in accordance with the subjects’ tidal volume and respiratory rate during exercise at each work rate. One rebreathing method was completed for each bout to ensure a consistent pressure gradient of inert gases in subsequent tests.

3.7 Pulmonary Blood Flow

Measurements of cardiac output (\(\dot{Q}\)) are often invasive, costly, and/or technically demanding (Agostoni et al., 2005; Saur et al., 2010), thereby limiting or burdening physiological investigations concerned with cardiac function and/or
oxygen delivery. Conversely, principles of gas transfer can be utilized by physiologists to measure pulmonary blood flow (PBF) easily and non-invasively with the inert gas rebreathing (IGR) method (Innocor™, Innovision, Odense, Denmark). After a deep expiration, a subject rebreathes from a bag for approximately five respiratory cycles, emptying the bag with each inspiration. The bag contains soluble nitrous oxide (N₂O) and insoluble sulfur hexafluoride (SF₆) supplied by a gas cylinder containing a mixture of 5% N₂O, 1% SF₆ and 94% O₂. Both inert gases are continuously measured by a photoacoustic infrared gas analyzer (Clemensen et al., 1994; Gonem et al., 2014). The equilibration of SF₆ between the lungs and rebreathing system is used to calculate the end-expiratory lung volume (EELV) at which the rebreathing maneuver began. The rate at which N₂O diffuses into the pulmonary circulation is proportional to PBF, which equals Ẑ in the absence of shunts (Laszlo, 2004; Peyton et al., 2005). Despite some assumptions and limitations (Petrini et al., 1978), the technique is agreeable with invasive gold-standard Fick and thermodilution methods, and allows physiologists to perform non-invasive hemodynamic assessments of subjects with cardiopulmonary disease at rest and during exercise (Agostoni et al., 2005; Saur et al., 2010; Cattadori et al., 2011).

3.7.1 Principles of Measurement

In 1870, the mathematician, physicist, and physiologist Adolf Fick presented what is now known as the Fick principle in the proceedings of the
Würzburg Physikalische Medizinische Gesellschaft. The principle states that an organ takes up or eliminates a substance from the bloodstream at a rate that is equal to the product of blood flow to that organ and the concentration gradient of that substance across the organ’s circulation (Fick, 1870; Hurst et al., 2000). Since the principle is derived from the conservation of mass, it is generalizable to accessible organs, circulatory beds, and measurable substances.

A derivative of the Fick principle can be utilized to calculate PBF based on gas transfer across the alveolar-capillary membrane. Attributed to Bornstein (Bornstein, 1910) by Krogh and Lindhard (Krogh & Lindhard, 1912), the principle states that the uptake of an inert and soluble gas that equilibrates across the alveolar-capillary membrane can be used to measure PBF. Equation 5 is a modification of the Fick equation in accordance with this principle (Cotes et al., 2006), and it can be applied to the IGR technique with corrections (Petrini et al., 1978) explained in section 3.7.2.

\[
PBF = \frac{\dot{V}_x}{\alpha(P_{AX} - P_{vX})} \tag{5}
\]

Where PBF (L min\(^{-1}\)) is equal to the volume of test gas “x” transferred into the blood each minute (\(\dot{V}_x\), mL min\(^{-1}\)) divided by the product of the partial pressure gradient between the alveolar [end-tidal] gas and mixed venous blood (\(P_{AX} - P_{vX}\), kPa) and the solubility of the gas in blood (mL L\(^{-1}\) kPa\(^{-1}\)) at body temperature (37\(^{\circ}\)C). Following from Henry’s Law, the denominator is equal to the concentration
gradient across the alveolar-capillary membrane, so this equation represents a derivative of the Fick principle (Lumb, 2012).

The properties of N₂O and SF₆ allow IGR to produce accurate measurements of PBF and EELV. Both gases are inert in that they do not react with hemoglobin, but SF₆ is so insoluble that the amount that diffuses into the blood is negligible. Nitrous oxide is relatively soluble and obeys Henry’s law, which states that the concentration of a gas dissolved in liquid under isothermic conditions is proportional to the partial pressure of the gas with which it is in equilibrium. In this case, PₐN₂O equilibrates with the mixed-venous blood before the end of the alveolar capillaries (i.e. PₐN₂O=PₐN₂O), and the amount that diffuses into the blood is proportional to the PₐN₂O − PᵥN₂O and a constant (steady-state) PBF. The assumption that SF₆ does not diffuse into blood allows its dilution and the principle of conservation of matter to be applied to calculate the end-expiratory lung volume at which the maneuver begins (Equation 6)(Peyton et al., 2005).

\[
V_L + V_{ds} + V_{rb} = \left( \frac{F_i^0 \times V_{rb}}{F_{leq}} \right)
\]

Where \(V_L\) (L) is lung volume at the beginning of the rebreathing maneuver, \(F_i^0\) is the initial fractional concentration of insoluble gas in the rebreathing bag, \(V_{rb}\) is the volume of the rebreathing bag (L) selected by the technician, \(F_{leq}\) is the fractional concentration of insoluble gas at equilibrium, and \(V_{ds}\) is the known
mechanical dead space volume (L) of the system when the rebreathing bag is empty.

3.7.2 Calculations

The practical measurement of PBF using IGR requires three major corrections to be applied to Equation 5. First, the solubility of N\textsubscript{2}O in lung tissue is taken into account to avoid an overestimation of PBF. Second, the dilution of N\textsubscript{2}O depends on its distribution throughout the ventilated lung regions and the volume of the rebreathing bag, which are both dynamic over the course of the test. Fractional concentrations of SF\textsubscript{6} are used to normalize the measured fractional concentration of N\textsubscript{2}O so its diffusion into the bloodstream can be isolated. Lastly, the mono-exponential decline in P\textsubscript{A}N\textsubscript{2}O and therefore N\textsubscript{2}O flux must be linearized in order to calculate PBF. These corrections (Petrini et al., 1978) are applied mathematically in Equation 7, which is analogous to Equation 5.

The first rebreathing inspirations are associated with a rapid disappearance of N\textsubscript{2}O that results from its solubility in lung tissue in addition to distribution throughout the lung and diffusion into the blood. A correction factor equal to the product of the solubility of N\textsubscript{2}O in the lung tissue and an assumed lung tissue volume is used to account for this phenomenon (Petrini et al., 1978).

With each expiration, the P\textsubscript{ET}N\textsubscript{2}O decreases as a result of its distribution throughout the lung and mechanical space in addition to its diffusion into the blood. Furthermore, the volume of the rebreathing bag can change throughout the
maneuver depending on the respiratory exchange ratio. For example, if $\dot{V}O_2$ is greater than $\dot{V}CO_2$, the system volume will decrease and the measured $P_{ET}N_2O$ will be greater than if $\dot{V}O_2$ and $\dot{V}CO_2$ were equal, even for a given PBF. It is assumed that the distributions of SF$_6$ and N$_2$O throughout the ventilated regions of the lung and mechanical space are identical. Therefore, the fractional concentration of N$_2$O throughout the maneuver can be normalized to the concentration of SF$_6$, thereby isolating the decline in measured N$_2$O to its diffusion into the blood (Petrini et al., 1978).

In a closed system with no recirculation and a constant PBF, $P_{AN_2O}$ and therefore $P_{A-\bar{v}}N_2O$ and N$_2$O transfer decline mono-exponentially as N$_2$O is absorbed by the blood. A natural logarithm is applied to the normalized N$_2$O concentration profile to make it linear on a semi-logarithmic plot. A linear regression line is plotted through the logarithmic normalized end-expiratory N$_2$O concentration values after SF$_6$ equilibration throughout the system has been achieved within acceptable standards (usually in 3-5 breaths)(Fontana et al., 2009; Saur et al., 2010; Perrault et al., 2016) (Figure 7). Only measurements in which the SF$_6$ concentration profile indicated complete mixing of gases were included in the analysis.

The slope of the regression line between two points is determined by Equation 7 (Petrini et al., 1978):
\[ \beta = \left( \frac{\ln \left( \frac{F_{SN+1}(t) \times F^0_i}{F_{LN+1}(t) \times F^0_s} \right) - \ln \left( \frac{F_{SN}(t) \times F^0_i}{F_{LN}(t) \times F^0_s} \right)}{T_{N+1} - T_N} \right) \] (7)

Where the slope of the regression line (\(\beta\)) is equal to the difference between the natural logarithmic (ln) normalized end-expiratory soluble (s) gas concentrations \(\left( \frac{F_{s}(t) \times F^0_i}{F_{i}(t) \times F^0_s} \right)\) at two points (N+1 and N) divided by the time between those same points \((T_{N+1} - T_N)\). \(F_s(t)\) and \(F_i(t)\) designate the fractional concentration of soluble and insoluble gas, respectively, as a function of time. \(F^0_s\) and \(F^0_i\) designate the initial fractional concentration of soluble and insoluble gas, respectively, in the rebreathing bag.

Figure 7. Pulmonary blood flow measurement at rest and exercise with Innocor™. Normalized soluble gas concentrations are shown throughout rebreathing maneuvers performed at rest and 50W cycle exercise by a 50-year-old sedentary male. The slope of the regression line through the logarithmic normalized end-expiratory soluble gas concentration points is proportional to pulmonary blood flow.
Pulmonary blood flow is calculated by the IGR system (Innocor™, Innovision, Odense, Denmark) with Equation 8, which is analogous to Equation 5:

\[
PBF = -\frac{\beta \times V_{s,\text{tot}}}{\alpha_b} - \frac{\beta \times \alpha_t V_t}{\alpha_b} \frac{P_B - 47}{760}
\]  \hspace{1cm} (8)

The slope of the regression line (\(\beta, \text{min}^{-1}\)) is multiplied by the total system volume (\(V_{s,\text{tot}}, \text{L, STPD}\)) to obtain the soluble gas uptake. This product is divided by the Bunsen solubility coefficient in blood at body temperature (\(\alpha_b, \text{mL STPD L}^{-1} \text{ atm}^{-1}\) at 37°C. Corrections are made for the ambient pressure (\(P_B, \text{mmHg}\)) and test gas solubility in a standard volume of lung tissue (\(\alpha_t V_t\)) to obtain PBF in L min\(^{-1}\) (Petrini et al., 1978).

3.8 Statistical Analysis

Values are reported as means ± SD unless otherwise specified (IBM® SPPS® Statistics version 22.0.0.0). Based on previous studies which contrasted ventilatory efficiency in COPD subjects with similar disease severity versus controls, we estimated that a sample size of 20 subjects in each group would be required (Ofir et al., 2008; Chin et al., 2013; Guenette et al., 2014; Elbehairy et al., 2015b, 2015a). According to a variable distribution (Kolmogorov-Smirnov), controls and COPD subjects were compared by non-paired \(t\) or Mann-Whitney’s test. A \(\chi^2\) test assessed differences in proportions. Pearson’s \(r\) correlation coefficient assessed linear association between continuous variables. The accepted risk for a type I error was less than 5% (p<0.05).
Chapter 4

Results

4.1 Subject Characteristics

Subjects with COPD and controls were well-matched by key demographic and anthropometric variables. Moreover, there were no between-group differences in the regular physical activity scores or co-morbidity burden as indicated by the Charlson index (Charlson et al., 1987) (Table 5). As expected, COPD subjects had higher modified Medical Research Council (mMRC) dyspnea scores and greater impairment on resting lung function compared to controls (Table 6). However, 15/19 (78.9%) COPD subjects had no or mild dyspnea in daily life (mMRC 0-1). Mild and moderate airflow obstruction were observed in 15 and 4 subjects, respectively. Therefore, most COPD subjects had GOLD grade 1A COPD severity.

The most prominent abnormalities from pulmonary function test results were mild absolute and relative pulmonary gas trapping, increased specific airway resistance and mild decrements in $D_L^{CO}$ and $K_{CO}$ (Table 6). In fact, 13/19 (68.4%) and 12/19 (63.2%) COPD subjects had RV and RV/TLC, respectively, above the upper limit of normality. Moreover, 11/19 (57.9%) COPD subjects had $D_L^{CO}$ test results below the LLN.
Table 5. Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>COPD (n=19)</th>
<th>Controls (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male: Female, n</td>
<td>9:10</td>
<td>14:12</td>
</tr>
<tr>
<td>Age, years</td>
<td>62±6</td>
<td>61±9</td>
</tr>
<tr>
<td>Height, cm</td>
<td>166±10</td>
<td>167±10</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>77.5±17.1</td>
<td>75.1±13.3</td>
</tr>
<tr>
<td>Body mass index, kg m²</td>
<td>27.4±5.2</td>
<td>26.8±4.1</td>
</tr>
<tr>
<td>Left ventricular ejection fraction</td>
<td>68.7±7.4</td>
<td>-</td>
</tr>
<tr>
<td>Smoking, pack-yrs</td>
<td>40.4±17.9*</td>
<td>5.6±11.6</td>
</tr>
<tr>
<td>Smoking status, n:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Current</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>- Never</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>- Ex-smoker</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>GOLD stages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>1B</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>2A</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>2B</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Modified MRC dyspnea scale</td>
<td>1.1±0.8*</td>
<td>0.1±0.3</td>
</tr>
<tr>
<td>Baecke’s Physical Activity score</td>
<td>7.1±1.9</td>
<td>7.6±1.9</td>
</tr>
<tr>
<td>Charlson Comorbidity Index</td>
<td>2.4±1.6</td>
<td>2.1±1.1</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD. *P<0.05. Abbreviations: GOLD= Global Initiative for Obstructive Lung Disease; MRC= Medical Research Council.
### Table 6. Resting Lung Function

<table>
<thead>
<tr>
<th></th>
<th>COPD (n=19)</th>
<th>Controls (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV$_1$, L</td>
<td>2.31±0.68* (82±13*)</td>
<td>2.92±0.62 (104±12)</td>
</tr>
<tr>
<td>FVC, L</td>
<td>3.80±1.03 (97±14)</td>
<td>3.76±0.88 (99±15)</td>
</tr>
<tr>
<td>FEV$_1$/FVC</td>
<td>62.3±8.3* (88±11*)</td>
<td>73.1±5.8 (104±9)</td>
</tr>
<tr>
<td>FEF$_{25-75%}$, L s$^{-1}$</td>
<td>1.10±0.42* (42±28*)</td>
<td>2.66±0.93 (96±22)</td>
</tr>
<tr>
<td>IC, L</td>
<td>2.88±0.64 (102±21)</td>
<td>2.95±0.73 (108±14)</td>
</tr>
<tr>
<td>FRC, L</td>
<td>3.38±0.62 (106±19)</td>
<td>-</td>
</tr>
<tr>
<td>TLC, L</td>
<td>6.13±1.11 (104±10)</td>
<td>-</td>
</tr>
<tr>
<td>RV, L</td>
<td>2.44±0.92 (117±29)</td>
<td>-</td>
</tr>
<tr>
<td>RV/TLC</td>
<td>38±9 (115±18)</td>
<td>-</td>
</tr>
<tr>
<td>D$_t$CO, ml min$^{-1}$ mmHg$^{-1}$</td>
<td>15.9±6.1 (80±17)</td>
<td>-</td>
</tr>
<tr>
<td>KCO, ml$^{-1}$ min$^{-1}$ mmHg$^{-1}$ L$^{-1}$</td>
<td>3.2±0.6 (86±15)</td>
<td>-</td>
</tr>
<tr>
<td>sRaw, cmH$_2$O s</td>
<td>9.6±4.1 (231±84)</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are means ± SD and expressed in brackets as a percent (%) of the predicted value. *P<0.05. Abbreviations: D$_t$CO= diffusing capacity of the lung for carbon monoxide; KCO = D$_t$CO divided by accessible ‘alveolar’ volume (transfer coefficient); FEF$_{25-75\%}$= forced expiratory flow between 25 and 75% of forced vital capacity; FEV$_1$= forced expired volume in 1 second; FVC= forced vital capacity; FEV$_1$/FVC= ratio between FEV$_1$ and FVC; FRC= functional residual capacity; IC= inspiratory capacity; RV=residual volume; sRaw= specific airway resistance; TLC= total lung capacity.

### 4.2 Imaging

All except three chest radiographs from the COPD group were considered free of abnormalities. The three abnormal chest radiographs indicated mild lung hyperinflation and increased interstitial markings. In contrast, evidence of emphysema was found in each subject, and 14/19 (73.7%) subjects had low attenuation areas greater than 5%. As shown in Table 7, there was a predominance ofcentrilobular over panlobular (18.4 ± 6.3%) and paraseptal (6.5 ± 3.1%) emphysema involving the upper lobes. Evidence of airway disease was less
extensive compared to emphysematous changes. No subjects had localized lobar hyperinflation or macroscopic bullae/cysts. Additionally, nodules greater than 1 cm, consolidations, bronchiectasis or pleural disease were not found in any subject.

Table 7. Chest HRCT results in subjects with COPD

<table>
<thead>
<tr>
<th></th>
<th>n=19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emphysema index (LAA&lt;sub&gt;950%&lt;/sub&gt;)</td>
<td>11.1 ± 8.0</td>
</tr>
<tr>
<td>Upper lobes/basal lobes ratio</td>
<td>0.82 ± 0.04</td>
</tr>
<tr>
<td>Centrilobular emphysema (% total emphysema)</td>
<td>76.8 ± 10.1</td>
</tr>
<tr>
<td>Airway thickness (Pi10, mm)</td>
<td>4.8 ± 0.5</td>
</tr>
<tr>
<td>Emphysema predominance over airway disease (n)</td>
<td>15</td>
</tr>
</tbody>
</table>

Values are means ± SD. Abbreviations: LAA= low attenuation areas; Pi10= Standardization of airway wall thickness to a theoretical airway with an internal perimeter of 10 mm.

4.3 Symptom-limited Incremental CPET

Subjects with COPD had significantly lower symptom-limited peak exercise capacity than healthy controls (p<0.05;Table 8). Higher relative ventilatory (VE, %MVV) but lower chronotropic (HR, % pred) and metabolic (RER) stresses were observed at exercise termination in COPD subjects compared to controls. There were no significant between-group differences in cardiovascular and arterial oxygenation variables. Variables typically related to deconditioning (e.g. low VO<sub>2</sub>GET and low peak O<sub>2</sub> pulse) also did not differ between COPD subjects and controls (p>0.05). However, a greater symptom burden (both in relation to peak dyspnea and leg discomfort scores) was found in the COPD group (Table 8).
Table 8. Symptom-limited Incremental CPET Measurements

<table>
<thead>
<tr>
<th>Variables</th>
<th>COPD (n=19)</th>
<th>Controls (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Power/Metabolism</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak work rate, W (% pred)</td>
<td>111±39 (79±20*)</td>
<td>134±37 (101±18)</td>
</tr>
<tr>
<td>Peak $\bar{V}O_2$, mL min$^{-1}$ kg$^{-1}$ (% pred)</td>
<td>19.3±5.0* (86±15*)</td>
<td>23.5±6.9 (100±21)</td>
</tr>
<tr>
<td>$\Delta \bar{V}O_2/\Delta$work rate, mL min$^{-1}$ W$^{-1}$</td>
<td>9.8±1.2</td>
<td>10.2±1.4</td>
</tr>
<tr>
<td>Peak RER</td>
<td>1.18±0.1*</td>
<td>1.22±0.1</td>
</tr>
<tr>
<td>$\bar{V}O_{2GET}$, L min$^{-1}$ (% pred peak $\bar{V}O_2$)</td>
<td>0.99±0.33 (47±14)</td>
<td>0.93±0.23 (46±15)</td>
</tr>
<tr>
<td><strong>Ventilatory</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak $\dot{V}$E, L min$^{-1}$ (%MVV)</td>
<td>57.7±20.8* (64±18*)</td>
<td>65.2±20.2 (51±17)</td>
</tr>
<tr>
<td>Peak $V_T$, L</td>
<td>1.90±0.62*</td>
<td>2.11±0.68</td>
</tr>
<tr>
<td>Peak $f$, breaths min$^{-1}$</td>
<td>31±5</td>
<td>32±8</td>
</tr>
<tr>
<td>Peak $\dot{V}$E/$\dot{V}$CO$_2$</td>
<td>33±3*</td>
<td>30±2</td>
</tr>
<tr>
<td><strong>Gas Exchange</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak SpO$_2$, %</td>
<td>95±3</td>
<td>96±3</td>
</tr>
<tr>
<td>$P_{ET}CO_2$ GET, mmHg</td>
<td>37±3*</td>
<td>40±4</td>
</tr>
<tr>
<td>Peak $P_{ET}CO_2$ mmHg</td>
<td>37±4</td>
<td>36±2</td>
</tr>
<tr>
<td><strong>Cardiovascular</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak HR, beats min$^{-1}$ (% predicted)</td>
<td>132±16* (84±13*)</td>
<td>146±14 (91±9)</td>
</tr>
<tr>
<td>Peak $O_2$ pulse, mL O$_2$ beat$^{-1}$</td>
<td>11.0±3.6</td>
<td>12.0±3.2</td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak dyspnea, Borg units†</td>
<td>5.8±1.7*</td>
<td>4.6±1.8</td>
</tr>
<tr>
<td>Peak leg discomfort, Borg units†</td>
<td>6.7±2.1*</td>
<td>5.3±1.7</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P<0.05. † n= 16 in COPD and n= 23 in controls, respectively.

*Abbreviations*: $f$ = breathing frequency; HR = heart rate; MVV = maximal ventilatory ventilation; $P_{ET}CO_2$ = partial pressure of end-tidal carbon dioxide; RER = respiratory exchange ratio; SpO$_2$ = arterial oxygen saturation by pulse oximetry; $\dot{V}$E = minute ventilation; $\bar{V}O_2$ = oxygen uptake; $V_T$ = tidal volume.

As shown in Figure 8, significantly steeper $\dot{V}$E-$\bar{V}$CO$_2$ slopes and marginally higher $\dot{V}$E-$\bar{V}$CO$_2$ intercepts led to greater $\dot{V}$E/$\bar{V}$CO$_2$ nadirs in COPD subjects compared to controls, i.e. poorer ventilatory efficiency (Neder et al., 2015a). Particularly low peak $\bar{V}O_2$ and high dyspnea scores were found in a subgroup of
COPD subjects (n=12) with $V_E/V_{CO_2}$ nadir $\geq$ 30 compared to their counterparts with lower nadirs (peak $VO_2 = 76 \pm 16$ % pred vs. $97 \pm 12$ % pred and dyspnea= 6.1 $\pm$ 1.6 vs. 4.4 $\pm$ 1.9, respectively; p<0.05).

![Graph showing parameters of the ventilation ($V_E$)–carbon dioxide output ($V_{CO_2}$) relationship obtained in the symptom-limited incremental CPET. Values are expressed as mean ± SD.](image)

**Figure 8.** Parameters of the ventilation ($V_E$)–carbon dioxide output ($V_{CO_2}$) relationship obtained in the symptom-limited incremental CPET. Values are expressed as mean ± SD.

### 4.4 Constant Work Rate Exercise Tests

Constant work rate tests were performed by 16 (25 W) and 13 (50W) COPD subjects and 26 controls. Technically-acceptable PBF measurements were obtained in 14 (25 W) and 12 (50 W) COPD subjects and 21 controls, respectively. Repeated tests were available in 12 COPD subjects and 21 controls (25W and 50W, respectively). Overestimation of EELV was the most common reason for
unacceptable IGR readings. The limits of agreement between test and re-test for both groups are shown in Figure 9. This analysis revealed limits of agreement of ~1 L min\(^{-1}\) at both 25 W and 50 W for COPD subjects and controls (~15% of mean value). Test-retest variability did not change as a function of the measured value, i.e., there was no significant heteroscedasticity (Figure 9).

Figure 9. Test-retest limits of agreement (Bland-Altman plot) for inert gas rebreathing (IGR) measurements of pulmonary blood flow (PBF) at 25W and 50W in subjects with mild-to-moderate COPD (n=12) and controls (n=21).

As expected from similar work rates in subjects with comparable body dimensions (Table 5), metabolic demand (expressed as \(\bar{V}O_2\) or \(\bar{V}CO_2\)) was
equivalent between COPD subjects and controls (Figure 10A). As was the case with the $\dot{V}_{E}/\dot{V}_{CO_2}$ nadir in the incremental test, $\dot{V}_{E}/\dot{V}_{CO_2}$ was higher in COPD subjects than controls at 25 W and at 50 W (Figure 11). COPD subjects with higher $\dot{V}_{E}/\dot{V}_{CO_2}$ nadir also had higher $\dot{V}_{E}/\dot{V}_{CO_2}$ values at 25 W and 50 W ($r=0.74$ and $r=0.69$, respectively; $p<0.01$). Moreover, $P_{ET}CO_2$ was significantly lower in COPD subjects than controls at both work rates (Figure 11B).

The key findings from these tests are depicted in Figure 10B: despite similar resting values, COPD subjects had lower PBF at both 25 W and 50 W. Thus, PBF increased to a lesser extent from rest to exercise in COPD subjects compared to controls ($p<0.05$). However, there were no between-group differences in PBF changes from 25 W to 50 W ($p>0.05$) (Figure 12A). As expected given similar metabolic demands, PBF values relative to $\dot{V}O_2$ closely followed the aforementioned pattern (Figure 12B).
Figure 10. Absolute metabolic (oxygen uptake, $\dot{V}O_2$) (panel A) and hemodynamic responses (pulmonary blood flow, PBF) (panel B) at two constant work rate exercise tests in subjects with mild-to-moderate COPD and controls. Values are mean ± SD. *$P<0.05$ for COPD subjects vs. controls at a given test condition.
Figure 11. Ventilation-gas exchange coupling in response to two constant work rate exercise tests in subjects with mild-to-moderate COPD and controls. $\dot{V}_{E}/\dot{V}_{CO_2}$ (panel A) is the ventilatory equivalent for carbon dioxide and $P_{ET}CO_2$ (panel B) is the end-tidal partial pressure for CO$_2$. Values are mean ± SD. *P<0.05 for COPD subjects vs. controls at a given test condition.
Figure 12. Exercise-induced changes in pulmonary blood flow (PBF) either in absolute units (panel A) or relative to oxygen uptake ($\dot{V}O_2$) (panel B) in subjects with mild-to-moderate COPD and controls. Values are mean ± SD. *$P<0.05$ for COPD subjects vs. controls.

4.5 Structural and Functional Correlates of Ventilatory Inefficiency in COPD

COPD subjects with low peak $\dot{V}O_2$ had greater ventilatory inefficiency (Figure 13A). Emphysema severity was positively related to $\dot{V}E/\dot{V}CO_2$ nadir (Figure 13B). In contrast, we did not find a significant correlation between airway wall thickness (Pi10) and $\dot{V}E/\dot{V}CO_2$ nadir ($p>0.05$). Low $D_l$CO (but not low FEV$_1$ or high RV; $p>0.05$) was associated with higher $\dot{V}E/\dot{V}CO_2$ nadir (Figure 13C) and
reduced PBF from rest to 25 W (r= 0.60; p<0.05). Of note, lower exercise-induced changes in PBF from rest to 25 W were associated with greater emphysema extent (r= -0.69, p<0.01) and higher \( \dot{V}_E/\dot{V}CO_2 \) nadir (Figure 13D); moreover, \( \dot{V}_E/\dot{V}CO_2 \) nadir was marginally related to changes in PBF from rest to 50 W (r= 0.44; p=0.07).

A summary of the key inter-measurement correlations is shown in Figure 14.

Figure 13. Correlates of ventilatory inefficiency (higher ventilation (\( \dot{V}_E \))/carbon dioxide output (\( \dot{V}CO_2 \) nadir) in subjects with mild-to-moderate COPD [(n=19) with exception on panel D (n=16)]: lower peak oxygen uptake (\( \dot{V}O_2 \) (panel A), higher emphysema extent by HRCT (panel B), lower lung diffusing capacity for carbon monoxide (\( D_LCO \)) (panel C) and blunted increase in pulmonary blood flow (PBF) from rest to mild exercise (25 W) (panel D).
Figure 14. Significant cross-correlations between ventilatory inefficiency (ventilation (\(\dot{V}_{E}\))/carbon dioxide output (\(\dot{V}CO_2\) nadir), exercise capacity (peak \(\dot{V}O_2\)), emphysema extent by HRCT and lung diffusing capacity for carbon monoxide (DLCO) in subjects with mild-to-moderate COPD.
Chapter 5

Discussion

This study investigated the structural [emphysema and airway wall thickness by high-resolution computed tomography (HRCT)] and resting functional correlates of exercise ventilatory inefficiency in COPD subjects with mild-to-moderate airflow obstruction. Our main results indicate that, compared to controls, subjects with COPD had:

1) Lower lung diffusing capacity for carbon monoxide ($D_LCO$) that was associated with emphysema severity but not airway wall thickness;

2) Lower maximal exercise capacity (peak $\dot{VO}_2$) and greater ventilatory inefficiency ($\dot{V}_E/\dot{V}CO_2$) nadir during incremental and constant work rate exercise;

3) Lesser increases in pulmonary blood flow (PBF) from rest to constant work rate exercise.

Moreover, within the COPD group, we found:

1) Significant correlations between ventilatory inefficiency and maximal exercise capacity, emphysema extent, decreased $D_LCO$ and reduced PBF responses to exercise;

2) A sub-group of COPD subjects with $\dot{V}_E/\dot{V}CO_2$ nadir $\geq 30$ had lower maximal exercise capacity and higher peak dyspnea scores that their counterparts with nadirs $<30$. 

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These data provide novel evidence that ventilation “wasted” in emphysematous areas has an important role in reducing resting $D_L$CO and exercise ventilatory efficiency in subjects with mild-to-moderate COPD. Considering that emphysema on chest CT and low resting $D_L$CO are both associated with meaningful patient-centered outcomes (poor exercise capacity and dyspnea) (Grydeland et al., 2010; Kirby et al., 2013; Yamasawa et al., 2015; Díaz et al., 2015), they should be clinically valued in COPD subjects with largely preserved FEV$_1$.

5.1 Subject Characteristics

Subjects with mild-to-moderate COPD in our study were younger than subjects with moderate-to-severe disease evaluated in most studies, an expected finding given the natural history of COPD (O’Donnell et al., 2014$a$). Most subjects were overweight or had only mild obesity; thus, we likely avoided the complex effects of obesity on exercise responses in subjects with COPD (Ora et al., 2009; O’Donnell et al., 2014). Moreover, the effect of obesity would have underestimated ventilatory inefficiency as determined by slope and nadir (Ora et al., 2009), which were significantly higher in the COPD group (Figure 8).

We excluded COPD subjects with major cardiovascular co-morbidities and impaired left ventricular systolic function. Moreover, COPD subjects and controls were well-matched by regular physical activity and the burden of co-morbidities as assessed by the Charlson index (Charlson et al., 1987). Thus, we avoided major confounding factors of peak exercise capacity and ventilatory inefficiency such as
heart failure (Arbex et al., 2016) and coronary artery disease (Thirapatarapong et al., 2014). Despite reduced exercise capacity and higher peak dyspnea scores compared to controls, it is noteworthy that COPD subjects typically reported only mild dyspnea on daily life. These results are in line with previous studies involving subjects with similar degrees of airflow obstruction (as reviewed by (O’Donnell et al., 2016)), suggesting that subjects with milder COPD either avoid dyspnea-generating activities or tend to ascribe their exertional symptoms to deconditioning and aging.

5.2 Resting Pulmonary Function

The COPD group had resting physiological abnormalities that closely resembled those found in previous studies involving mild-to-moderate COPD subjects. These included absolute and relative pulmonary gas trapping, increased airway resistance, and low DLCO and KCO (Ofir et al., 2008; Chin et al., 2013; Guenette et al., 2014; Elbehairy et al., 2015b, 2015a). There is growing interest in defining resting functional and imaging characteristics that are associated with clinically-meaningful outcomes such as dyspnea and reduced exercise capacity (Kirby et al., 2013, 2015; Kirby & Parraga, 2014; Díaz et al., 2015). Our data adds to the recent evidence that a low DLCO is associated with impaired maximal exercise capacity in subjects with mild-to-moderate COPD (Fujimoto et al., 2011; Kirby et al., 2015). The low DLCO does not indicate diffusion-limitation of oxygen in subjects with COPD, and is reflective of the underlying emphysema and abnormal distribution
of ventilation that can reduce $D_{L}CO$ independent of alveolar-capillary membrane loss (Baldi et al., 2006). Therefore, the correlation is likely due to the contribution of underlying emphysema and airway abnormalities to abnormal pulmonary mechanics that limit ventilation and reduce exercise capacity (section 2.6). However, a low $D_{L}CO$ is also a consistent (but non-significant) signal for greater peripheral vascular impairment assessed by flow-mediated dilation (Barr et al., 2007). This topic warrants additional investigation in subjects with milder COPD.

As expected by the inclusion criteria, most of the COPD subjects had a $FEV_{1} \geq 80\%$ predicted, a cut-off proposed by the GOLD report to indicate normality (Vogelmeier et al., 2016). Thus, albeit characterized as having “mild-to-moderate” airflow obstruction, our sample was predisposed to represent COPD subjects at the less extensive end of the spectrum of disease severity. Also in line with the GOLD recommendations, we used the 0.7 $FEV_{1}/FVC$ threshold to indicate the presence of airway obstruction (Vogelmeier et al., 2016). Although this might lead to a false-positive diagnosis of COPD in older subjects (Hoesein et al., 2011), all but 5 COPD subjects also had $FEV_{1}/FVC < LLN$. Of note, these 5 subjects had reduced $D_{L}CO$ and increased residual volume, thereby indicating the presence of physiological abnormalities consistent with COPD (O’Donnell et al., 2014).

5.3 Imaging

There is growing evidence that subjects with preserved flows or only mild airflow obstruction might have relatively extensive structural abnormalities,
particularly emphysema as detected by HRCT (Kirby et al., 2013, 2015; Tan et al., 2015; Pike et al., 2016; Bhatt et al., 2016). Our study confirms these previous findings as most of the COPD subjects in our study had > 5% emphysema (less than -950 HU attenuation areas) on chest CT scans. Although the prevalence of subjects with low attenuation areas varies widely according to the specific sample characteristics, several previous studies indicate that the majority of subjects with mild-to-moderate airflow obstruction have low attenuation areas in the range of 5% to 25% (Patel et al., 2008; Kirby et al., 2013, 2015; Pike et al., 2016).

The 5% low attenuation area threshold has been used to differentiate a trivial amount of emphysema from mild emphysema (Patel et al., 2008). A recent study also confirmed that the -950 HU threshold represents the best tomographic combination of sensitivity and specificity to indicate the presence of emphysematous areas (Wang et al., 2013). Thus, we are confident that our methodological approach provided a sound estimate of the extent of emphysema.

There is recent evidence that subjects with mild-to-moderate COPD that have an “emphysema-dominant” phenotype – as in the present study - have increased lung volumes and more impaired gas exchange in comparison with the “airways disease-dominant” phenotype (Subramanian et al., 2016). The landmark “MESA COPD study” found a greater prevalence of emphysema compared to airway disease in smokers with or without airway obstruction (Smith et al., 2014). In line with an inconsistent history of chronic bronchitis symptoms, only 4/19 of
the COPD subjects showed a more dominant feature of airway disease than emphysema. The MESA study also found that, compared to subjects without COPD, smokers with centrilobular emphysema had greater dyspnea, reduced walk distance in 6 minutes, greater hyperinflation, and lower $D_{L}CO$ (Smith et al., 2014). These results (and ours) confirm a time-honored concept that emphysema is more likely than airway disease to be associated with negative physiological outcomes pertinent to reduced exercise capacity.

It is noteworthy that we found a predominance of centrilobular emphysema involving the upper lobes. Similarly, Pike et al. found that the majority of subjects with mild-to-moderate COPD had centrilobular emphysema in the upper lobes rather than the lower lobes (Pike et al., 2016). Of note, Wang et al. found that $D_{L}CO$ and pulmonary gas trapping were primarily affected by the percentage of low attenuation areas in the upper lobes (Wang et al., 2015). As discussed in section 5.6, this pattern of structural abnormalities might have relevant implications for the exercise ventilatory inefficiency in the COPD subjects.

5.4 Responses to Incremental Exercise

Consistent with previous studies involving subjects with similar resting functional impairment, the COPD subjects had lower maximal exercise capacity compared to healthy controls (Chin et al., 2013; Elbehairy et al., 2015b, 2015a). It is noteworthy that some of those previous studies included many control subjects with peak $\dot{V}O_2$ values greater than 100% predicted (Elbehairy et al., 2015b, 2015a).
In contrast, the control group in our study had a mean peak $V_O^2$ that was equal to the predicted value, indicating that differences observed between groups were not due to a high number of fit subjects in the control group. Other physiological findings associated with poor conditioning did not differ between COPD subjects and controls (Table 8). Thus, we minimized the confounding influence of high and exceptionally low fitness on the exercise responses of the control group (Wasserman et al., 2011).

In line with the premise of our study, we found poorer ventilatory efficiency in the COPD group. Interestingly, higher nadirs in COPD subjects were secondary to steeper slopes and to higher intercepts (Figure 8). These data corroborate the notion that all parameters of the $V_E/VCO_2$ relationship are able to reflect the presence of ventilatory inefficiency in mild-to-moderate COPD (Neder et al., 2015a). The nadir represents a combined effect of the slope and intercept. Therefore, the nadir might constitute a more sensitive index of ventilatory inefficiency in COPD subjects with positive intercepts and limited mechanical constraints below the ventilatory compensation point (Palange et al., 2007). As discussed in section 1.3, however, it is not clear whether the slope and intercept represent different pathophysiological constructs in COPD (Poon & Tin, 2013; Ward, 2013; Gargiulo et al., 2014). This topic merits further scrutiny in subjects with mild-to-moderate airflow obstruction. Greater dyspnea scores were found in COPD subjects with higher nadirs. A large volume of research has shown that this
finding largely stems from the combined effects of higher neural drive and greater erosion of the mechanical reserves induced by an excessive ventilatory response to exertion (Ofir et al., 2008; Chin et al., 2013; O’Donnell et al., 2014b; Guenette et al., 2014; Elbehairy et al., 2015b, 2015a).

5.5 Exercise Pulmonary Blood Flow

We found a downward displacement of the PBF-\(\dot{V}O_2\) relationship in subjects with COPD (Figure 12B). Thus, reduced absolute PBF in COPD subjects (Figure 12A) is unlikely to reflect lower cardiac output due to diminished metabolic demands. The subjects in this study did not have major cardiovascular co-morbidities and, considering the presence of only mild resting pulmonary gas trapping, had a low probability of negative cardiopulmonary interactions (O’Donnell et al., 2014a). In other words, it is also improbable that they had lower cardiac output/\(\dot{V}O_2\) ratios than controls. Of note, impairments in PBF/\(\dot{V}O_2\) did not worsen with exercise progression as \(\Delta PBF\) 50W-25W did not differ between the groups (Figure 12B). It has been well-established that the pulmonary vasculature is already intensely recruited from rest to mild exercise (Reeves & Taylor, 2010). Thus, increasing exercise work rate does not seem to have uncovered further impairments in exercise PBF in the COPD subjects.

The exact mechanism behind low exercise PBF in the COPD subjects remains elusive. However, it is noteworthy that emphysema severity was associated with lower PBF/\(\dot{V}O_2\). On one hand, this might reflect the inherent
limitations of IGR in subjects with ventilation distribution abnormalities (see below) (Perrault et al., 2016). On the other hand, emphysema-related vascular destruction may have compromised PBF under the stress of exercise, i.e. when distension and recruitment of vasculature is important (Reeves & Taylor, 2010; Hilde et al., 2013). Indirect compressive effects on lung microvasculature due to over-distension of expanded air spaces and less tethering effects secondary to loss of alveolar attachments cannot be excluded (West, 2012). Whether PBF was also impaired due to microvascular abnormalities beyond those induced by emphysema per se should be investigated using advanced vascular imaging during exercise (Hueper et al., 2015).

It should be acknowledged that the interpretation of lower PBF by IGR is fraught with complexities in subjects with COPD. IGR (Innocor™, Innovision, Odense, Denmark) would underestimate PBF by the amount of blood flow through intrapulmonary shunts and poorly ventilated alveoli (areas of low ventilation-perfusion ratios ($\dot{V_A}/\dot{Q}$)). As the extent of emphysema increases in mild COPD, the dispersion of blood flow increases as does the absolute amount supplying regions with low $\dot{V_A}/\dot{Q}$ (0.005-0.1) (Barbera et al., 1990). However, hypoxic pulmonary vasoconstriction helps maintain ventilation-perfusion matching such that these regions only receive 0-12% of the total pulmonary blood flow (Barbera et al., 1990, 1994). Subjects with severe obstructive disease predominantly characterized by emphysema have less perfusion to low $\dot{V_A}/\dot{Q}$.
regions than their counterparts with chronic bronchitis (Wagner et al., 1977). Therefore, IGR underestimates pulmonary blood flow to poorly ventilated regions, but likely to a small degree in subjects with only mild obstruction, functional hypoxic pulmonary vasoconstriction, and an emphysematous phenotype.

The calculation used by the IGR system assumes that the disappearance of N₂O into the blood is represented by a mono-exponential decline in end-expiratory gas concentration. This relies on a constant pulmonary blood flow, the absence of recirculated test gas, and that the decline in end-expiratory gas concentration is representative of the decline in alveolar gas concentration. Subjects must therefore achieve a steady state at rest or during exercise in order for the IGR test to provide a valid measurement. If exercise intensity increases to the extent that recirculation of test gas decreases the alveolar-capillary partial pressure gradient, pulmonary blood flow through ventilated lung regions will be underestimated. The end-expiratory gas concentration is not necessarily representative of alveolar gas concentration in the presence of alveolar dead space, and this will reduce the accuracy of IGR at rest and exercise as COPD severity increases (Perrault et al., 2016).

The Innocor™ system has been validated against gold-standard Fick and thermodilution techniques. In a study with healthy subjects and heart failure patients, the correlations between Innocor™ and Fick, Innocor™ and
thermodilution, and Fick and Thermodilution from rest to maximal exercise were r=0.95, 0.94, and 0.95, respectively. The methods also agreed according to Bland-Altman analysis (Agostoni et al., 2005). Other rebreathing techniques have also been shown to agree with thermodilution despite abnormal distribution of ventilation (Reinhart et al., 1979), and InnocorTM is agreeable with cardiac magnetic resonance imaging at rest on subjects with lung disease (Saur et al., 2010).

In addition, the use of inert gas rebreathing is advantageous over non-invasive CO2 rebreathing above the anaerobic threshold (Sun et al., 2000). In the present study, we observed limits of agreement between test and retest (Figure 9) which compare favorably with those observed from other non-invasive techniques (Jakovljevic et al., 2008). However, important errors were observed in a sizeable number of observations in subjects with COPD. This shed negative light on the technique as a clinically-viable method for routine use in this population. In a study with a cohort of subjects with more severe COPD, inadequate gas mixing led to the underestimation of PBF by inert gas rebreathing (Perrault et al., 2016). In summary, we acknowledge that the observed association between emphysema extent and low exercise PBF might represent the combination of a truly physiological phenomenon (poor perfusion due to capillary destruction in emphysematous areas) (Hueper et al., 2015) with unwanted consequences of methodological caveats of IGR in subjects with COPD (Perrault et al., 2016).
5.6 Ventilatory Inefficiency and its Correlates

Increased “wasted” ventilation represents a major challenge to the lungs as an efficient gas exchanger (Robertson, 2015). The significant cross-correlations between emphysema severity, lower $D_{l, CO}$ and greater ventilatory inefficiency (Figure 14) provide compelling evidence that increased alveolar dead space, and therefore physiological dead space ($V_D$) due to emphysema, is a relevant mediator of impaired ventilatory efficiency in COPD subjects. As discussed in Chapter 1 and Chapter 2, the $V_D/V_T$ ratio is strongly influenced by ventilation-perfusion mismatching. Centrilobular (centriacinar) emphysema is associated with loss of the respiratory bronchioles in the proximal portion of acini while distal alveoli are spared (Takahashi et al., 2012). Structure-function correlation studies in subjects with mild-moderate COPD have shown that emphysema results in ventilation-perfusion mismatching with a wide spectrum of $\dot{V}_A/\dot{Q}$ heterogeneity, including: a) areas of low $\dot{V}_A/\dot{Q}$ ratio, due to reduced ventilation secondary to airway narrowing and distortion and b) areas of high $\dot{V}_A/\dot{Q}$ ratio due to more extensive microvascular destruction than loss of alveolar units (Hueper et al., 2015), as well as mechanical compression by over-distended air spaces in the distal acini (Barbera et al., 1990, 1991). It is rather interesting that emphysema predominated in the upper lobes, an area with particularly high $\dot{V}_A/\dot{Q}$ ratios at rest and during exercise (Harf et al., 1978). In other words, areas of centrilobular emphysema in the
upper lobes are more prone to increase “wasted” ventilation upon exertion than better perfused areas of the lower lobes.

In this context, Barbera and colleagues found that in subjects with “milder” COPD (FEV$_1$ = 76 ± 15%) the severity of emphysema was the major morphologic correlate of the increase in resting P$_{A-a}$O$_2$ (Barbera et al., 1990). These authors subsequently found that despite improvements in $\dot{V}_A/\dot{Q}$ distributions during exercise in subjects with mild-to-moderate airflow obstruction (emphysema score = 16 ± 4 %), the severity of emphysema was correlated with exertional P$_a$O$_2$ ($r = -0.54$, $p < 0.05$) (Barbera et al., 1991). Capderou and co-workers used pulmonary distribution of transit times of a $^{99m}$Tc intravascular tracer to show the presence of abnormal transit times in mild COPD which could not be explained by cardiac output, hypoxemia, hypercapnia, or acidosis. The authors raised the hypothesis that this could be related to structural abnormalities (including early emphysema) or dysfunctional regulatory responses (Capderou et al., 2000). Collectively, our data suggest that $\dot{V}_A/\dot{Q}$ mismatch, which is influenced by emphysema severity (Barbera et al., 1990), may have contributed to decreased exercise ventilatory efficiency and $D_l$CO beyond alveolar-capillary membrane destruction. Considering that ventilation delivered to unperfused alveolar spaces has an even greater impact on alveolar dead space, microvascular destruction in non-emphysematous areas may have also contributed to reduced ventilatory efficiency (Hueper et al., 2015). Thus, inflammatory/hyperoxidative processes
involved in the genesis of emphysema may have also damaged the lung microvasculature in non-emphysematous areas and contributed to “wasted” ventilation (Hueper et al., 2015).

The present study provided new insight into the mechanisms behind the reported associations between emphysema extent, impaired gas transfer, and poor exercise capacity (lower 6-minute walk distance and lower peak VO2) (Kirby et al., 2013; Yamasawa et al., 2015; Díaz et al., 2015). Thus, the inter-relationships between these measurements (Figure 14) indicate that excessive exercise ventilation links structural abnormalities (emphysema) and resting physiological impairment (gas transfer) to a key negative outcome (reduced exercise capacity) despite unremarkable spirometric abnormalities. From a clinical standpoint, they indicate that evidence of early emphysema on HRCT and/or decrements in Dl,CO should be clinically valued in COPD subjects with largely preserved FEV1.

5.7 Study Limitations

As a relatively small clinical physiology study, our investigation has some relevant limitations. Functional assessment was restricted to the conventional lung function tests as more sophisticated tests of small airway function and ventilation distribution (e.g. single-breath nitrogen washout test) were not performed (Fowler, 1949; Elbehairy et al., 2015b). Given the peripheral airway abnormalities that can occur in smokers without COPD (see Chapter 2), an impulse oscillometry system could be useful for excluding controls with a history of smoking (Brashier
& Salvi, 2015). We were also unable to separate the membrane and vascular components of gas transfer, which precluded the determination of their relative contributions to low \(D_L\text{CO}\) in COPD subjects. Future studies using methods able to provide estimates of capillary blood volume (such as \(D_L\text{NO}/D_L\text{CO}\) measurements) (Hughes & Lee, 2013) might be informative.

Potential structural abnormalities in addition to emphysema were not investigated, including small airway disease/pulmonary gas trapping and pulmonary microvascular disease. Thus, we cannot rule out that they are also mechanistically linked to exercise ventilatory inefficiency in these subjects. More advanced imaging techniques that can assess ventilation distribution abnormalities (such as hyperpolarized \(^3\)He magnetic resonance imaging (MRI) might have uncovered important abnormalities with a potential to increase “wasted” ventilation (Kirby et al., 2013; Pike et al., 2016). Although the study was adequately powered to uncover key physiological abnormalities in the COPD group, the number of observations (particularly PBF) likely weakened the calculated correlations. Moreover, we were unable to assess the putative association between physiological outcomes with other features of disease expression such as daily life dyspnea scores. Finally, we did not measure operating lung volumes or arterial blood gases because the causes and consequences of mechanical-ventilatory and pulmonary gas exchange abnormalities have already
been extensively investigated in subjects with mild COPD (Chin et al., 2013; Guenette et al., 2014; Elbehairy et al., 2015a).

5.8 Conclusions

Results from the present study, which investigated the structural and resting functional correlates of exercise ventilatory inefficiency in subjects with mild-to-moderate COPD, corroborated previous findings (Chin et al., 2013; Guenette et al., 2014; Elbehairy et al., 2015a) that indicated:

1) Ventilatory inefficiency is associated with reduced exercise capacity and exertional dyspnea.

Moreover, they provided novel evidence that:

2) Centrilobular emphysema in the upper lobes is an important structural correlate of impaired resting $D_{L}CO$ and exercise ventilatory inefficiency in subjects with mild-to-moderate COPD.

Consequently, evidence of emphysema on HRCT and/or low resting $D_{L}CO$ should be clinically-valued as they are associated with meaningful patient-centered outcomes in individuals with COPD with largely preserved FEV$_1$ (Grydeland et al., 2010; Kirby et al., 2013; Yamasawa et al., 2015; Díaz et al., 2015).
Literature Cited


Appendix A

A Primer on the Ventilation-Carbon Dioxide Output Relationship

The relationship between ventilation and carbon dioxide output is an excellent indicator of the adequacy of cardiopulmonary coupling and ventilatory control mechanisms, particularly during moderate exercise (Whipp & Ward, 1982). When ventilation is not mediated by psychogenic factors, humoral factors, hypoxemia, or changes in arterial hydrogen ion concentration, the output of carbon dioxide is determined by the regulated arterial partial pressure of carbon dioxide and the fraction of tidal volume comprised of physiological dead space (Whipp & Ward, 1982). Thus:

\[ V_T = V_A + V_D \]  

(9)

Where tidal volume \((V_T)\) is equal to the sum of alveolar volume \((V_A)\) and physiological dead space volume \((V_D)\). Tidal volume is the volume of air displaced between the atmosphere and the lungs in a given ventilatory cycle. Alveolar volume is the component of tidal volume that changes composition as a result of gas exchange with the pulmonary circulation. Physiological dead space volume is the volume of the conducting airways down to the location at which a large change in gas composition occurs due to gas exchange with blood (Fowler, 1948).

\[ \dot{V}_E = V_T \times f \]  

(10)

Where minute ventilation \((\dot{V}_E)\) is the flow of gas exhaled from the lungs in liters per minute at body temperature and saturated with water at ambient pressure (L...
min⁻¹, BTPS). It is equal to the product of tidal volume in liters ($V_T$, L) and the frequency of exhalations ($f$, breaths min⁻¹).

$$\dot{V}_E = \dot{V}_A + \dot{V}_D$$  \hspace{1cm} (11)

Where minute ventilation ($\dot{V}_E$) is equal to the sum of alveolar ventilation ($\dot{V}_A$, L min⁻¹, BTPS) and physiological dead space ventilation ($\dot{V}_D$, L min⁻¹, BTPS). Alveolar ventilation and dead space ventilation have equations analogous to Equation 10 with alveolar volume and physiological dead space volume, respectively, in place of tidal volume.

The primary function of ventilation is to exchange air between the alveoli and the atmosphere in order to facilitate gas exchange at the alveolar-capillary membrane. The equivalents of ventilation for removing carbon dioxide ($\dot{V}_E/\dot{V}$ CO₂) can be monitored non-invasively, while the factors that contribute to $\dot{V}_E/\dot{V}$ CO₂ during moderate and heavy exercise must be measured using invasive techniques. These factors will be introduced, and practical and (patho)physiological factors will be taken into account so that high $\dot{V}_E/\dot{V}$ CO₂ in disease can be understood even when there are deviations from a strictly mathematical relationship.

$$\dot{V}_A = k \frac{\dot{V}_{CO_2}}{P_{a\text{-}CO_2}}$$  \hspace{1cm} (12)

Where alveolar ventilation ($\dot{V}_A$, L min⁻¹, BTPS) is the fraction of ventilation that has participated in gas exchange with the pulmonary microcirculation. It is equal to the product of a coefficient ($k$) and the rate of carbon dioxide output at standard
temperature and pressure ($\dot{V}_{CO_2}$, L min$^{-1}$, STPD) divided by the regulated arterial partial pressure of carbon dioxide in millimeters of mercury ($P_{a}CO_2$, mmHg). The coefficient ($k$) is necessary for the conversion between the aforementioned conventional units. For the purposes of this document, it will be taken as a constant with a value of 863 mmHg.

When $\dot{V}_A$ is plotted on the ordinate and $\dot{V}_{CO_2}$ is plotted on the abscissa (Figure 15), they are directly proportional when the slope of their relationship is constant and equal to $k/P_{a}CO_2$. That is, they have a linear relationship that passes through the origin when a given $P_{a}CO_2$ is tightly regulated. Moreover, when $\dot{V}_A/\dot{V}_{CO_2}$ is plotted on the ordinate and $\dot{V}_{CO_2}$ is plotted on the abscissa (Figure 16), the function will be equal to $k/P_{a}CO_2$. Two indexes of abnormally high (excessive) $\dot{V}_A$, and therefore $\dot{V}_E$, can be appreciated even without taking into account the normal physiological condition of dead space ventilation. When $P_{a}CO_2$ is regulated at an abnormally low level independent of hypoxemia, arterial pH, or humoral factors, then $\dot{V}_A/\dot{V}_{CO_2}$ will be high across all values of $\dot{V}_{CO_2}$. That is, the linear relationship between $\dot{V}_A$ and $\dot{V}_{CO_2}$ will have a high slope. Secondly, $\dot{V}_A/\dot{V}_{CO_2}$ as a function of $\dot{V}_{CO_2}$ will equal a higher constant ($k/P_{a}CO_2$) value across the spectrum of $\dot{V}_{CO_2}$ when $P_{a}CO_2$ is low. When $k/P_{a}CO_2$ remains constant, the linear function will still pass through origin, and the two indicators of excessive $\dot{V}_A$ (slope and ratio) will be equal regardless of the value of $\dot{V}_{CO_2}$. Since $\dot{V}_D$ is not changing
in the circumstances described, higher ventilation is not “inefficient”, but may be considered “excessive” (Neder et al., 2016).

Figure 15. Steeper $\bar{V}_A$ versus $\dot{V}_{CO_2}$ relationships at lower regulated values of $P_{aCO_2}$ across the spectrum of $\dot{V}_{CO_2}$ in the absence of other mediators of ventilation.

Figure 16. Higher and constant $\bar{V}_A/\dot{V}_{CO_2}$ values at lower regulated values of $P_{aCO_2}$ across the spectrum of $\dot{V}_{CO_2}$ in the absence of other mediators of ventilation.
\[ \dot{V}_E = k \frac{\dot{V}CO_2}{P_aCO_2} + \dot{V}_D \] (13)

Equation 13 is obtained by the substitution of Equation 12 into Equation 11.

Three physiological conditions cause the measured \( \dot{V}_E/\dot{V}CO_2 \) to differ from the values produced from Equation 13 and displayed in Figure 15 and Figure 16. First, the ventilation and carbon dioxide output can never be zero for a given \( P_aCO_2 \). Second, psychogenic mediators of ventilation are prominent at rest and low levels of exercise such that ventilation is not entirely regulated by the \( P_aCO_2 \) set point. Third, ventilation is not just regulated by the \( P_aCO_2 \) set point in the upper domain of \( \dot{V}CO_2 \). As power output increases beyond that at the ventilatory compensation point, the reduction in buffering capacity for protons produced from anaerobic metabolism causes \( \dot{V}_E \) to increase out of proportion to \( \dot{V}CO_2 \) (Wasserman et al., 2011). The first two physiological conditions are taken into account during test interpretation, while the third can be avoided practically by disregarding data points generated above the ventilatory compensation point (Sun et al., 2002).

Ventilation and carbon dioxide output can never be zero at a constant \( P_aCO_2 \), so the intercept is calculated from an extrapolation of a linear regression line through real data points. The non-zero intercept is a consequence of a non-zero resting \( \dot{V}CO_2 \) and dead space ventilation(\( \dot{V}_D \)), but its magnitude is affected by the slope of the function and therefore by the \( P_aCO_2 \) set point. High positive intercepts in the absence of abnormal pulmonary mechanics may indicate greater
amounts of $\dot{V}_D$ during exercise (Chin et al., 2013; Gargiulo et al., 2014). Although $\dot{V}_D$ is always positive, the intercept extrapolated from recorded data is very near or below the origin in 10% of healthy individuals (Sun et al., 2002), and is commonly negative in subjects with pulmonary arterial hypertension. The latter phenomenon is due to the regulated $P_aCO_2$ being so low in subjects with pulmonary arterial hypertension that the $\dot{V}_E$-$\dot{V}CO_2$ slope is often high enough to produce a negative intercept (Vicenzi et al., 2016). In COPD, 96.8% of subjects’ data across all grades of COPD produced a positive intercept (Neder et al., 2015a).

The minimum $\dot{V}_E/\dot{V}CO_2$ over the $\dot{V}CO_2$ domain is influenced by both the slope and the intercept of the function. A non-zero intercept will cause the function of $\dot{V}_E/\dot{V}CO_2$ to be non-linear over the carbon dioxide output domain. Furthermore, negative and positive shifts will cause downward and upward shifts, respectively, in the minimum $\dot{V}_E/\dot{V}CO_2$ value below the ventilatory compensation point. When the intercept is zero, $\dot{V}_E/\dot{V}CO_2$ minimum will not provide information that is not already given by the slope, because it will be constant across all values of $\dot{V}CO_2$. As previously explained, an increased slope also contributes to an upward shift in the minimum $\dot{V}_E/\dot{V}CO_2$. The non-zero intercept causes the $\dot{V}_E/\dot{V}CO_2$ vs. $\dot{V}CO_2$ relationship to be hyperbolic below the ventilatory compensation point (Figure 17). The minimum or “nadir” of the function can therefore be used as another index of ventilatory inefficiency or excessive ventilation.
Figure 17. The combined effects of slope and positive intercept (i) on ventilatory equivalents for carbon dioxide ($V_{E}/V_{CO2}$) as a function of carbon dioxide ($V_{CO2}$) output.
APPENDIX

Questionnaire, codes, and method of calculation of scores on habitual physical activity

1) What is your main occupation? .................................................. 1 - 3 - 5

2) At work I sit
never/seldom/sometimes/often/always 1 - 2 - 3 - 4 - 5

3) At work I stand
never/seldom/sometimes/often/always 1 - 2 - 3 - 4 - 5

4) At work I walk
never/seldom/sometimes/often/always 1 - 2 - 3 - 4 - 5

5) At work I lift heavy loads
never/seldom/sometimes/often/very often 1 - 2 - 3 - 4 - 5

6) After working I am tired
very often/often/sometimes/seldom/never 5 - 4 - 3 - 2 - 1

7) At work I sweat
very often/often/sometimes/seldom/never 5 - 4 - 3 - 2 - 1

8) In comparison with others of my own age I think my work is physically
much heavier/heavier/as heavy/lighter/much lighter 5 - 4 - 3 - 2 - 1

9) Do you play sport? yes/no

If yes:
- which sport do you play most frequently?
  how many hours a week? <1/1-2/2-3/3-4/>4
  how many months 1 year? <1/1-3/3-4/>7

If you play a second sport:
- which sport is it?
  how many hours a week? <1/1-2/2-3/3-4/>4
  how many months 1 year? <1/1-3/3-4/>7

10) In comparison with others of my own age I think my physical activity during leisure time is
more/more/the same/less/much less 5 - 4 - 3 - 2 - 1

11) During leisure time I sweat
very often/often/sometimes/seldom/never 5 - 4 - 3 - 2 - 1

12) During leisure time I play sport
never/seldom/sometimes/often/very often 1 - 2 - 3 - 4 - 5

13) During leisure time I watch television
never/seldom/sometimes/often/very often 1 - 2 - 3 - 4 - 5

14) During leisure time I walk
never/seldom/sometimes/often/very often 1 - 2 - 3 - 4 - 5

15) During leisure time I cycle
never/seldom/sometimes/often/very often 1 - 2 - 3 - 4 - 5

16) How many minutes do you walk and/or cycle per day to and from work, school and shopping?
<3/3-5/5-15/15-30/30-45/>45 1 - 2 - 3 - 4 - 5

Calculation of the simple sport-score (I_s)
(a score of zero is given to people who do not play a sport)

\[ I_s = \sum (\text{intensity} \times \text{time} \times \text{proportion}) \]
\[ = 0.01 \times <4/4-8/8-12/>12 \]

Calculation of scores of the indices of physical activity:

[Work index \( = [I_w + (6 - I_s) + I_s + I_s + I_s + I_s + I_s]/8\)]

[Sport index \( = [I_s + I_s + I_s + I_s]/4\)]

[Leisure-time index \( = (6 - I_s) + I_s + I_s + I_s)/4\)]
Appendix C

Advertisement 1

RESEARCH VOLUNTEERS NEEDED

LUNG FUNCTION STUDY

YOU WILL BE COMPENSATED FOR YOUR TIME

- Men & Women
- Age: 50 and over
- Smokers & Non-smokers
- Physically Inactive

(613) 549-6666 ext:3197

This study in the Laboratory of Clinical Exercise Physiology (LACEP) has Health Sciences Ethics Board approval as of March 24, 2015 [DMED-1704-14 Resnr460129708]

LACEP

Queens

KGH

Kingston General Hospital - Connell 2-2000
76 Stuart Street, Kingston, ON, K7L 2V7
Appendix D
Advertisement 2

Research Volunteers Needed!

Queen's University & KGH Lung Function Study

*You will be compensated for your time*

**Inclusion Criteria:**
- Men & Women
- Aged: 50 and over
- Smokers & Non-smokers
- Physically Inactive

This study conducted in the Laboratory of Clinical Exercise Physiology (LACEP) has Health Sciences Research Ethics Board approval as of March 28, 2013 (SHED: 17041; AHEC: 96012758)

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Appendix E
Informed Consent Form

Study Information and Consent: COPD Form

Do Abnormal Cardiocirculatory Responses Contribute to Exercise Impairment in COPD Patients with Mild-to-Moderate Airflow Obstruction?

Principal Investigator: J. Alberto Neder-Seraphini, MD, FRCP(C)
Laboratory of Clinical Exercise Physiology (LACEP)
Division of Respiratory and Critical Care Medicine
Queen's University and Kingston General Hospital

Co-Investigator: Denis E. O'Donnell, MD, FRCP(C)
Respiratory Investigation Unit
Division of Respiratory and Critical Care Medicine
Queen's University and Kingston General Hospital

Research Team:
Daniel M. Hirai, PT, PhD (Post-Doctoral Fellow)
Joshua Jones, BSc (Master's Student)
Joel Zelt, BSc (Master's Student)
Luiza Castanhas, PT (Research Assistant)
Ingrid Szaigyarto, MSc (Research Associate)

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Version date: May 25, 2015

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# Appendix F

## Manuscript

**COPD: Journal Of Chronic Obstructive Pulmonary Disease**

### Emphysema on Thoracic CT and Exercise Ventilatory Inefficiency in Mild-to-Moderate COPD

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| Complete List of Authors: | Jones, Joshua; Queen’s University  
Zalt, Joel; Queen’s University  
Hirai, Daniel; Pulmonary Function and Clinical Exercise Physiology Unit (SEFEC), Respiratory Division, Federal University of São Paulo (UNIFESP)  
Fisher, John; Queen’s University  
O’Donnell, Denis; Queen’s University,  
Neder, J Alberto; Queen’s University, |
| Keywords: | Cardiopulmonary exercise testing, emphysema, computed tomography, gas exchange, chokes, ventilation |

URL: [http://me.manuscriptcentral.com/eopd](http://me.manuscriptcentral.com/eopd)
Appendix G

Health Sciences Research Ethics Board Approval

QUEEN’S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS
RESEARCH ETHICS BOARD

August 12, 2014

Dr. Neda Nader Serafini
Department of Medicine
Respiratory and Critical Care
Dear Dr. Neda Nader Serafini,

Study Title: Do Abnormal Cardiopulmonary Responses Contribute to Exercise Impairment in Patients with Mild COPD?
Co-Investigators: Dr. D. O’Donnell, Mr. J. Jones, Mr. J. Zelt, Miss N. Puri, Miss N. Abu-Ghazaleh,
Mme. L. Castanho, Dr. D. Mulher Hirai
Full Board Meeting Date: May 12, 2014

The members of the Queen’s University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board have examined the revised protocol, Baechle’s questionnaire, Borg scale, revised information/consent form – Controls, revised information/consent form – Patients for your project as stated above and consider it to be ethically acceptable. This approval is valid for one year from the date of this letter. Please attend carefully to the following list of ethics requirements you must fulfill over the course of your study:

Reporting of Amendments: If there are any changes to your study (e.g. consent, protocol, study procedures, etc.), you must submit an amendment to the Research Ethics Board for approval.

Reporting of Serious Adverse Events: Any unexpected serious adverse event occurring locally must be reported within 2 working days or earlier if required by the study sponsor. All other serious adverse events must be reported within 15 days after becoming aware of the information.

Reporting of Complaints: Any complaints made by participants or persons acting on behalf of participants must be reported to the Research Ethics Board within 7 days of becoming aware of the complaint. Note: All documents supplied to participants must have the contact information for the Research Ethics Board.

Annual Renewal: Prior to the expiration of your approval (which is one year from the date of the Chair’s signature below), you will be reminded to submit your renewal form along with any new changes or amendments you wish to make to your study. If there have been no major changes to your protocol, your approval may be renewed for another year.

Yours sincerely,

Chair, Health Sciences Research Ethics Board
Study Code: DMEC-1701.14Rome 6601759

Investigators: Please note that if your trial is registered by the sponsor, you must take responsibility to ensure that the registration information is accurate and complete
Appendix H
Health Sciences Research Ethics Board Amendment Approval

QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS
RESEARCH ETHICS BOARD (HSREB)

HSREB Amendment Acknowledgment/Ethics Clearance

August 06, 2015

Dr. Jose Neder Serafini
Department of Medicine
Richardson House

ROMEOTRAQ: #6012750
Department Code: DMED.1701.14
Study Title: DMED-1701-14 Do Abnormal Cardiocirculatory Responses Contribute to Exercise Impairment in COPD Patients with Mild-to-Moderate Airflow Obstruction?
Review Type: Delegated
Date Ethics Clearance Issued: August 06, 2015

Dear Dr. Neder Serafini,

The Queen’s University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board (HSREB) has reviewed the amendment application and granted ethics clearance/acknowledgement for the documents listed below.

- Addition of Ingrid Snijgynarte (Research Associate) to the study team
- Letter dated May 25 regarding requested amendments
- Change in title from “Do Abnormal Cardiocirculatory Responses Contribute to Exercise Impairment in Patients with Mild COPD?”
- Request to allow appointment of qualified physicians to supervise exercise tests in the absence of the P.I.
- Revised protocol
- General Information Package
- Consent to Contact - Smoking Cessation
- Consent to Contact - Clinics
- Clinical Recruitment Chart
- Information/Consent Form – Controls – Version date: May 25, 2015
- Information/Consent Form – Participants with COPD – Version date: May 25, 2015
- Addition of Paula Etienne (Research Assistant) as a team member
- Additional funding from the Department of Medicine Innovation Fund

Yours sincerely,

[Signature]
Chair, Health Sciences Research Ethics Board