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Cerebrovascular and Ventilatory Function in Chronic Obstructive Pulmonary Disease

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Cerebrovascular and Ventilatory Function in Chronic Obstructive Pulmonary Disease

by

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A THESIS

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Abstract

Chronic Obstructive Pulmonary Disease (COPD) is a disease primarily affecting the pulmonary system, most commonly resulting from prolonged exposure to cigarette smoke. While systemic respiratory disturbances in COPD are apparent, the chemical regulation within the brainstem and at the carotid bodies is unclear. Furthermore, the role that cerebral blood flow contributes to respiratory disturbances is poorly understood. Recent studies have demonstrated peripheral vascular impairments in COPD, which may have further implications for exercise –hyperemia. Oxidative stress serves as a plausible mechanism leading to vascular impairments, and the overall increased risk of stroke and cardiovascular disease.

This collection of studies set out to define the ventilatory and cerebrovascular responses to acute alterations in PCO_2 and PO_2 in COPD patients, and the relationship to oxidative stress. A secondary focus was to characterize both cerebral and peripheral blood flow during exercise. The thesis begins by investigating the cerebrovascular and ventilatory responses to acute euoxic-hypercapnia in mild-moderate COPD patients, and the relationship between these physiological parameters, and markers systemic of oxidative stress. Second, the cerebrovascular responses during moderate cycling exercise are defined. The final three studies collectively investigate the effect of the antioxidant, vitamin C, on the cerebrovascular and ventilatory responses to acute hyperoxic-hypercapnia, isocapnic-hypoxia, and on forearm blood flow during handgrip exercise, in COPD patients and healthy controls.

This thesis demonstrates that the cerebrovascular and ventilatory responses to hypercapnia, but not hypoxia, are impaired in COPD. Vitamin C was found to augment the ventilatory response to hyperoxic-hypercapnia suggesting oxidative stress contributes to the overall ventilatory limitations in COPD. Dynamic cycling exercise (at a matched

relative intensity), evoked a similar cerebrovascular response between COPD and controls, however, a modest increase in workload increased cerebral blood flow in COPD to levels greater than controls, reducing the cerebrovascular reserve capacity. Lastly, forearm blood flow in COPD patients during exercise was similar to controls, and was not affected by vitamin C. Overall, this series of experiments provides a better understanding of the complex systemic consequences of COPD in an integrated nature, thereby advancing knowledge in this important area of study.

Preface

This thesis contains a brief introduction (chapter 1), followed by five manuscript-style chapters (chapter 2-6), and a general discussion (chapter 7). Chapter 2 assesses the cerebrovascular and ventilatory responses to hypercapnia in women with COPD, and the role of systemic oxidative stress in the regulation of this response. Chapter 3 determines the cerebrovascular response in COPD during submaximal cycling exercise. Chapters 4 and 5 determine the effect of an antioxidant intervention on the cerebrovascular and ventilatory responses to acute hypercapnia and hypoxia in COPD patients, and in healthy aging. Lastly, Chapter 6 assesses endothelial function, and the peripheral blood flow response in COPD patients.

All scientific papers (chapter 2-6) are multi-author collaborations, and my role is described in the preface section of each manuscript. Each of these chapters begins with a preface to set the context of the research, declaration of my role in the work, and (where appropriate) the publisher's statement of permission to use reproduced published material.

The concepts for the studies described in Chapter 2-3 (*i.e.*, Studies #1-2) originated together. Similarly, Studies #3-5 (described in Chapters 4-6) were designed and performed at consecutive times. As such, many patients overlap in these studies. Study #3 and #4 were performed on the same day and thus utilized the same subjects for both studies.

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My experience would not have been the same without the great lab members that have helped in many ways. Mr. Andrew Beaudin for his expertise in Excel macros that I'm sure saved me hundreds of hours! Also to Dr. Craig Steinback for his knowledge and guidance in peripheral vascular ultrasound measurements. Last, but certainly not least, are all of the volunteers that took part in these studies. I would particularly like to thank the COPD patients, for whom much of what I asked them to do was challenging. Their willingness and desire to help is an inspiration.

I would also like to acknowledge the financial support that I have received along the way, including the Hotchkiss Brain Institute (Dr. Chen Fong Doctoral Scholarship), Alberta Lung Association, and the University of Calgary. This research was supported by the Brenda Strafford Foundation, and the Heart and Stroke Foundation of Canada.

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List of Symbols, Abbreviations and Nomenclature

Symbol	Definition
6MWD	Six-minute walking distance
8-OHdG	8-hydroxy-2'-deoxyguanosine
ACh	Acetylcholine
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AOPP	Advanced oxidation proteins product
ATS	American Thoracic Society
BA	Brachial artery
B*f*	Breathing frequency
BMI	Body mass index
CBF	Cerebral blood flow
$CMRO_2$	Cerebral metabolic rate of oxygen
CNS	Central nervous system
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
COx	Cerebral oxygenation
CPET	Cardiopulmonary exercise test
ctHb	Concentration of total hemoglobin
CV	Coefficient of variation
CVC	Cerebrovascular conductance
CVD	Cardiovascular disease
CVR	Cerebrovascular resistance
DBP	Diastolic blood pressure
DH	Dynamic hyperinflation
D_{LCO}	Diffusing capacity of the lung for carbon monoxide
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
EDD	Endothelial dependent dilation
ELISA	Enzyme-linked immunosorbent assay
EMGd	Electromyogram activity of the diaphragm
eNOS	Endothelial nitric oxide synthase
FBF	Forearm blood flow
FEV_1	Forced expiratory volume in one second
FEV_1/FVC	Ratio of forced expiratory volume in one second / forced vital capacity
FMD	Flow-mediated dilation
FRC	Functional residual capacity
FVC	Forced vital capacity or Forearm vascular conductance
GOLD	Global initiative for obstructive lung disease

GPX	Glutathione peroxidase
H^+	Hydrogen ion
H_2O_2	Hydrogen peroxide
HCO_3^-	Bicarbonate ion
Hct	Hematocrit
HCVR	Hypercapnic ventilatory response
HDL	High-density lipoprotein
HG	Handgrip
HR/HRT	Heart rate
HVR	Hypoxic ventilatory response
IC	Inspiratory capacity
ICA	Internal carotid artery
ICC	Intraclass correlation coefficient
$P_{0.1}$	Mouth occlusion pressure
MBP/MAP	Mean arterial blood pressure
MCA	Middle cerebral artery
MCAv	Middle cerebral artery velocity
MDA	Malondiadehide
METS	Metabolic equivalents
MVV	Maximum voluntary ventilation
NIRS	Near infrared spectroscopy
NIV	Non-invasive ventilation
NMD	Nitroglycerin-mediated dilation
n.p	Data not presented
eNOS	Endothelial nitric oxide synthase
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOx	Index of nitric oxide production (sum of nitrate and nitrite)
NPV	Negative predictive value
NTG	Nitroglycerine
O_2	Oxygen
O_2^-	Superoxide ion
O_2Hb	Oxy-hemoglobin
O_2 pulse	$\dot{V}O_2$ / HR
$ONOO^-$	Peroxynitrite
OS	Oxidative stress
$\overline{P}$	Mean power index (index of cross-section area of middle cerebral artery)
$Paco_2$	Arterial partial pressure of carbon dioxide
Pao_2	Arterial partial pressure of oxygen

$PcCO_2$	Partial pressure of carbon dioxide in capillary blood
PcO_2	Partial pressure of oxygen in the capillary blood
$P_{ET_{CO_2}}$	End-tidal partial pressure of carbon dioxide
$P_{ET_{O_2}}$	End-tidal partial pressure of oxygen
PPV	Positive predictive value
Q	Cardiac output
RER	Respiratory exchange ratio
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RV	Residual volume
Sa_{O_2}	Arterial oxyhemoglobin saturation
SBP	Systolic blood pressure
SD	Standard deviation
SE	Standard error
TCD	Transcranial Doppler ultrasound
T_I/T_{TOT}	Ratio of inspiratory time to total breath time
TLC	Total lung capacity
u/s	Ultrasonography
VA	Vertebral artery
$\dot{V}CO_2$	Rate of carbon dioxide production
$\dot{V}_E$	Minute ventilation
$\dot{V}O_2$	Rate of oxygen uptake
$\dot{V}O_{2peak}$	Peak oxygen uptake
$\bar{V}_P$	Mean peak blood flow velocity of the middle cerebral artery
V_{TE}	Tidal volume
V_{TI}/T_I	Inspiratory flow (index of inspiratory drive)
W	Watt
WR	Work rate

Chapter One: **Introduction**

1.1 Chronic obstructive pulmonary disease

Chronic Obstructive Pulmonary Disease (COPD) is a chronic lung disease, and is Canada's fourth leading cause of mortality, constituting 4% of all deaths (1). Cigarette smoking is the main etiological factor for developing COPD (2), which is thought to evoke an abnormal chronic inflammatory response in the lungs, leading to airway inflammation and remodeling. This inflammatory response induces heterogeneous morphological changes in three regions of the lungs: central airways (chronic bronchitis), peripheral airways (small airway disease), and the lung parenchyma (emphysema). Irreversible expiratory flow limitation is the pathophysiological hallmark of COPD. Loss of elastic recoil and an increase in airway resistance throughout the bronchioles are the causative factors related to flow limitation in COPD. Clinically, the diagnosis of airway obstruction is identified by the ratio of the forced expiratory volume in one second (FEV_1) divided by the forced vital capacity (FVC), as determined by spirometer measures. With respect to this ratio, (*i.e.*, FEV_1/FVC), a post-bronchodilator ratio of less than 0.70 signifies airflow obstruction (3). Disease severity, however, is internationally classified according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification system, according to the age- and height- corrected value for FEV_1 (4), (Table 1, *page 16*). In addition to pulmonary flow limitation, accumulating pathophysiological changes occur in COPD, such as lung hyperinflation (represented by an increase in total lung capacity [TLC], residual volume [RV], and/or functional residual capacity [FRC]). Figure 1 (*page*

16) demonstrates a typical expiratory-flow volume loop in a COPD patient, with evidence of hyperinflation.

COPD is classically defined as a disease affecting the pulmonary system, however systemic complications including increased sympathetic activation (5), endothelial dysfunction (6, 7) , oxidative stress (8), and systemic inflammation (9) may contribute to early disturbances of the cerebrovascular and cardio-respiratory system.

Despite the classic definition of COPD as a respiratory disease, epidemiological evidence certainly points to COPD as a risk factor for cardiovascular disease (CVD) and stroke. Mortality rates from CVD in COPD patients are increased 3-4 fold, and strong evidence links reduced FEV_1 as a marker for cardiovascular mortality, independent of smoking status (10). Furthermore, cardiac events account for nearly 30% of deaths in patients with moderate-COPD (whereas respiratory consequences only account for 4%) (11). Despite the epidemiological evidence suggesting a link between CVD and COPD, the mechanism by which these diseases interact remains unclear.

One common factor that may be involved in the underlying systemic pathologies associated with COPD involves oxidative stress. Oxidative stress (OS) results from the imbalance between pro-oxidants and protective antioxidants, and is considered a major etiology of both "normal" aging, and many clinical diseases. Accumulating evidence suggests that COPD patients have increased burden of systemic OS. It is well known that increased reactive oxygen species (ROS) can interact with nitric oxide (NO) to form peroxynitrite ($ONOO^-$) in the endothelium, thereby reducing NO bioavailability, and consequently endothelial-mediated relaxation of the vessel. While recent evidence suggests oxidant-related vascular dysfunction in COPD (12), the implications for this may extend to the cerebral circulation, however this remains to be investigated. OS may also

be involved in the regulation of the altering the chemosensitivity to CO_2, as evidence in healthy individuals have shown an augmented ventilatory response to following antioxidants (13). Antioxidants may therefore be particularly beneficial in patients with COPD, with a speculated redox imbalance.

The integrated aspects of these pathophysiological changes is poorly understood. Specifically, the independent and co-dependent features of the cerebrovascular and respiratory systems in COPD remains to be determined. A comprehensive review of the literature was undertaken to summarize 1) respiratory function and 2) cerebrovascular regulation in COPD.

1.2 Assessment of respiratory function in humans: techniques and literature findings

1.2.1 Methods to assess the control of breathing

The homeostatic regulation of blood gases and pH is an important result of ventilatory gas exchange, achieved through the interaction of chemical sensors (*i.e.*, central and peripheral chemoreceptors), effector organs (*e.g.*, respiratory muscles), and the neural network within the brainstem. The significance of acute alterations in arterial PO_2 (Pa_{O_2}) and arterial PCO_2 (Pa_{CO_2} on breathing dates back almost 140 years ago to the initial work of Pleüger (14) who showed that breathing could be stimulated by reductions in oxygen content or by an increase in carbon dioxide. Since this time, significant advances to our understanding of the chemical control of breathing and technical methodologies have been made. One such technique that has led to these advances is dynamic endtidal forcing (DEF), first developed by Swanson and Belleview (15) in the 1970's. DEF utilizes three elements: 1) fast, precise control of endtidal PO_2 ($P_{ET_{O_2}}$) and PCO_2 ($P_{ET_{CO_2}}$) with

simultaneous measures of ventilation (and its components); 2) complex modelling parameters to predict and compensate for respiratory responses to a given inspiratory gas concentration on a breath-by-breath basis; and 3) an estimated model parameter to account for input-output noise. The main advantage of this system is the independent control of $P_{ET_{O_2}}$ and $P_{ET_{CO_2}}$, allowing isolation of the peripheral and central chemoreceptors. The utilization of the DEF system in populations with lung disease (*e.g.*, obstruction and ventilation-perfusion limitations) however, presents challenges which need to be recognized. In health, endtidal measurements provide a good estimation of arterial blood gases. Patients with lung disease that have increased deadspace ventilation and/or intrapulmonary shunting increase the arterial-endtidal difference, thus making endtidal measures less reliable. Secondly, chemosensitivity (either central or peripheral) is determined by the change in $\dot{V}_E$ divided by the change in the stimulus (such as Pa_{O_2} or Pa_{CO_2}). In cases of mechanical lung impairment (*e.g.*, COPD), $\dot{V}_E$ may not provide the best indication of chemoreceptor activation, and is reflected by a "wants to breathe" *vs.* "can't breathe" scenario. Alternate techniques have been employed to quantify the "drive to breathe" where mechanical impairment may limit pulmonary ventilation, including electrical activation of the diaphragm (*i.e.*, neuromuscular activation) (EGMd), mechanical work of breathing, and inspiratory muscle activation. Inspiratory mouth occlusion pressure ($P_{0.1}$) generated at 0.1 seconds serves as a non-invasive index of neuromuscular inspiratory drive (16), and since its conception, has been widely used in the COPD literature to characterize the "drive to breathe".

1.2.2 Ventilatory responses to hypercapnia and hypoxia in healthy humans

It is now well established that in healthy individuals ventilation ($\dot{V}_E$) increases linearly with Pa_{CO_2}, and decreases in Pa_{O_2}. Under hypoxic conditions, the chemical-drive to breathe is initiated predominantly via the peripheral chemoreceptors, located within the carotid bodies while the ventilatory response to hypercapnia is largely dictated by the central chemoreceptors, located within the brain stem. Hypercapnia, or more specifically, increases in CO_2/H^+ in the arterial blood and brain tissue, stimulate the central chemoreceptors, resulting in increased ventilation in an effort to decrease or "blow off" CO_2 stores, thereby returning to homeostasis (termed hypercapnic ventilatory response, HCVR).

1.2.3 Chemical regulation of breathing in COPD: a review of the literature

1.2.3.1 Search strategy

A sensitive search strategy was conducted in PubMed and Google Scholar using a combination of MeSH terms and keywords. PubMed was searched using MeSH terms "Hypercapnia" or "Hypoxia" and "Respiration" and "Pulmonary Disease, Chronic Obstructive ", using filter for "English" articles. Titles in Google Scholar were searched using terms: "COPD hypoxic ventilatory response" and "COPD hypercapnic ventilatory response". Duplicates were removed, and only full-text articles were included.

1.2.3.2 Search results

The search returned 177 hits. After screening titles and abstracts for relevance, the number of relevant articles remaining was 19. Articles were included if they were directly related to the chemical regulation of breathing in COPD. The articles most pertinent to the

chemical control of breathing in COPD are summarized in Table 2 (*page 18*), and discussed in more detail below.

Central to the pathophysiology of COPD, are complications associated with respiratory control. Disease progression results in blood gas abnormalities in some patients (but not all), including chronic hypoxemia and hypercapnia. The earliest studies in COPD focused on the origin of chronic blood gas alterations under resting conditions. Traditionally, hypercapnia was believed to results from a decreased ventilatory drive, which would be indicative of a reduced $P_{0.1}$. Sorli *et al.* (17) refuted this hypothesis, reporting a normal P0.1, but a reduced resting tidal volume. Similarly, the findings of Montes de Oca *et al.* (18) report a higher $P_{0.1}$ at rest in COPD. Others have since reported an increased neural drive in the respiratory muscles by EMGd in hypercapnic patients, and an overall increase in EMGd in COPD compared to healthy controls (19). Further studies aiming to characterize the central chemosensitivity to increased CO_2 report a lower HCVR in chronically hypercapnic COPD patients (20-23). Importantly, as Montes de Oca *et al.* (18) has shown, COPD have a lower HCVR compared to controls, however, the $P_{0.1}$ was not found to be different between groups, suggesting reduced HCVR is a result of mechanical impairment rather than decreased chemosensitivity.

Chronic hypoxemia in severe COPD has been found to play an important role in determining the peripheral chemosensitivity, as determined by the ventilatory response to hypoxia (HVR). The HVR has been found to be similar to controls when patients exhibit normal levels of Pa_{O_2} (23). Exceptions are found in chronically hypoxemic COPD patients, who exhibit lower HVR compared to normoxic COPD patients (23, 24). Others, however, have provided evidence for a low HVR in severe COPD patients even with normal Pa_{O_2} (25). Healthy individuals with a low HCVR and HVR improved these responses following administration of doxapram, a respiratory stimulant. Interestingly,

COPD patients did not exhibit a similar response suggesting important differences in the physiology of chemoreceptors. Fenoterol, however, has been found to improve the HCVR in COPD patients (26), providing evidence that central chemosensitivity can be improved in COPD. In addition to studying the physiological adaptations of the ventilatory responses, various sensations of breathlessness in response to hypoxia *vs*. hypercapnia have been found, such that COPD patients report higher levels of dyspnea during hypoxia compared to hypercapnia (27), highlighting different somatosensory factors related to the drive to breathe.

Perhaps due to the interest in the effect of chronic blood gas alterations, the majority of studies have been conducted in severe to very severe COPD patients (*see* Table 2, *page 18*), thus leaving a major gap in the literature and questions surrounding the implications for patients in mild-moderate stages of the disease. Further, the majority of studies have been conducted in men. As such, we performed a study in mild-moderate COPD patients, and found a trend towards a decreased HCVR ($P = 0.07$) (28). Similar data characterizing the HVR response in mild-moderate COPD is not presently available.

To summarize, it appears that in severe COPD, the HVR is better preserved than the HCVR. Pulmonary ventilation in response to hypercapnia is often decreased in COPD, however, it is likely that the drive to breathe is preserved. Decreased tidal volume and inspiratory muscle strength may play an important role in determining the HCVR. Chronic hypoxemia likely has a role in the decreased HVR response in COPD patients.

1.3 Assessment of physiological factors affecting cerebral blood flow: techniques and literature findings

1.3.1 Anatomy and techniques to measure cerebral blood flow

The brain has high metabolic demands, considering it requires 15-20% of cardiac output, despite weighing only 2% of the total body weight. As such, two large neck arteries, the internal carotid artery (ICA) and vertebral artery (VA), direct supply oxygenated blood to the brain. The ICA is the main blood supply to the cerebellum, and the left and right VA join to form the basilar artery. The ICA enters the cranial cavity and divides to form the middle cerebral artery (MCA) and anterior cerebral artery. Together with communicating arteries, these form a complete anastomotic ring at the base of the brain, the Circle of Willis (29). These arteries later divide into arterioles and penetrate the cortex to provide blood supply.

Early understandings of cerebral blood flow (CBF) date back to the 1770's. It was at this time the Monro-Kellie hypothesis was formed suggesting, 'if the skull is intact, then the sum of the volumes of the brain, cerebrospinal fluid (CSF) and intracranial blood volume is constant'. It wasn't until two centuries later that Kety and Schmidt (30) quantified for the first time, global CBF, based on the Fick principle that utilized nitrous oxide as an inert gas tracer. While much of the basis for understanding around cerebrovascular physiology was formed using this technique, several limitations exists, such as the inability to measure dynamic changes of CBF, the invasiveness of the procedure, and the global measurement of CBF, rather than regional.

The utilization of Doppler ultrasound to measure blood flow velocity of basal arteries was first reported by 1965 by Miyazaki *et al*. (31). Since this time, transcranial Doppler ultrasound (TCD) has become widely popular in both research and clinical settings, as it

overcomes some of the limiting factors described by the Kety-Schmidt technique. As an indication of the wide-spread use of TCD, the frontier method-paper first described by Aaslid *et al*. (32) has been cited >2600 times. At low frequencies (~2 MHz), ultrasound can penetrate the skull in areas where bone is relatively thin, such as the temporal window. The temporal window allows for insonnation of the major cerebral arteries, to obtain cerebral blood flow velocities in major cerebral arteries, such as the MCA. TCD is a non-invasive technique which with high temporal resolution, allowing for the quantification of dynamic changes in blood flow velocity. However, TCD is a measure of blood flow velocity, not cerebral blood flow, because of the poor spatial resolution, and the inability to obtain information on the vessel diameter. However, with moderate changes in blood gases, the MCA vessel diameter is not expected to change, thus allowing any change in velocity to infer a change in volumetric flow.

1.3.2 Cerebrovascular responses to alterations in Pa_{CO_2} and Pa_{O_2}

The dynamic regulation of cerebrovascular tone is primarily regulated at the pial vessels. The cerebral vessels quickly adapt to changes in perfusion pressure, metabolic demands, and humoral factors. The physiological responses to hypoxia and hypercapnia have been studied extensively and are well established. In healthy individuals, cerebral blood flow is proportionally related to Pa_{CO_2}, and inversely proportional to Pa_{O_2}. Cerebral vessels are highly sensitivity to increases in Pa_{CO_2} (termed, "cerebrovascular reactivity"), and to a lesser extent, hypoxia.

Recent literature suggests that cerebrovascular sensitivity and ventilatory response are tightly linked in healthy individuals (33). It is unclear if a reduced cerebrovascular response, secondary to disease, could augment the ventilatory response to CO_2. In healthy

individuals, indomethacin has been used to pharmacologically decrease CBF during hypercapnia, thereby potentiating the ventilatory response to CO_2 (34), due to decreased washout of H^+ at the central chemoreceptors. The tightly linked relationship between the central control of breathing and CBF remains of interest in COPD.

1.3.3 Cerebrovascular responses in COPD: a review of the literature

1.3.3.1 Search strategy

A comprehensive review of the literature was undertaken to summarize the regulation of cerebral blood flow and in COPD patients. A sensitive search strategy was conducted in PubMed and Google Scholar using a combination of MeSH terms and keywords. PubMed was searched using keywords: MeSH terms "Cerebral Circulation" and Pulmonary Disease, Chronic Obstructive ", using filter for "English" articles. Titles in Google Scholar were searched using terms: "COPD" and "cerebral" or "cerebrovascular". References lists from relevant articles were cross-referenced to include relevant articles that were omitted using the main search techniques. Duplicates were removed, and only full-text articles were included.

1.3.3.2 Search findings

The search returned 55 hits. After screening titles and abstracts for relevance, the number of relevant articles remaining was 18. Articles were included if they were directly related to the regulation of cerebral blood flow, or oxygenation in COPD. Two distinct areas of interest were revealed in the search, which related to 1) basic cerebrovascular

characterization in COPD, and 2) cerebrovascular responses to exercise in COPD. These articles are summarized in Table 3 (*page 20*).

1.3.3.3 Cerebrovascular responses to Pa_{CO_2} and Pa_{O_2} in COPD

The scientific interest in studying CBF in COPD patients can often be related to chronic alterations in blood gases in COPD. The first study to provide insight into this area of research was in the early 1950s, using the Kety-Schmidt nitrous oxide technique (35). These authors found patients with emphysema to have higher CBF, compared to individuals without lung disease, likely as a consequence of chronic hypoxemia and hypercapnia in the patients. Patients were also reported to have decreased cerebral metabolic rate of O_2 ($CMRO_2$). Using similar techniques, Sari *et al*. (36) confirmed the finding of a reduction in $CMRO_2$ in COPD, and extended these findings to suggest a preserved CBF response to CO_2 (using hyperventilation as the stimulus). It is important to highlight the patients studied in the aforementioned studies. Despite not providing precise lung function measures, we can assume that patients were very severe, as the authors reported the death of 3/7 patients (36), and evidence congestive heart failure in 5/7 patients (35).

Until the report of Clivati *et al*. (37) the effect of chronic elevations of Pa_{CO_2} on the cerebral circulation was unclear. COPD patients with chronic hypercapnia were found to have decreased responsiveness to an acute hypercapnic stimuli. Further, patients showed decreased responsiveness to acetazolamide, indeed suggesting reduced responsiveness to CO_2 in severe COPD patients. Additional support of the importance of Pa_{CO_2} in determining CBF is found when correction of hypoxemia in COPD patients appears to have little effect on CBF velocity (38), whereas a reduction in Pa_{CO_2} (as a consequence of non-

invasive ventilation) reduced cerebral blood flow velocity. Van de Ven *et al*. (21) was the first to conduct an integrative $\dot{V}_E$-CBF study, hypothesizing that a high cerebrovascular response would lead to a low Pa_{CO_2}, thereby reducing the ventilatory drive to hypercapnia. COPD patients were in fact found to have a reduced CBF and $\dot{V}_E$ response to hypercapnia, compared to controls, and were not different between normo- and hyper-capnic COPD patients.

Decreased cerebrovascular response to CO_2 in COPD is not a universal finding, as patients with mild COPD were not found to have reduced responses to either hypercapnia or hypoxia (39). Lastly, we have indeed found that mild-moderate women with COPD have reduced cerebrovascular responses, which were found to be related to systemic markers of oxidative stress (28). Clearly, the current literature investigating cerebrovascular control in COPD lacks generalizability across disease subsets, and is furthermore limited by the lack control groups and non-standardized techniques.

1.3.3.4 Cerebrovascular responses to exercise in COPD

The role of cerebrovascular regulation during exercise is of interest for two immediate reasons. Firstly, a reduction in Pa_{O_2} during exercise is a common occurrence in COPD, which may in turn compromise cerebrovascular oxygenation (COx), and secondly, a reduction in COx has been found to affect motor recruitment, and in turn may limit performance. Studies collectively show a reduction in COx in moderate-severe COPD patients who exhibit exercise-induced desaturation (40, 41). Supplemental O_2 has been found to maintain COx in these patients, serving as a favorable therapy to utilize. O_2 utilization during exercise prolongs duration time in COPD, but was not found to be explained by the increased COx (42). We recently investigated the cerebrovascular

response to submaximal exercise, and found that during matched intensities, COPD patients had a similar blood flow velocity response compared to healthy controls (43). However, when individuals worked at the same absolute intensity (to simulate daily activities), COPD patients had elevated CBF. While the direct implications for this are not clear, it is possible that COPD patients have a reduced reserve capacity during normal activities, secondary to reduced physical capacity.

In summary, it appears that very severe (~<30% FEV_1) COPD patients exhibit cerebrovascular consequences, as evident by reduced $CMRO_2$, a reduction in the hypercapnic response, and reduced COx during exercise. Blood gas alterations, particularly chronic hypercapnia appear to influence basal CBF and potentially CO_2 reactivity. Less clear, however, is the progression of these disturbances, as very few studies are undertaken in mild-moderate COPD. Additionally, the cerebrovascular response to acute hypoxia is unclear, as only one study in mild patients has investigated this.

1.4 Overall Objectives:

To determine whether moderate-COPD patients have reduced ventilatory and cerebro- and peripheral-vascular responses to alterations in blood gases at rest and during exercise; and to identify whether the responses are related to systemic oxidative stress.

1.5 Specific Aims:

Experiment #1:

To determine whether COPD patients have a decreased cerebrovascular response to CO_2, and if this response is related to systemic oxidative stress.

Experiments #2:

To determine if COPD patients have altered cerebrovascular responses during cycling exercise compared to healthy adults.

Experiment #3:

To determine whether an antioxidant intervention can augment the cerebrovascular and ventilatory responses to CO_2 in COPD patients.

Experiment #4:

To determine the cerebrovascular and ventilatory responses to acute hypoxia in COPD patients, and whether these responses are modulated by oxidative stress.

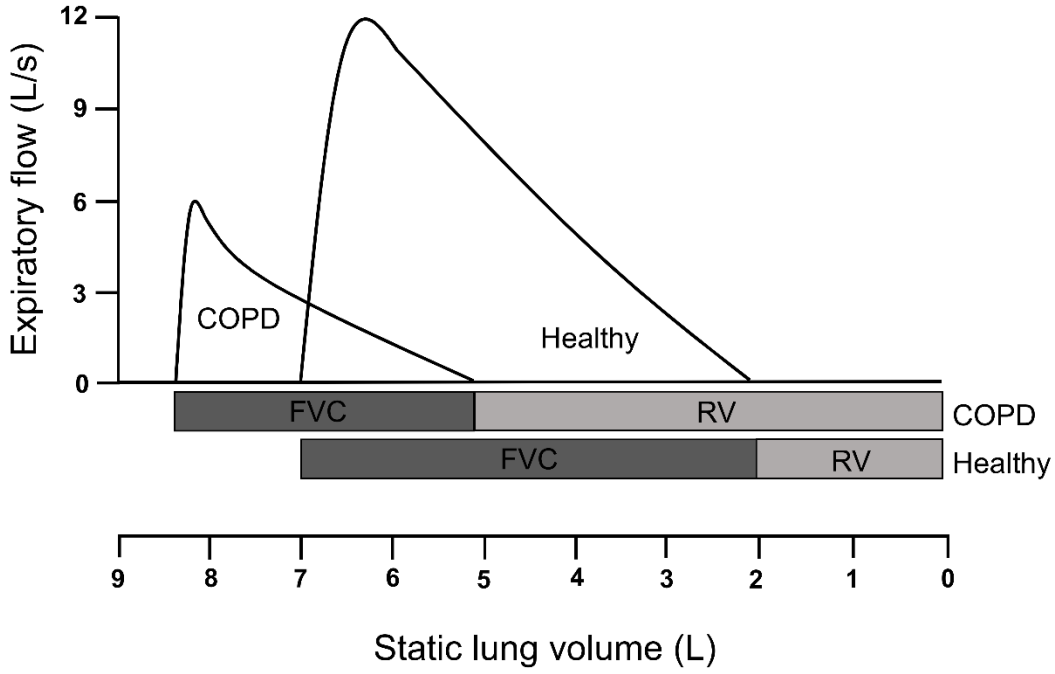
Experiment #5:

To determine whether the forearm blood flow response is diminished in COPD patients during mild exercise, and to determine the relationship of this response to endothelial function.

These studies are novel, and provide a strong physiological understanding of the integrated nature of the cerebro- , cardio-vascular, and respiratory processes in COPD.

1.6 Chapter One Figures and Tables

Figure 1. Representation of a flow-volume loop as a function of lung volumes, in a COPD patient and healthy individual.



Footnote*:* The COPD patient has reduced expiratory flow and hyperinflation as indicated by the increased lung volume. FVC: forced vital capacity; RV: residual volume.

Table 1. Classification of severity of airflow limitation in COPD.

GOLD Classification	Severity	FEV_1/FVC	FEV_1 (%)
0	At risk	Normal	Normal
I	Mild	< 0.70	≥80
II	Moderate	< 0.70	50-79
III	Severe	< 0.70	30-49
IV	Very severe	< 0.70	<30

Footnote: spirometry based on post-bronchodilator values.

Table 2. Studies describing respiratory chemosensitivity in COPD patients.

Reference	n	Male, %	Age, yr	FEV_1	Control group[A]	Stimulus	Objective or outcome	Main findings
(23) Flenley *et al.* (1970)	12	n.p	61±9	23±8%	No	Hypercapnia and hypoxia	HCVR and HVR	↓ HCVR in hypoxemic-hypercapnic patients; Ø HVR, but ↓ in hypoxemic patients
(22) Altose *et al*. (1977)	14	n.p	59±8	~40%	Yes	Hypercapnia	$\dot{V}_E$ and $P_{0.1}$ in normo- and hypercapnic COPD *vs.* controls	↓ $\dot{V}_E$ in normo- and hypercapnic COPD; Ø $P_{0.1}$ in COPD
(17) Sorli *et al*. (1978)	15	93	60±12	31±14%	Yes*	Rest/air breathing	Determine respiratory control differences in hyper-and normo-capnic COPD	↓ tidal volume in hypercapnic COPD; Ø $P_{0.1}$, $\dot{V}_E$ between groups
(24) Bradley *et al*. (1979)	20	n.p	n.p	25±12%	No	Hypoxia and hypercapnia	HCVR and HVR in hypoxemic and non-hypoxemic COPD	↓ HVR in hypoxemic COPD
(44) Yoshikawa *et al*. (1987)	15	80	53±18	n.p	Yes	Hypercapnia and hypoxia	$\dot{V}_E$ and $P_{0.1}$ response to hypoxia and hypercapnia in COPD; effect of doxapram	↓ chemosensitivity in COPD; doxapram ↑ HVR in controls but not COPD
(19) Gorini *et al*. (1990)	15	87	67±7	29±7%	Yes	Measurements obtained at rest/air breathing	Neural respiratory drive and neuromuscular activation in COPD	↓ inspiratory muscle strength and ↑ EMGd in COPD *vs.* control; ↑ neural drive/activation in hypercapnic *vs*. normocapnic COPD patients
(25) Eberland *et al*. (1990)	25	100	68	0.7±0.2L	Yes*	Hypercapnia and hypoxia	Effect of acute hypercapnia during hypoxia on respiratory drive in COPD	↓ hypoxic drive in COPD (normocapnic); hypoxic drive is ↑ with addition of CO_2 in COPD

Table continued on *page 19*

Table continued from *page 18*

Reference	n	Male, %	Age, yr	FEV_1	Control group[A]	Stimulus	Objective or outcome	Main findings
(20) Scano *et al*. (1995)	17	94	~66±4	~30±8%	Yes	Hypercapnia	Central *vs*. mechanical ventilatory impairment in COPD	↓ HCVR and inspiratory muscle strength in COPD chemoresponsiveness; preserved in normocapnic, but ↓ in hypercapnic COPD
(27) Kobayashi *et al*. (1996)	15	100	65±11	1.1±0.6L	No	Hypercapnia and hypoxia	Determine if breathlessness is related to $\dot{V}_E$ in COPD	↑ breathlessness with hypoxia *vs*. hypercapnia
(45) Celli *et al*. (1997)	8	75	65±6	0.8±0.3L	Yes	Hypercapnia	If ↓ lung volume ↓ ventilatory drive ($P_{0.1}$)	Lung reduction surgery ↓ ventilatory drive
(26) Suzuki *et al*. (1997)	19	100	67±8	63±25%	No	Hypercapnia and hypoxia	HVR and HCVR. response to fenoterol.	Fenoterol ↑ HCVR in COPD
(18) Montes *et al* (1998)	33	70	62±13	<35%	Yes	Hypercapnia	Differences in HCVR in hyper- and normo-capnic COPD	↓ HCVR in COPD *vs*. controls, but Ø $P_{0.1}$ across COPD groups or controls
(21) van de Ven *et al*. (2001)	33	76	~60	<29%	Yes	Hypercapnia	Differences in HCVR in hyper- and normo-capnic COPD	↓ HCVR in COPD *vs*. controls; lowest HCVR found in hypercapnic-COPD
(46) van de Ven *et al*. (2002)	33	76	~60	<29%	No	Hypercapnia	HCVR during induced acidosis/alkalosis in COPD	Chronic alterations in pH do not effect HCVR in COPD
(28) Hartmann *et al*. (2012)	11	0	70±6	~67%	Yes	Hypercapnia	HCVR in women with COPD	Trend to ↓ HCVR in COPD

Abbreviations: Ø: not different from controls; EMGd: electromyographic activity of the diaphragm; HCVR: hypercapnic ventilatory response; HVR: hypoxic ventilatory response; n: COPD group sample size; n.p: (data) not presented; $P_{0.1}$: mouth occlusion pressure or inspiratory effort; $\dot{V}_E$: minute ventilation. [A]Control group refers to a non-lung disease comparison group; * not age-matched comparison.

Table 3. Studies describing cerebrovascular regulation in COPD patients.

Reference	n	Male, %	Age, yr	FEV_1, %	Control group[A]	Technique	Objective or outcome	Main finding
A. Regulation of CBF								
(35) Patterson *et al*. (1952)	9	n.p	50±12	n.p	Yes	Kety-Schmidt	Basal CBF	↑ CBF and ↓$CMRO_2$ in emphysema *vs*. control
(37) Clivatti *et al*. (1992)	8	n.p	60-70	33±15	Yes*	TCD	MCAv response to CO_2 in COPD with chronic hypercapnia	COPD patients with chronic hypercapnia show ↓ MCAv in response to CO_2 challenge
(36) Sari *et al*. (1992)	7	100	63±15	n.p	Yes	Kety-Schmidt	CBF sensitivity to CO_2 during mechanical ventilation	CBF sensitivity to CO_2 is preserved in COPD on mechanical ventilation; ↓ $CMRO_2$
(38) Cannizzaro *et al*. (1997)	13	77	63±7	27±5	No	TCD	MCAv in response to correcting hypoxemia/hypercapnia in ventilated COPD patients	In ventilated, chronically hypercapnic COPD patients, CO_2 is main determinant of MCAv; correction of hypoxemia has no effect on MCAv
(21) van de Van *et al*. (2001)	33	76	~60	<29	Yes	NIRS	CBF response to CO_2 in COPD (chronically hyper- *vs.* nomo-capnic)	↓ CBF in COPD at rest *vs*. controls; ↓ CBF sensitivity to CO_2 *vs*. controls; no differences between normo- and hyper-capnic patients
(46) van de Van *et al*. (2002)	33	76	~60	<29	No	NIRS	CBF response to CO_2 during induced states of mertabolic alkalosis or acidosis in COPD patients with chronic hypercapnia	Normo- and hyper-capnic COPD patients have similar CBF responsiveness to CO_2 irrespective of induced acidosis or alkalosis
(47) Albayrak *et al*. (2006)	20	n.p	~63±10	~32	Yes	u/s	ICA and VA hemodynamics in COPD patients	COPD patients had ↑ extracranial blood flows *vs*. controls
(39) Bernardi *et al*. (2008)	15	67	52±3	76±3	No	TCD	MCAv sensitivity to CO_2 and hypoxia	MCAv response to CO_2 and hypoxia is preserved in mild COPD

Table continued on *page 21*

Table continued from *page 20*

Reference	n	Male, %	Age, yr	FEV_1, %	Control group[A]	Technique	Objective or outcome	Main finding
(28) Hartmann *et al*. (2012)	8	0	69±4	63	Yes	TCD	Cerebrovascular CO_2 reactivity	↓ MCAv sensitivity to CO_2 in women with COPD; related to ↑ oxidative stress
B. Exercise								
(48) Jensen *et al*. (2002)	13	8	55±7	33±19	No	NIRS, TCD	COx during exercise	Hyperoxia increases COx during exercise in patients with terminal lung disease
(41) Oliveira *et al*. (2012)	20	100	~63	~47	No	NIRS	Effect of hyperoxia on COx during exercise in COPD	↓ COx in COPD patients that desaturate with exercise; Hyperoxia improves COx in these patients
(42) Vogiatzis *et al*. (2013)	12	n.p	66±5	42±13	No	NIRS	Effect of hyperoxia and heliox on exercise duration and COx in COPD	Hyperoxia and heliox ↑exercise time in COPD but this is not explained by ↑COx
(49) Rodrigues *et al*. (2013)	13	100	65±8	49±15	No	NIRS	Effect of NIV on COx during exercise in COPD	NIV + hyperoxia ↑cardiac output and COx during exercise in COPD
(43) Hartmann *et al*. (2014)	11	0	70±6	~67	Yes	TCD	MCAv response to exercise	COPD patients have preserved CBF during exercise at 50% $\dot{V}O_{2peak}$
C. Additional clinical studies								
(50) Jasani *et al*. (2003)	6	100	64±11	Severe; 50%	No	NIRS	COx during sleep	COPD have ↓COx during periods of arterial desaturations during sleep
(51) Yildiz *et al*. (2012)	21	100	64±8	54±14	No	u/s	ICA and VA hemodynamics following a COPD exacerbation	COPD exacerbation ↑ ICA and ↑ VA flow

Abbreviations: CBF: cerebral blood flow; $CMRO_2$: cerebral metabolic rate of oxygen; COx: cerebral oxygenation; ICA: internal carotid artery; MCAv: middle cerebral artery blood velocity; n: COPD group sample size; NIV: non-invasive ventilation; n.p: (data) not presented; NIRS: near infrared spectroscopy; TCD: transcranial Doppler ultrasound; u/s: ultrasonography; VA: vertebral artery. [A]Control group refers to a healthy comparison group, without lung disease; * not age-matched comparison.

Chapter Two: **Decreased cerebrovascular response to CO_2 in post-menopausal women with COPD: role of oxidative stress**

Sara E. Hartmann, Vincent Pialoux, Richard Leigh and Marc J. Poulin. (2012) Decreased Cerebrovascular Response to CO_2 in Post-menopausal Women with COPD: Role of Oxidative Stress. *European Respiratory Journal*. **40**(6): pp 1354-1361.

Preface

COPD patients are at increased risk for cardiovascular disease and stroke, however, the physiological mechanisms are unclear. Increased oxidative stress may play a role in the vascular pathologies associated with COPD. The purpose of the study was to investigate the cerebrovascular responses to CO_2 in COPD patients. Furthermore, the study aims to investigate the role of oxidative stress in the cerebrovascular regulation in COPD.

Declaration

The original scientific article was written by myself, with guidance from co-authors' Drs. Pialoux, Leigh, and Poulin. The original study conception was developed by Dr. Leigh, Poulin, Pialoux, and myself. I was responsible for all data collection and analyses performed in the Laboratory of Human Cerebrovascular Physiology. Dr. Pialoux led the data collection for the biological assays, and I assisted. I recruited all study participants and coordinated study visits. I submitted and received ethical approvals. The pulmonary function tests were performed by staff in the Foothills Hospital. The data were first presented in abstract form (52) at the European Respiratory Society Annual Congress in September, 2010 and later published in its final form in the *European Respiratory Journal*.

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Permission was granted by Kay Sharpe, on November 27, 2014. See APPENDIX A, *page 192*.

2.1 Abstract

Chronic obstructive pulmonary disease (COPD) is associated with cerebrovascular abnormalities and an overproduction of reactive oxygen species. We hypothesized that COPD patients have oxidant-related cerebrovascular dysfunction. The main objective was to evaluate cerebrovascular reactivity and its relationship to oxidative stress in women with COPD.

We studied eight women with moderate-COPD, and ten healthy women control subjects of similar age. Transcranial Doppler ultrasound assessed cerebral blood flow velocity during hypercapnia. Plasma was assessed at rest for DNA oxidation, advanced oxidation protein products, lipid peroxidation, nitrotyrosine, antioxidant enzyme activity (glutathione peroxidase and catalase), and end-product of nitric oxide metabolism.

Moderate-COPD patients showed decreased cerebrovascular sensitivity to CO_2 (COPD: 1.17 ± 0.54 *versus* Control: 2.15 ± 0.73 $cm \cdot sec^{-1} \cdot mmHg^{-1}$; $P < 0.01$). COPD patients to have higher levels of DNA and lipid oxidation, advanced oxidation protein products, and higher glutathione peroxidase activity ($P<0.05$). Controlling for measures of oxidative stress (DNA and lipid oxidation, advanced oxidation protein product) statistical differences between the COPD and control groups are eliminated in the cerebral blood flow sensitivity to CO_2.

Women with moderate COPD have cerebrovascular dysfunction. Our results suggest that increased levels of systemic oxidative stress may have implications in the cerebrovascular dysfunction observed during hypercapnia in COPD.

2.2 Introduction

Most recently, COPD is gaining attention for the consequential systemic manifestations and co-morbidities resulting from the disease (53). Although these patients are at a 3-4 fold increase in the risk of developing cardio- and cerebro-vascular disease, limited information is available regarding the pathophysiology relating to vascular diseases in COPD. Extra-pulmonary consequences, including altered arterial blood gas levels, pH imbalance, increased oxidative stress, vascular dysfunction and autonomic disturbances, all have potential to alter the regulation of cerebral blood flow (CBF). In healthy individuals, CBF and ventilation increase linearly with arterial P_{CO_2} (Pa_{CO_2}). It is well established that COPD patients have a decreased ventilatory output to CO_2 (20-22). However, few studies have investigated the cerebrovascular response to CO_2 in these subjects, and how decreased ventilation can affect this response. Recent literature suggests that cerebrovascular sensitivity and ventilatory response are tightly linked in healthy individuals (33). However, evidence suggests that COPD patients exhibit cerebrovascular disturbances (21, 37, 39), although the pathologic onset and cause of these disturbances have not yet been comprehensively studied.

Furthermore, the role of oxidative stress pertaining to cerebrovascular dysfunction is of particular interest in COPD patients. COPD is associated with an overproduction of reactive oxygen and nitrogen species (ROS and RNS, respectively), leading to an imbalance of oxidants-antioxidants, resulting in oxidative stress (54). ROS have been implicated in the role of vascular dysfunction both directly (via dilatory effects of H_2O_2), and indirectly through decreased bioavailability of nitric oxide (NO) via the promotion of superoxide anion quenching to form peroxynitrite (55). By the reduction of available NO,

this reaction (*i.e.* $O_2^{\cdot -}$ + NO → $ONOO^-$) can subsequently affect cerebrovascular tone, resulting in vasoconstriction (56).

While a few studies have looked at cerebrovascular health in COPD patients (21, 38, 39, 57), no study has investigated how oxidative stress and antioxidant activity could be involved in the CBF regulation observed in COPD. Estrogen is a sex-hormone with beneficial vasoactive and antioxidant properties. After menopause estrogen sharply declines, and oxidative stress is reported to be increased (58). This may have an impact on women with COPD who are already at increased risk of exposure to oxidants.

Furthermore, the present collection of literature relating to cerebrovascular physiology and COPD represents exclusively male, or mixed gender studies. Recent commentary states the impact of COPD in women is significantly understudied, and evidence that does exist, suggests important gender differences, raising debate as to the role of gender as a potential risk factor for developing COPD (59). Evidence suggests that women are at increased susceptibility for the development of the disease (e.g., incur COPD after fewer number of cigarettes/lifetime compared to men), have increased prevalence rates, and exhibit the poorest outcomes associated with COPD (60).

We therefore choose to exclusively study women to gain better understanding of certain physiological effects of COPD in this under-represented group. Thus, we sought to determine: 1) the extent to which cerebrovascular regulation is altered in women with moderate-COPD, and whether there is a relationship to ventilation, and 2) whether the expected differences in cerebral blood flow are explained by systemic oxidative stress markers, or antioxidant activity. Some of these results have been previously presented in an abstract form (52).

2.3 Methods

2.3.1 Study participants

Ten post-menopausal women with smoking-related COPD, and twelve healthy, non-smoking post-menopausal women (controls) were recruited for participation in this study. COPD subjects were recruited from participating outpatient medical clinics within the Calgary Health Region, and controls were recruited from the community. All study participants visited the Laboratory of Human Cerebrovascular Physiology at the University of Calgary, Alberta (1103 m elevation above sea level) for two testing sessions. Subjects were instructed to refrain from eating or drinking 4 hours prior to each testing session. The study was approved by the institutional Conjoint Health Research Ethics Board and conformed to the Declaration of Helsinki, and all participants provided written, informed consent.

2.3.1.1 Major inclusion criteria

Patients had physician-diagnosed COPD, with a smoking history >10 pack-years and airflow obstruction (FEV_1/FVC <70%; $FEV_1 \leq 70\%$ predicted). Patients were all ex-smokers (>1 year), post-menopausal for ≥ 12 months, able to walk independently outside or on stairs, and had a BMI < 35 $kg \cdot m^{-2}$. Participating controls were healthy volunteers, with no history of lung disease, or regular cigarette smoking (< 1 pack-year). Complete exclusion criteria are listed in the online supplement.

2.3.2 Experimental protocol

Participants visited the laboratory on two occasions. The first day consisted of a medical screening questionnaire, pulmonary function testing, and venous blood collection. After approximately 1 week, subjects returned for a CO_2-challenge test, which was conducted in most participants between 11:00 am - 2:00pm.

2.3.2.1 Pulmonary function test

Spirometry, measures of lung volumes and single-breath diffusion capacity were completed in all subjects, as per ATS guidelines (61-63).

2.3.2.2 Protocol to measure the cerebrovascular and ventilatory response to euoxic hypercapnia

Subjects were comfortably seated in a semi-recumbent position at rest for 10 minutes for collection of baseline vascular and ventilatory variables. An arterialized capillary blood sample was taken from the middle finger after warming for 3-minutes. Blood samples were immediately analyzed for PO_2, PCO_2, and acid-base balance (Radiometer ABL 725, Denmark). Briefly, using dedicated software (BreatheM v2.38, University Laboratory of Physiology, Oxford, UK), the technique of dynamic end-tidal forcing (64), was used to precisely target the desired end-tidal pressure CO_2 ($P_{ET}CO_2$) and end-tidal pressure of oxygen ($P_{ET}O_2$). The ~10-minute hypercapnic protocol progressed to hypercapnia at +9 mmHg above the resting $P_{ET}CO_2$, while $P_{ET}O_2$ was held constant at baseline values. The protocol was designed as 5 x 2-minute stages of increasing $P_{ET}CO_2$, in a stepwise fashion (*i.e.*, +1, +3, +5, +7, +9 mmHg above eucapnia).

Heart rate, oxygen saturation, and continuous beat-beat blood pressure via finger pulse photoplethysmography was measured. Respiratory volumes were measured with a turbine and volume transducer (VMM-400; Interface Associates, Laguna Niguel, CA), and respiratory flow direction and timing were obtained with a pneumotachograph (RSS100-HR, Hans Rudolph, Kansas City, MO, USA). Middle cerebral artery (MCA) blood flow velocity was continuously measured using a 2-MHz pulsed Doppler Ultrasound system (TC22, SciMed, Bristol, England). Peak blood flow velocity ($\overline{V}_P$) was used as a surrogate for global cerebral blood flow (CBF). The power ($\overline{P}$) signal acquired from the Doppler system is used as an indicator for changes in vessel diameter. Therefore, without a change in $\overline{P}$, $\overline{V}_P$ is considered to be a reliable index of flow.

2.3.2.3 Biochemical analyses

Venous blood was collected at rest into two EDTA, and two SST tubes for biochemical analysis. Blood was centrifuged at 3200 rpm for 10 minutes at 4˚C. Plasma and serum were separated into appropriate aliquots and frozen at -80˚C until assays were performed. Assays to measure plasma levels of oxidative stress (8-hydroxy-2'-deoxyguanosine [8-OHdG], malondiadehide [MDA], advanced oxidation proteins product [AOPP]) nitrosative stress (nitrotyrosine), antioxidant enzyme activity (glutathione peroxidase [GPX] and catalase activities), and end-product of nitric oxide (nitrites and nitrates) were performed. Hormone and cholesterol analysis was performed by Calgary Laboratory Services. Further procedural details are provided in the Online Supplement.

2.3.3 Data and statistical analysis

The main outcome variable is the cerebrovascular sensitivity to CO_2. Secondary outcomes include the ventilatory and the blood pressure response to CO_2, and molecular markers related to vascular function (oxidative stress, antioxidants, and NOx). An estimate of the cerebrovascular sensitivity (*i.e.*, $\bar{V}_P$ sensitivity) to CO_2 was calculated for each individual as the slope of the regression relating $\bar{V}_P$ *versus* $P_{ET}CO_2$, during hypercapnia. Likewise, the ventilatory sensitivity (*i.e.*, $\dot{V}_E$ Sensitivity) to CO_2 was calculated as the regression slope of $\dot{V}_E$ against $P_{ET}CO_2$ during hypercapnia. The last 30-seconds of data of each hypercapnic stage was averaged and used in this calculation. Where percent changes are reported, this data is normalized to the last 3-minutes of the isocapnia euoxia baseline period preceding the hypercapnic steps.

Using the variability (SD = 0.57 $cm \cdot sec^{-1} \cdot mmHg^{-1}$) and expected between-group difference of 0.75 $cm \cdot sec^{-1} \cdot mmHg^{-1}$ from a previously published study (39), we determined that to detect a significant difference in the cerebrovascular sensitivity to hypercapnia, each group would consist of 11 participants (using a two-tailed test and setting $\alpha = 0.05$ and $\beta = 0.80$).

Tests of normality and homogeneity of variance were performed and confirmed the appropriate use of parametric statistical procedures (SPSS Version 17.0, SPSS Inc., Chicago, IL). The CO_2-sensitivity indices for CBF and $\dot{V}_E$ were compared between groups using an independent t-test. The main effect of "hypercapnia" and the "group x hypercapnia" interaction was evaluated on physiological variables (respiratory [$P_{ET}CO_2$, $P_{ET}O_2$, $\dot{V}_E$, B*f*, V_T, $\dot{V}_E$/MVV], cardiovascular [MAP, HR, Sa_{O_2}], and cerebrovascular [$\bar{V}_P$, CVC]) using a repeated measure analysis of variance. The repeated effect of each CO_2

stage (*i.e.*, +1, +3, +5, +7, +9) was evaluated on the aforementioned physiological variables. Individual cerebrovascular and ventilatory sensitivities were ranked, and Spearman's correlation coefficient was used to evaluate the strength of the relationship between $\dot{V}_E$ and $\bar{V}_P$ sensitivity. Group differences in molecular markers (*i.e.*, oxidants, antioxidants, and NOx metabolism) were assessed using independent t-tests. Post-hoc analysis assessed relationships between these molecular markers and main cardio- and cerebro-vascular outcome variables using Pearson's correlations. Using exploratory analyses, we used several baseline factors (blood pressure, age, oxidative stress, antioxidant enzyme status, NO-metabolism) as covariates in a one-way ANCOVA to find the best "adjusted" model to explain the variance in $\bar{V}_P$ sensitivity to CO_2 between groups. Data are presented as mean ± SD, and significance was set at α-level ≤ 0.05. Confidence intervals are calculated at 95%.

2.4 Results

2.4.1 Subject characteristics

Data were not obtained in 2 subjects (1 control and 1 COPD) due to lack of a suitable MCA signal. Furthermore, data from 1 COPD patient was excluded because the patient did not complete the CO_2 challenge to entirety, due to overwhelming breathlessness. A blood sample was not obtained in 1 control. Therefore, we analyzed data from 10 controls, and 8 COPD patients.

Physical characteristics and pulmonary function tests of participants are summarized in Table 4 (*page 49*). Measurements of resting blood gases acquired by end-tidal, and via capillary blood is summarized in Table 5 (*page 50*).

2.4.2 Physiological responses to hypercapnia

At rest, there were no significant differences between main vascular outcomes (*i.e.*, $\bar{V}_P$, MAP, heart rate) between COPD patients and controls (Table 6, *page 51*). In response to hypercapnia, the vascular response in COPD patients was blunted, as evident by only a 19% increase in $\bar{V}_P$ (*vs* 37% in controls; $P < 0.01$), and an 8% increase in MAP (*vs* 12% in controls; $P > 0.05$) from baseline (Figure 2, *page 47*). The mean $\bar{V}_P$ sensitivity (unadjusted) for COPD patients is significantly decreased (COPD: 1.17 ± 0.54 *versus* Control: 2.15 ± 0.73 $cm \cdot sec^{-1} \cdot mmHg^{-1}$; $P < 0.01$) (Figure 3, *page 48*).

Ventilatory output was decreased in COPD patients at the end of the CO_2 challenge ($P \leq 0.05$) (Figure 2, *page 47*), however, there was only a trend towards a statistical difference when considering the $\dot{V}_E$ sensitivity to CO_2 (0.90 ± 0.61 *versus* 1.83 ± 1.26 $L \cdot min^{-1} \cdot mmHg^{-1}$; $P = 0.07$) (Figure 3, *page 48*). Increases in ventilation were achieved

primarily by an increase in tidal volume (V_{TE}), rather than breathing frequency (B*f*) (Table 6, *page 51*). Using Inspiratory flow (V_{TI}/T_I) as an indication of respiratory drive, COPD patients showed a similar drive to breathe at rest between compared to controls, whereas V_{TI}/T_I was reduced during hypercapnia in COPD patients (Table 3, *page 51*).

Each individual $\bar{V}_P$ and $\dot{V}_E$ sensitivity score was ranked amongst all participants. There was a significant positive correlation between the ranking of ventilatory and cerebrovascular sensitivity to hypercapnia. Subjects with low cerebrovascular sensitivity to CO_2 had a corresponding low ventilatory sensitivity, and vice versa ($r = 0.61$; $P < 0.01$).

2.4.3 Biochemical analysis: oxidative stress, antioxidant enzyme activity, and NO level

2.4.3.1 Oxidative stress and antioxidant activity

Results of biochemical assays are summarized in Table 7 (*page 52*). COPD patients showed significantly higher levels of oxidative stress as indicated by increased plasma 8-OHdG, MDA, and AOPP ($P \leq 0.05$). These patients also had significantly higher antioxidant enzyme activity in the form of GPX ($P \leq 0.01$). The ratio between oxidative stress and antioxidant activity (*i.e.*, 8-OHdG and GPX) was significantly higher in COPD patients than controls (1.03 ± 0.50 *versus* 0.62 ± 0.23, respectively) ($P \leq 0.05$).

2.4.3.2 Vascular parameters and oxidative stress

We performed (with-in group) correlation analysis between plasma oxidative stress/antioxidant markers, and vascular outcomes to measure the strength of the relationship between these variables. We did not find any significant relationships between oxidative stress (8-OHdG, AOPP, MDA, and nitrotyrosine) and $\bar{V}_P$ sensitivity, MAP, or

cerebrovascular conductance. However, COPD patients with higher catalase activity were found to be associated with higher $\dot{V}_P$ sensitivity (r^2 = 0. 59; P < 0.05).

A one-way ANCOVA was conducted using "group" (COPD or control) as the fixed factor, and cerebrovascular sensitivity as the dependent variable. A preliminary analysis evaluating the homogeneity of regression (slopes) assumption indicated the relationship between the co-variates and dependent variable did not differ significantly ([8-OHdG: F(1,14) = 1.45, p = 0.248]; AOPP: F(1,14) = 0.466, p = 0.506]; [MDA: F(1,14) = 0.081, p = 0.780]). Using these co-variates, the ANCOVA was non-significant, F(1,13) = 0.033, p = 0.858, thus eliminating the difference in cerebrovascular sensitivity previously observed between groups (COPD: 1.65 ± 1.08 $cm \cdot sec^{-1} \cdot mmHg^{-1}$ *versus* controls: 1.76 ± 1.01 $cm \cdot sec^{-1} \cdot mmHg^{-1}$) (Figure 3, *page 48*).

Based on our findings that suggest a potential role between OS and cerebrovascular dysfunction in women with COPD, we incorporated additional statistical analyses to offer a clinical perspective. Using the adjusted model, participants were assigned to either "COPD", or "control" group, according to the plasma levels of oxidative stress. Positive predictive, and negative predictive values (PPV and NPV, respectively) were calculated, where the prevalence rate of COPD was 44% (8/18). Individuals were identified to have "COPD" if the level of plasma oxidative stress was above the expected mean concentration (of *either* 8-OHdG, MDA, or AOPP). Individuals were identified as "healthy/control" if *all three* levels of OS markers were below the adjusted mean OS concentration. Results indicate the PPV to be 93.5%, and the NPV to be 100%.

2.5 Discussion

We present novel data indicating a link between cerebrovascular reactivity, and measures of systemic oxidative stress in COPD patients. The major finding in this study is an impaired cerebrovascular response to hypercapnia in women with COPD. We report a blunted cerebrovascular dilatory response in most, but not all COPD patients, even at modest levels of CO_2 administration. As predicted, higher levels of systemic oxidative stress markers were found in the COPD patient cohort. As suggested in other populations (56, 65), in this context increased oxidative stress could explain the differences observed between the cerebrovascular sensitivity to hypercapnia observed between COPD patients, and healthy control subjects.

2.5.1 Physiologic response to hypercapnia

It is well known that in a healthy population, increased Pa_{CO_2} induces cerebrovascular dilation, leading to an increase in cerebral blood flow (66). This response depends on several co-operative pathways: 1) the chemical stimulus (pH/H^+) at the central chemoreceptors, 2) the ventilatory response (e.g., respiratory muscles), and 3) the vasodilatory response in the small vessels of the cerebral circulation. Insufficiencies at any one of these levels, can lead to an abnormal hypercapnic response.

Our findings show COPD patients to have a lower CBF response to CO_2 compared to healthy controls (+19% *versus* +41%, respectively). We did not find evidence for chronic cerebrovascular dilation in COPD patients. Our results are supported in both an animal model (67), as well as in hypercapnic patients, with severe COPD (57). Cigarette smoking is known to induce both acute and chronic cerebrovascular dilation in healthy individuals, but cerebrovascular reactivity appears to be maintained. In young adults,

cerebrovascular deficiencies are only observed following acute smoking (1-minute) (68). Similarly, in a healthy older population, smoking status was not a significant factor in determining the sensitivity to hypercapnia (39). Interestingly, in this same study, Bernardi *et al*. found that individuals who were current smokers *and* had mild COPD, had significantly lower cerebrovascular sensitivity to hypercapnia compared to individuals who only had mild airflow obstruction, *without* a smoking history. Overall, however, the cerebrovascular reactivity in mild COPD patients did not differ from matched healthy controls. We now show that normocapnic patients with moderate COPD show cerebrovascular abnormalities. We believe that frequent stimuli specific to individuals with COPD (e.g. cigarette smoking, occurrence of frequent arterial oxygen desaturations) may exhaust the normal vascular response via constant vaso- dilation/constricting cycles. Acute effects of smoking cause an immediate constriction of the pial arteries, followed by vasodilation which is likely mediated by nicotine that stimulates NO release (69). Extensive reviews on the topic suggest that increased oxidative stress may lead to either a decreased generation, or bioavailability of NO leading to vasomotor dysfunction, specific to the vascular endothelium (70, 71).

We found that patients with COPD showed a trend towards decreased ventilatory response to hypercapnia. Both mechanical (respiratory) limitations and desensitization of the central chemoreceptor have been implicated in the explanation of this pathology. A decreased ventilatory response provides an avenue for increased cerebral dilation in COPD participants. This is contrary to what we observed in patients, as the cerebrovascular response was blunted. In healthy individuals, Xie and colleagues (34) showed that decreased cerebrovascular responsiveness to CO_2 stimulates the ventilatory response, suggesting that cerebrovascular sensitivity to CO_2 has great influence on the $\dot{V}_E$ responsiveness of the central chemoreceptors. It is possible that the mechanisms involved

in the control of breathing and cerebrovascular regulation in COPD patients are independently altered (*i.e.*, two separate mechanisms affecting these outcomes).

2.5.2 Molecular markers

2.5.2.1 Oxidative stress and antioxidant status

Oxidative stress represents an unfavorable imbalance between reactive oxygen species and antioxidants, either from the overproduction of oxidants, or the depletion of antioxidants. In addition to endogenous sources of ROS (normal cellular metabolism), COPD patients are exposed to exogenous forms of free radicals from environmental pollutants and/or cigarette smoke. Our findings indicate a significant increase in both systemic oxidative stress markers (8-OHdG, MDA, and AOPP), and increased antioxidant enzyme activity (GPX) in COPD patients, compared to healthy control subjects, and are in similar agreement with other published data (8, 72-74). In contrast to our results, increased glutathione has previously been shown to be negatively correlated with lung function in patients with chronic airflow limitation (73). We suspect that there may be an adaptive response involved in the oxidative stress-antioxidant enzyme response, and that the high level of oxidants stimulates the antioxidant enzymatic system in an effort to counteract the high burden of oxidants. Recent reports suggest a decrease in antioxidant capacity in both healthy smokers and patients with COPD, when compared to non-smoking controls (72, 75). Furthermore, antioxidant status was not different between current or ex-smokers in either the healthy or those with COPD in these groups, implying that the disease state itself is a determinant of systemic oxidative stress, rather than current smoking habit (75) .

2.5.2.2 Nitric oxide and vascular parameters

Smoking is known to alter NO bioavailability (76) and cause endothelial dysfunction (77), however, less is known how this affects COPD patients. NO is produced by the conversion of L-Arginine to L-citrulline in the presence of NO synthases. One possible explanation for the decrease in NOx that we observed is that increased ROS (superoxide anion, $O_2^{\cdot -}$) reacts with NO, forming peroxynitrite ($ONOO^-$), consequently leading to the formation of 3-nitrotyrosine, thereby reducing the availability of NO. Although nitrotyrosine tended to be higher in COPD ($P = 0.11$), we did not find a significant negative correlation between NOx and nitrotyrosine, as expected, suggesting that other mechanisms regulate NO metabolism such as NO-synthase (78). We have previously shown in healthy older women that higher levels of NOx are associated with a decrease in resting arterial blood pressure (65), however, resting MAP did not differ between COPD patients and control subjects, and thus no relationship was found between NOx and MAP. This same study (65) suggests that in a healthy aging population, increased ROS and peroxynitrite may in part be a detrimental contributor in the determination of cerebrovascular tone. We anticipated NOx to have a greater involvement in the cerebrovascular indices measured, since it is known that tone of cerebral blood vessels is influenced by NO under resting conditions, and the loss of NO bioavailability produces vasoconstriction (56). Furthermore, increases in CBF during hypercapnia also appear to be dependent on production on NO (79).

2.5.3 Limitations

A limitation of our study is the use of end-tidal measurements used as an indication of arterial gas concentrations. Caution should be taken in making this comparison,

particularly in elderly populations and individuals with chronic lung disease due to widened alveolar-arterial gradients. To account for this limitation, we obtained capillary blood samples to measure blood gases at rest. When considering PCO_2, H^+, and HCO_3^-, good agreement has been shown between arterial and capillary samples in patients with chronic lung disease (80). In COPD patients, we found that end-tidal PCO_2 was significantly lower than capillary PCO_2 ($PcCO_2$), consistent with increased alveolar deadspace, and a widened alveolar-arterial gradient. However, based on the $PcCO_2$, we can conclude that on average, the COPD group is not chronically hypercapnic. Direct arterial blood samples are invasive and at the risk of compromising patient recruitment, were not included in our study.

Our sample size was calculated to detect differences in our main outcome variable (*i.e.*, $\dot{V}_P$ sensitivity to CO_2). We do however acknowledge that our sample size may be insufficient to detect correlational differences between oxidative stress markers and measures of cerebrovascular function, and the possibility of a type II error does exist. As our sample size addresses our main outcome variable, it is our view that larger scale studies need to be undertaken to further investigate these relationships, which would furthermore include comparison between men and women, offering important information in regards to sex-related differences.

We considered the confounding effects of current smoking status and past smoking history between our two groups in deciding on selection criteria for the study. We ruled out the known immediate autonomic and cardiovascular effects of nicotine on vascular tone by requiring all COPD subjects to have quit smoking >1 year prior to entering the study. Because little is known about the cerebrovascular effects of smoking in COPD patients, we first wanted to identify outstanding differences between a COPD patient and

a matched control. Indeed, a third ex-smoker control group would provide a valuable comparison.

2.6 Conclusion

This study is the first to show altered cerebrovascular responses to hypercapnia in women with moderate, smoking-related COPD. We show that increased oxidative stress in COPD patients, and we believe that this may contribute to the cerebrovascular impairments observed in these patients. Future research is needed to address interventional strategies aimed at minimizing systemic oxidative stress, thus providing direct evidence of the relationship between oxidative stress and cerebrovascular function.

2.7 Acknowledgements

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2.8 Online Data Supplement

Participants

Recruitment of patients for this study was mainly through collaboration with physicians who were part of the *Calgary COPD & Asthma Program*. Past and present patient information was stored in a clinic database, and female patients with COPD were identified. A total of 94 female patients with COPD were identified through these means. Of these patients, 71 did not meet the study criteria (medical history, age, co-morbidities, and FEV_1 criteria [$30\% \leq FEV_1 \leq 70\%$]). Of the remaining 23 patients, we were unable to contact 4 individuals, and 12 declined to participate. We therefore had a participation rate of 7/94 (7.4%). Additional patients who had recently participated in other COPD clinical studies were recruited through the Heritage Medical Research Clinic at the University of Calgary. Five patients were asked to participate, and 3 gave consent.

Control participants were recruited from two sources: 1) a database search, and by 2) word-of-mouth. The database held contact details (and information of age and sex) of individuals from the community that had previously expressed interest in our research studies. Sixty-one women were identified from this database. From these women, 13 were excluded, and we were unable to obtain contact with 39 of these individuals. Three individuals declined to participate, and 6 individuals gave consent. Furthermore, 6 individuals were recruited through word-of-mouth, and gave informed consent.

Inclusion/exclusion criteria

Study patients were allowed to be on long- or short-acting bronchodilators, either alone, or in combination with inhaled corticosteroids. Exclusion criteria included

heart/chest pain upon physical exertion, surgery or trauma within previous 6 months, history of myocardial infarction, angina, arrhythmia, valve disease, chronic heart failure, history of stroke, diabetes, uncontrolled hypertension, cardiovascular or cerebrovascular disease, history of chronic headaches/migraines, history of blood clots/thrombosis, and patients on domiciliary oxygen.

Protocol to measure the cerebral blood flow response to euoxic hypercapnia

Experimental set-up

O_2 and CO_2 were sampled continuously at the mouth via a fine catheter to obtain a measure of $P_{ET}O_2$ and $P_{ET}CO_2$. Respiratory gas concentrations were sampled at 100 Hz using mass spectrometry (AMIS 2000; Innovision, Odense, Denmark). Following a 5 minute lead-in period of isocapnic euoxia the protocol progressed with 4 levels of hypercapnia, each lasting 2-minutes; in the order of +3.0, +5.0, +7.0 and +9.0 mmHg above baseline $P_{ET}CO_2$. $P_{ET}O_2$ was held constant at baseline values throughout the test. The transition between each stage was obtained within 2-3 breaths. Breath-by-breath oscillations of the inspired partial pressures of O_2, CO_2, and N_2 were controlled via a fast gas mixing system for precise accuracy and stability of the desired end-tidal values.

A 3-lead electrocardiogram was used to monitor heart rate (Micromon 7142B monitor; Kontron Keynes, UK), and continuous beat-beat blood pressure was measured using finger pulse photoplethysmography (Portapress; TPD Biodemical Instrumentation, Amsterdam, The Netherlands). Arterial oxygen saturation was monitored using finger pulse oximetry (Model 3900; Datex-Ohmeda, Louisville, CO).

Specific search techniques were used to locate the MCA, and the signal was refined by adjustments to the depth, power, and angle of insonnation. A headband was used to secure the Doppler probe for the duration of the test.

Biochemical assay methodology

Plasma levels of oxidative stress (*i.e.* 8-hydroxy-2'-deoxyguanosine [8-OHdG], Malondiadehide [MDA], advanced oxidation proteins product [AOPP], nitrotyrosine, antioxidant enzymatic activities (*i.e.* plasma glutathione peroxidase [GPX] and catalase activities), end-product of nitric oxide (nitrites and nitrates) were measured at rest.

Concentrations of plasma 8-OHdG was determined using an ELISA kit from Cell BioLabs (Cell Biolabs, Inc. San Diego, CA).The limits of detection for this assay are 1-200µg.l^{-1}.

Concentrations of plasma MDA were determined as thiobarbituric reactive substances by a modified method of Ohkawa *et al* (81), as previously described (82). Although MDA assay is often associated with relevant methodological limitations (83), it was the most common lipid peroxidation marker and it still widely used as marker of oxidative stress in the area of blood pressure regulation.

Concentrations of plasma AOPP were determined using the semi-automated method described by Witko-Sarsat *et al*. AOPP concentrations were expressed as micromoles per litre of chloramine-T equivalents.

Concentrations of plasma Nitrotyrosine, as end product of protein nitration by ONOO-, were measured using an ELISA kit from Cell BioLabs (Cell Biolabs, Inc. San Diego, CA). The limits of detection for this assay are 1-8000 nmol.l^{-1}.

GPX in the plasma was determined by the modified method of Paglia and Valentine (84) using hydrogen peroxide (H_2O_2) as a substrate. GPX was determined by the rate of oxidation of NADPH to NADP+ after addition of glutathione reductase, reduced glutathione, NADPH.

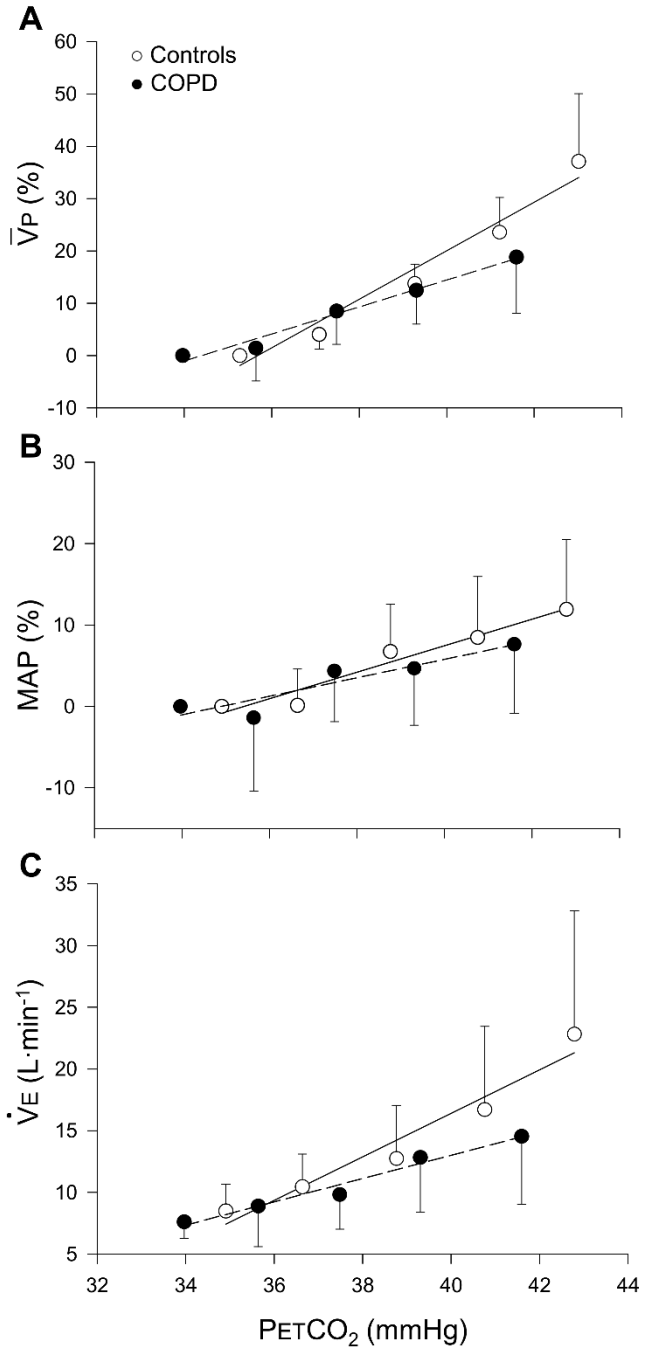
Catalase activity in the plasma was determined by the method of Johansson and Borg (85) using hydrogen peroxide (H_2O_2) as substrate, and formaldehyde as standard. Catalase activity was determined by the formation rate of formaldehyde induced by the reaction of methanol and H_2O_2 using catalase as enzyme.

The end-products of endothelium nitric oxide, nitrites and nitrates, were measured in the serum using a commercially available kit (Cayman Chemical Company, Ann Arbor, MI, USA) based on methods previously described (86). The sum of nitrite and nitrate in the plasma (NOx) is considered an index of nitric oxide production (87).

Hormone and cholesterol measurements used serum blood. Estradiol was measured by chemiluminescent immunoassay (Elecsys 2010, Roche Diagnostics). Progesterone was measured by chemiluminescent immunoassay (Advia Centaur, Siemens/Bayer Diagnostics). HDL-cholesterol was measured by enzymatic colorimetric assay (Roche/Hitachi, HDL-C Plus), and total cholesterol was measured by enzymatic colorimetric assay (Roche/Hitachi, Cholesterol CHOD-PAP).

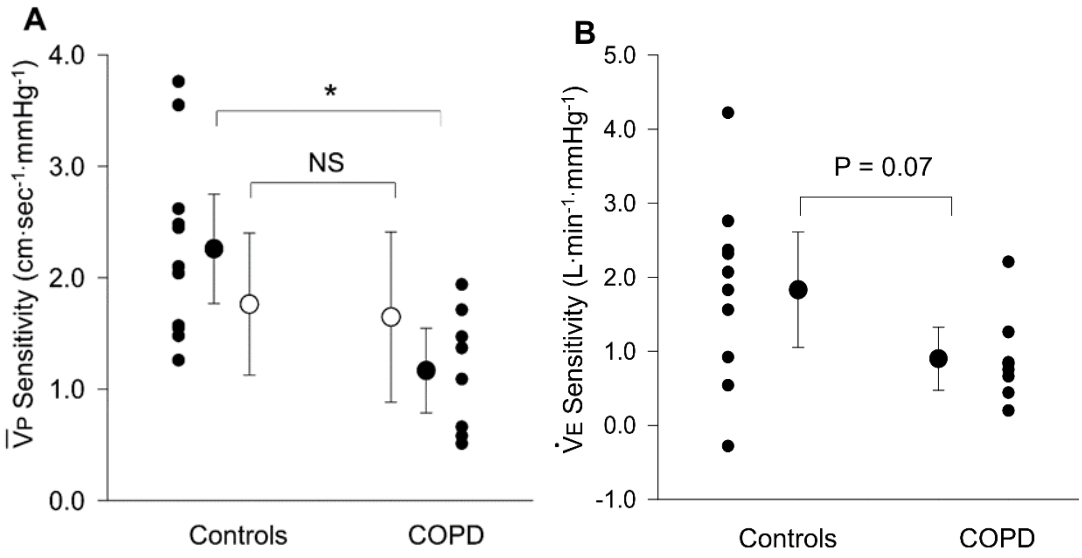
2.9 Chapter Two Figures and Tables

Figure 2. Cerebral blood flow velocity, mean arterial pressure, and ventilatory responses with acute euoxic- hypercapnia in COPD patients and controls.



Footnote: Change in A) peak cerebral blood flow velocity, B) mean arterial pressure and C) minute ventilation during incremental steps of euoxic hypercapnia. Chronic obstructive pulmonary disease patients demonstrate a decreased cerebral blood flow response to hypercapnia ($p<0.05$). *Error bars* represent standard deviation.

Figure 3. Cerebrovascular and ventilatory sensitivity to hypercapnia in COPD patients and controls.



Footnote: Cerebrovascular sensitivity indices were calculated as the slope of the line relating peak cerebral blood flow velocity ($\overline{V}_P$) or minute ventilation ($\dot{V}_E$), respectively, to the increases in end-tidal carbon dioxide (+9 mmHg above rest). *Mean values* are presented with 95% confidence intervals. A) Cerebrovascular sensitivity to carbon dioxide. Individual (small closed circles) and unadjusted mean (large close circles) responses are plotted for both COPD and controls. The cerebrovascular sensitivity is decreased in the COPD group when comparing the unadjusted means (COPD: y = 51.22x + 5.11; controls: y = 52.16x + 31.03). Once means are adjusted for oxidative stress (8- hydroxy-2'-deoxyguanosine, malondialdehyde and advanced oxidation proteins product) (open circles), no significant difference (NS) between groups exists. B) Ventilatory sensitivity to carbon dioxide. Individual (small closed circles) and mean (large closed circles) responses are plotted for both COPD and controls. There is a trend to decreased ventilatory response during hypercapnia in COPD patients compared with controls (COPD: y=50.89x+6.28; controls: y=51.75x+5.50).

Table 4. Subject characteristics.

	COPD (n=8)		Controls (n=10)	
Physical Characteristics	Mean		Mean	
Age, years	69.4 ± 4.3		64.3 ± 6.2	
BMI, $kg \cdot m^{-2}$	26.5 ± 6.4		25.4 ± 2.3	
Total Cholesterol ($mmol \cdot L^{-1}$)	5.16 ± 0.86		5.21 ± 0.87	
HDL Cholesterol ($mmol \cdot L^{-1}$)	1.66 ± 0.41		1.46 ± 0.38	
Estradiol ($pmol \cdot L^{-1}$)	69 ± 32		52 ± 16	
Progesterone ($nmol \cdot L^{-1}$)	2.2 ± 3.5		1.2 ± 0.5	
Smoking Pack-years	42.9 ± 11.2 †		0.16 ± 0.25	
Lung Function		% Predicted		% Predicted
FEV_1, L	1.28 ± 0.28 †	63	2.52 ± 0.33	106
FVC, L	2.55 ± 0.51 †	95	3.24 ± 0.38	107
TLC, L	5.55 ± 0.90	119	5.11 ± 0.48	100
RV, L	3.04 ± 0.87 †	159	1.78 ± 0.29	87
FRC, L	3.57 ± 0.85 †	134	2.47 ± 0.36	85
IC, L	1.89 ± 0.35 †		2.58 ± 0.40	
DL_{CO}, $ml \cdot min^{-1} \cdot mmHg^{-1}$	12.1 ± 3.6 †	64	21.8 ± 2.3	109

***Definition of abbreviations*:** BMI, Body mass index; HDL, High-density lipoprotein; FEV_1, Forced vital capacity in 1 second; FVC, Forced vital capacity; TLC, total lung capacity; RV, Residual volume; FRC, Functional residual capacity; IC, Inspiratory capacity; DL_{CO}, Diffusion lung capacity. Significantly different from Controls at $P \leq 0.05$ (*) and $P \leq 0.01$ (†).

Table 5. Comparison of end-tidal and capillary blood gases, hematocrit, and acid-base balance between COPD and control groups.

	COPD (n=7)	Controls (n=8)
pH	7.44 ± 0.02	7.46 ± 0.09
Pc_{CO_2}, mmHg	37.1 ± 4.8	34.9 ± 4.3
Pc_{O_2}, mmHg	59.5 ± 7.8	59.8 ± 6.8
$P_{ET_{CO_2}}$, mmHg	32.5 ± 2.7	33.6 ± 2.8
$P_{ET_{O_2}}$, mmHg	90.3 ± 4.0	89.4 ± 6.2
$[HCO_3^-]$, $mmol \cdot L^{-1}$	25.7 ± 2.9	24.7 ± 4.9
ctHb, $g \cdot dL^{-1}$	16.1 ± 0.7	13.5 ± 2.7

***Definition of abbreviations*:** Pc_{CO_2}, Pressure of CO_2 from capillary blood; Pc_{O_2}, Pressure of O_2 from capillary blood; $P_{ET_{CO_2}}$, Pressure of end-tidal CO_2; $P_{ET_{O_2}}$, Pressure end-tidal O_2; HCO_3^-, Bicarbonate ion; ctHb, concentration total hemoglobin

Table 6. Physiological variables at rest and during hypercapnia.

	Baseline Isocapnia		Hypercapnia		
	COPD n = 8	Controls n = 10	COPD n = 8	Controls n = 10	
Respiratory					
$P_{ET_{CO_2}}$, mmHg	34.0 ± 2.6	35.2 ± 3.1	41.6 ± 2.7	43.0 ± 3.3	†
$P_{ET_{O_2}}$, mmHg	90.1 ± 3.9	88.8 ± 6.6	90.3 ± 4.3	89.0 ± 7.5	
$\dot{V}_E$, L·min^{-1}	7.6 ± 1.3	8.2 ± 1.7	14.5 ± 5.5	23.3 ± 9.5	† ‡
B*f*, breaths·min^{-1}	14 ± 4	12 ± 4	16 ± 3	16 ± 4	†
V_{TE}, L·breath^{-1}	0.59 ± 0.18	0.72 ± 0.22	0.95 ± 0.38	1.50 ± 0.61	† ‡
V_{TI}/T_I, L·sec^{-1}	0.34 ± 0.11	0.34 ± 0.07	0.59 ± 0.21	0.82 ± 0.33	† ‡
T_I/T_{TOT}	0.38 ± 0.05	0.41 ± 0.05	0.39 ± 0.05	0.43 ± 0.04	
$\dot{V}_E$/MVV, %	18 ± 5 *	10 ± 3	32 ± 8	26 ± 10	† ‡
Cardiovascular					
MAP, mmHg	86.6 ± 9.8	87.6 ± 12.3	92.8 ± 9.2	97.7 ± 12.2	†
HR, beats min^{-1}	67 ± 11	67 ± 9	70 ± 11	71 ± 10	
Sa_{O_2}, %	94 ± 2 *	96 ± 1	95 ± 2	96 ± 1	
Cerebrovascular					
$\bar{V}_P$, cm·sec^{-1}	46.9 ± 6.9	46.6 ± 15.0	55.8 ± 9.6	63.3 ± 19.6	†
CVC, cm·sec^{-1}·mmHg^{-1}	0.55 ± .08	0.54 ± 0.16	0.60 ± 0.08	0.65 ± 0.17	† ‡

***Definition of abbreviations*:** $P_{ET_{CO_2}}$, Pressure of end-tidal CO_2; $P_{ET_{O_2}}$, Pressure end-tidal O_2; $\dot{V}_E$, Expired ventilation rate; B*f*, Breathing frequency; V_{TE}, Volume of expired tidal breath; V_{TI}, Volume of inspired tidal breath; T_I, Inspiratory time; T_{TOT}, Total time of respiratory duty cycle; $MVV_{predicted}$, Predicted maximum voluntary ventilation; MAP. Mean arterial blood pressure; HR, Heart rate; Sa_{O_2}, Arterial oxygen saturation; $\bar{V}_P$; Peak cerebral blood flow velocity; CVC, cerebrovascular conductance (CVC = $\bar{V}_P$/MAP). Significantly different from Controls at baseline; $P \leq 0.05$ (*). Significant main effect of hypercapnia at $P \leq 0.05$ (†). Significant group interaction with hypercapnia at $P \leq 0.05$ (‡).

Table 7. Plasma oxidative stress markers, antioxidant enzyme activity, and end-products of nitric oxide metabolism in COPD and controls.

	COPD (n=8)	Controls (n=10)
Oxidative Stress Markers		
8-OHdG, $\mu g \cdot L^{-1}$	9.6 ± 0.7 *	8.5 ± 1.3
MDA, $\mu mol \cdot L^{-1}$	23.4 ± 4.7 †	13.1 ± 4.7
AOPP, $\mu mol \cdot L^{-1}$	292.1 ± 125.1 †	134.8 ± 70.0
Nitrotyrosine, $nmol \cdot L^{-1}$	93.1 ± 102.0	38.7 ± 34.2
Antioxidant enzyme activity		
Catalase, $\mu mol \cdot L^{-1} \cdot min^{-1}$	10.5 ± 6.6	9.0 ± 7.1
GPX, $\mu mol \cdot L^{-1} \cdot min^{-1}$	16.9 ± 4.9 †	9.5 ± 3.2
NO End-products		
NOx, $\mu mol \cdot L^{-1}$	4.9 ± 2.2 *	10.9 ± 6.4

***Definition of abbreviations*:** 8-OHdG, 8-Hydroxy-2'-deoxyguanosine; MDA, malondialdehyde; AOPP, advanced oxidation protein products; GPX, glutathione peroxidase; NOx, end-product of nitric oxide metabolism ($[NO_3] + [NO_2]$). Significantly different from Controls at $p \leq 0.05$ (*). Significantly different from controls at $P \leq 0.01$ (†).

Chapter Three: **Cerebrovascular responses to submaximal exercise in women with COPD**

Sara E. Hartmann, Richard Leigh, and Marc J. Poulin (2012) Cerebrovascular Responses to Submaximal Exercise in Women with COPD. *BMC Pulmonary Medicine* **14**:99. http://www.biomedcentral.com/1471-2466/14/99

Preface

Cerebrovascular disturbances have been described in patients with COPD, however, whether these disturbances exist during exercise are unknown. This study set out to assess cerebral blood flow responses in COPD patients during submaximal cycling exercise.

Declaration

The original manuscript contained within this chapter was written by myself, with guidance by co-author's Dr. Leigh and Poulin. The original study conception was initiated by Drs. Leigh and Poulin, and myself. I obtained ethical approvals, and performed all of the data collection and analysis. Dr. Leigh provided medical coverage. I submitted and received ethical approvals. The lung function test were performed by staff at the Foothills Hospital. Preliminary data were presented in abstract form (88) at the Canadian Society for Exercise Physiology Conference in November 2010 before being published in its final form in *BMC Pulmonary Medicine*.

Open Access Declaration to Reproduce Published Material

3.1 Abstract

Background: COPD patients have decreased physical fitness, and have an increased risk of vascular disease. In the general population, fitness is positively associated with resting cerebral blood flow velocity, however, little is known about the cerebrovascular response during exercise particularly in COPD patients. We hypothesized that COPD patients would have lower cerebral blood flow during exercise secondary to decreased physical fitness and underlying vascular disease.

Methods: Cardiopulmonary exercise testing was conducted in 11 women with GOLD stage I-II COPD, and 11 healthy controls to assess fitness. Cerebro- and cardio-vascular responses were compared between groups during two steady-state exercise tests (50% peak O_2 consumption and 30W). The main outcome variable was peak middle cerebral blood flow velocity ($\bar{V}_P$) during exercise using transcranial Doppler ultrasonography.

Results: Physical fitness was decreased in COPD patients. $\bar{V}_P$ was comparable between COPD and controls (25 ± 22% *versus* 15 ± 13%, respectively; P>0.05) when exercising at the same relative intensity, despite patients having higher blood pressure and greater arterial desaturation. However, $\bar{V}_P$ was elevated in COPD (31 ± 26% *versus* 13 ± 10%; $P \leq 0.05$) when exercising at the same workload as controls.

Conclusions: Our results are contradictory to our *a-priori* hypothesis, suggesting that during matched intensity exercise, cerebral blood flow velocity is similar between COPD and controls. However, exercise at a modestly greater workload imposes a large physical demand to COPD patients, resulting in increased CBF compared to controls. Normal activities of daily living may therefore impose a large cerebrovascular demand in COPD patients, consequently reducing their cerebrovascular reserve capacity.

3.2 Introduction

The regulation of cerebral blood flow (CBF) is imperative for adequate delivery of O_2 and energy supply to the brain. During cerebral activation and increased metabolism, cerebral arterioles dilate, thereby increasing CBF, and ultimately O_2 delivery. This process of neurovascular coupling (*i.e.*, neural activation coupled with an adequate increase in blood flow) is challenged during dynamic exercise. Factors such as age and physical fitness have been suggested to play an important role in this regulatory process both at rest (89), and during exercise (90).

The reduction of physical activity levels and exercise capacity in COPD patients (91-93) may pose a risk to patients' cerebrovascular health. Even in the early stages of COPD, patients with mild and moderate obstruction are reported to have reduced exercise capacity(94). Evidence suggests that patients with moderate COPD are already at an increased risk of cardiovascular disease and stroke, thereby suggesting an underlying vascular pathology. We have previously reported decreased CBF responsiveness to CO_2 in women with COPD, suggesting reduced cerebral dilatory function (28).

Cycle ergometry testing offers a functional and translational tool to test integrated responses during exercise. COPD patients may exhibit arterial hypercapnia (95) and hypoxemia, exaggerated vasopressor response (96), and ventilatory limitations (97) during exercise – which all have implications on the regulation of CBF. Furthermore, greater distribution of cardiac output to respiratory or peripheral muscles could impact the blood flow delivery to the brain. Presently, few studies have focused on the cerebrovascular response of COPD patients during exercise (40, 42), and of these studies, focus has been on exercise limitations. It is currently unknown if the cerebrovascular response to exercise in COPD patients differs from that of a healthy control group.

We therefore tested the hypothesis that women with mild-moderate COPD would have reduced cerebral blood flow during exercise, secondary to reduced physical fitness. Women were of particular interest in this study due to a shift in the disease burden from men to women (98), and comparatively, women report worse symptoms for a similar severity of disease to men, and for any given age, have lower aerobic capacity. Additionally, we chose to focus on patients with mild-moderate COPD because this demographic represents approximately half of all cases of COPD (99).

3.3 Methods

3.3.1 Study subjects

Twenty-three postmenopausal women (COPD = 11, Healthy Control = 12) between the ages of 55-79 years old were recruited for participation in this study. Control subjects were recruited from the community, and COPD patients were recruited from participating outpatient medical clinics within the Calgary Health Region. All study participants visited the Laboratory of Human Cerebrovascular Physiology at the University of Calgary (1103 m elevation) for two testing sessions. Subjects were instructed to refrain from eating or drinking 4 hours prior to each testing session. All study participants provided written, informed consent. Ethical approval was obtained by the Conjoint Health Research Ethics Board at the University of Calgary (Ethics ID: E-22138).

Since there are currently no published studies describing the CBF response in COPD during exercise, compared to healthy controls, we based our sample size using data from our previous study (28) describing changes in $\bar{V}_P$ during hypercapnia. We expected a similar difference in $\bar{V}_P$ between groups (Control = 37% vs. COPD = 19%). Therefore, a sample size of n = 10 in each group was needed to detect a significant difference with a power of 95%, and a two-tailed α of 0.05. It is likely that there are similar physiological mechanisms involved in the regulation of blood flow during exercise and hypercapnia, and reason that this power calculation would be an appropriate estimation of sample size.

3.3.1.1 Inclusion criteria

Patients were eligible for inclusion into the COPD cohort if they had physician-diagnosed GOLD stage I-II COPD, a smoking history >10 pack-years and chronic airflow obstruction

(FEV_1/FVC <70%; $FEV_1 \geq$ 50% predicted). To control for the immediate and lasting cardiovascular effects of nicotine, patients needed to be ex-smokers (>1 year). Additional criteria included: post-menopausal for ≥ 12 months, able to walk independently outside or on stairs, and a BMI < 35 $kg \cdot m^{-2}$. Study subjects were allowed to be on long- or short-acting bronchodilators, either alone, or in combination with inhaled corticosteroids. Exclusion criteria included heart/chest pain upon physical exertion, surgery or trauma within previous 6 months, history of myocardial infarction, angina, arrhythmia, valve disease, chronic heart failure, history of stroke, cardiovascular or cerebrovascular disease, history of chronic headaches/migraines, history of blood clots/thrombosis, and patients who were on domiciliary oxygen.

Participating control subjects were sedentary (< 3 planned exercise sessions/week), post-menopausal, and who had no history of lung disease, and no significant history of smoking (< 1 pack-year lifetime smoking history). Additional exclusion criteria conformed to that as outlined above for COPD patients.

3.3.2 Study design

Subjects visited the laboratory on two occasions, and followed the same study protocol and procedures. On the first visit, volunteers completed a medical screening questionnaire, pulmonary function test, and cardiopulmonary exercise test (CPET) to assess physical fitness. Volunteers returned to the laboratory to complete the main experimental session involving CBF measurements during exercise. The main exercise protocol consisted of two bouts of 6-minutes each of exercise on a modified semi-supine cycle ergometer (~70 degrees upright) (Exercise 1 [EX1 = 50% $\dot{V}O_{2peak}$ and Exercise 2 [EX2 = 30 Watts]), as previously described (100, 101). The workload at 50% $\dot{V}O_{2peak}$ was

intended to observe the peak CBF response, while an absolute value of 30 Watts was selected to represent the intensity of common activities of daily living, comparable to household work and light gardening (~3 metabolic equivalents; METS). The exercise periods were separated by a 6-minute rest/recovery period to allow physiological variables to return to baseline (BL).

3.3.2.1 Pulmonary function testing

Spirometry, measures of lung volumes and single-breath diffusion capacity were completed according to ATS guidelines (61-63).

3.3.2.2 Cardiopulmonary exercise test

Peak oxygen uptake ($\dot{V}O_{2peak}$) was assessed by a CPET performed on a modified semi-reclined cycle ergometer, according to ATS guidelines (102). The test was initiated with a 3-minute warm-up period of unloaded cycling, immediately followed by an increase in workload to 20 W, with progressive increments of either 10 or 15 $W \cdot min^{-1}$ (COPD patients or controls, respectively). Ratings of perceived dyspnea and leg fatigue were scored using a modified Borg Scale every 2 minutes by the participant. Breath-by-breath metabolic and respiratory variables were collected using dedicated software (BreatheM v2.38, Oxford) and measured by mass spectrometry (AMIS 2000; Innovision, Odense, Denmark). Continuous measures of heart rate via 3-lead ECG (Micromon 7142 monitor; Kontron Medical, Milton Keynes, UK), and arterial oxygen saturation (Sa_{O_2}) (Model 3900; Datex-Ohmeda, Louisville, CO) were recorded.

3.3.2.3 Protocol to measure the cerebral blood flow response to submaximal exercise

Middle cerebral artery (MCA) blood flow velocity was continuously measured using a 2-MHz pulsed Doppler Ultrasound system (TC22, SciMed, Bristol, England) as previously described (101). Peak middle cerebral artery velocity ($\bar{V}_P$) was used as a surrogate for CBF. Heart rate was collected using a 3-lead ECG (Micromon 7142B monitor; Kontron Keynes, UK), and continuous beat-beat blood pressure was measured using finger pulse photoplethysmography (Portapress; TPD Biomedical Instrumentation, Amsterdam, The Netherlands). Arterial oxygen saturation was collected using finger pulse oximetry (Model 3900; Datex-Ohmeda, Louisville, CO).

3.3.2.4 Respiratory measures

Subjects breathed through a mouthpiece with their nose occluded. Respiratory gas concentrations were sampled at the mouth at 100 Hz using mass spectrometry (AMIS 2000; Innovision, Odense, Denmark). Respiratory volumes were measured with a turbine and volume transducer (VMM-400; Interface Associates, Laguna Niguel, CA), and respiratory flow direction and timing were obtained with a pneumotachograph (RSS100-HR, Hans Rudolf, Kansas City, MO, USA).

3.3.3 Data analysis

3.3.3.1 Analysis

Physiological data were collected on a beat-beat and breath-breath basis using dedicated software (BreatheM v2.38, University Laboratory of Physiology, Oxford, UK)

and later averaged into 15-second bins. Baseline data were averaged over 2-minutes immediately preceding exercise. To achieve steady state parameters, the last 60-seconds of exercise (*i.e.*, minute 5 to 6) were used in the stage averages. The main outcome variable in this study was $\bar{V}_P$. Secondary outcomes were $\dot{V}O_{2peak}$, mean blood pressure (MBP), and cerebrovascular conductance (CVC = $\bar{V}_P$/MBP).

3.3.3.2 Statistical procedures

Using statistical software (SPSS Version 20.0, SPSS Inc., Chicago, IL, USA), mean between-group differences in subject characteristics, pulmonary function data, and endpoints of the maximal cardiopulmonary exercise test were determined using independent t-tests. Paired t-tests were used to assess baseline (BL) differences within groups (*i.e.*, COPD BL1 vs. COPD BL2). A 2x2 mixed factorial repeated measures analysis of variance was used to assess the main effect of independent variables "*condition"* (*i.e.*, BL1 vs. EX1 or BL2 vs. EX2), and "*grouping*" (*i.e.*, COPD or CONTROL), and the interaction between *condition* x *grouping* on physiological outcomes (*i.e.*, $\bar{V}_P$, CVC, MBP, HR, Sa_{O_2}).

Relationships were assessed exclusively within groups using Pearson's correlation coefficient to investigate fitness status ($\dot{V}O_{2peak}$), disease severity (FEV_{1pred}, D_{LCO}), Sa_{O_2}, $P_{ET}CO_2$, and MBP on cerebrovascular variables at rest and during exercise. Data are presented as mean ± SD, with significance set at α-level ≤ 0.05. Two tailed tests were performed in all analyses.

3.4 Results

Two COPD patients were not able to complete the EX2 protocol; however, their data from EX1 was included in the study analysis. One control participant was excluded due to lack of a suitable CBF signal. We therefore compared the exercise responses between 11 COPD and 11 control participants in EX1, and 9 COPD and 11 control participants in EX2.

3.4.1 Baseline characteristics

Physical characteristics and results of pulmonary function tests are listed in Table 8 (*page 75*). Groups were well matched for age, weight and BMI ($P > 0.05$). By study design, COPD patients had a significantly greater smoking history than controls ($P<0.01$). Based on post-spirometric FEV_1 predicted values, disease severity ranged from GOLD I-II (mild to moderate) (51% to 86%) in COPD patients (n = 9, GOLD II). COPD patients had reduced diffusion capacity (D_{LCO}), and increased hyperinflation (FRC, IC/TLC) compared to control subjects ($P<0.01$), who all had normal lung function.

3.4.2 Cardiopulmonary exercise test

Results of the cardiopulmonary exercise test are summarized in Table 9 (*page 76*). Mean $\dot{V}O_{2peak}$ and peak power output was lower in patients with COPD. Arterial oxygen saturation was significantly decreased in COPD patients with exercise, but not in controls ($P<0.01$). At end of exercise, COPD patients achieved similar predicted heart rate values to controls, suggesting a ventilatory limitation to exercise, rather than any cardiovascular impairment ($P>0.05$).

3.4.3 Responses to exercise at a relative workload: 50% $\dot{V}O_{2peak}$ (EX1)

The target intensity for EX1 was set at 50% $\dot{V}O_{2peak}$ for 6 minutes (EX1). Accordingly, groups exercised at a similar exercise intensity ($\dot{V}O_{2peak}$: 69% (COPD) *versus* 50% (controls); $P>0.05$) (Table 10, *page 77*). Metabolic cost was not different between groups in EX1 ($\dot{V}O_2$: 0.69 $L \cdot min^{-1}$ (COPD) *versus* 0.74 $L \cdot min^{-1}$ (controls); $P>0.05$). Despite similar workrates between groups (COPD: 15W *versus* Controls: 23W; $P>0.05$), COPD patients exercised at a greater capacity of their WR_{peak} (COPD: 25% *versus* Controls: 18%; $P \leq 0.05$). Patients and controls had similar $P_{ET}CO_2$ and $P_{ET}O_2$ at rest, and in response to EX1 ($P>0.05$).

The cerebro-and cardiovascular responses to EX1 are summarized in Table 11 (*page 78*). There were no differences between resting cerebro- or cardio-vascular variables between groups ($P>0.05$). The two-factor analysis of variance revealed that there was a significant main effect of exercise in increasing $\bar{V}_P$, HR, and MBP ($P \leq 0.05$). CVC was not affected by exercise ($P>0.05$). There was a significant group x exercise interaction for MBP (mmHg) ($P \leq 0.05$), but not $\bar{V}_P$ ($cm \cdot s^{-1}$) ($P=0.17$). Sa_{O_2} decreased with exercise, and this effect was the greatest in COPD patients, as exhibited by an exercise x group interaction ($P \leq 0.05$).

In summary, exercise increased $\bar{V}_P$ similarly in COPD (42.8 $cm \cdot s^{-1}$ to 50.2 $cm \cdot s^{-1}$) and controls (44.2 to 50.2 $cm \cdot s^{-1}$), while working at a similar exercise intensity ($P>0.05$), despite COPD patients exhibiting a greater exercise-pressor response and greater arterial oxygen desaturation.

3.4.4 Responses to exercise at absolute workload: 30 Watts (EX2)

Physiological description of the exercise protocol is summarized in Table 10 (*page 77*). All subjects cycled at a workrate of 30 W for 6 minutes (EX2). By design, there was no difference between $\dot{V}O_2$ between groups ($\dot{V}O_2$: 0.78 $L \cdot min^{-1}$ (COPD) *versus* 0.74 $L \cdot min^{-1}$ (controls); $P>0.05$). There was a trend for COPD patients to be exercising at a greater relative exercise intensity than controls ($\dot{V}O_2$ peak: 74% (COPD) *versus* 52% (controls); $P=0.08$). Despite the same workrate between groups, COPD patients exercised at a greater capacity of their WR_{peak} (COPD: 48% *versus* Controls: 25%; $P \leq 0.01$). Patients and controls had similar $P_{ET}CO_2$ and $P_{ET}O_2$ at rest, and in response to EX2.

The cerebro-and cardiovascular responses to EX2 are summarized in Table 11 (*page 78*), and individual stage means are presented in Figure 4 (*page 74*). There were no differences between resting cerebro- or cardio-vascular variables between groups ($P>0.05$). The two-factor analysis of variance revealed that there was a significant main effect of exercise in increasing $\bar{V}_P$, HR, and MBP, and decreasing Sa_{O_2} ($P \leq 0.05$). There was a significant group by exercise interaction for $\bar{V}_P$ ($cm \cdot s^{-1}$) ($P \leq 0.05$). Exercise significantly decreased Sa_{O_2} overall ($P<0.01$), but had the greatest effect in COPD patients whom desaturated at a greater rate than controls as exhibited statistically by a grouping by exercise interaction effect ($P<0.01$).

In summary, exercise increased $\bar{V}_P$ to a greater extent in COPD patients (40.2 $cm \cdot s^{-1}$ to 51.1 $cm \cdot s^{-1}$) compared to controls (43.8 to 48.7 $cm \cdot s^{-1}$), while working at 30 Watts (and an equivalent $\dot{V}O_2$). At this workload, however, there was a trend for COPD patients to be working at a higher percentage of maximum capacity than the control subjects. Blood pressure increased with exercise, but changes were similar between groups.

3.4.5 Relationships between fitness, disease severity, and cerebrovascular measurements during exercise

At rest, there was no relationship between fitness status, disease severity, Sa_{O_2}, $P_{ET_{CO_2}}$, and blood pressure on cerebrovascular measures. In both groups, and in both exercise conditions, participants with lower baseline $\bar{V}_P$ were more likely to have low exercise $\bar{V}_P$, and *vice versa* (EX1: R^2 = 0.75, P<0.01; EX2: R^2 = 0.78, P <0.01). Furthermore, individuals with lower baseline $\bar{V}_P$ were more likely to have a greater absolute increase in exercise $\bar{V}_P$ (EX1: R^2 = 0.18, $P \leq 0.05$; EX2: R^2 = 0.29, P<0.01). Percent change in MBP was positively associated with change in $\bar{V}_P$ (EX1: R^2 = 0.36, P<0.01; EX2: R^2 = 0.22, $P \leq 0.05$). There was no relationship between cerebrovascular variables and Sa_{O_2} during exercise. In EX2, the change in $P_{ET_{CO_2}}$ with exercise was positively related to the changes in $\bar{V}_P$ and CVC ($\bar{V}_P$: R^2 = 0.37, P<0.01; CVC: R^2 = 0.40, respectively, P<0.01). There was no significant correlations between $\dot{V}O_{2peak}$ and $\bar{V}_P$, MBP, or CVC (P>0.05) in either EX1 or EX2 (all subjects combined).

3.5 Discussion

Our main findings are that 1) when patients with mild to moderate COPD exercise at the same intensity to healthy controls, the CBF response is similar; and 2) when comparing CBF at a matched O_2 demand (*i.e.*, 30 Watts), CBF is elevated in this cohort of COPD patients. From these results, it is apparent that COPD does not have a detrimental effect on cerebral blood flow during moderate intensity exercise. The mechanisms underlying the increased CBF in COPD at higher workloads needs to be investigated further.

Cerebral blood flow velocity has consistently been shown to increase in healthy individuals during mild to moderate exercise intensity (101, 103-105), and seems to be intensity dependent (106). Although mechanisms are not fully elucidated (107), important regulators of the exercise-induced CBF response include: cortical activation (108), arterial blood pressure (109), cardiac output (103), and blood gases (particularly Pa_{CO_2}) (110). Comparative studies are particularly lacking in the area of ageing and disease. Consequential alterations of CBF are apparent with age, however, physical fitness has been shown to offset these changes (89, 100). Aerobic capacity appears to be an important determinant in cerebrovascular health with ageing, as it has been shown to be positively correlated with cerebrovascular CO_2 reactivity (111), and better cognitive outcomes (100). Current opinion suggests that COPD represents a case of advanced ageing (112), which may make these patients particularly susceptible to alterations in cerebrovascular responses.

We reasoned that the known vascular consequences associated with COPD, including endothelial dysfunction (6), and increased arterial stiffness (113) would play a detrimental role in the CBF response to exercise. A recent study suggests that physical fitness (*i.e.*, 6MWD) has a favorable effect on endothelial function in COPD patients (114).

We have furthermore shown that women with moderate COPD exhibit a nearly two-fold reduction in the expected CBF response to CO_2 (28). It is likely that during a more modest cerebrovascular challenge (e.g., submaximal exercise *versus* hypercapnia), the cerebrovascular response remains intact, despite underlying vascular pathologies that may exist.

We found patients to have decreases in Sa_{O_2} during exercise in response to maximal and submaximal exercise. As hypoxia is known to stimulate blood flow, it is interesting that we did not find a relationship between individuals with lower Sa_{O_2} during exercise and increased CBF. However, it is possible that increased CBF, as seen in COPD during more intense exercise (e.g., EX2), could be partially driven by decreased Sa_{O_2}, in a regulatory effort to increase cerebral oxygen delivery.

Results from a study investigating cerebral hemodynamics in severe COPD during exhaustive exercise suggest increased frontal cortex O_2 delivery at the end of exhaustive normoxic exercise, in conjunction with increased CBF, despite exercise induced hypoxemia (42). These results would suggest a regulatory difference, as it has alternatively been shown in healthy hypoxemic individuals that CBF is decreased (from rest) during exhaustive exercise (115). The differences between these studies was explained by the lack of hyperventilation in COPD which maintained (and even increased) Pa_{CO_2}, causing a vasodilatory response. However, caution is warranted as this study does not allow for an accurate comparison with an age-matched control group. The "normal" hyperventilation-induced hypocapnia evident during high-intensity exercise has been shown to be blunted in healthy older individuals, which in turn leads to maintenance of Pa_{CO_2}, and CBF (116). In patients with terminal lung disease, Jensen *et al*.(40) has shown continuous increases in CBF, with a slight reduction in cerebral oxygenation at end-exercise in room air, without

changes in Pa_{CO_2}. It is therefore likely, that the CBF response in COPD depends on several aspects, such as disease severity, blood gases, exercise intensity, and physical fitness. We are hesitant to draw conclusions based on Pa_{CO_2}, as this variable was not measured in our study. However, COPD patients continue to increase $\dot{V}_E$ in EX2, which would not support the hypoventilation/increased Pa_{CO_2} theory.

Cardio-pulmonary interactions during exercise in COPD have the potential to have an adverse influence on CBF. Increased expiratory loading and dynamic hyperinflation (DH) during exercise may impair venous return, and consequently lead to a reduction in right heart preload, and ultimately stroke volume. O_2 pulse is commonly used as a surrogate measure of stroke volume (and cardiac efficiency). Studies have shown DH to be negatively correlated with O_2 Pulse during incremental exercise in COPD (117, 118). Furthermore, resting hyperinflation (IC/TLC) was associated with a reduction of O_2 pulse at peak exercise. We did not find a difference in O_2 pulse at peak exercise between COPD patients and controls (Table 9, *page 76*), and take this to indicate that cardiac function was not impaired in our patients. Despite COPD patients in this study having significantly lower IC/TLC ratio at rest (37% *versus* 49%), this level of hyperinflation is noticeably less when compared to results of the aforementioned studies which reported DH during exercise. Furthermore, the role of DH in mild-moderate COPD remains to be clearly defined. Lastly, although cardiac output remains an important determinant in cerebrovascular regulation, reductions in cardiac output by (by lower body negative pressure) reduce CBF, but seem to be less important during exercise compared to the resting state, which has been explained as a counter-regulatory activation of the sympathetic nervous system (119). Overall, we speculate, that DH is not a major

contributor to the determinants of CBF in our sample population. As this remains beyond the scope of this study, it should be considered in future studies.

One strength of our study is that it includes data comparing the physiological response during both as exercise challenge that is a fixed percentage of each person's maximal workload, and an absolute workload - which represents a value that may be a more realistic occurrence in activities of daily living. Although we found that patients with mild and moderate COPD have a normal CBF response at 50% $\dot{V}O_{2peak}$, it is important to highlight that this workload is equivalent to ~2.9 METS, which would equate to light housework such as self-care, washing dishes, and walking to a parking lot(120). When physical demands are slightly increased to 30 Watts (~3.2 METS), these COPD patients further increase CBF. The importance of absolute requirements are highlighted by Paterson *et al*.(121) who report that a relative $\dot{V}O_2$ of ~15 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ is required for independent living in the aging population. During moderate activities (e.g., vigorous cleaning, sweeping, etc.), COPD patients would be exposed to increased aerobic demand, which may have cerebrovascular implications, including a reduced cerebrovascular reserve capacity compared to healthy controls. This remains highly speculative, and the implications of this are unclear at this time. Although we do not have measures of habitual activity, we have previously shown a significant, positive agreement between self-reported physical and $\dot{V}O_{2max}$ in healthy, older women (122). In this same study, fitness was correlated with better vascular outcomes during exercise (100). We therefore suspect that individuals in the present study who have a higher $\dot{V}O_{2max}$ are also more physically active, although this needs to be confirmed in future research. Conversely, it is possible that frequent increases in CBF may pose a vascular benefit to COPD patients, whereby increases in CBF result in shear stress on the vascular endothelium, thus providing beneficial adaptations through the up-regulation of nitric oxide synthase (123). In contrast,

modest activities increase CBF in COPD and may limit the supply of blood flow available for additional challenges, such as cognitive tasks.

Our selection of mild – moderate COPD patients includes 9 of 11 patients with GOLD II, moderate COPD. Of these 11 patients, 2 could not complete a 6-minute workload of 30 Watts. We believe that more severe patients would have further difficulty completing 2x6-min bouts of cycling exercise. Further, our selected cohort of COPD patients represents the patients that are most likely either still physically active, or still have the potential to become more active, and we would therefore gain the greatest insight into studying this population. Women in particular have lower $\dot{V}O_{2max}$, smaller lung volumes, and exhibit greater dyspnea at a similar $\dot{V}O_2$ compared to men (124). Finally, a homogeneous sample of women reduces additional heterogeneity between subjects, and is particularly important in this smaller sample size.

3.5.1 Limitations

Transcranial Doppler ultrasound is a commonly used, non-invasive tool to measure the velocity of blood in the MCA. The MCA provides ~ 80% of the blood flow to the brain, and perfuses the frontal and temporal lobe, including the motor-sensory cortex, which is activated during exercise. This technique offers several advantages over other methodologies because of its non-invasive properties, and high temporal resolution (64, 125). One drawback of using this method, however, is the assumption that the MCA diameter remains constant, allowing us to infer that velocity represents flow. The regulation of CBF is thought to occur in the small arterioles, such that the large basal cerebral arteries maintain a constant diameter thus, providing an accurate representation of CBF (101, 125).

We firstly chose to compare the steady-state CBF response at a relative exercise intensity. Secondarily, we studied the physiological response between COPD and controls at an absolute workload. We acknowledge that at an absolute workload participants are working at a different relative exercise capacity, which introduces confounding variables. It is therefore difficult to interpret the specific mechanisms involved in the observed differences in CBF during this stage. However, we believe this exercise workload to be of significant importance and these findings notable.

Lastly, a limitation of our study was the absence of arterial blood gases as part of our methodology. We therefore are precluded from making a conclusion regarding the influence of Pa_{CO_2} on the regulation of CBF during exercise in COPD.

Conclusion

Cerebral blood flow responsiveness appears to be normal in patients with mild to moderate COPD compared to healthy controls when exercising at a moderate intensity (50% $\dot{V}O_{2peak}$), but not when exercising at a slightly greater, matched workload (*i.e.*, 30 Watts). It is likely that decreased physical fitness contribute to this. These results have implications for normal activities of daily living such as household activities in which COPD patients would likely experience greater increases in CBF, thereby decreasing the cerebral vascular reserve.

3.6 Acknowledgements

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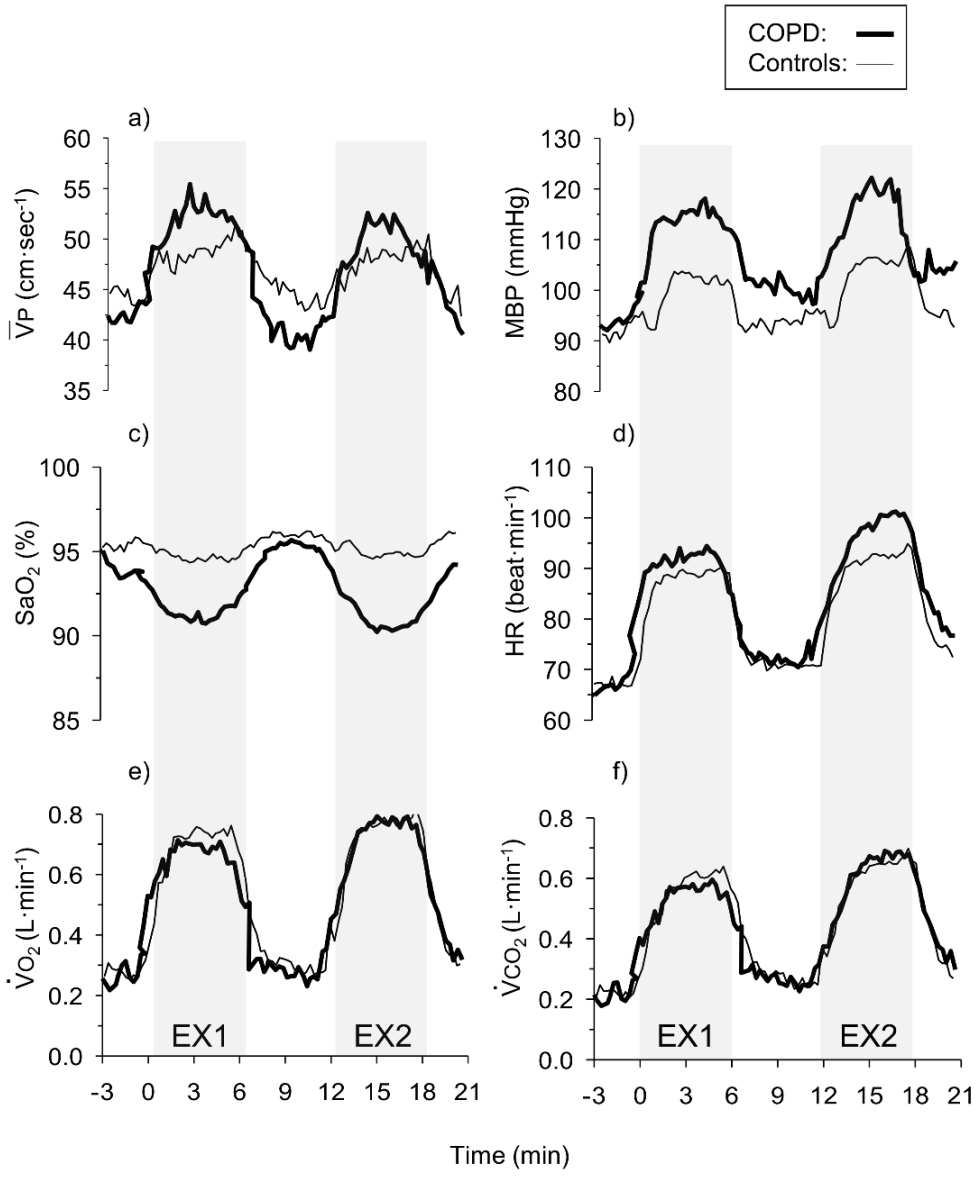
3.7 Chapter Three Figures and Tables

Figure 4. Continuous representation of the physiological responses to two, 6-minute exercise periods in women with moderate COPD and healthy age-matched controls.



Footnote: Exercise 1 (EX1; 50% $\dot{V}O_{2peak}$) and Exercise 2 (EX2; 30 Watts) were separated by 6-minutes of resting recovery. a) Peak cerebral blood flow velocity in the middle cerebral artery ($\bar{V}_P$); b) mean arterial blood pressure (MBP); c) arterial oxygen saturation (Sa_{O_2}); d) heart rate (HR); e) volume of oxygen consumption ($\dot{V}O_2$); and f) volume of carbon dioxide production ($\dot{V}CO_2$). *Thick* line represents COPD, and *thin* line represents Controls. Data represent 15-second averages.

Table 8. Subject characteristics.

	COPD (n=11)		Controls (n=11)	
Physical Characteristics	Mean		Mean	
Age, years	69.6 ± 5.6		64.2 ± 7.1	
Weight, kg	68.8 ± 12.8		69.4 ± 9.6	
BMI, $kg \cdot m^{-2}$	26.8 ± 4.6		25.6 ± 2.7	
Smoking Pack-years	38.4 ± 12.9 †		0.2 ± 0.3	
Lung Function		% Predicted		% Predicted
FEV_1, L	1.38 ± 0.24 †	67	2.50 ± 0.34	107
FVC, L	2.64 ± 0.51 *	97	3.24 ± 0.38	108
TLC, L	5.53 ± 1.04	115	5.12 ± 0.45	101
RV, L	2.97 ± 0.78 †	146	1.81 ± 0.30	88
FRC, L	3.40 ± 0.77 †	124	2.48 ± 0.34	86
IC, L	2.07 ± 0.50 *		2.52 ± 0.43	
IC/TLC, %	37 †		49	
RV/TLC, %	53 †		35	
D_{LCO}, $ml \cdot min^{-1} \cdot mmHg^{-1}$	14.2 ± 3.3 †	72	21.1 ± 3.1	107

***Definition of abbreviations*:** BMI, Body mass index; FEV_1, Forced vital capacity in 1 second; FVC, Forced vital capacity; TLC, Total lung capacity; RV, Residual volume; FRC, Functional residual capacity; IC, Inspiratory capacity; D_{LCO}, Diffusion lung capacity. Significantly different from Controls at $P \leq 0.05$ (*) and $P \leq 0.01$ (†).

Table 9. Summary of cardiopulmonary exercise test endpoints in COPD and controls.

	COPD (n=11)	Controls (n=11)
$\dot{V}O_{2\ peak}$		
$L \cdot min^{-1}$	1.10 ± 0.34 *	1.52 ± 0.41
$mL \cdot min^{-1} \cdot kg^{-1}$	16.1 ± 4.6 *	22.6 ± 7.0
$\dot{V}O_{2peak\ pred}$, %	85 ± 29	106 ± 28
$\dot{V}CO_{2\ peak}$, $L \cdot min^{-1}$	1.08 ± 0.37 †	1.64 ± 0.50
$\dot{V}_{E\ peak}$, $L \cdot min^{-1}$	40.7 ± 9.5 *	62.5 ± 21.1
$\dot{V}_{E\ peak}$/ MVV, %	84 ± 16	71 ± 19
B*f*, $breaths \cdot min^{-1}$	31 ± 5	35 ± 8
Sa_{O_2}, %	89.5 ± 4.3 †	94.5 ± 2.0
Heart rate		
$HR_{\ peak}$, $beats \cdot min^{-1}$	121 ± 14 *	140 ± 20
$HR_{\ age\ pred}$, %	81 ± 10	90 ± 13
O_2 Pulse ($ml \cdot beat^{-1}$)	9.1 ± 2.7	10.8 ± 2.0
Workrate		
$WR_{\ peak}$, Watts	57 ± 13 †	117 ± 34
$WR_{\ pred}$, %	63 ± 21 †	103 ± 20
Borg dyspnea (0-10)	5.2 ± 1.1	5.8 ± 1.8
Borg leg Fatigue (0-10)	4.3 ± 1.2	5.6 ± 1.6

***Definition of abbreviations*:** $\dot{V}O_{2peak}$, Peak volume of O_2 consumed; $\dot{V}CO_{2peak}$, Peak volume of CO_2 produced; $\dot{V}_{E\ peak}$, Peak expired ventilation rate; B$f_{\ peak}$, Peak breathing frequency; Sa_{O_2}, Arterial oxygen saturation; $HR_{\ peak}$, Peak heart rate; O_2 Pulse: Oxygen pulse ($\dot{V}O_2$ / HR); $WR_{\ peak}$, Peak work rate. Significantly different from controls at $P \leq 0.05$ (*) and $P \leq 0.01$ (†).

Table 10. Workrate, metabolic and respiratory parameters during rest and submaximal exercise.

	Exercise 1 (Relative Workload)				Exercise 2 (Absolute Workload)			
	COPD (n=11)		Control (n=11)		COPD (n=9)		Control (n=11)	
	BL1	EX1	BL1	EX1	BL2	EX2	BL2	EX2
Workrate, W	-	15 ± 20	-	23 ± 19	-	30	-	30
WR_{max}, %	-	25 ± 37 †	-	18 ± 14	-	48 ± 7 †	-	25 ± 11
Metabolic and Respiratory								
$\dot{V}_E$, L·min^{-1}	8.9 ± 2.3	22.4 ± 7.1	7.3 ± 1.4	20.5 ± 5.1	11.8 ± 2.8*	27.9 ± 5.8	9.0 ± 1.4	21.0 ± 6.6
$\dot{V}O_2$, L·min^{-1}	0.28 ± 0.12	0.69 ± 0.28	0.27 ± 0.05	0.74 ± 0.17	0.27 ± 0.04	0.78 ± 0.22	0.28 ± 0.05	0.74 ± 0.19
$\dot{V}O_{2max}$, %	28 ± 10*	69 ± 31	19 ± 6	50 ± 11	25 ± 9	74 ± 30	20 ± 7	52 ± 20
$\dot{V}CO_2$, L·min^{-1}	0.23 ± 0.09	0.58 ± 0.24	0.22 ± 0.04	0.63 ± 0.17	0.26 ± 0.05	0.69 ± 0.18	0.25 ± 0.04	0.63 ± 0.17
$P_{ET_{CO_2}}$, mmHg	32.5 ± 3.2	34.9 ± 4.1	33.8 ± 2.1	36.0 ± 3.0	31.1 ± 3.3	34.4 ± 3.7	33.1 ± 1.9	35.4 ± 2.7
$P_{ET_{O_2}}$, mmHg	89.3 ± 5.0	87.6 ± 4.9	88.2 ± 4.8	86.3 ± 5.1	95.2 ± 5.2	89.4 ± 5.5	92.5 ± 5.2	88.0 ± 5.8

***Definition of abbreviations*:** WR_{max}, Work rate maximum; $\dot{V}_E$, minute ventilation; $\dot{V}O_2$, volume of oxygen uptake; $\dot{V}CO_2$, Volume of carbon dioxide production; $P_{ET_{CO_2}}$, Pressure of end-tidal CO_2; P_{ETCO_2}, Pressure of end-tidal O_2. *$P \leq 0.05$ (different from controls at baseline). † $P \leq 0.05$ (different between groups).

Table 11. Cerebro- and cardio-vascular responses to submaximal exercise in COPD and controls.

	EXERCISE 1					EXERCISE 2				
	COPD		Control			COPD		Control		
	BL1	EX1	BL1	EX1	*P*	BL2	EX2	BL2	EX2	*P*
Cerebrovascular										
$\bar{V}_P$, cm·s^{-1}	42.8 ± 10.4	52.4 ± 10.3	44.2 ± 13.6	50.2 ± 13.3	*	40.2 ± 12.1	51.1 ± 11.5	43.8 ± 12.5	48.7 ± 11.9	* †
%		25 ± 22		15 ± 13	*		31 ± 26		13 ± 10	* # †
CVC, cm·s^{-1}·mmHg^{-1}	0.46 ± 0.11	0.47 ± 0.09	0.49 ± 0.15	0.50 ± 0.15		0.45 ± 0.13	0.46 ± 0.90	0.47 ± 0.14	0.49 ± 0.15	
%		5 ± 15		3 ± 8			9 ± 27		4 ± 8	
Cardiovascular										
HR, bpm	67 ± 7	93 ± 11	66 ± 8	90 ± 14	*	69 ± 8	101 ± 10	71 ± 12	92 ± 15	* †
MBP, mmHg	94.5 ± 8.7	113.7 ± 18.1	92.0 ± 13.2	101.8 ± 12.7	* †	95.4 ± 9.6	112.9 ± 27.0	94.5 ± 15.2	103.7 ± 24.2	*
%		20 ± 13		11 ± 8	*		18 ± 22		9 ± 10	*
Sa_{O_2}, %	94 ± 2	91 ± 4	95 ± 1	94 ± 2	* # †	95 ± 1	91 ± 4	96 ± 1	95 ± 1	* # †

***Abbreviations*:** $\bar{V}_P$; Peak cerebral blood flow velocity; CVC, cerebrovascular conductance (CVC = $\bar{V}_P$/MBP); MBP, Mean arterial blood pressure; HR, Heart rate; Sa_{O_2}, Arterial oxygen saturation; * $P \leq 0.05$ (main effect of e*xercise*); # $P \leq 0.05$ (main effect of g*rouping*); † $P \leq 0.05$ (e*xercise* x g*rouping* interaction).

Chapter Four: **Increased ventilatory response to hyperoxic-hypercapnia in COPD patients following vitamin C administration**

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Preface

Previous reports have found antioxidants to improve vascular function, and additionally augment the ventilatory response to CO_2 in healthy adults. The aims of the present study were to test the hypothesis that vitamin C would increase the cerebrovascular reactivity and ventilatory response in COPD patients. This study assesses potential mechanisms relating to both the cerebrovascular and ventilatory responses in COPD patients.

Declaration

The original manuscript was written by myself with guidance from Drs. Anderson, Leigh and Poulin. I conceived the study design with guidance from Drs. Anderson, Leigh and Poulin. I performed all data collection and analyses in the Laboratory for Human Cerebrovascular Physiology. Dr. Kissel performed arterial blood gas procedures. I recruited the majority of participants, and coordinated study visits. I submitted and received institutional ethical approval, and through Health Canada's Research Ethics Board approval.

4.1 Abstract

Background: COPD patients have decreased ventilatory and cerebrovascular responses to hypercapnia. Antioxidants have been found to increase the ventilatory response to hypercapnia in healthy adults. Cerebral blood flow is an important determinant of $[H^+]$ at the central chemoreceptors, and may be affected by antioxidants. It is unknown if antioxidants can improve the ventilatory and cerebral blood flow response in individuals in whom these responses are diminished. Thus, the aim of this study was to determine the effect of a vitamin C intervention on the ventilatory and cerebrovascular responses to hypercapnia in healthy older adults, and in COPD patients.

Methods: We measured the ventilatory and cerebral blood flow responses, using transcranial Doppler ultrasound, to hyperoxic-hypercapnia before and after an intravenous vitamin C infusion in healthy *Young* (n=12) and *Older* (n=15) subjects, as well as in patients with moderate smoking-related COPD (n=11).

Results: Vitamin C increased the ventilatory response in COPD patients (1.1±0.4 *vs*. 1.5 ± 0.7 $L \cdot min^{-1}$ per mmHg [mean ± SD]; $P<0.01$), but not in *Young* (2.5±1.0 *vs*. 2.4±0.80; $P>0.05$) or *Older* (1.3±0.7 *vs*. 1.3±0.7; $P>0.05$) healthy subjects. Vitamin C did not affect the cerebral blood flow response in the *Young* (2.9±1.2 *vs*. 2.8±1.2 $cm \cdot s^{-1}$ per mmHg), *Older* (2.2±1.0 *vs*. 2.3±1.1 $cm \cdot s^{-1}$ per mmHg), or COPD (2.0±1.0 vs. 2.1±0.7 $cm \cdot s^{-1}$ per mmHg) ($P>0.05$) cohorts.

Conclusions: Vitamin C increases the ventilatory, but not cerebrovascular, response to hyperoxic-hypercapnia in patients with moderate, smoking-related COPD.

4.2 Introduction

Adequate pulmonary ventilation is essential to effectively regulate CO_2/H^+ and regulate pH within normal physiological balance. An increase in arterial PCO_2 stimulates the central chemoreflex loop, which leads to an increase in pulmonary ventilation. This homeostatic mechanism relies on the integration of several processes, including the sensors (central chemoreceptors) located within the brainstem and the effector-pathways to the respiratory muscles. While this robust mechanism remains intact in health, aberrations may arise in the presence of respiratory disease, where the chemoreflex loop may be compromised. Chronic obstructive pulmonary disease (COPD) is known to involve many respiratory disturbances, including chemical control, and mechanical impairment. Studies have shown lower ventilatory responses to CO_2 in COPD patients (20, 21). While pulmonary constraints alone (126), as observed in COPD, lower the ventilatory response to CO_2, chemosensitvity and respiratory weakness have been suggested to play a role. An initial study from Zakynthinos and colleagues (13) found that oral antioxidant supplementation increased the ventilatory sensitivity to CO_2 in healthy adults, suggesting a role for oxidative stress in regulating the sensitivity of the central chemoreceptors. Furthermore, the load (of CO_2) to the central chemoreceptors is largely determined by cerebral blood flow (CBF), due to the proximity of the cerebral vessels to the chemosensitive areas in the brainstem medulla.

The oxidant scavenging properties of the antioxidant, vitamin C, have been recognized for decades (127). Most recently, an intervention of vitamin C was shown to reduce oxidative stress, and restore vascular endothelial function in COPD patients (12). It is presently unknown if this same vascular benefit evident in the peripheral vessels is

applicable to the cerebral circulation. We and others have previously shown decreased cerebrovascular responsiveness to CO_2 in COPD patients (28, 37), and on this basis, it may thus be possible for vitamin C supplementation to improve this response.

The purpose of this study was therefore to evaluate the effect of a vitamin C intervention on the ventilatory and cerebrovascular responses to hyperoxic-hypercapnia in patients with established, smoking-related COPD of at least moderate severity, compared to a healthy older control group (*Older*). Furthermore, we sought to study the effect of vitamin C on these responses in a healthy young (*Young*) cohort to establish the effect of aging which, to date, remains unknown. We hypothesized that a single infusion of vitamin C would have the greatest effect in increasing the ventilatory response in COPD patients. Furthermore, we postulated that a blunted cerebrovascular response to hypercapnia in patients with COPD would be normalized following a single vitamin C infusion.

4.3 Methods

4.3.1 Subjects

Healthy volunteers between 20 and 80 years were recruited from the community, and patients with established, smoking related COPD of at least moderate severity (FEV_1 <80% of predicted value and FEV_1/FVC <0.7) were recruited from outpatient clinics in Calgary between April 2013-June 2014. Participants were asked to refrain from the use of short- and long- acting bronchodilators (24 hours), blood pressure medication (24 hours), and any combination of vitamin-antioxidant supplementation (72 hours) before laboratory sessions.

4.3.2 Measurements and procedures

Participants in the COPD and *Older* groups completed a pulmonary function test. All individuals completed a hypercapnic test before and after vitamin C administration. Tests were separated by a 45-minute wash-out period. Normal saline was infused in the first experiment as a non-active control substance, and vitamin C was infused in the second test. Complete methodological details are described in the *Online supplement (page 96).*

4.3.2.1 Measurement of vascular variables

Peak cerebral blood flow velocity ($\bar{V}_P$) in the middle cerebral artery was continuously measured using transcranial Doppler Ultrasound, as previously described.(64, 101) Heart rate, beat-beat blood pressure, and finger pulse oximetry, was measured continuously.

4.3.2.2 Hyperoxic-hypercapnia

Testing was conducted in the supine position, while breathing through a face mask. The pressure of end-tidal PO_2 ($P_{ET}O_2$) and CO_2 ($P_{ET}CO_2$) was controlled using the technique of dynamic end-tidal forcing (64), and dedicated software (BreatheM v2.38, University Laboratory of Physiology, Oxford, UK). The protocol progressed as follows: 5-minutes of baseline isocapnic euoxia ($P_{ET}CO_2$ = +1.5 mmHg above rest and $P_{ET}O_2$ held at 88.0 mmHg), 2-minutes of isocapnic-hyperoxia ($P_{ET}O_2$ = 300 mmHg), and 5-minutes of hyperoxic hypercapnia ($P_{ET}O_2$ = 300 mmHg and $P_{ET}CO_2$ = +8 mmHg). Expired PO_2 and PCO_2 were sampled continuously (100Hz) using mass spectrometry (AMIS 2000; Innovision, Odense, Denmark) via a fine catheter. Respiratory volumes were measured with a turbine and volume transducer (VMM-400; Interface Associates, Laguna Niguel, CA), and respiratory flow direction and timing were obtained with a pneumotachograph (RSS100-HR, Hans Rudolf, Kansas City, MO, USA). Breath-by-breath oscillations of the inspired partial pressures of O_2, CO_2, and N_2 were controlled via a fast gas mixing system for precise accuracy and stability of the desired end-tidal values.

4.3.2.3 Vitamin C administration

A 3g loading dose (200mg/min; 75ml) of vitamin C (Ascorbic acid injection, Alveda Pharma, Canada) was administered intravenously over 15-minutes, followed by a continuous maintenance dose for 24 minutes (40mg/min; 13ml) during the experiment. This dosage of vitamin C has previously been shown to be effective at acutely increasing plasma ascorbic acid concentrations more than 15 fold (128). Isotonic normal saline (0.9% NaCl) was infused at an identical flow-rate to ascorbic acid.

4.3.2.4 Arterial blood gas analysis

In a subset of participants (N=13), a catheter was inserted into the radial artery to obtain multiple blood gas measurements. Arterial blood samples were drawn into a pre-heparinized syringe (BD Preset) and sampled at the end of each stage. Blood was processed immediately (ABL800 FLEX, Radiometer, Copenhagen, Denmark).

4.3.3 Data analysis

The response slopes (*i.e.*, sensitivities) to hypercapnia were determined by linear regression using the last minute of isocapnic-hyperoxia, and hyperoxic-hypercapnia, and relating the change in the dependent variable to the change in $P_{ET}CO_2$. Mean blood pressure (MBP) was calculated as:

$$MBP = (1/3) \times SBP + (2/3) \times DBP$$

where SBP represents systolic blood pressure, and DBP represents diastolic blood pressure. Cerebrovascular conductance (CVC) was calculated as:

$$CVC = \bar{V}_P / MBP.$$

To assess the demographic and physiological differences between groups at baseline, data were assessed by comparing 1) "*Young vs. Older*", and 2) "*Older vs.* COPD" using independent t-tests. Main effects and interactions were determined using a mixed design 2x2 repeated measurers ANOVA [time x grouping] (SPSS Version 20.0, SPSS Inc.,

Chicago). Dependent t-tests (within group) and independent t-tests (between-group) were applied if a significant *F* ratio was detected. The Bonferroni post-hoc correction factor was used to determine significance in the case of multiple comparisons. All data are presented as mean ± SD, and significance is determined at α-level ≤ 0.05.

4.4 Results

4.4.1 Study participants

Thirty-eight subjects (*Young* = 12, *Older* = 15, COPD = 11) completed the study. One COPD patient could not complete the experiment due to fatigue and breathlessness, and two *Older* control subjects had spirometric evidence of mild airflow obstruction (FEV_1/FVC <0.70) and were therefore excluded from the study. Cerebrovascular data for one *Older* control were excluded due to a lack of a suitable MCA signal. All subjects received the intended dose of vitamin C, without adverse effects, and all data were included. Physical characteristics and pulmonary function results are shown in Table 12 (*page 105*).

4.4.2 Effect of vitamin C at rest

Ventilatory variables were not affected by vitamin C at rest in either *Young*, *Older*, or COPD (Table 13, *page 106*). *Young* individuals had higher $\bar{V}_P$ and CVC, and lower MBP than *Older* (Table 14, *page 107*). COPD patients had similar $\bar{V}_P$, CVC, MBP, but higher heart rate, compared to *Older* before and after vitamin C. COPD patients had higher diastolic blood pressure only after vitamin C infusion (Table 14, *page 107*). Results of the

air breathing data and the arterial blood gas analyses are included in the O*nline supplement* (Table 15, *page 108;* and Table 16, *page 109;* respectively).

4.4.3 Effect of vitamin C during hyperoxic-hypercapnia

4.4.3.1 Ventilatory variables

$\dot{V}_E$ sensitivity was significantly greater in *Young* compared to *Older*, but was not altered by vitamin C (Figure 5, *page 102*). COPD patients did not have a significantly different $\dot{V}_E$ sensitivity from *Older* during the saline control. Following vitamin C, $\dot{V}_E$ sensitivity significantly increased in COPD patients only ($P<0.05$) (Figure 5, *page 102;* and Figure 7, *page 104*). Contrary to what was observed in *Older*, inspiratory flow and breathing frequency were increased in COPD following vitamin C (Table 13, *page 106*).

4.4.3.2 Cerebro- and cardiovascular variables

Absolute $\bar{V}_P$ and CVC were greater in the *Young* compared to *Older*, during hypercapnia (Table 14, *page 107*). However, the $\bar{V}_P$ sensitivity was not different between *Young* and *Older*, under saline conditions (Figure 5, *page 102*). Vitamin C did not have an effect on $\bar{V}_P$ sensitivity in the *Young* or *Older* groups (Figure 5, *page 102*). $\bar{V}_P$ sensitivity did not differ between COPD and the *Older* cohort, and were not different following vitamin C administration (Figure 5, *page 102;* and Figure 7, *page 104*).

4.4.3.3 Disease severity and response to vitamin C infusion

There was a significant negative correlation between FEV_1 (% predicted) and the change in $\dot{V}_E$ sensitivity observed with vitamin C ($r = -0.53$; $P \leq 0.001$), indicating that

individuals with lower FEV_1 (% predicted) had the greatest increase in $\dot{V}_E$ sensitivity after vitamin C (Figure 6, *page 103*). We did not observe any relationships between the change in $\bar{V}_P$ sensitivity and FEV_1 following vitamin C (Figure 6, *page 103*). No relationship were was observed between $\bar{V}_P$ and $\dot{V}_E$ sensitivity.

4.5 Discussion

This is the first study to investigate the role of antioxidants in the regulation of cerebral blood flow and the chemical control of breathing in healthy individuals and those with moderate, smoking-related COPD. We found that an acute, intravenous vitamin C intervention increase the ventilatory sensitivity to hyperoxic-hypercapnia in COPD patients, but not in healthy *Young* and *Older* subjects. The increase in ventilation was achieved by both an increase in breathing frequency and inspiratory drive. The mechanisms for this observation remain unclear, although they appear to be unrelated to differences in the regulation of cerebral blood flow.

4.5.1 Ventilatory response to hyperoxic-hypercapnia: Effect of vitamin C

Perhaps the most striking finding of our study is that vitamin C increased the ventilatory sensitivity to CO_2 in patients with established, smoking-related COPD of at least moderate severity, and that the greatest improvement were observed in the more severe patients. The ventilatory response to hypercapnia represents the functionality of the *entire* chemoreflex arc, including chemosensitivity, neuromechanical coupling, and respiratory muscle function. In agreement with previous reports (129-131) citing a ~25-50% decrease in the hypercapnic ventilatory response, we also found a decreased response with aging. Contrary to previous findings (13), antioxidant administration did not increase the ventilatory sensitivity to CO_2 in either the *Young* or *Older* groups. Zakynthinos and colleagues (13) found that an antioxidant cocktail improved the ventilatory response to CO_2 by nearly two-fold in healthy adults. One possible explanation for the discrepancy is the difference in antioxidants used, as well as the administration protocol. Our rationale for using vitamin C specifically as an intervention was based on numerous studies which

have shown the ability of vitamin C to acutely decrease systemic oxidative stress and improve vascular function due its free radical scavenging properties (128, 132, 133). While these authors speculated that oxidative stress is involved in the central chemosensitivity to CO_2 sensing, we did not find any evidence for this. Conceivably, the *Young* and *Older* individuals were not ventilatory limited during the hypercapnic challenge, and we would have expected an antioxidant mediated increase in ventilatory sensitivity of the chemoreceptors to be reflected by increased ventilation. NO is suggested to be involved in the control of breathing during hypercapnia. Specifically, neuronal NOS (nNOS) has been implicated in the neuromodulation within the ventral surface of the medulla (134). We would expect vitamin C to increase the bioavailability of NO (by reduction of superoxide), thereby enhancing the neuromodulation properties of NO within the CNS. However, the specific contribution of NOS in the ventilatory response to hypercapnia remains undetermined. In two separate studies, Kline and colleagues (135, 136) did not find any difference in the ventilatory responses to CO_2 between wild-type and mutant mice (deficient in either eNOS or nNOS), suggesting that NOS has a limited role in the regulation of the ventilatory response to hyperoxic-hypercapnia. Conversely, Teppema and colleagues (137) found a reduced central and peripheral CO_2 sensitivity following systemic NOS-blockade in cats (N-nitro-L-arginine).

Theoretically, the increase in the ventilatory response following vitamin C infusion could be a result of either 1) increased sensitivity and neural output of the central chemoreceptor via redox signaling, and/or 2) improvement of the respiratory muscles function. As we did not see an increase in the ventilatory sensitivity in either the *Young* or *Older* groups, we hesitate in concluding a direct effect of vitamin C on the central chemoreceptors. An alternate explanation involves increased activity of the respiratory muscles, either by an increase in blood flow or contractility. Free radicals have been

implicated in the development of diaphragmatic fatigue, resulting from strenuous breathing. Furthermore, free radical scavengers have been found to reduce the rate of development of fatigue of the diaphragm (138).

4.5.2 CBF response to hyperoxic-hypercapnia: effect of vitamin C

Arterial P_{CO_2} is an important regulator of the cerebral circulation. The dilatory response of the cerebral vasculature in response to increases in Pa_{CO_2} is termed "cerebrovascular reactivity", and is often used as an index of cerebrovascular health (139). The cerebrovascular response to CO_2 depends on the availability of endothelial NO (140). Alterations in cerebrovascular regulation have been linked to ROS-related endothelial dysfunction (141), and/or arterial stiffness (142). In our cohort of older healthy volunteers, resting CBF was reduced by ~25% compared to the *Young* cohort. However, the cerebrovascular responsiveness to CO_2 was not different with aging, nor in COPD. We were also surprised that mean arterial blood pressure increased ~5% in COPD patients (Table 15, *page 108*). This was not found in either *Young*, or *Older* volunteers. Conceivably, we would expect an improvement in vascular endothelial function with vitamin C, and thus a subsequent decrease in vascular resistance. It is possible that the intervention also affected the central cardiovascular function, and increased sympathetic outflow. Following vitamin C administration, COPD patients had an increase in heart rate, which may suggest increased cardiac output, contributing to the observed increase in MBP.

While we have previously found women with moderate COPD to have increased systemic oxidative stress and reduced responsiveness to euoxic-hypercapnia (28), it is not entirely clear why results of the current study are contrary to this. However, several possibilities exist such as a difference in protocols (euoxic- *vs*. hyperoxic-hypercapnia),

anatomical position (supine *vs.* upright), and sex differences. It is clear that heterogeneity exists between studies, protocols, and disease severity, as others have also reported either a decreased (57), or normal (39) cerebrovascular response to CO_2 in COPD patients.

Previous studies have found cerebrovascular reactivity to be restored in patients with elevated cardiovascular risk, secondary to increased NO availability (143, 144). Our study reveals that vitamin C does not increase basal CBF, or cerebrovascular sensitivity to CO_2. The precise mechanism underlying these findings is not clear at this time. Firstly, it is possible that the brain is well protected against oxidative stress, as the brain has one of the greatest concentrations of vitamin C in the body. It also remains possible that the brain has alternate pathways for vasodilation, and in a state where NO is compromised, other pathways (e.g., prostaglandins) predominate. This is supported by previous findings in our laboratory (145) where NOS inhibition did not alter the cerebrovascular response to hypercapnia in healthy humans. Lastly, it is also possible that vitamin C does not adequately cross the blood brain barrier, with the preferred method of transport being in the oxidized form, dehydroascorbic acid (146).

4.5.3 Methodological considerations

Because plasma concentrations of vitamin C remains elevated for several hours following i.v. infusion (147), we were unable to perform a randomized-control trial of the active and control interventions within the same day. Therefore, the order of trials was always saline, followed by vitamin C. Although subjects were not blinded to the intervention, the specific study objects were not revealed. To reduce day-day variability of physiological variables, and optimize volunteer retention, the study was completed on the same day. Carry-over effects were ruled out by adding a "time-control" experiment

(n=4) (*see Online Data Supplement*). Secondly, although the plasma concentration of vitamin C is lower in COPD patients than *Controls*, and is improved with vitamin C intervention (12), we do not have a measurement of these variables, nor do we have an indication of the antioxidant/oxidative stress balance within the brain. Thirdly, end-tidal PCO_2 values were used as an estimate of arterial PCO_2; the accuracy of end-tidal measurements decreases with aging (and lung disease), as the physiological deadspace increases, resulting in a widening of the $P_{ET}CO_2$–Pa_{CO_2} difference, and an underestimation of $P_{ET}CO_2$. In a subset of volunteers we validated the end-tidal measurements against arterial measurements, and found that during hypercapnia, $P_{ET}CO_2$ was lower than Pa_{CO_2} in the COPD and *Older* cohorts, but not in *Young*. This has the greatest impact on between-group comparisons, but does not affect the interpretation of the effect of antioxidants on the physiological outcomes.

Lastly, transcranial Doppler ultrasound is the most common technique used in humans to measure CBF velocity in the middle cerebral artery due to its non-invasive properties and high temporal resolution. It is believed that during moderate hypercapnia, the diameter of the MCA does not change, allowing to infer that relative changes in CBF velocity represent similar changes in global CBF (125, 148, 149).

4.5.4 Clinical implications

The role of oxidative stress in COPD, and its relationship to cardiovascular disease has received significant attention recently (12). While we have previously suggested a link between oxidative stress and cerebrovascular function in COPD (28), we were unable to modify the cerebrovascular response with an antioxidant intervention in this study. However, our present results do show an involvement of a high-dose antioxidant

intervention in regulating the central ventilatory response to CO_2 in COPD. This is an important finding, as a lower ventilatory sensitivity experienced in more advanced stages of COPD can lead to more severe disturbances in acid-base regulation. While our findings implicate a positive role of antioxidants on the ventilatory response, these findings need to be corroborated with future studies that use a more practical dosing regimen that would be available to patients, such as large oral doses.

In conclusion, we found that intravenous vitamin C administration in COPD patients increased the ventilatory, but not cerebrovascular sensitivity to hyperoxic-hypercapnia. We did not find any influence of vitamin C administration on ventilatory or cerebrovascular sensitivity in healthy *Young* or *Older* healthy volunteers.

4.6 Acknowledgements

The authors are especially grateful for the volunteers that participated in the study. We also thank Drs. Angela Kealey and Anna Bizios for their clinical support for placement of arterial catheters, Mr. Curtis Dumonceaux for pulmonary function testing, Ms. Linda Knox and Dr. Jean Rawling for their help with medical coverage and patient recruitment, and Mr. Brad Hansen for technical support.

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4.7 Online Data Supplement

Detailed methodology

Subjects were instructed to fast for 4 hours prior to testing. All participants completed the main experimental session in the Laboratory of Human Cerebrovascular Physiology, University of Calgary

Inclusion/exclusion criteria

Participants were excluded from participating in the study if they met any of the following criteria: current smoker, BMI > 35 $kg \cdot m^{-2}$, pre-menopausal status (excluding *Young*), heart/chest pain upon physical exertion, surgery or trauma within previous 6 months, history of myocardial infarction, angina, arrhythmia, valve disease, chronic heart failure, additional lung disease other than smoking-related COPD, history of stroke, diabetes, cardiovascular or cerebrovascular disease, history of chronic headaches/migraines, history of blood clots/thrombosis, uncontrolled hypertension, domiciliary oxygen therapy, or a recent COPD exacerbation.

Pulmonary Function

Spirometry (pre- and post- bronchodilator), lung volume measurements and single-breath diffusing capacity (D_{LCO}) were completed by a trained respiratory therapist, as per ATS guidelines (61-63).

Vitamin C administration

An intravenous catheter was inserted in a vein near the antecubital fossa for drug infusion. Vitamin C (Ascorbic acid injection, Alveda Pharma, Canada) was diluted in normal saline, and administered intravenously using the following dosing regimen; before the start of the experiment, a loading dose of 3g ascorbic acid (200mg/min) was administered over 15-minutes, followed by a continuous maintenance dose (40mg/min) during the experiment. This dosage of vitamin C has previously been shown to be effective at acutely increasing plasma ascorbic acid concentrations more than 15 fold (128). Isotonic normal saline (0.9% NaCl) was infused at an identical flow-rate to ascorbic acid. The total volume infused (Trial 1 + Trial 2) was 176 ml.

Time control group

An additional group (n=4) was included to rule out the "time" effect of repeated (non-randomized) study interventions within the same day. These subjects completed an identical protocol (Trial 1 and Trial 2, separated by 45 minutes) below, without vitamin C infusion, to evaluate the effect of repeated tests on physiological variables. The test-retest reliability coefficient (*i.e.*, intervention 1 *vs.* intervention 2) for $\dot{V}_E$ and $\bar{V}_P$ sensitivity was r = 0.90 and r = 0.76 for $\dot{V}_E$ and $\bar{V}_P$ sensitivity, respectively. We did not observe any significant differences between interventions. See APPENDIX B (*page 195*) for expected repeatability for the hypercapnic responses, based on previously published and work-in-progress from the Laboratory of Human Cerebrovascular Physiology.

Measurement of middle cerebral artery blood flow velocity

Peak cerebral blood flow velocity ($\bar{V}_P$) in the middle cerebral artery was measured continuously using a 2-MHz pulsed Doppler Ultrasound system (TOC2M, Multigon Industries, Inc., Yonkers, NY), as previously described (64, 101). $\bar{V}_P$ was used as a surrogate for cerebral blood flow. The power ($\bar{P}$) signal acquired from the Doppler system is used as an indicator for changes in vessel cross-sectional area. Therefore, without a change in $\bar{P}$, $\bar{V}_P$ is considered to be a reliable index of flow.

Measurement of cardiovascular variables

Heart rate was determined from the R-R interval using a 3-lead electrocardiogram (Micromon 7142B monitor; Kontron Keynes, UK) which was continuously monitored and data collected at 1000 Hz. Beat-beat blood pressure was monitored continuously, and data collected at 100 Hz using finger pulse photoplethysmography (Portapress; TPD Biodemical Instrumentation, Amsterdam, The Netherlands). Finger pulse oximetry was used to monitor arterial oxygen saturation (Model 3900; Datex-Ohmeda, Louisville, CO) and data collected at 100 Hz.

Hyperoxic-hypercapnia protocol

Resting end-tidal partial pressures of O_2 ($P_{ET_{O_2}}$) and CO_2 ($P_{ET_{CO_2}}$) were measured with subjects in a supine position, breathing room air through a face mask which allowed for nose and/or mouth breathing. Expired PO_2 and PCO_2 was sampled continuously (100Hz) using mass spectrometry (AMIS 2000; Innovision, Odense, Denmark) via a fine catheter, and was collected using dedicated software (Chamber v2.26; University of Oxford Laboratory of Physiology, Oxford, UK) over a period of 10 minutes to collect baseline

values. Following a 5 minute lead-in period of isocapnic euoxia ($P_{ET}CO_2$ = +1.5 mmHg above resting values and $P_{ET}O_2$ held constant at 88.0 mmHg), the protocol progressed with 2-minute stage of isocapnic-hyperoxia ($P_{ET}O_2$ = 300 mmHg), followed by a 5 minute period of hyperoxic hypercapnia ($P_{ET}O_2$ = 300 mmHg and $P_{ET}CO_2$ = +8 mmHg). The technique of dynamic end-tidal forcing (64), was used to control the desired $P_{ET}CO_2$ and $P_{ET}O_2$ values (BreatheM v2.38, University Laboratory of Physiology, Oxford, UK). Respiratory volumes were measured with a turbine and volume transducer (VMM-400; Interface Associates, Laguna Niguel, CA), and respiratory flow direction and timing were obtained with a pneumotachograph (RSS100-HR, Hans Rudolf, Kansas City, MO, USA). Breath-by-breath oscillations of the inspired partial pressures of O_2, CO_2, and N_2 were controlled via a fast gas mixing system for precise accuracy and stability of the desired end-tidal values.

Arterial blood gas analysis

After an Allen's test was performed, the skin at the wrist above the radial artery was infiltrated with Xylocaine 1% for local anesthesia. A 3F radial catheter (Cook Medical) was inserted into the radial artery via standard Seldinger technique in sterile fashion. A pressurized bag with heparinized saline (1000 IU heparin /500ml 0.9% sodium chloride) was used to keep the arterial line open in between blood gas sampling. The arterial line was removed after completion of the hyperoxic-hypercapnic protocol and manual pressure was held for 5 minutes.

We include only a subset of individuals for the arterial blood gas analysis due to the invasiveness of the procedure, and logistics involved. Participants were selected based on

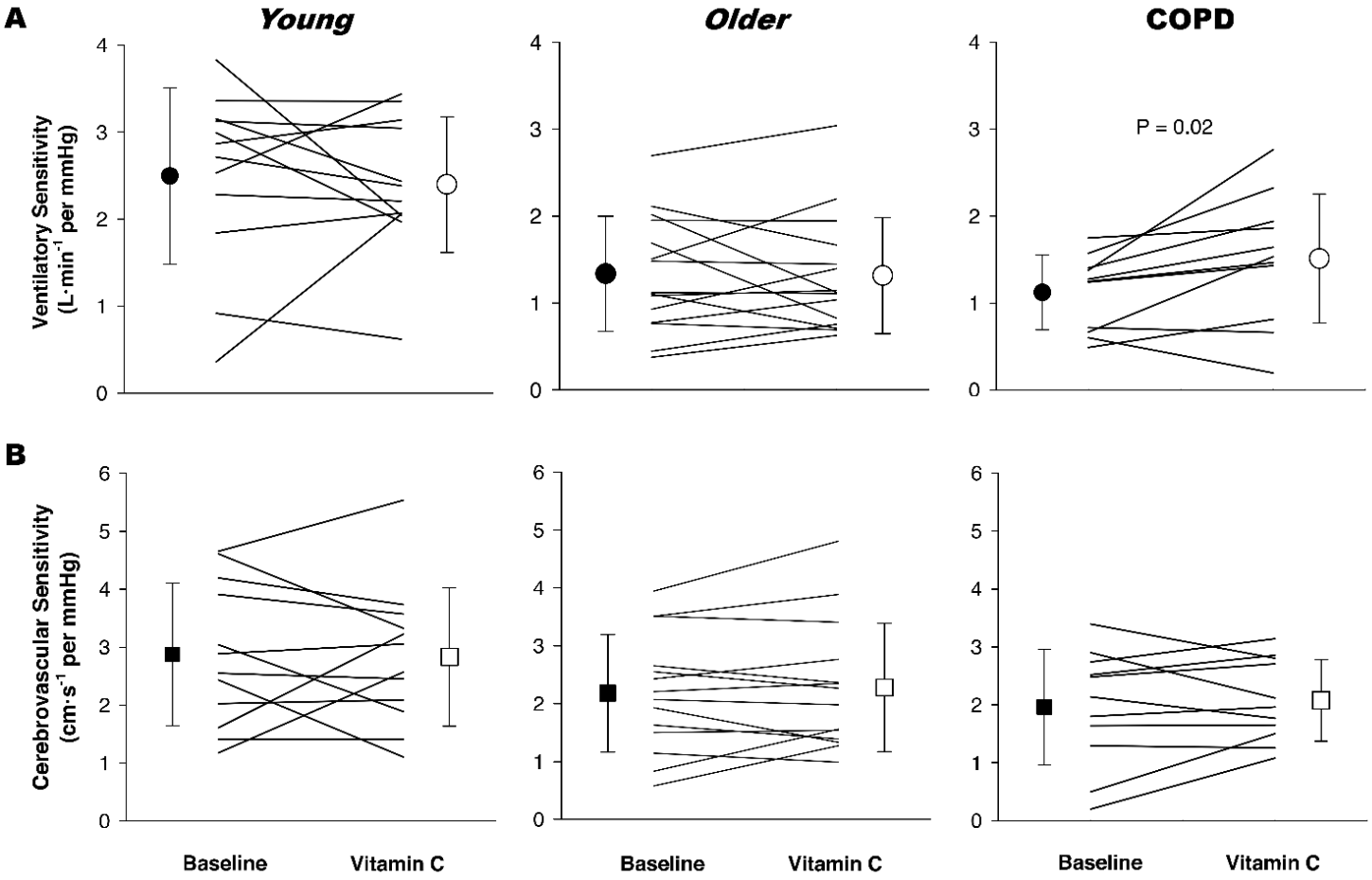
the availability of medical personnel, and their willingness to undergo this additional procedure.

Data analysis

To identify differences in the ventilatory responses, an a-priori power calculation was conducted based on previous data (13) in young adults. To detect an increase of 1.5 $L \cdot min^{-1} \cdot mmHg^{-1}$ in the ventilatory response to CO_2 following an antioxidant intervention, with a power of 0.95 for a one-tailed, paired t-test, a sample size of 3 was required to achieve a power of 0.95. We prospectively increased this sample size to accommodate our co-primary outcome of cerebral blood flow. Increases in peripheral blood flow of 34% have been observed with vitamin C in older adults (133). Using these data, it is anticipated that 18 subjects (9 COPD patients, 9 controls) were required in order to provide a power of 80%, using two-sided tests with $\alpha = 0.05$.

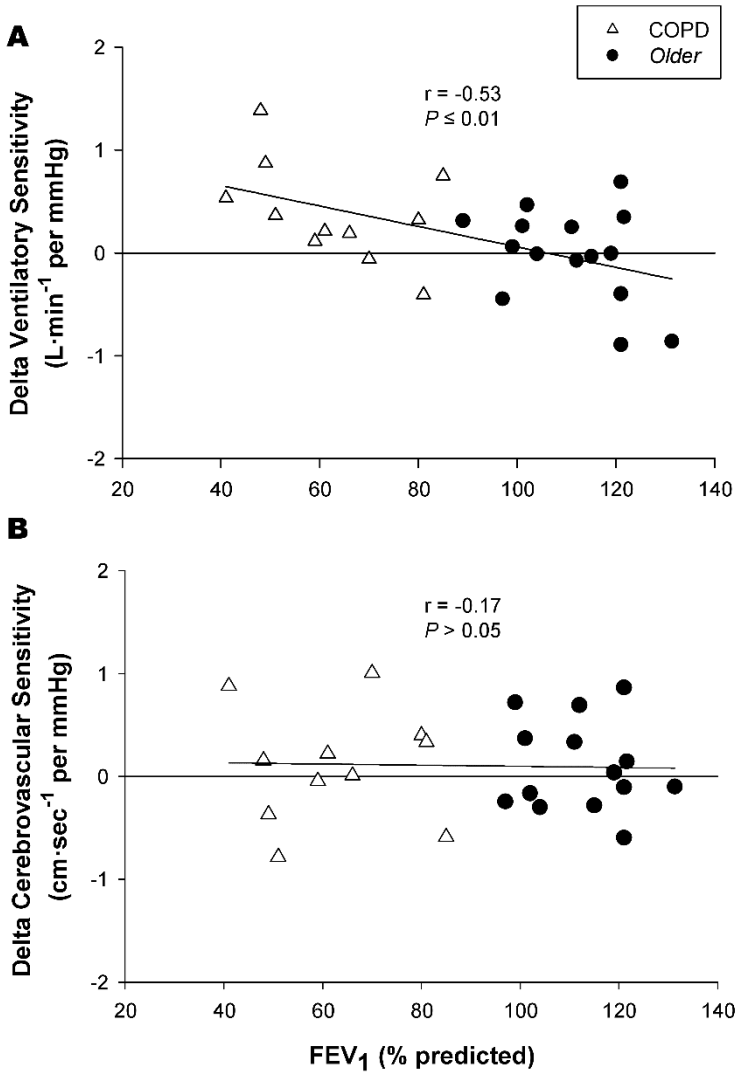
4.8 Chapter Four Figures and Tables

Figure 5. Ventilatory and cerebrovascular responses to hyperoxic-hypercapnia.



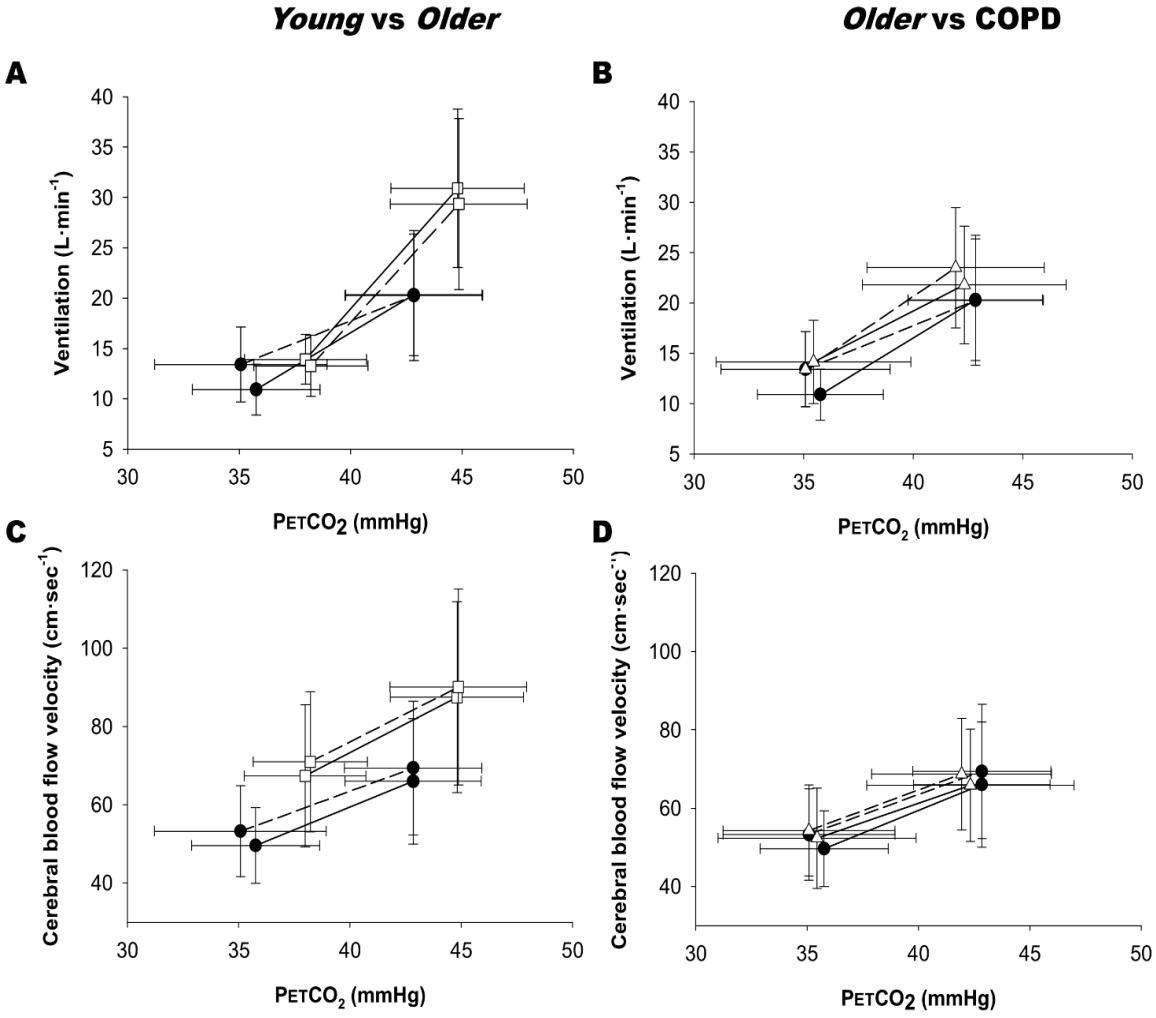
Footnote: Individual (*solid lines*) and mean values (*symbols*) for the (A) ventilatory (*circles*) and (B) cerebrovascular (*square*) sensitivity to hyperoxic-hypercapnia in *Young*, *Older*, and COPD patients, during baseline control (*closed symbol*) and vitamin C (*open symbol*). Note the increased ventilatory response in COPD patients following vitamin C infusion ($P \leq 0.05$). *Symbols* represent group means ± SD.

Figure 6. Relationship between the change in ventilatory and cerebrovascular sensitivity to hyperoxic-hypercapnia following vitamin C in COPD patients and *Older* controls.



Footnote: Pearson correlation between the change in the (A) ventilatory sensitivity and (B) cerebrovascular sensitivity in *Older* (●) and COPD patients (Δ), according to lung function. Note the significant relationship between increasing disease severity (FEV_1, %), and a larger change in ventilatory, but not cerebrovascular sensitivity, after vitamin C infusion.

Figure 7. Ventilatory and cerebrovascular reactivity to acute hyperoxic-hypercapnia in *Young*, *Older*, and COPD patients, before and after vitamin C infusion.



Footnote: The ventilatory and cerebrovascular responses to hyperoxic–hypercapnia in (A,C) *Young* (□) and *Older* (●) and (B,D) *Older* and *COPD* (Δ) before (*solid line*) and after vitamin C infusion (*dashed line*). The ventilatory sensitivity to CO_2 for only the COPD group was significantly greater following the vitamin C infusion ($P \leq 0.05$) (B). No significant changes were observed following vitamin C in the cerebrovascular response. *Values* represent mean data ± SD.

Table 12. Subject characteristics and pulmonary function data.

	Young	*Older*	COPD
Subjects (n)	12	15	11
Sex (M / F)	7 / 5	7 / 8	4 / 7
Age, yr	30.3 ± 5.5 *	68.3 ± 5.0	67.8 ± 8.1
Weight, kg	71.4 ± 15.2	75.8 ± 13.3	70.6 ± 16.6
Height, m	1.78 ± 0.05 *	1.70 ± 0.07	1.64 ± 0.07 *
BMI, $kg \cdot m^{-2}$	24.1 ± 2.6	26.1 ± 3.5	26.3 ± 5.6
Smoking history, pack yr	0*	16 ± 11	44 ± 17*
Pulmonary function			
(% predicted)			
FEV_1, L	-	2.98 ± 0.82 (109 ± 11)	1.52 ± 0.33* (63 ± 15)*
FEV_1 / FVC	-	0.78 ± 0.51 (104 ± 7)	0.49 ± 0.10* (65 ± 13)*
FRC, L	-	3.38 ± 0.65 (107 ± 15)	4.25 ± 1.04* (143 ± 24)*
RV, L	-	2.27 ± 0.36 (99 ± 15)	3.38 ± 0.83* (156 ± 36)*
TLC, L	-	6.18 ± 1.19 (102 ± 7)	6.35 ± 1.33 (116 ± 17)*
IC/TLC	-	0.45 ± 0.06 (95 ± 12)	0.33 ± 0.05* (73 ± 11)*
D_LCO, $mL \cdot min^{-1}/mmHg$	-	25.2 ± 7.6 (95 ± 17)	16.3 ± 6.7* (69 ± 28)*

Definition of abbreviations: BMI : body mass index; FEV_1 : forced expiratory volume in one second; FEV_1 / FVC : ratio between forced expiratory volume in one second to the forced vital capacity; FRC: functional residual capacity; RV: residual volume; TLC: total lung capacity; IC / TLC: ratio between inspiratory capacity and total lung volume; D_LCO: diffusion capacity of carbon monoxide. Values in *parenthesis* indicate percent predicted. Data presented as means ± SD. *$P \leq 0.05$ statistically different from *Older* group

Table 13. Ventilatory responses to euoxic-isocapnia and hyperoxic-hypercapnia in *Young*, *Older*, and COPD.

	Young n=12		*Older* n=15		COPD n=11	
	Control	**Vitamin C**	**Control**	**Vitamin C**	**Control**	**Vitamin C**
Euoxic-Isocapnia						
$P_{ET}CO_2$ (mmHg)	38.6 ± 3.0	38.8 ± 3.0	36.7 ± 3.0	36.9 ± 2.9	35.3 ± 5.2	35.1 ± 4.2
Sa_{O_2} (%)	97 ± 1	97 ± 1	96 ± 1	96 ± 1	94 ±1	94 ± 1
V_T/T_I ($L \cdot s^{-1}$)	0.57 ± 0.13*	0.58 ± 0.11*	0.46 ± 0.08	0.45 ± 0.13	0.66 ± 0.12*	0.67 ± 0.18*
T_I/T_{TOT} (%)	42 ± 3	43 ± 3	41 ± 3	42 ± 5	35 ± 3*	35 ± 3*
V_T ($L \cdot breath^{-1}$)	1.1 ± 0.3	1.0 ± 0.2	0.9 ± 0.4	1.0 ± 0.6	1.0 ± 0.3	0.9 ± 0.2
B*f* ($breath \cdot min^{-1}$)	15 ± 4	16 ± 3	14 ± 4	14 ± 4	16 ± 5	16 ± 3
$\dot{V}_E$ ($L \cdot min^{-1}$)	15.0 ± 3.3*	15.3 ± 3.2*	11.5 ± 2.2	12.0 ± 3.4	14.8 ± 4.6*	14.5 ± 4.4
Hyperoxic-Hypercapnia						
$P_{ET}CO_2$ (mmHg)	44.8 ± 3.0	44.9 ± 3.1	42.8 ± 4.0	42.8 ± 3.1	42.3 ± 4.7	41.9 ± 4.0
Sa_{O_2} (%)	99 ± 1	99 ± 1	99 ± 1	99 ± 1	99 ± 1	99 ± 1
V_T/T_I ($L \cdot s^{-1}$)	1.05 ± 0.27*	1.01 ± 0.27*	0.75 ± 0.23	0.74 ± 0.19	0.97 ± 0.26*	1.04 ± 0.26*†
T_I / T_{TOT} (%)	46 ± 4*	46 ± 4*	43 ± 3	43 ± 3	36 ± 3*	36 ± 3*
V_T ($L \cdot breath^{-1}$)	1.9 ± 0.4	1.8 ± 0.4	1.6 ± 0.7	1.6 ± 0.7	1.3 ± 0.3	1.3 ± 0.3
B*f* ($breath \cdot min^{-1}$)	17 ± 5	17 ± 5	14 ± 3	14 ± 4	17 ± 4*	19 ± 4*†
$\dot{V}_E$ ($L \cdot min^{-1}$)	30.9 ± 7.9*	29.4 ± 8.5*	20.3 ± 6.1	20.3 ± 6.5	21.8 ± 5.8	23.5 ± 6.0†

***Definition of abbreviations*:** $P_{ET}CO_2$: pressure of end-tidal CO_2; Sa_{O2}: oxygen saturation; V_T: expired tidal volume; B*f*: breathing frequency; V_{TI} / T_I : Mean inspiratory flow; T_I / T_{TOT}: Ratio of inspiratory time to total breath time; $\dot{V}_E$: minute ventilation. Data presented as means ± SD. *Different from *Older* under the same condition †Different from saline, within-group.

Table 14. Cardio- and cerebro-vascular responses to euoxic-isocapnia, and hyperoxic-hypercapnia in *Young*, *Older*, and COPD.

	Young **n=12**		***Older*** **n=14**		**COPD n=11**	
	Control	**Vitamin C**	**Control**	**Vitamin C**	**Control**	**Vitamin C**
Euoxic-Isocapnia						
$\bar{V}_P$ (cm·s^{-1})	67.0 ± 18.1*	69.1 ± 18.1*	50.9 ± 9.2	51.7 ± 9.9	50.1 ± 11.9	52.7 ± 11.2
CVC (cm·s^{-1}/ mmHg)	0.74 ± 0.21*	0.78 ± 0.24*	0.50 ± 0.13	0.51 ± 0.12	0.48 ± 0.12	0.48 ± 0.10
SBP (mmHg)	123 ± 8*	121 ± 9*	140 ± 17	142 ± 14	138 ± 11	144 ± 14
DBP (mmHg)	73 ± 7*	74 ± 7*	86 ± 10	83 ± 8	91 ± 8	95 ± 11*
MBP (mmHg)	90 ± 6*	90 ± 6*	104 ± 11	103 ± 8	107 ± 7	111 ± 11
HR (beat·min^{-1})	64 ± 9	64 ± 9	61 ± 8	63 ± 9	73 ± 11*	75 ± 12*
Hyperoxic-Hypercapnia						
$\bar{V}_P$ (cm·s^{-1})	87.5 ± 24.4*	90.1 ± 25.0*	65.5 ± 16.5	68.7 ± 17.3	65.8 ± 14.3	68.7 ± 14.3
CVC (cm·s^{-1}/ mmHg)	0.92 ± 0.24*	0.97 ± 0.30*	0.62 ± 0.18	0.63 ± 0.16	0.57 ± 0.12	0.57 ± 0.11
SBP (mmHg)	128 ± 10*	125 ± 11*	142 ± 15	150 ± 12	150 ± 14*	157 ± 16*
DBP (mmHg	77 ± 8*	78 ± 7*	91 ± 8	90 ± 5	98 ± 8*	102 ± 8*
MBP (mmHg)	94 ± 7*	94 ± 6*	108 ± 9	110 ± 4	115 ± 9	121 ± 9*
HR (beat·min^{-1})	67 ± 10	67 ± 10	63 ± 9	65 ± 12	74 ± 10*	76 ± 11*†

***Definition of abbreviations*:** $\bar{V}_P$: peak cerebral blood flow velocity; CVC: cerebrovascular conductance (CVC = $\bar{V}_P$ / MBP); MBP: mean arterial blood pressure; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; Data presented as means ± SD. *Different from *Older* under the same condition †Different from saline, within-group.

Table 15. Respiratory, cardio- and cerebro-vascular variables during air breathing, before and after vitamin C infusion.

	Young (n=12)		*Older* (n=15)		COPD (n=11)	
	Saline	**Vitamin C**	**Saline**	**Vitamin C**	**Saline**	**Vitamin C**
Respiratory						
$P_{ET_{CO_2}}$ (mmHg)	36.6 ± 2.7	36.1 ± 2.2	35.1 ± 2.8	35.1 ± 3.5	33.6 ± 4.1	33.1 ± 3.8
$P_{ET_{O_2}}$ (mmHg)	86.0 ± 4.7	87.7 ± 5.3	88.3 ± 5.1	89.7 ± 6.9	86.5 ± 9.3	89.7 ± 6.9
Sa_{O_2} (%)	96 ± 1	96 ± 1	95 ± 2	96 ± 1	93 ± 2*	94 ± 1*
V_T (L)	0.8 ± 0.2	0.8 ± 0.3	0.9 ± 0.4	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3
Bf (breath·min^{-1})	14 ± 3	15.4 ± 3.6†	12 ± 3	13.3 ± 3.2†	14 ± 4	14 ± 4
V_{TI} / T_I (L·s^{-1})	0.41 ± 0.07	0.44 ± 0.05	0.35 ± 0.07	0.40 ± 0.10†	0.49 ± 0.18*	0.50 ± 0.19
T_I/T_{TOT} (%)	42 ± 3	41 ± 4	40 ± 6	38 ± 4†	34 ± 4	35 ± 6
$\dot{V}_E$ (L·min^{-1})	10.3 ± 1.6*	11.0 ± 1.7	8.8 ± 1.7	9.4 ± 2.1	10.1 ± 3.3	11.0 ± 3.3†
Cardio- and cerebro- vascular						
HR, (beats·min^{-1})	64 ± 8	65 ± 9	61 ± 9	62 ± 9†	71 ± 11*	73 ± 13*
MBP (mmHg)	86 ± 8*	87 ± 6*	98 ± 9	99 ± 7	102 ± 11	107 ± 10*†
$\bar{V}_P$ (cm·s^{-1})	66 ± 16*	68 ± 19*	51 ± 10	52 ± 11	50 ± 10	52 ± 11
CVC (cm·s^{-1}/ mmHg)	0.77 ± 0.16*	0.79 ± 0.27*	0.52 ± 0.13	0.54 ± 0.12	0.50 ± 0.14	0.49 ± 0.11

***Definition of abbreviations*:** $P_{ET_{CO2}}$: end-tidal partial pressure of carbon dioxide; $P_{ET_{O2}}$: end-tidal partial pressure of oxygen; Sa_{O2}: oxygen saturation; V_T: expired tidal volume; B*f*: breathing frequency; V_{TI} / T_I : Mean inspiratory flow = Inspired tidal volume/Duration time of inspiration; T_I / T_{TOT}: Ratio of inspiratory time to total breath time; $\dot{V}_E$: minute ventilation; HR: heart rate; MBP: mean arterial blood pressure; $\bar{V}_P$: peak cerebral blood flow velocity; CVC: cerebrovascular conductance (CVC = $\bar{V}_P$ / MBP). Data presented as means ± SD. *Different from *Older* under the same condition †Different from saline, within-group.

Table 16. Arterial blood gas analysis during conditions of air, euoxic-isocapnia, and hyperoxic-hypercapnia in *Young*, *Older*, and COPD.

	AIR		
	***Young* (n=5)**	***Older* (n=4)**	**COPD (n=4)**
Pa_{O_2} (mmHg)	83.0 ± 5.0	74.5 ± 4.1	80.5 ± 10.9
$P_{ET_{O_2}}$ (mmHg)	84.0 ± 4.7	88.5 ± 4.8	93.8 ± 9.9
$Pa_{O_2} - P_{ET_{O_2}}$ (mmHg)	-1.0 ± 2.9	-14.0 ± 8.1	-13.3 ± 4.6
Pa_{CO_2} (mmHg)	34.0 ± 3.1	38.0 ± 2.7	37.2 ± 4.8
$P_{ET_{CO_2}}$ (mmHg)	35.0 ± 2.3	33.7 ± 3.1	30.6 ± 2.0
$Pa_{CO_2} - P_{ET_{CO_2}}$ (mmHg)	-1.0 ± 1.1	4.3 ± 2.3	3.1 ± 4.0
	EUOXIC-ISOCAPNIA		
	***Young* (n=5)**	***Older* (n=4)**	**COPD (n=4)**
Pa_{O_2} (mmHg)	89.5 ± 3.5	80.4 ± 6.2	76.8 ± 4.0
$P_{ET_{O_2}}$ (mmHg)	88.2 ± 0.4	87.1 ± 1.8	87.3 ± 1.1
$Pa_{O_2} - P_{ET_{O_2}}$ (mmHg)	1.3 ± 3.6	-6.7 ± 6.3	-10.5 ± 3.7
Pa_{CO_2} (mmHg)	35.0 ± 2.0	37.3 ± 2.7	38.1 ± 3.1
$P_{ET_{CO_2}}$ (mmHg)	36.4 ± 3.2	36.1 ± 1.7	32.8 ± 5.1
$Pa_{CO_2} - P_{ET_{CO_2}}$ (mmHg)	-1.4 ± 3.3	1.1 ± 2.3	3.7 ± 2.4
	HYPEROXIC-HYPERCAPNIA		
	***Young* (n=5)**	***Older* (n=4)**	**COPD (n=4)**
Pa_{O_2} (mmHg)	293.0 ± 8.5	271.0 ± 15.6	268.5 ± 15.3
$P_{ET_{O_2}}$ (mmHg)	300.4 ± 1.5	300.0 ± 0.9	300.1 ± 0.6
$Pa_{O_2} - P_{ET_{O_2}}$ (mmHg)	-7.4 ± 8.5	-29.0 ± 16.0	-31.6 ± 15.5
Pa_{CO_2} (mmHg)	41.1 ± 4.2	42.3 ± 1.9	41.4 ± 3.7
$P_{ET_{CO_2}}$ (mmHg)	42.9 ± 3.4	41.3 ± 2.0	38.9 ± 4.7
$Pa_{CO_2} - P_{ET_{CO_2}}$ (mmHg)	-1.8 ± 2.3	1.0 ± 1.2	2.5 ± 2.3

***Abbreviations*:** Pa_{O_2}: pressure of arterial O_2; $P_{ET_{O_2}}$: pressure of end-tidal O_2; Pa_{CO_2}: pressure of arterial CO_2; $P_{ET_{CO_2}}$: pressure of end-tidal CO_2. Values represent mean ± SD.

Chapter Five: **Cerebrovascular and ventilatory responses to acute isocapnic-hypoxia in healthy aging and chronic obstructive pulmonary disease: effect of vitamin C**

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Preface

This manuscript addresses the underlying mechanisms of the cerebrovascular and ventilatory responses to hypoxia in health and disease in older adults, which have previously not been investigated. We tested the hypothesis that vitamin C would augment the cerebrovascular and ventilatory responses to hypoxia. Additionally, this was tested in patients with COPD, a group of patients with known cerebrovascular and ventilatory disturbances whom may particularly benefit from an antioxidant intervention. It is important to recognize patho-physiological responses with aging, and test mechanisms that will contribute to our understanding of these responses.

Declaration

The original scientific manuscript contained in this chapter was written by myself with guidance from my supervisor, Dr. Poulin, and by senior co-authors, Drs. Leigh and Anderson. I performed all of the data collection related to cerebrovascular and ventilatory measurements in the Laboratory of Human Cerebrovascular Physiology. Dr. Kissel performed the arterial blood gas procedure. Drs. Szabo, Walker and Leigh provided medical support. Lung function tests were performed by a trained respiratory therapist in the Teaching, Research and Wellness Building. I recruited the majority of volunteers for the study, with the assistance of Drs. Walker and Leigh. I submitted and received institutional ethical approval, and through Health Canada's Research Ethics Board.

5.1 Abstract

Acute hypoxia results in increased cerebral blood flow and ventilation. It is unknown if these physiological responses are impacted with normal aging, or in individuals with chronic obstructive pulmonary disease (COPD). Oxidative stress may induce pathological responses to hypoxia. We investigated the cerebrovascular and ventilatory responses to hypoxia in thirty-nine study participants at baseline, and during an acute intravenous infusion of vitamin C. Dynamic end-tidal forcing was used to induce a 5-minute period of isocapnic hypoxia ($P_{ET_{O_2}}$ = 50 mmHg). We used transcranial Doppler ultrasound to continuously measure peak cerebral blood flow velocity ($\bar{V}_P$) in the middle cerebral artery, and breath-by-breath ventilation ($\dot{V}_E$). *Older* individuals had a significantly reduced $\bar{V}_P$ compared to *Young* (*Older* = 0.33 ± 0.40; *Young* = 0.79 ± 0.68 cm·s^{-1} per % desaturation; P = 0.039) and $\dot{V}_E$ (*Older*: 0.7 ± 0.5 *vs*. *Young*: 2.0 ± 1.2 L·min^{-1} per % desaturation; P = 0.003) responses to hypoxia. COPD patients had similar responses to *Older* individuals. Vitamin C did not improve either the cerebrovascular or ventilatory responses in any group. Aging appears to be an important determinant in the cerebrovascular response to acute hypoxia. Despite support for vitamin C in restoring peripheral endothelial function, we do not find similar benefits in the cerebral circulation in humans.

5.2 Introduction

Aging and disease are widely acknowledged as risk factors capable of disrupting normal physiological responses to hypoxia. With an acute decrease in arterial PO_2 (Pa_{O_2}), ventilation briskly increases, with similar increases in cerebral blood flow (CBF). The hypoxic ventilatory response (HVR) is a coordinated response determined by peripheral chemoreceptor activation/sensitivity, arterial PO_2 (Pa_{O_2}), arterial PCO_2 (Pa_{CO_2}), and lung function. Although not a universal finding (150), advanced age has been associated with a decrease in the HVR in humans (131, 151, 152), however the specific mechanisms by which this occurs remains unclear. A brisk HVR seems particularly important in patients with lung disease, where they may be exposed to hypoxic episodes. Despite this important clinical relevance, relatively little information is available regarding the HVR in patients with Chronic Obstructive Pulmonary Disease (COPD). Previous studies have shown both an increase (25), and decrease (23) in the HVR in COPD patients, but how these responses compare to age-related healthy control subjects is unclear. Furthermore, an attenuated cerebrovascular response to hypoxia may compromise cerebral oxygenation, and pose a risk for cerebral ischemia. While the requirement of increased cerebral perfusion during conditions of low PO_2 is apparent, the mechanisms regulating increased pial vessel dilation remain incompletely defined. Currently, we are unaware of any studies that specifically investigated age-related effects on the cerebrovascular response to acute hypoxia in humans.

Oxidative stress has been implicated in aging and COPD, and is associated with cardiovascular and respiratory disturbances. Several studies have shown positive benefits on peripheral blood vessels through mechanisms aimed at acutely reducing a state of pro-oxidants via high-dose antioxidant interventions (153). Improvements in vascular

endothelial function are believed to play a dominant role in the translation of these benefits. Furthermore, the antioxidant, vitamin C, has been shown to enhance the ventilatory response to acute hypoxia in healthy elderly women (154), suggesting the role for oxidative stress in the hypoxic ventilatory response. We have previously reported increased systemic biomarkers of oxidative stress in smoking-related COPD patients (28). COPD is known to contribute to cardio-vascular disturbances (12) and, therefore, COPD patients in particular, may benefit the greatest from an antioxidant intervention.

We therefore set out to determine 1) the cerebrovascular and ventilatory responses to acute isocapnic-hypoxia in healthy older adults, and in COPD patients, and 2) test the hypothesis that vitamin C increases the ventilatory and cerebrovascular response to hypoxia in COPD patients. Age-related questions were tested by comparing the physiological responses between healthy *Young* and *Older* study participants. To identify any physiological differences with COPD patients, we compared these responses to the *Older* control group, who were of a similar age.

5.3 Methods

5.3.1 Ethical approval

All participating subjects who met the inclusion/exclusion criteria for the study provided written, informed consent that conformed to the *Declaration Helsinki*. Approvals were obtained from the University of Calgary's Institutional Conjoint Health Research Ethics Board and the Health Protection Branch of Health Canada prior to commencement.

5.3.2 Study participants

Healthy volunteers between the ages of 20 and 79 years (*Young*: 20-39 years; *Older* and COPD: 55-79 years) were recruited from the community, and COPD patients were recruited from participating outpatient clinics in Calgary. Subjects were excluded from participating in the study for any of the following reasons: *current* smoker, BMI > 35 $kg \cdot m^{-2}$, pre-menopausal status (excluding *Young*), chest pain upon physical exertion, surgery or trauma within previous 6 months, history of myocardial infarction, angina, arrhythmia, valvular heart disease, chronic heart failure, other/additional lung disease, history of stroke/cerebrovascular disease, diabetes, peripheral arterial disease, history of chronic headaches/migraines, history of blood clots/thrombosis, uncontrolled hypertension, oxygen therapy, or COPD exacerbation within previous 8 weeks. Patients were characterized as having smoking-related COPD on the basis of a physician-diagnosis, with a smoking history >10 pack-years and at least moderate airflow obstruction (FEV_1/FVC <0.70; $FEV_1 \leq 80\%$ predicted) evident on spirometry.

5.3.3 Experimental protocol

Study participants in the *Older* and COPD groups first completed pulmonary function testing to either rule out undiagnosed lung disease (*Older*), or to confirm and characterize the COPD diagnosis and severity. The main physiological testing session was conducted on a separate day, in the Laboratory of Human Cerebrovascular Physiology, University of Calgary. Prior to testing, subjects were instructed to refrain from the following: eating or drinking (4 hours), caffeine (12 hours), vigorous exercise (12 hours), vitamin supplementation (72 hours), blood pressure medication (24 hours), and short- and long-acting bronchodilators (12 and 24 hours, respectively in COPD patients).

5.3.4 Measurements and procedures

To determine the effects of antioxidants on the acute hypoxic responses, all individuals completed a 5-minute hypoxic challenge before and after Vitamin C administration. Tests were separated by a 45-minute wash-out period. Normal saline was infused in the first experiment as a non-active sham control, and vitamin C was infused in the second test. A "time control" group was included to insure no carry-over effects between the two hypoxic tests. These subjects underwent an identical protocol to the main study, without an intravenous intervention.

5.3.4.1 Pulmonary volumes and function

Spirometry, measures of lung volumes and single-breath diffusing capacity (DL_{CO}) were completed in all *Older* and COPD subjects by a trained respiratory therapist, as per ATS guidelines (61-63).

5.3.4.2 Protocol to measure acute responses to hypoxia

The subject's resting end-tidal partial pressures of O_2 ($P_{ET}O_2$) and CO_2 ($P_{ET}CO_2$) were measured using dedicated software (Chamber v2.26; University of Oxford Laboratory of Physiology, Oxford, UK) over a period of 10 minutes with the subject in a supine position, breathing via a fitted face mask that allowed for nose and/or mouth breathing. Expired PO_2 and PCO_2 was sampled continuously (100Hz) using mass spectrometry (AMIS 2000; Innovision, Odense, Denmark) via a fine catheter. Respiratory volumes were measured with a turbine and volume transducer (VMM-400; Interface Associates, Laguna Niguel, CA), and respiratory flow direction and timing were obtained with a pneumotachograph (RSS100-HR, Hans Rudolf, Kansas City, MO, USA).

Using dedicated software (BreatheM v2.38, University Laboratory of Physiology, Oxford, UK), the technique of dynamic end-tidal forcing was used to precisely control the desired $P_{ET}CO_2$ and $P_{ET}O_2$ values (64). Breath-by-breath oscillations of the inspired partial pressures of O_2, CO_2, and N_2 were controlled via a fast gas mixing system for precise accuracy and stability of the desired end-tidal values.

Following a 5 minute lead-in period of isocapnic euoxia ($P_{ET}CO_2$ = +1.5 mmHg above resting values and $P_{ET}O_2$ = 88.0 mmHg), the protocol progressed with an acute stage of isocapnic hypoxia ($P_{ET}O_2$ = 50.0 mmHg and $P_{ET}CO_2$ = +1.5 mmHg) for a further 5 minutes. This was then followed by a 2-minute stage of isocapnic-hyperoxia ($P_{ET}O_2$ = 300 mmHg), aimed to quickly re-oxygenate patients.

5.3.4.3 Measurement of middle cerebral artery blood flow velocity

Peak cerebral blood flow velocity ($\overline{V}_P$) was continuously measured in the middle cerebral artery (MCA) during the entire protocol using a 2-MHz pulsed Doppler Ultrasound system (TOC2M, Multigon Industries, Inc., Yonkers, NY). Specific search techniques were used to locate the MCA, and the signal was optimized by adjustments to the depth, power, and angle of insonnation (32). A fitted headband secured the Doppler probe for the duration of the test. $\overline{V}_P$ was used as a surrogate for global cerebral blood flow (CBF). Without changes in the power ($\overline{P}$) index acquired from the Doppler system, vessel diameter is assumed to be constant. Therefore, without a change in $\overline{P}$, $\overline{V}_P$ is considered to be a reliable index of CBF.

5.3.4.4 Measurement of cardiovascular variables

Heart rate (HRT) was monitored and determined from the R-R interval using a 3-lead electrocardiogram (Micromon 7142B monitor; Kontron Keynes, UK), and finger pulse photoplethysmography (Portapress; TPD Biodemical Instrumentation, Amsterdam, The Netherlands) was used to measure beat-beat blood pressure. Arterial oxygen saturation (Sp_{O_2}) was monitored using finger pulse oximetry (Model 3900; Datex-Ohmeda, Louisville, CO). To increase perfusion to the finger, the hand was warmed with an electric heating pad.

5.3.4.5 Vitamin C administration

An intravenous catheter was inserted in a vein near the antecubital fossa for drug infusion. Vitamin C (Ascorbic acid injection, Alveda Pharma, Canada) was diluted with

normal saline, and administered intravenously. Before the start of the experiment, a loading dose of 3g ascorbic acid (200mg/min) was administered over 15-minutes, followed by a continuous maintenance dose (40mg/min; 13ml) during the experiment. This dosage of vitamin C has previously been shown to be effective at rapidly increasing plasma ascorbic acid concentrations more than 15 fold (128). Isotonic normal saline (0.9% NaCl) was infused at an identical flow-rate to ascorbic acid.

5.3.4.5.1 Arterial blood gas analysis

In a subset of participants (*Young*: n=5, *Older*: n=4; COPD: n=4), a 3F arterial catheter (Cook Medical) was inserted into the distal radial artery to obtain blood gas measurements. Arterial blood samples were drawn into a preheparinized syringe (BD Preset) at the end of each stage. Blood was processed immediately (ABL800 FLEX, Radiometer, Copenhagen, Denmark).

5.3.5 Data analysis

Euoxic-isocapnic (Baseline) breath-breath and beat-beat data was taken as a 1-minute average of the last minute of the 5-minute euoxic-isocapnic period. Since the peak hypoxic response occurs within 2-3 minutes, a 1-minute average around the peak hypoxic cerebrovascular and ventilatory response was used to indicate the response to isocapnic-hypoxia. The sensitivity to hypoxia was calculated as the change (in either $\dot{V}_E$ or $\bar{V}_P$) from isocapnic euoxia to isocapnic hypoxia divided by the change in Sp_{O_2}. Sp_{O_2} (as determined by finger-pulse oximetry) provided the most accurate estimate of arterial O_2 saturation (*i.e.*, direct measures of arterial oxyhemoglobin saturation). Mean blood pressure (MBP) was

calculated as (1/3) x systolic blood pressure + (2/3) x diastolic blood pressure. Cerebrovascular resistance (CVR) was calculated as MBP/$\bar{V}_P$.

5.3.6 Statistics

Comparisons were separated to include 1) "*Young vs. Older*" (*i.e.*, aging comparison), and 2) "*Older vs. COPD*". Planned comparisons to identify group differences between the ventilatory and cerebrovascular sensitivity to hypoxia were conducted using independent t-tests. Main effects and interactions were determined using a mixed design 2x2 repeated measurers ANOVA [time x grouping] (SPSS Version 20.0, SPSS Inc., Chicago, IL). Dependent t-tests (within group) and independent t-tests (between-group) were applied if a significant *F* ratio was detected. The Bonferroni correction factor was used in the case of multiple comparisons. All data are presented as mean ± SD, and significance is determined at α-level ≤ 0.05.

5.4 Results

5.4.1 Study participants

Thirty-nine subjects (*Young* = 12, *Older* = 15, COPD = 12) completed the study. In addition to these subjects, two older controls had a reduced FEV_1/FVC ratio (*i.e.*, <0.70) and were therefore excluded from the study. Cerebrovascular data for one *Older* control were excluded due to a lack of suitable MCA signal. Physical characteristics and pulmonary function results are summarized in Table 17 (*page 134*). *Older* participants were of similar age and BMI, but had less smoking history, compared to COPD patients. COPD patients had moderate airflow obstruction (mean FEV_1 predicted 62 ± 14%), greater lung volumes, and lower diffusing capacity, each of which are consistent with the physiological impairment seen in this condition (Table 17, *page 134*).

5.4.2 Time control group

A separate sub-group (n = 4) was tested to rule out the effect of repeated hypoxic tests within same day. The test-retest reliability coefficient (*i.e.*, Test 1 *vs.* Test 2) for $\dot{V}_E$ and $\bar{V}_P$ sensitivity was r = 0.81 and r = 0.86 for $\dot{V}_E$ and $\bar{V}_P$ sensitivity, respectively. No significant differences were noted (specifically, $\bar{V}_P$ and $\dot{V}_E$) between trials. See APPENDIX B (*page 195*) for expected repeatability for the hypoxic responses, based on previously published and work-in-progress from the Laboratory of Human Cerebrovascular Physiology.

5.4.3 Arterial blood gases

Thirteen volunteers participating in the study underwent the procedure of arterial blood gas analysis to validate the use of $P_{ET_{O_2}}$ during hypoxia. During hypoxia, the $P_{ET_{O_2}}$ was 50.0 mmHg in all groups (*Young* = 50.2 ± 0.9; *Older* = 49.7 ± 1.0, COPD = 49.7 ± 0.5 mmHg; $P > 0.05$). There was a progressive decline in Pa_{O_2} across the groups, particularly between *Young* and *Older* (*Young* = 53.2 ± 2.6; *Older* = 48.4 ± 3.7, COPD = 46.1 ± 1.2 mmHg; [*Young vs*. *Older*: $P = 0.053$; *Older vs.* COPD: $P = 0.279$]). During hypoxia, *Young* and *Older* had similar Sp_{O_2} (86%); however, COPD patients experienced greater arterial desaturation (80%) ($P < 0.001$). The best (non-invasive) indication of the hypoxic stimulus was finger pulse oximetry (Sp_{O_2}) (direct measures of Sa_{O_2} *vs* Sp_{O_2}: r = 0.96). A poor relationship was found between $P_{ET_{O_2}}$ and Pa_{O_2} (r = 0.49). Therefore, hypoxic sensitivity values were calculated based on finger pulse oximetry.

5.4.4 Cerebro- and cardio-vascular responses to isocapnic hypoxia: effect of vitamin C

5.4.4.1 Control experiment (saline)

Cerebro- and cardiovascular responses to acute isocapnic hypoxia are summarized in Table 18 (*page 135*). Basal $\bar{V}_P$ was significantly lower with normal aging at Baseline (saline) (*Older*: 52.5 ± 11.3 *vs*. *Young*: 68.5 ± 18.5 $cm \cdot s^{-1}$; $P = 0.024$), and during hypoxia (*Older*: 55.8 ± 11.8 *vs*. *Young*: 76.1 ± 23.3 $cm \cdot s^{-1}$; $P = 0.018$). CVR and blood pressure were lower in *Young* both at rest and during hypoxia ($P < 0.01$; Table 18, *page 135*). The $\bar{V}_P$ sensitivity to hypoxia was less in *Older* compared to *Young* (0.33 ± 0.40 *vs*. 0.79 ± 0.68 $cm \cdot s^{-1}$ per % desaturation, respectively; $P = 0.039$) (Figure 8A, *page 133*).

Absolute $\bar{V}_P$ was similar between COPD and *Older* at Baseline (51.2 ± 12.3 *vs.* 52.5 ± 11.3 cm·s^{-1}, respectively; P = 0.794) and during hypoxia (56.3 ± 12.5 *vs.* 55.8 ± 11.8 cm·s^{-1}, respectively; P = 0.919) (Table 18, *page 135*). Similarly, the $\bar{V}_P$ sensitivity to hypoxia was not different between COPD and *Older* (COPD: 0.74 ± 0.48 *vs. Older*: 0.91 ± 0.63 cm·s^{-1} per % desaturation; P = 0.824; Figure 8A, *page 133*). COPD patients had greater HRT than *Older* at baseline (73 ± 10 *vs.* 61 ± 8 beats·min^{-1}, respectively; P = 0.004) and during hypoxia (81 ± 11 *vs.* 69 ± 11 beats·min^{-1}, respectively; P = 0.018). Blood pressure and CVR were similar between COPD and *Older* at baseline and during hypoxia (P > 0.05; Table 18, *page 135*). There was a trend for a main effect of hypoxia reducing CVR (P = 0.087), with COPD patients specifically showing a trend to lower CVR during hypoxia (P = 0.094; Table 18, *page 135*).

5.4.4.2 Effect of vitamin C

There was a trend for vitamin C to increase $\bar{V}_P$ by ~ 4% in *Young* during euoxic-isocapnia (68.5 ± 18.5 to 71.5 ± 18.8 cm·s^{-1}) (P = 0.08; Table 18, *page 135*). CVR and blood pressure indices were not affected by vitamin C at baseline or during hypoxia in *Young* or *Older* (P > 0.05; Table 18, *page 135*). In *Young* only, the $\bar{V}_P$ sensitivity to hypoxia was decreased with vitamin C (0.79 ± 0.68 *vs.* 0.44 ± 0.51 cm·s^{-1} per % desaturation) (P = 0.039; Table 19, *page 136;* and Figure 8A, *page 133*).

In COPD, absolute $\bar{V}_P$ at Baseline did not change with vitamin C (P = 0.157), however, $\bar{V}_P$ was increased during hypoxia (56.3 ± 12.5 to 60.3 ± 11.7 cm·s^{-1} (P = 0.019; Table 18, *page 135*). However, the $\bar{V}_P$ sensitivity was not different following vitamin C in COPD (P = 0.247; Figure 8A, *page 133*). MBP was increased at Baseline following vitamin C only in COPD (106 ± 7 to 111 ± 9 mmHg; P = 0.007; Table 18, *page 135*).

5.4.5 Ventilatory responses to isocapnic hypoxia: effect of vitamin C

5.4.5.1 Control experiment (saline)

Ventilatory responses to euoxic isocapnia and isocapnic hypoxia are summarized in Table 19 *(page 136).* *Young* had greater Baseline and hypoxic ventilation than *Older* and, similarly, had a significantly greater $\dot{V}_E$ sensitivity to hypoxia (*Young*: 2.0 ± 1.2 *vs.* *Older*: 0.7 ± 0.5 $L \cdot min^{-1}$ per % desaturation) ($P = 0.003$; Table 19, *page 136;* and Figure 8B, *page 133*). Inspiratory flow and tidal volume were greater in *Young*, than *Older* ($P <$ 005; Table 19, *page 136*).

COPD patients had a greater peak $\dot{V}_E$ response to hypoxia compared to *Older* (COPD: 22.5 ± 6.9 *vs. Older*: 17.0 ± 5.2 $L \cdot min^{-1}$; $P = 0.054$), and had greater breathing frequency (B*f*), inspiratory flow, and reduced fraction of T_I / T_{TOT} ($P < 0.01$; Ventilatory responses to acute euoxic-isocapnia and isocapnic-hypoxia in *Young*, *Older*, and COPD, before and after vitamin C infusion.). However, Sp_{O_2} was significantly less in COPD compared to *Older* (COPD: 80 ± 3% *vs. Older*: 86 ± 3%; $P < 0.001$), despite similar $P_{ET_{O_2}}$ (COPD: 49.8 ± 0.7 *vs. Older:* 48.8 ± 1.6 mmHg; $P = 0.126$) (Table 19, *page 136*). Taken together, the ventilatory sensitivity to hypoxia was not different between COPD and *Older* (COPD: 0.7 ± 0.4 *vs. Older*: 0.7 ± 0.4 $L \cdot min^{-1}$ per % desaturation; $P = 0.977$) (Figure 8B, *page 133*).

5.4.5.2 Effect of vitamin C

Vitamin C did not have an effect on the ventilatory sensitivity to isocapnic hypoxia in the *Young*, *Older*, or COPD cohorts (Figure 8, *page 133*), nor on any ventilatory

parameters (*i.e.*, frequency, timing, and inspiratory drive) ($P > 0.05$; Table 19, *page 136*), with the exception of reduced tidal volume in *Young* during hypoxia ($P = 0.008$; Table 19, *page 136*). However, the overall ventilation in *Young* during hypoxia was not different between conditions (32.9 ± 13.9 *vs*. 31.0 ± 14.5 $L \cdot min^{-1}$; (Table 19, *page 136*; $P = 0.344$).

5.5 Discussion

5.5.1 Major findings

The main findings of the present study are: 1) the cerebrovascular and ventilatory responses to acute hypoxia are reduced in healthy aging, 2) compared to *Older* control subjects, COPD patients were found not to have a reduced ventilatory or cerebrovascular response, 3) vitamin C did not augment either the ventilatory or cerebrovascular responses during acute hypoxia.

5.5.2 Influence of aging and COPD on the cerebrovascular response to acute hypoxia

To our knowledge, no previous study has directly assessed the effect of aging on the cerebrovascular responses to hypoxia in humans. Similarly, we are aware of only one previous study investigating these responses in COPD patients (39). The present data suggest that healthy aging significantly reduces the hypoxic cerebrovascular dilatory response by approximately 50%. The CBF response to isocapnic hypoxia in *Young* subjects is similar to previous studies (64, 155). Initial experiments performed in rats showed little difference in CBF with aging during *moderate*-hypoxia (156-158). However, direct comparison is cautioned, as several important aspects differ, such as a difference in species, duration of hypoxic stimulus, and control of Pa_{CO_2}. Contrary to these findings, however, reduced pial vessel dilation has been reported in older rats in response to adenosine (159) - a primary factor regulating hypoxic vasodilation (160). Furthermore, adenosine blockade eliminated the age-related differences previously found during severe hypoxia (158). The hypoxic CBF responses of COPD patients in our study were similar to a previous study (39), which reported a preserved cerebrovascular response in COPD patients to isocapnic-hypoxia. In our present study, COPD patients experienced a greater

arterial desaturation (80%) compared to controls (86%), despite $P_{ET}O_2$ being tightly controlled at 50 mmHg, signifying either an increased $\dot{V}_A/\dot{Q}$ ratio, and/or a right shift in the O_2 dissociation curve, in COPD. The implications for this are important, and expand beyond the laboratory to situations commonly encountered in daily life such as travelling to higher altitude or flying.

5.5.3 Effect of vitamin C on the cerebrovascular regulation of acute hypoxia in aging and COPD

A well-characterized consequence to aging is impaired vascular endothelial function, and hence, a reduced availability of nitric oxide (NO) (132). While experimental evidence in various species suggests the importance of NO in this response (161), human studies have produced variable findings. Previously, our laboratory showed that NOS-inhibition did not have an overall effect on the cerebrovascular response during isocapnic-hypoxia (145), whereas contradictory findings were reported in another study (162). Hypoxia is thought to increase systemic levels of oxidative stress, and the brain and cerebrovascular endothelium have been implicated in contributing to this overall accumulation of free radicals during hypoxia (163). While numerous studies have shown vitamin C to restore vascular endothelial function in older (164) and patient populations (12), the present study is the first to test the efficacy of this intervention on improving CBF during hypoxia. Our findings do not, however, suggest a role of vitamin C in augmenting the CBF response to hypoxia in individuals with a previously reduced responsiveness (*i.e.*, *Older* and COPD). This is consistent with recent findings that showed no effect of vitamin C in enhancing peripheral blood flow in older individuals, despite blunted hypoxic vasodilation (165). It is possible that the vascular responses to hypoxia do not solely rely on an intact endothelium, as Headrick and Berne (166) have suggested that adenosine

mediates relaxation by both endothelial-dependent and -independent mechanisms. Secondly, it is possible the vitamin C would have had a greater effect in *active* smokers as previous studies suggest a greater improvement in endothelial function, presumably due to elevated oxidative stress (167). The fact that vitamin C decreased the sensitivity of the hypoxic CBF response in *Young* volunteers was an unexpected finding in the present study. It is likely that there is an "absolute" requirement for CBF to maintain adequate O_2 delivery and metabolism during hypoxia. Although the sensitivity to hypoxia decreased, the absolute blood flow during hypoxia was similar. We reason that the decreased sensitivity is, rather, an artifact of a trend towards a higher baseline CBF velocity following vitamin C in *Young* subjects.

5.5.4 Influence of aging and COPD on the acute hypoxic ventilatory response

Normal aging is associated with several important physiological adaptations. Nonetheless, relatively little consistent information and consensus is available regarding the impact that healthy aging and disease have on the HVR. Similar to others (151), we found a 50% lower HVR in healthy *Older* subjects compared to *Younger*. Conversely, a recent large scale study (combined cross-sectional and longitudinal study design) showed an increase in the HVR with aging (150). Despite a similarity in the hypoxic stimulus between studies, we believe the discrepancy in these findings are likely due to the difference in protocols; mainly the control of Pa_{CO_2}. When Pa_{CO_2} falls with hyperventilation (during hypoxia), the ventilatory sensitivity is only 16% of what would be achieved when Pa_{CO_2} is held constant (155). The aforementioned findings (150) represent combined stimulation of the peripheral and central chemoreceptors, whereas the present data represents isolated peripheral chemoreceptor stimulation.

We found that COPD patients had a preserved HVR compared to *Older* control subjects. Despite the clinical importance of defining the hypoxic response, similar data for comparison is lacking. We expected a blunted HVR in COPD patients, secondary to mechanical and ventilatory constraints. Conversely, it could be argued that the HVR in COPD patients would be increased due to presumed episodes of intermittent hypoxia encountered with exertional activities of daily living, and becuase HVR has been found to be augmented following intermittent hypoxia (168).

5.5.5 Effect of vitamin C on the acute hypoxic ventilatory response in aging and COPD

Our findings are in contrast to our hypothesis, which predicted increased peripheral chemoreceptor activity in COPD patients, leading to increased HVR. Putative mechanisms of O_2 sensing involve redox-sensitive signaling processes in Type I cells of the carotid body. A reduced O_2 tension inhibits potassium-channel activity, thereby increasing influx of calcium and hence, increased nerve traffic to the carotid-sinus nerve (169). The reducing properties of vitamin C on carotid body functioning are presently not clear, as studies have produced mixed findings. Pokorski and Marczak (154) found oral supplementation of vitamin C to increase the HVR by 31% in healthy older women (60-80 years), suggesting the reducing properties of vitamin C affected the redox-sensitive potassium channels. Conversely, the effect of an antioxidant intervention (vitamin C + E) on HVR in young men only appeared to have an effect under various conditions of ventilatory depression, including anesthetic, and acetazolamide (170-172). It is unclear why our results differ from those of (154), although overall, the ventilatory responses were more variable in our study, which may be related to including both men and women in our study cohorts. It is also clear from their results that not all individuals "responded" to vitamin C (only 67%), signifying additional mechanisms.

5.5.6 Experimental considerations

Cigarette smoking is a primary factor in contributing to cardiovascular disease. While it is possible that an underlying smoking history contributed to the observed differences between *Young* and *Older*, we doubt this to be the case due to the prolonged period that had elapsed between subjects previously being "active" smokers (24-36 years). This is supported by evidence that suggests at least partial reversibility in endothelial function following smoking cessation (173), although the effects on ventilation are less certain.

We employed the technique of "dynamic end-tidal forcing" to independently control the desired level of $P_{ET}O_2$ and $P_{ET}CO_2$ (174). While the target level of hypoxia was predetermined at $P_{ET}O_2$ = 50 mmHg for all individuals, we found the *actual* stimulus (*i.e.*, Pa_{O_2} and subsequently, Sa_{O_2}) to vary between groups. Sensitivities (for $\bar{V}_P$ and $\dot{V}_E$) were therefore calculated based on Sp_{O_2} values, which were found to highly correlated with the true Sa_{O_2}.

Cerebral blood flow velocity was measured using transcranial Doppler ultrasound (TCD) of the MCA. The non-invasive properties and high temporal resolution of TCD make this a desirable technique to study cerebrovascular physiology. We believe that the MCA velocity provides a reasonable index of CBF given that there is little expected diameter change with moderate hypoxia. In support of this (125, 175), previously showed that during isocapnic hypoxia (50 mmHg) the relative cross sectional area of the MCA does not change after 20 minutes of moderate hypoxia (50 mmHg).

5.5.7 Clinical implications

We provide evidence of reduced cerebrovascular and ventilatory sensitivity to hypoxia in older adults. Importantly, a cohort of patients with well characterized COPD of at least moderate severity did not have a further reduced responsiveness. Older adults, and especially COPD patients, are at increased risk of sleep apnea (176, 177), ischemic stroke (178), and acute hypoxic events (*i.e.*, COPD exacerbation), all potentially leading to a reduction in cerebral PO_2 without adequate responsiveness. Furthermore, these data may provide insight into older adults travelling to altitude, as little is known regarding the cerebrovascular effects of aging at altitude. Clinically, these findings highlight the effect of lung disease on travel to altitude, as COPD patients would be expected to experience lower Pa_{O_2} for a given altitude, and thus, present a further challenge to maintain adequate Pa_{O_2}. These findings may be extended to included studies of poikilocapnic hypoxia, which would provide further insight into the integrated blood gases on the overall effect of altitude.

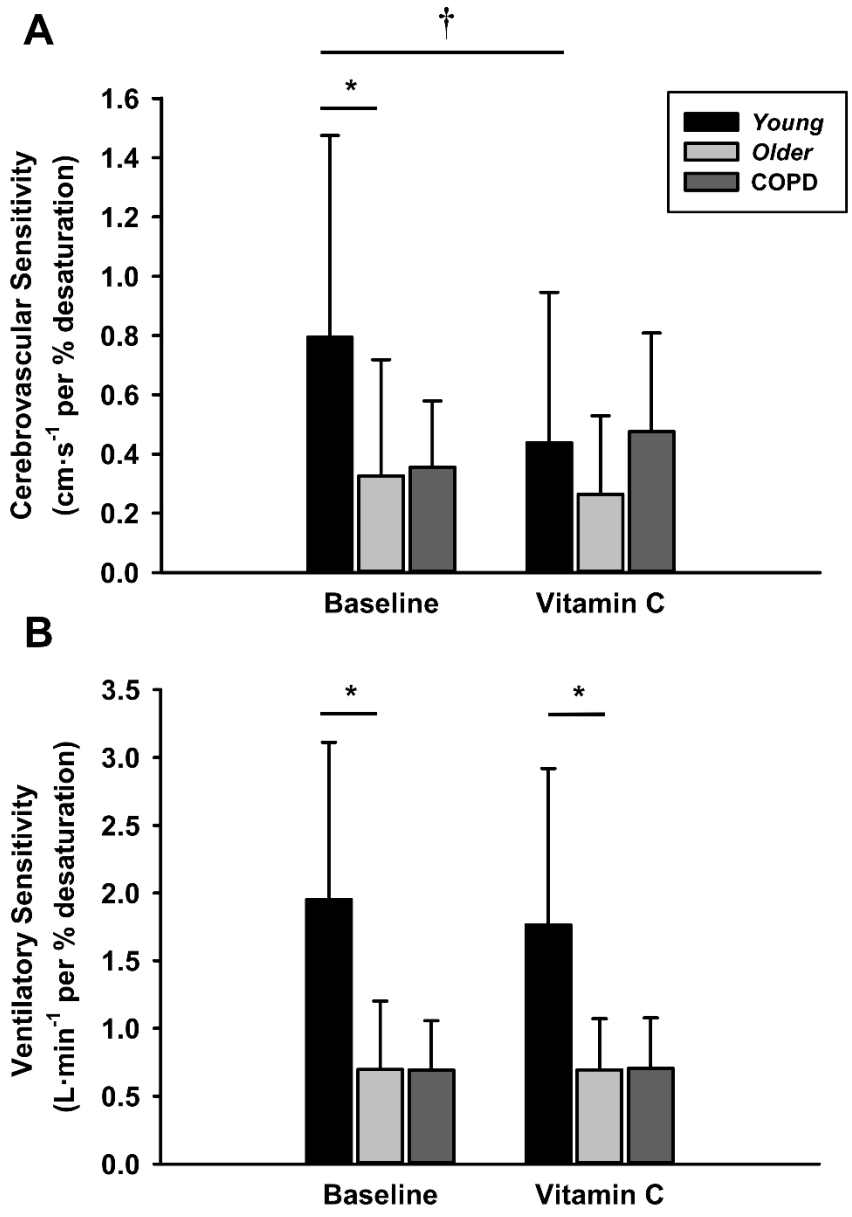
In summary, this is the first study to investigate the effects of aging on the cerebrovascular response to acute hypoxia in healthy humans. In agreement with some animal studies, we show a decreased cerebrovascular response to acute hypoxia with healthy aging. We furthermore confirmed a decreased ventilatory response with aging. Patients with COPD were not found to have a further decrease in either the ventilatory or cerebrovascular responses. Vitamin C did not improve either the ventilatory or cerebrovascular response to hypoxia, in either *Young* or *Older* subjects, or in COPD patients, indicating a limited role of oxidative stress in the regulation of hypoxic ventilation and CBF.

5.6 Acknowledgments

The authors are especially grateful to out subjects for their time and enthusiasm. We would also like to thank Drs. A. Kealey and A. Bizios for their clinical support for placement of arterial catheters, Mr. C. Dumonceaux for pulmonary function testing, Ms. L. Knox and Dr. J. Rawling for their help with medical coverage and patient recruitment, and Mr. B. Hansen for technical support. Study support was provided by the CIHR (PI: MJ Poulin) and the Heart and Stroke Foundation of Canada (PI: MJ Poulin). S.E.H. received support from Dr. Chen Fong Doctoral Scholarship (Hotchkiss Brain Institute) and a CIHR operating grant. T.J.A is a senior scholar of the Alberta Heritage Foundation for Medical Research. R.L is the GSK-CIHR Professor of Inflammatory Disease. MJP is the Brenda Strafford Foundation Chair in Alzheimer Research.

5.7 Chapter Five Figures and Tables

Figure 8. Group means representing the (A) cerebrovascular and (B) ventilatory sensitivity to isocapnic hypoxia in *Young, Older,* and *COPD* patients during sham-control and vitamin C infusion.



Footnote: *Error bars* represent SD. * $P < 0.05$, Different from *Older*, under same condition; † $P < 0.05$, Different from saline, within-group.

Table 17. Subject characteristics and pulmonary function data.

	Young	*Older*	COPD
Subjects (n)	12	15	12
Sex (M / F)	7 / 5	7 / 8	4 / 8
Age, yr	30.3 ± 5.5 *	68.3 ± 5.0	68.6 ± 8.1
Weight, kg	71.4 ± 15.2	75.8 ± 13.3	71.1 ± 16.0
Height, m	1.78 ± 0.05 *	1.70 ± 0.07	1.64 ± 0.07*
BMI, kg·m^{-2}	24.1 ± 2.6	26.1 ± 3.5	26.3 ± 5.4
Smoking history, pack yr	0*	6 ± 11	43 ± 16*
Pulmonary function			
(% predicted)			
FEV_1, L	-	2.98 ± 0.82 (109 ± 11)	1.49 ± 0.33* (62 ± 14)*
FEV_1 / FVC	-	0.78 ± 0.51 (104 ± 7)	0.50 ± 0.10* (66 ± 14)*
FRC, L	-	3.38 ± 0.65 (107 ± 15)	4.17 ± 1.03* (141 ± 24)*
RV, L	-	2.27 ± 0.36 (99 ± 15)	3.35 ± 0.80* (154 ± 36)*
TLC, L	-	6.18 ± 1.19 (102 ± 7)	6.23 ± 1.33 (114 ± 17)*
IC/TLC	-	0.45 ± 0.06 (95 ± 12)	0.33 ± 0.05* (73 ± 10)*
DL_{CO}, mL·min^{-1}·mm Hg^{-1}	-	25.2 ± 7.6 (95 ± 17)	16.14 ± 6.39* (68 ± 27)*

*P < 0.05 compared to *Older* group. Data presented as means ± SD. ***Definition of abbreviations:*** BMI : body mass index; FEV_1: forced expiratory volume in one second (post-bronchodilator); FEV_1 / FVC : ratio between forced expiratory volume in one second to the forced vital capacity (post-bronchodilator); FRC: functional residual capacity; RV: residual volume; TLC: total lung capacity; IC / TLC: ratio between inspiratory capacity and total lung volume; DL_{CO}: diffusion capacity of the lung for carbon monoxide. Values in *parenthesis* indicate lung function as, % predicted.

Table 18. Cardio- and cerebro-vascular responses to euoxic-isocapnia and isocapnic-hypoxia in *Young*, *Older*, and COPD, before and after vitamin C infusion.

	Young **n=12**		***Older*** **n=14**		**COPD n=12**	
	Control	**Vitamin C**	**Control**	**Vitamin C**	**Control**	**Vitamin C**
Euoxic-Isocapnia						
$\bar{V}_P$ (cm·s^{-1})	68.5 ± 18.5*	71.5 ± 18.8*	52.5 ± 11.3	54.9 ± 10.6	51.2 ± 12.3	54.0 ± 10.7
CVR (mm Hg·cm·s^{-1})	1.3 ± 0.2*	1.3 ± 0.3*	2.0 ± 0.5	1.9 ± 0.4	2.2 ± 0.4	2.1 ± 0.4
SBP (mm Hg)	118 ± 9*	119 ± 7*	139 ± 16	140 ± 9	137 ± 9	147 ± 12
DBP (mm Hg)	72 ± 5*	74 ± 5*	83 ± 7	82 ± 4	90 ± 8	94 ± 10
MBP (mm Hg)	87 ± 5*	89 ± 5*	101 ± 10	102 ± 5	106 ± 7	111 ± 9*†
HRT (beats·min^{-1})	65 ± 8	64 ± 10	61 ± 8	62 ± 9	73 ± 10*	74 ± 11*
Isocapnic-Hypoxia						
$\bar{V}_P$ (cm·s^{-1})	76.1 ± 23.3*	75.9 ± 21.8*	55.8 ± 11.8	57.7 ± 12.0	56.3 ± 12.5	60.3 ± 11.7†
CVR (mm Hg·cm·s^{-1})	1.2 ± 0.5*	1.3 ± 0.3*	2.0 ± 0.5	1.9 ± 0.4	2.0 ± 0.4	2.0 ± 0.4
SBP (mm Hg)	125 ± 12*	124 ± 9*	145 ± 19	145 ± 14	144 ± 11	151 ± 17
DBP (mm Hg)	76 ± 10*	77 ± 9*	87 ± 11	85 ± 8	95 ± 8	98 ± 13*
MBP (mm Hg)	92 ± 10*	93 ± 8*	107 ± 13	105 ± 9	111 ± 9	116 ± 13*
HRT (beats·min^{-1})	79 ± 12	78 ± 15	69 ± 11	72 ± 13	81 ± 11*	85 ± 11*

*P < 0.05, compared to *Older*, between group under the same condition; †P < 0.05, compared to saline, within-group. Data presented as means ± SD. ***Definition of abbreviations:*** $\bar{V}_P$: peak cerebral blood flow velocity; CVR: cerebrovascular resistance; SBP: systolic blood pressure, DBP: diastolic blood pressure, MBP: mean arterial blood pressure; HRT: heart rate.

Table 19. Ventilatory responses to acute euoxic-isocapnia and isocapnic-hypoxia in *Young*, *Older*, and COPD, before and after vitamin C infusion.

	Young n=12		*Older* n=15		COPD n=12	
	Control	**Vitamin C**	**Control**	**Vitamin C**	**Control**	**Vitamin C**
Euoxic Isocapnia						
$P_{ET_{CO_2}}$ (mm Hg)	38.5 ± 3.0	38.6 ± 2.8	37.0 ± 3.2	37.0 ± 2.9	35.2 ± 5.3	35.1 ± 4.1
$P_{ET_{O_2}}$ (mm Hg)	87.8 ± 0.4	87.7 ± 0.7	88.1 ± 1.0	87.9 ± 0.9	88.1 ± 0.6	87.9 ± 0.4
Sp_{O_2} (%)	97 ± 1	96 ± 1	96 ± 1	96 ± 1	94 ± 1*	94 ± 1*
V_T/T_I ($L \cdot s^{-1}$)	0.53 ± 0.11*	0.51 ± 0.07	0.41 ± 0.07	0.43 ± 0.10	0.62 ± 0.15	0.62 ± 0.17
T_I/T_{TOT} (%)	43 ± 4	43 ± 3	41 ± 4	41 ± 4	35 ± 4	35 ± 4
V_T ($L \cdot breath^{-1}$)	1.01 ± 0.26	0.89 ± 0.17	0.85 ± 0.44	0.81 ± 0.27	0.87 ± 0.25	0.89 ± 0.28
B*f* ($breaths \cdot min^{-1}$)	15 ± 3	16 ± 3	14 ± 3	14 ± 4	17 ± 6	16 ± 3
$\dot{V}_E$ ($L \cdot min^{-1}$)	14.3 ± 3.1*	13.7 ± 2.2*	10.5 ± 2.5	10.7 ± 2.5	13.2 ± 3.0*	13.4 ± 3.8
Isocapnic Hypoxia						
$P_{ET_{CO_2}}$ (mm Hg)	38.1 ± 3.1	38.4 ± 3.2†	36.5 ± 3.1	36.6 ± 3.0	35.0 ± 5.5	34.9 ± 4.0
$P_{ET_{O_2}}$ (mm Hg)	50.4 ± 1.3*	50.0 ± 1.2	48.8 ± 1.6	48.9 ± 1.9	49.8 ± 0.7	49.8 ± 0.4
Sp_{O_2} (%)	87 ± 2	87 ± 3	86 ± 3	86 ± 4	80 ± 3*	80 ± 4*
V_T/T_I ($L \cdot s^{-1}$)	1.12 ± 0.46*	1.05 ± 0.41*	0.65 ± 0.19	0.64 ± 0.13	1.02 ± 0.38*	1.03 ± 0.38*
T_I / T_{TOT} (%)	46 ± 4	46 ± 4*	43 ± 3	42 ± 4	36 ± 4*	35 ± 4*
V_T ($L \cdot breath^{-1}$)	2.02 ± 0.60*	1.70 ± 0.40†	1.35 ± 0.59	1.37 ± 0.60	1.22 ± 0.36	1.24 ± 0.35
B*f* ($breaths \cdot min^{-1}$)	17 ± 7	18 ± 6	14 ± 3	14 ± 4	19 ± 6*	19 ± 6*
$\dot{V}_E$ ($L \cdot min^{-1}$)	32.9 ± 13.9*	31.0 ± 14.5*	17.0 ± 5.2	17.3 ± 4.5	22.5 ± 6.9*	22.6 ± 8.1

*$P < 0.05$, compared to *Older*, between group under the same condition; †$P < 0.05$, compared to saline, within-group. Data presented as means ± SD. ***Definition of abbreviations:*** $P_{ET_{CO_2}}$: end-tidal partial of carbon dioxide; $P_{ET_{O_2}}$: end-tidal partial of oxygen; Sp_{O_2}: arterial oxyhaemoglobin saturation; V_{TI} / T_I : Mean inspiratory flow; T_I / T_{TOT}: Ratio of inspiratory time to total breath time; V_T: expired tidal volume; B*f*: breathing frequency; $\dot{V}_E$: minute ventilation.

Chapter Six: **Forearm blood flow during moderate handgrip exercise in patients with COPD: effect of vitamin C**

Sara E. Hartmann, Xavier Waltz, Richard Leigh, Todd J. Anderson, and Marc J. Poulin

Preface

Exercise limitation is multi-factorial in COPD patients, however, the blood flow response has not been investigated during moderate handgrip exercise. Endothelial dysfunction in COPD may contribute to vascular abnormalities. An acute reduction of oxidative stress may improve nitric oxide bioavailability, and lead to improvements in exercise.

Declaration

The original scientific manuscript contained in this chapter was written by myself with guidance from my supervisor, Dr. Poulin. The study conception was developed by myself, with input from all members of my advisory committee. I was responsible for all data collection, including the vascular ultrasound measurements and subsequent analysis. Dr. Xavier Waltz monitored the physiological recordings the for quality assurance. The lung function tests were performed by a trained respiratory therapist (Teaching, Research and Wellness Building). I recruited the volunteers for the study, and I submitted and received institutional ethical approval, and Health Canada's Research Ethics Board approval.

6.1 Abstract

Background: Patients with chronic obstructive pulmonary disease (COPD) have reduced endothelial function, possibly related to increased oxidative stress. This may have implications for the blood flow response during exercise.

Objective: We set out to determine whether forearm blood flow (FBF) was reduced during moderate handgrip exercise in COPD, compared to controls, and if this response would be improved following vitamin C infusion.

Methods: Doppler vascular ultrasound was used to continuously measure FBF and forearm vascular conductance (FVC) during 5-min of moderate, rhythmic handgrip exercise (EX) under conditions of saline and vitamin C. Measures of flow mediated dilation (FMD) and nitroglycerine-mediated dilation were used to assess endothelial -dependent and independent dilation, respectively.

Results: Ten COPD patients with moderate COPD (FEV_1 = 60 ± 5%) and ten healthy age-matched controls participated. Under saline conditions, COPD patients had similar increases in FBF and FVC during EX, compared to controls (FBF: 426 ± 100% *vs*. 550 ± 82%, respectively; $P > 0.05$). Vitamin C was not found to improve exercise-mediated FBF or FVC in either COPD or control group ($P > 0.05$). FMD and NMD were similar between COPD and controls at baseline (saline), however, FMD responses were augmented with vitamin C similarly in both groups.

Conclusions: Moderate-COPD patients have a preserved FBF response during moderate handgrip exercise. Despite augmented FMD following vitamin C infusion, FBF responses were during exercise were not changed, suggesting that endothelial dysfunction does not

play a predominant role in the exercise-mediated blood flow response to moderate, small-muscle mass exercise.

6.2 Introduction

Exercise hyperemia is closely matched to metabolism in the active skeletal muscle. As such, at moderate intensities, the increase in blood flow is directly related to vasodilation in the vascular bed (179), matching the O_2 supply with the required O_2 demand. This response is achieved through a complex interaction among mechanical, metabolic, and neural stimuli.

While the precise mechanism(s) contributing to the increase in blood flow remains speculative, exercise hyperemia is achieved through local regulation of the microvasculature, and feed arteries (180). Healthy aging has been shown to decrease limb blood flow during exercise (133, 181-183). Acute vitamin C infusion, however, mitigates the overall aging difference in exercise hyperemia, which has been confirmed to act through an improvement in nitric oxide (NO) availability (184). These findings suggest oxidative stress, may contribute to exercise-blood flow impairments.

Patients with chronic obstructive pulmonary disease (COPD) have several factors that may pose a risk for reduced blood flow regulation during exercise, including endothelial dysfunction (6, 7, 12), increased oxidative stress (8, 28), arterial stiffness (113, 185), and reduced physical activity levels (186). While a recent study showed improvement in endothelial function (by flow-mediated dilation) following an antioxidant intervention in COPD, it is unknown if these benefits would be extended to exercise hyperemia (12). It is alternatively possible that COPD patients may experience a reduction in exercise hyperemia due to augmented sympathetic vasoconstriction (5). Supplemental oxygen may be utilized by COPD patients to enhance O_2 delivery during dynamic exercise.

Experimentally, hyperoxia has been shown to transiently enhance muscle blood flow in young healthy individuals, secondary to a reduction in the sympathetic response (187). Alternatively, hyperoxic conditions may increase oxidative stress, thereby potentially reducing NO-availability (188).

The present study tested the hypothesis that patients with moderate-COPD would have reduced brachial blood flow responses to handgrip exercise, compared to healthy age-matched controls. Since vitamin C has previously been shown to improve exercise hyperemia in healthy older adults through an endothelial-dependent mechanism (133), we used this intervention to test if indeed, an improvement in NO-availability would increase hyperemia in COPD. We further hypothesized that COPD patients would exhibit the greatest increase in exercise blood flow during the combined hyperoxia + vitamin C condition. Using intravenous saline (sham-control) and vitamin C infusions (active-intervention) in all participants, we tested the aforementioned hypotheses using the following outcomes: 1) brachial blood flow during mild rhythmic handgrip exercise (normoxia and hyperoxia); and 2) vasodilator responses to flow- and nitroglycerine-mediated dilation.

6.3 Methods

6.3.1 Study participants

A total of 20 [10 COPD (67±3 yr); 10 controls (66±2 years)] volunteers completed the study. COPD patients were recruited from outpatient clinics in the Calgary area, and control participants were recruited from the community. Exclusion criteria included: current smoker, BMI > 35 $kg \cdot m^{-2}$, additional lung disease (including asthma), uncontrolled hypertension, current domicillary oxygen, history of: diabetes, cerebrovascular disease, myocardial infarction, angina, arrhythmia, valvular heart disease, chronic heart failure, peripheral arterial disease, chronic headaches/migraines, blood clots/thrombosis. COPD patients were required to have a past smoking history > 10 pack-years, and moderate-severe airflow obstruction (FEV_1/FVC <0.70; 50% ≤ FEV_1 ≤ 80% predicted) evident on spirometry. COPD patients must not have had an exacerbation within previous 8 weeks.

All participants provided written, informed consent and ethical approval was obtain from University of Calgary's Institutional Conjoint Health Research Ethics Board and the Health Protection Branch of Health Canada prior to commencement.

6.3.2 Experimental design

Study participants were required to visit the University of Calgary on two separate occasions. The first session consisted of a pulmonary function test, followed by a 6-minute walking distance test (6MWD). The main experimental session was conducted on the second visit, and involved a peripheral vascular assessment. Prior to the main experimental session, participants were instructed to refrain from eating/drinking (6 hours), caffeine (12

hours), vitamin supplements (72 hours), vigorous exercise (12 hours), blood pressure medication (24 hours), and COPD patients were asked to refrain from short- and long-acting bronchodilators (12 and 24 hours, respectively).

Upon arrival to the laboratory, subjects were seated for 10 minutes with their hand warmed (10 min at ~42^{o}C) prior to obtaining a capillary blood sample from the finger (ABL800 FLEX, Radiometer, Copenhagen, Denmark). An intravenous catheter was inserted in the non-dominant arm for delivery of either normal saline solution or vitamin C. All physiological measurements were obtained with the participant in the supine position, resting quietly in a dimly lit room. Briefly, subjects underwent a test of flow-mediated dilation (FMD), followed by rhythmic handgrip exercise (HG), during infusion of saline (sham-control). After a 30 min period of rest, the protocol was repeated with the infusion of vitamin C. Lastly, nitroglycerine (NTG) was administered to assess endothelial-independent dilation. An overview of the experimental protocol is provided in Figure 9 (*page 161*).

6.3.3 Measurements and Procedures

6.3.3.1 Maximal Voluntary Contraction (MVC)

Handgrip strength was assessed in the dominant hand using a strain gauge dynamometer (Lafayette 78010, IN, USA) and recorded as the greatest of three isometric contractions. Forearm volume was assessed using the water displacement method (189).

6.3.3.2 Dynamic handgrip exercise

Handgrip exercise (HG) was performed with a weighted pulley at an intensity of 10% MVC for 7 min. Audio feedback was used to coordinate a duty cycle of 20 contractions per minute (1:2 seconds; contraction-relaxation). Previous reports have shown this intensity effective in increasing blood flow, without an increase in heart rate, blood pressure, or brachial diameter (190). A face mask was fit over the nose and mouth to collect baseline end-tidal partial pressures of O_2 and CO_2 ($P_{ET_{O_2}}$ and $P_{ET_{CO_2}}$, respectively) for a period of 10 minutes using dedicated software (Chamber v2.26; University of Oxford Laboratory of Physiology, Oxford, UK). Expired P_{O_2} and P_{CO_2} were analyzed at 100Hz using mass spectrometry (AMIS 2000; Innovision, Odense, Denmark) via a fine catheter. Respiratory volumes were measured with a turbine and volume transducer (VMM-400; Interface Associates, Laguna Niguel, CA), and respiratory flow direction and timing were obtained with a pneumotachograph (RSS100-HR, Hans Rudolph, Kansas City, MO, USA). During HG, dynamic end-tidal forcing (64) was used to control $P_{ET_{CO_2}}$ and $P_{ET_{O_2}}$ during the first 5 minutes near baseline conditions ($P_{ET_{CO_2}}$ = +1.5 mmHg above rest, and $P_{ET_{O_2}}$ = 88.0 mmHg). After which, isocapnic hyperoxia was administered for the final 2 min of exercise ($P_{ET_{O_2}}$ = 300.0 mmHg).

6.3.3.3 Endothelial dependent and independent dilation

The technique of flow-mediated dilation (FMD) was employed to assess endothelial-dependent dilation, according to guidelines (191, 192). Briefly, a manual pneumatic cuff was rapidly inflated on the forearm to 250 mmHg for 5 min, after which the cuff was rapidly deflated to initiate a hyperemic blood flow response, measured in the

brachial artery (BA). Physiological variables were recorded for 60s at rest (BL), immediately before cuff inflation. Continuous measurements were resumed 30s prior to cuff release, and thereafter for 5min. Sublingual nitroglycerin (0.4 mg) was administered to assess endothelial-independent dilation. Baseline diameter of the brachial artery was recorded using 2D ultrasound for 60s prior to drug administration, and continuously for a 5min period thereafter.

6.3.3.4 Ascorbic acid infusion

Vitamin C (Ascorbic acid injection, Alveda Pharma, Canada) was diluted with normal saline, and administered intravenously. A loading dose of 3g ascorbic acid (200mg/min) was administered over 15 minutes, followed by a maintenance dose (40mg/min) during the experiment. This dosage of vitamin C has previously been shown to be effective at rapidly increasing plasma ascorbic acid concentrations more than 15 fold (128). Isotonic normal saline (0.9% NaCl) was infused at an identical flow-rate to ascorbic acid.

6.3.3.5 Cardiovascular measurements

Cardiovascular parameters were recorded to a personal computer at 100Hz, and signals were integrated using a data acquisition system (PowerLab 16/35, ADInstruments Inc., Colorado Springs, USA). Data was collected and stored offline for later analysis using accompanying software (LabChart 7.0 Pro; ADInstruments). Heart rate (HR) was determined by 3-lead electrocardiography (Micromon 7142B monitor; Kontron Keynes, UK), and finger pulse photoplethysmography was used to measure beat-beat blood

pressure (Portapress; TPD Biodemical Instrumentation, Amsterdam, The Netherlands). Arterial oxy-hemoglobin saturation (SpO_2) was determined by finger pulse oximetry (Model 3900; Datex-Ohmeda, Louisville, CO).

6.3.3.6 Vascular Doppler ultrasound measurements

A pulsed Doppler 4-MHz probe was fixed over the brachial artery, immediately superior to the antecubital fossa (4 MHz; Multigon Industries Inc., Yonkers, NY). Signal depth and power were adjusted to optimize the signal. The analog signal was integrated into the data acquisition system, allowing for continuous, beat-beat velocity measurements. A 12-MHz linear array ultrasound probe in B-mode was used to obtain brachial diameters (Vivid 7, GE; Milwaukee, WI). Video imaging was continuously recorded to a personal computer (GrabBee Software) and stored offline for later analysis.

6.3.4 Data Analysis

Diameters were analyzed at 30 Hz, using semi-automated edge-detecting software (Brachial Analyzer; Medical Imaging Applications, Coralville, Iowa, USA). The peak diameters (for FMD and NTG) were recorded as the highest 3-sec average (*i.e.*, 90 frames).

The mean blood flow velocity (MBV) was taken as a 60s average to reduce variance associated with contraction-relaxation cycles during handgrip exercise. Averages were taken at the end of the 5^{th} minute (normoxia), and at the end of the 7^{th} minute (hyperoxia). Forearm blood flow (FBF) and forearm vascular conductance (FVC) during exercise were calculated using the following equations:

$$FBF\ (ml \cdot min^{-1}) = MBV \times \pi r^2 \times 60$$

where MBV is mean brachial blood flow velocity ($cm \cdot s^{-1}$), r is the radius (cm). Forearm vascular conductance was calculated as:

$$FVC = FBF/MBP \cdot (100\ mmHg)^{-1}$$

and expressed as millilitres per minute per 100 mmHg. The FMD was expressed as percent change in vessel diameter, compared with baseline:

$$FMD\% = (BA_{peak\ diam} - BA_{BL\ diam})/(BA_{BL\ diam}) \times 100\%$$

Absolute FMD(mm) was also analyzed from the change in vessel diameter, as above.

6.3.5 Statistics

The Shapiro-Wilk test was used to confirm a normal distribution of data. Statistical comparisons between groups during Trial 1 (saline) were performed using a mixed 2x3 repeated- measures ANOVA to identify any physiological differences with exercise, under baseline-sham conditions. Significant main effects and interactions were further examined using t-tests. To assess the effect of the intervention [Trial 1 *vs.* Trial 2] during exercise, a within-group 2x3 repeated measures was performed [Baseline, HG, HG+O_2]. Where appropriate, t-tests were conducted to identify the effect of exercise (Rest *vs.* EX) and the effect of hyperoxia (EX *vs.* EX+O_2), and were corrected for multiple comparisons using

the Bonferroni correction method. All statistical analyses were performed using the statistical software SPSS version 20.0 (Chicago, IL). Data are presented as means ± SE. Significance was determined at $P \leq 0.05$.

6.4 Results

6.4.1 Study Participants

Ten patients with COPD and 10 healthy controls completed the study and were included in analysis. Four control subjects were excluded from analysis due to technical challenges encountered during the hyperoxic-exercise period. Two COPD patients had incomplete data for the NTG measurements due to an adverse reaction (acute headache). Subject characteristics are listed in Chapter Six Figures and Tables

Table 20 (*page 156*). Groups were well matched on age and BMI ($P > 0.05$). COPD patients had a shorter 6-minute walking distance, and a greater smoking history, than controls ($P \leq 0.05$). COPD and controls subjects had similar blood gases and hematological characteristics (Table 21, *page 158*). Blood measurements were not obtained in two participants.

6.4.2 Rhythmic handgrip exercise

6.4.2.1 Trial 1: Differences between COPD and controls during sham-saline exercise.

Physiological responses to 10% handgrip exercise resulted in a similar increase in absolute FBF, FVC, and HR between COPD patients and controls during the sham-saline condition (Table 22, *page 159*; and Figure 10, *page 162*; $P > 0.05$). Similarly, when expressed as a percent change from Rest, there was no difference in ΔFBF between COPD and controls (426 ± 100% *vs*. 550 ± 82%, respectively*; P* > 0.05). Hyperoxia increased SpO_2, and decreased HR (Table 22, *page 159*). There was no difference between normoxic and hyperoxic FBF or FVC during exercise, in either COPD or controls (Figure 11, *page 163;* and Table 22, *page 159*; $P > 0.05$).

6.4.2.2 Trial 2: Effect of vitamin C on exercise-blood flow

From Rest to EX, the increases in peripheral blood flow parameters (FBF, FVC) and cardiovascular indices (HR, MBP were not different from Trial 1 (Table 22, *page 159;* and Figure 10, *page 162*). No interaction effects were observed within either the COPD or Control group ($P > 0.05$). In controls only, hyperoxia (combined with vitamin C) reduced

FBF during exercise (Figure 10 *page 162*), and there was a trend for a reduction in FVC (P=0.092, after corrected for multiple comparisons) (Table 22, *page 159*). Similarly, as shown in Figure 11A (*page 163*), vitamin C decreased ΔFBF% in controls (vitamin C: -10.6 ± 5.1%; saline: +4.7 ± 4.5%; $P \leq 0.05$). Hyperoxia did not influence FBF or FVC in COPD patients. Similar to Trial 1, hyperoxia decreased HR and increased SpO_2 in both groups (Table 22, *page 159*).

6.4.3 Endothelial dependent- and -independent dilation

As shown in Figure 12 (*page 164*), FMD% response was not different between COPD and Controls during the sham-saline condition (6.0 ± 0.9% *vs.* 5.9 ± 1.0%, respectively). A main effect for the intervention [saline *vs.* vitamin C] was observed ($P = 0.013$), but no interaction ($P = 0.740$) or group effect ($P = 0.766$) were found. Table 23 (*page 160*) summarizes additional FMD endpoints. Nitroglycerine-mediated dilation, initiated similar changes between groups (COPD: 25.6 ± 1.6% *vs*. 23.5 ± 2.3%, $P > 0.05$). Similar changes were found between groups when comparing the absolute change in BA diameter with NTG (COPD: +0.85 ± 0.08 mm; controls: +0.85 ± 0.04 mm). No relationship was found between FMD% and exercise FBF (P>0.05).

6.5 Discussion

This study is the first to directly investigate the peripheral blood flow response to exercise (*i.e.*, moderate, rhythmic handgrip exercise; EX) in COPD patients, compared to healthy controls. We hypothesized that COPD patients would have a decreased blood flow response to exercise, which would be augmented by vitamin C. Secondly, we hypothesized that hyperoxia would enhance exercise blood flow in COPD patients. The main findings were that: 1) moderate-COPD patients have a similar exercise blood flow response to healthy older controls; 2) exercise blood flow responses are *not* improved by a high-dose vitamin C infusion (in either COPD patients or controls); 3) exercise blood flow in aging/COPD is independent of the improvement in endothelial function, as indicated by an augmented FMD response with vitamin C, but an unchanged exercise hyperemic response; 4) hyperoxia was not found to augment exercise blood flow response in either COPD or controls. Collectively, these findings provide evidence for an overall preserved vascular response during exercise in COPD patients.

6.5.1 Exercise-hyperemia in COPD patients

During moderate, 10% MVC steady-state handgrip exercise, FBF and FVC were similar between COPD patients and older controls. Patients and controls had similar MVC and forearm volume. Resting brachial blood flow in COPD patients was similar to resting forearm blood flow in controls (albeit greater, but non-significant). We selected a moderate EX challenge to minimize the systemic changes associated with whole-body exercise and investigate an "isolated" vascular response. Despite the modest challenge, HR significantly increased (~4 $beats \cdot min^{-1}$) to a similar magnitude in both groups. Contrary to

our hypothesis, vitamin C did not augment FBF or FVC in either COPD or control groups, indicating additional mechanisms involved in the regulation of exercise blood flow with aging/disease. While no previous study has investigated the role of vitamin C in muscle blood flow in COPD, a previous report suggests restorement of vascular endothelial redox balance (by vitamin C) in older adults, leading to increased exercise blood flow. These results are supported by a concomittent augmentation of ACh-mediated blood flow (133), which later confirmed that indeed, this improvement in endothelial function was mediated by increased NO derived from the NOS pathway (184). We are surprised that vitamin C did not improve muscle blood flow during exercise, particularly because FMD responses were augmented following vitamin C (discussed later). This provides additional evidence that skeletal muscle blood flow response is not solely mediated by NO-mechanisms. With aging, there is ~40% reduction in NO- and complete loss of prostaglandin- mediated contribution to exercise-related vasodilation (193). In general, evidence suggests the activation of redundant pathways contributing to exercise-hyperemia. Our findings of preserved exercise hyperemia in COPD are consistent with a previous study (194), despite different study designs utilizing different exercise challenges (*i.e.*, moderate *vs.* maximal exercise), and vascular segments (forearm *vs.* lower limb). It is likely that the difference in limbs specified for research study is not trivial, as important differences exist in both endothelial-dependent and –independent dilation between the forearm and lower limb vasculature (195).

A secondary outcome in our study was utilizing hyperoxia to reduce sympathetically-mediated vasoconstriction, which may limit blood flow in older adults, and in particular COPD patients. It is possible that hyperoxia exhibits diverging effects on

the vasculature. Firstly, hyperoxia has been shown to inhibit peripheral chemoreceptor outflow, and transiently increase muscle blood flow in younger humans (187). Secondly, hyperoxia may increase accumulation of free radicals, leading to increased oxidative stress and reduction of NO. During sham-saline condition, hyperoxia did not have a significant effect on FBF. It is likley that there would only be modest influence of the sympathetically-mediated constriction during a moderate exercise challenge, utilizing a small muscle mass (compared to cycling). Secondly, as we were interested in steady-state blood flow, it is possible that the transient increase in blood flow would have occurred with the immediate onset of hyperoxic-exercise (<60s), before returning to normoxic levels, as observed the aforementioned study (187). Our findings of a reduced exercise FBF response (~10%) during combined hyperoxia and vitamin C was contrary to our hypothesis. However, this may partially be explained by previous studies that have shown hyperoxia to reduce exercise blood flow, due to increased O_2 content (196, 197). The resulting interaction seen between hyperoxia and vitamin C is unclear, as it could be reasoned that these factors would act in opposition.

6.5.2 Endothelial function in COPD patients

There has been a developing interest in the investigation of vascular endothelial function in COPD in recent years. FMD is a widely accepted technique used to non-invasively measure endothelial function in humans. The conclusion of the FMD test is a measure of conduit artery vasomotion, brought about by hyperemic-induced shear stress on the vascular endothelium. Putative mechanisms suggests this response to be largely NO-dependent (198). Increased reactive oxygen species (ROS) may decrease NO-bioavailability, thereby attenuating the FMD response. We did not find COPD patients to

have reduced endothelial function, as determined by FMD, when compared to controls. Contrary to our findings, the general consensus appears to support the contention that COPD patients have lower vascular endothelial function, as assessed by FMD (6, 7, 12, 114). However, Maclay *et al*., (185) assessed peripheral vascular responses in COPD patients (and controls) using the technique of venous occlusion plethysmography, a measure of resistance arterial (arterioles) function. Blood flow responses to both endothelial-dependedent and -independent dilators were similar between COPD patients and matched controls, thereby refuting the hypothesis that COPD patients have systemic endothelial dysfunction. Collectively, these findings would suggest that heterogeneity exists within the vascular tree, as conduit artery relaxation is often found to be decreased, whereas, resistance vessels remain intact.

In our study, vitamin C was found to increase FMD in COPD and controls, alike, suggesting a ROS-mediated suppression of NO-availability, influencing arterial relaxation. We did not see an interaction effect of vitamin C, suggesting that the overall pathophysiology affecting COPD is similar to healthy older adults. Indeed, a decline in FMD with "Normal" aging is evident (>40-50 years), and has previously shown to be restored with vitamin C (128, 153), similar to our findings. However, results from a recent study (12) suggests COPD patients (FEV_1 = 55%) in fact do have decreased FMD, which are restored following an antioxidant intervention, thus indeed suggesting a ROS-related mechanism affecting vascular function in COPD. One plausible explanation for the discrepancy between our findings and those of Ives *et al*. (12) relates to the status of co-morbidities affecting patients. Our aim was to isolate the effect of COPD, thereby excluding patients with co-existing disease, whereas the previous did not.

6.5.3 Study limitations

A limitation of the current study is study by not employing a randomized design. To maximaize voluteer retention, and reduce day-to-day variability associated associated with the vascular measurements, we completed Trial 1 and Trial 2 on the same day. Repeated measurements of FMD have been shown to be reproducible after a period of 5-10 min, or until vessel diameter returns to baseline (199). Secondly, because of the persisting effects of systemic vitamin C administation, we were not permitted to randomize the Trials. Lastly, we do not have an indication of the basal vitamin C, nitric oxide or oxidative stress status of individuals.

6.6 Conclusions

To our knowledge, this is the first study to investigate exercise hyperemia in COPD patients, compared to controls during conditions of moderate, handgrip exercise. We furthermore testsed the effect of vitamin C on these response. Collectively, these results suggest that COPD patients have a preserved exercise blood flow responses, and endothelial function when compared to age-matched controls. Despite an improvement in endothelial function with vitamin C (as indicated by flow-mediated dilation), this age-related impairment does not appear to affect the peripheral blood flow response.

6.7 Chapter Six Figures and Tables

Table 20. Subject characteristics and pulmonary function measurements.

	COPD	Controls
Subjects (n)	10	10
Sex (M / F)	4 / 6	4 / 6
Age (yr)	67 ± 3	66 ± 2
Height (m)	1.65 ± 0.02	1.68 ± 0.09
Weight (kg)	69.0 ± 5.1	70.9 ± 3.9
BMI ($kg \cdot m^{-2}$)	25.3 ± 1.7	25.0 ± 0.9
Smoking history (pack yr)	45 ± 14*	5 ± 4
6MWD (m)	461 ± 21* (78 ± 5)*	639 ± 21 (103 ± 3)
MVC (kg)	34 ± 7	35 ± 5
10% MVC (kg)	2.9 ± 0.4	3.5 ± 0.5
Forearm volume (mL)	1261 ± 105	1337 ± 82
Lung Function		
FEV_1 (L)	1.51 ± 0.10* (60 ± 5)*	2.99 ± 0.28 (107 ± 4)
FEV_1 / FVC (%)	46 ± 3* (61 ± 5)*	79 ± 2 (105 ± 2)
FRC (L)	4.6 ± 0.3* (153 ± 7)*	3.3 ± 0.2 (108 ± 5)
RV (L)	3.7 ± 0.2* (169 ± 11)*	2.2 ± 0.1 (101 ± 5)
TLC (L)	6.8 ± 0.3 (123 ± 5)	5.9 ± 0.4 (101 ± 3)
D_LCO ($mL \cdot min^{-1}/mmHg$)	15.5 ± 2.3* (63 ± 9)*	25.6 ± 2.7 (98 ± 6)

Values are means ± SE; * Significantly different from controls ($P \leq 0.01$). ***Definition of abbreviations:*** BMI: body mass index; 6MWD: six-minute walking distance test; MVC: maximal voluntary contraction; FEV_1: forced expiratory volume in one-second; FVC: forced vital capacity; FRC: functional residual capcity; RV: residual volume; TLC: total lung capacity; D_LCO: diffusion capacity of carbon monoxide. *Parentheses* indicate % predicted, mean values.

Table 21. Resting hematological and oximetry parameters measured from arterialized capillary blood.

	COPD (n=9)	Controls (n=9)
P_cO_2 (mmHg)	66.3 ± 2.4	67.8 ± 1.9
P_cCO_2 (mmHg)	33.3 ± 1.2	35.9 ± 0.5
pH	7.45 ± 0.01	7.43 ± 0.01
[HCO_3^-] (mmol/L)	22.6 ± 0.8	23.1 ± 0.3
ctHb (g/dL)	15.3 ± 0.4	14.9 ± 0.4
Hct (%)	46.7 ± 1.2	45.5 ± 1.3
O_2Hb (%)	91.7 ± 0.8	92.4 ± 0.5

Values are means ± SE; * Significantly different from controls ($P \leq 0.05$). ***Definition of abbreviations:*** P_cO_2 : pressure of O_2 in capillary blood; P_cCO_2 : pressure of CO_2 in capillary blood; [HCO_3^-]: bicarbonate ion concentration; ctHb: total hemoglobin; Hct: hematocrit; O_2Hb: oxy-hemoglobin.

Table 22. Brachial vascular conductance and cardiovascular responses to moderate, rhythmic handgrip exercise before and after vitamin C infusion in COPD patients and healthy controls.

	Rest		10% HG EX		10% HG EX + O_2	
	Saline	Vit C	Saline	Vit C	Saline	Vit C
FVC (ml/min 100 mmHg)						
COPD	40±12	28±5	112±24*	94±19*	115±25	96±18
Control	25±8	30±7	94±17*	121±20	97±17	105±18
MBP (mmHg)						
COPD	99±2	102±4	103±3	108±5	102±4	110±5
Control	99±4	102±3	103±4	106±3	102±4	107±4
HR (beats/min)						
COPD	69±3	67±3	73±3*	73±3	71±3†	70±3†
Control	62±2	62±2	66±2*	65±2	63±2†	63±2†
SpO_2 (%)						
COPD	94±1	93±0	94±0§	94±0	99±0†	99±0†
Control	94±1	95±1	95±0*	95±0	99±0†	99±0†

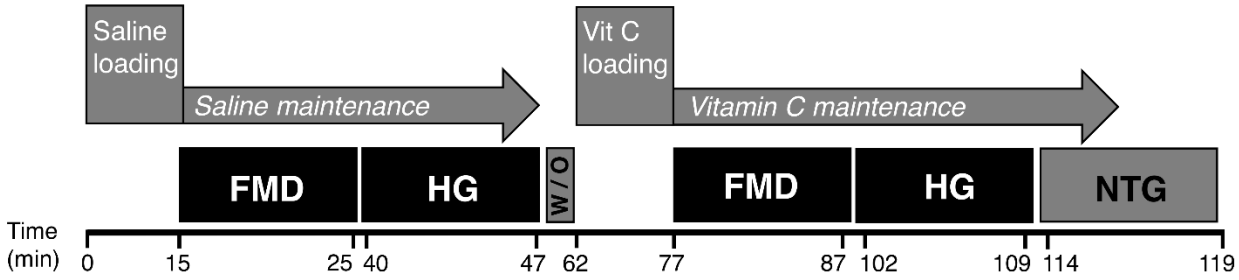
Definition of abbreviations: FVC: forearm vascular conductance; MBP: mean blood pressure; HR: heart rate; SpO_2: arterial oxy-hemoglobin saturation. *$P \leq 0.05$ vs. rest (within trial [*e.g., saline or vitamin C*]); †$P \leq 0.05$ vs. 10% HG EX (within trial [*e.g., saline or vitamin C*]; § $P \leq 0.05$ vs. controls [*same condition*]. *Values* are mean ± SE.

Table 23. Vascular responses to flow-mediated and nitroglycerine-mediated dilation in COPD and older healthy controls.

	COPD		Control		
	Saline	Vitamin C	Saline	Vitamin C	*P*
FMD Measurements					
Baseline (mm)	3.69 ± 0.16	3.52 ± 0.18	3.78 ± 0.23	3.62 ± 0.20	†
FMD (%)	6.0 ± 0.9	8.1 ± 1.3	5.9 ± 1.0	7.4 ± 0.8	†
FMD (Δmm)	0.22 ± 0.03	0.21 ± 0.03	0.27 ± 0.03	0.27 ± 0.03	†
NMD Measurement [a]					
Baseline (mm)	-	3.40 ± 0.2	-	3.68 ± 0.2	NS
NMD (%)	-	25.6 ± 1.6	-	23.5 ± 2.3	NS
NMD_{peak} (mm)	-	4.26 ± 0.16	-	4.53 ± 0.18	NS

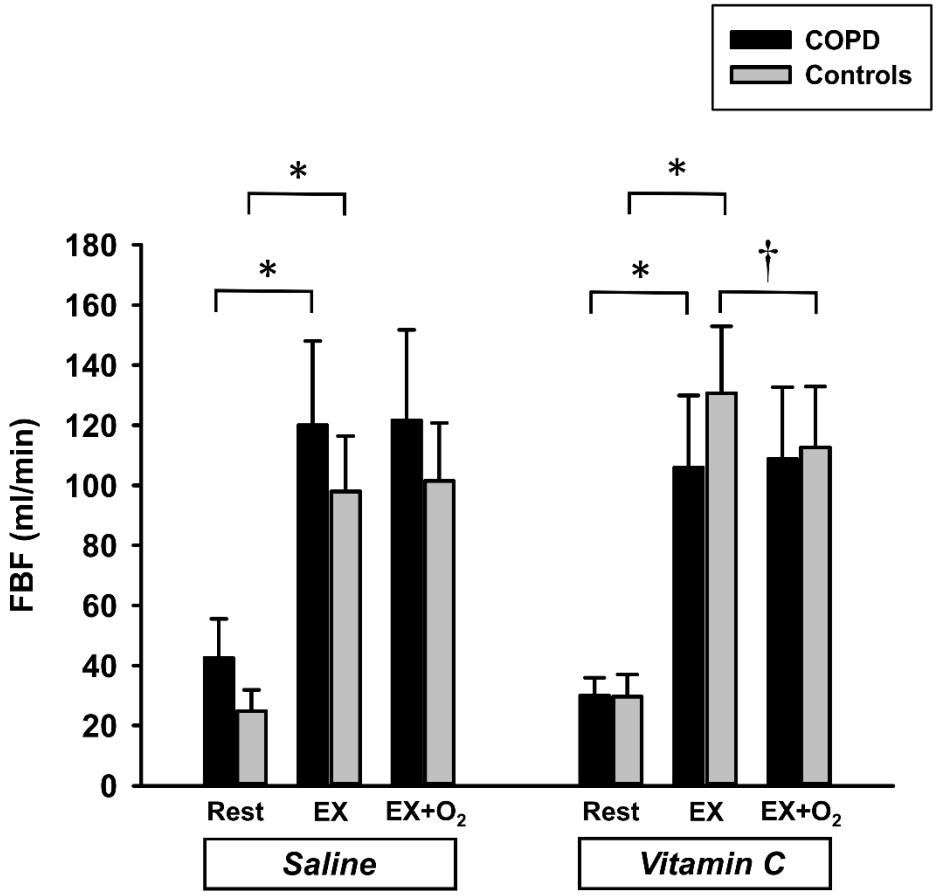
[a] COPD: n=8. ***Definition of abbreviations:*** FMD: flow-mediated dilation; NMD: nitroglycerine-mediated dilation. *Values* are mean ± SE. †P ≤ 0.05 main effect of intervention, NS non-significant.

Figure 9. Experimental design.



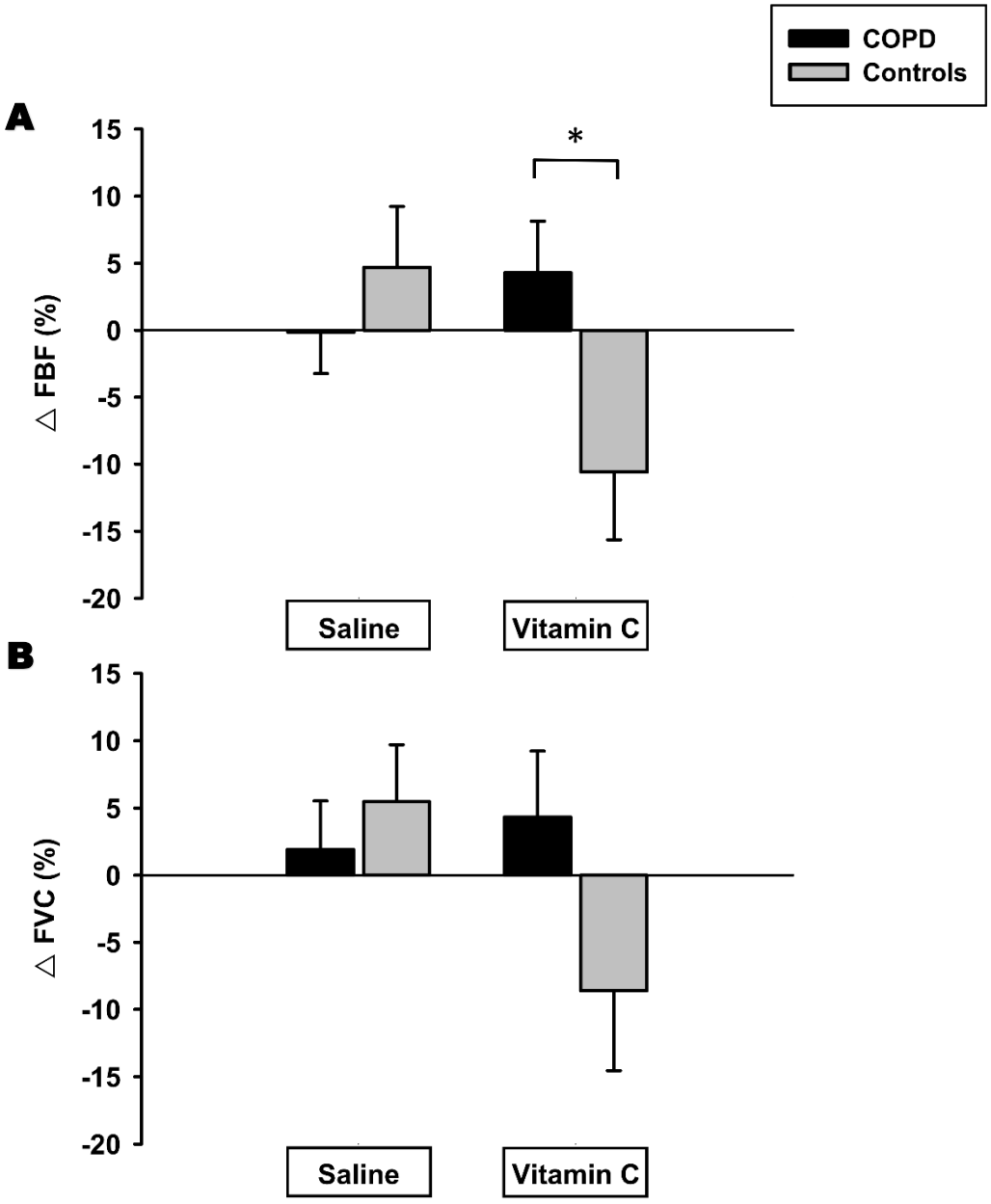
Footnote: All subjects completed a test of flow-mediated dilation (FMD) and handgrip exercise (HG). Participants first received saline, followed by a washout (w/o), and repeated the tests during a vitamin C infusion. Nitroglycerine (NTG) was administered after the completion of FMD and HG.

Figure 10. Forearm blood flow in COPD and healthy controls at rest and during moderate, handgrip exercise under conditions of sham-saline and vitamin C infusion.



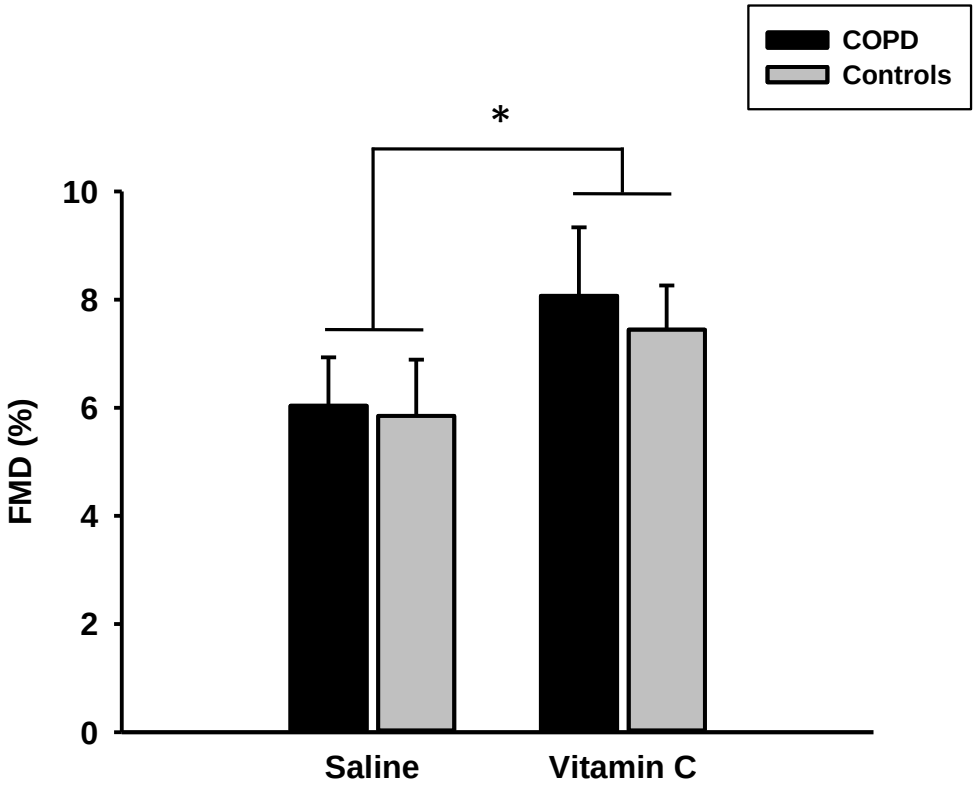
Footnote: FBF: Forearm blood flow; EX: handgrip exercise under normoxic conditions; $EX+O_2$: handgrip exercise under hyperoxic conditions. Data represent means ± SE. *$P \leq 0.05$ vs. rest (with-in condition). †$P = 0.056$ vs. EX (with-in condition).

Figure 11. Effect of hyperoxia on forearm blood flow and forearm vascular conductance during handgrip exercise in COPD patients and controls.



Footnote: Data represent the change in exercise (A) forearm blood flow (FBF), and (B) forearm vascular conductance (FVC) from conditions of normoxia to hyperoxia during saline and vitamin C infusion. Data represent means ± SE. *$P \leq 0.05$.

Figure 12. Flow mediated dilation response in COPD patients and controls.



Footnote: Flow-mediated responses in the brachial artery in COPD patients and healthy controls, before and after vitamin C infusion. Vitamin C significantly improves the dilatory response (main effect). Note no significant group or interaction effect. Data represent means ± SE. * $P \leq 0.05$.

Chapter Seven: **General Discussion**

7.1 Main Findings

The overall goal of this thesis was to gain a better understanding of the integrated-nature of the pathophysiology in COPD. When work on this thesis began, there was a large gap in the literature, and very little information was available regarding cerebrovascular physiology, and the integrity of the chemoreflex. With this in mind, the early studies (chapter 2 and 3) were designed to characterize the cerebrovascular and ventilatory responses, using modern techniques. Chapters 4 and 5 were follow-up studies (to chapter 2), with the aim of investigating the role of oxidative stress on the cerebrovascular and ventilatory responses to blood gas alterations, using an interventional approach. The final chapter (chapter 7) followed a similar theme, however, was an investigation of peripheral vascular function.

Chapter 2 set out to determine the relationship between cerebrovascular reactivity to CO_2 and oxidative stress in women with mild-moderate COPD. Previous studies have suggested an altered cerebrovascular regulation to CO_2 (21, 37, 39, 200). However, mechanisms contributing to this were unresolved. We postulated that increased oxidative stress would contribute to cerebrovascular dysfunction. The main findings of the study were that: 1) women with mild-moderate COPD had reduced cerebrovascular and ventilatory (trend) sensitivity to CO_2 compared to healthy older controls; 2) COPD patients had greater levels of systemic oxidative damage (AOPP, MDA, 8-OHdG), and lower NO metabolites; 3) controlling for oxidative stress markers eliminated the overall difference observed in the cerebrovascular sensitivity to CO_2 between groups, implying oxidative stress may play a role in decreased response to CO_2 in COPD patients.

Chapter 3 investigated the cerebrovascular responses to moderate cycling exercise. In healthy individuals, cerebral blood flow (CBF) increases during submaximal exercise, however, it is unknown whether COPD patients with known exercise limitations would have lower cerebrovascular responses. The main findings were 1) mild-moderate COPD patients have a preserved cerebrovascular response during moderate exercise (50% $\dot{V}O_{2\,peak}$); 2) at an absolute workload (30W), COPD patients have a higher CBF response compared to controls. These findings highlight the effect of low physical fitness on the cerebrovascular response in COPD patients. These findings may provide some insight into the cerebrovascular demands encountered during normal activities of daily living for COPD patients.

In chapters 4 and 5 we addressed the findings from the original study (chapter 2) that implicated oxidative stress in the cerebrovascular dysfunction encountered in moderate COPD patients. We attempted to follow-up this study with an acute antioxidant intervention (vitamin C) to acutely reduce oxidative stress, thereby increasing NO bio-availability. This was a novel approach when applied to the cerebrovascular circulation. Additionally, a promising study in healthy humans had provided evidence for an antioxidant intervention augmenting the ventilatory response to CO_2 (13). Our main findings in chapter 4 were: 1) vitamin C did not augment the cerebrovascular response in COPD patients, nor did it have an effect healthy individuals (either young or older adults); 2) vitamin C increased the ventilatory response to CO_2 only in COPD patients. These findings provide novel insight into the cerebrovascular and ventilatory regulation in health and COPD. These findings suggest that oxidative stress may restrain the activity of the central chemoreceptors, and that the breathing limitations in COPD may at least partially be mediated by the central-reflex.

Chapter 5 is also an extension of the original study (chapter 2). To gain a comprehensive understanding of the cerebrovascular and ventilatory responses in moderate COPD, we examined these responses during acute hypoxia. A similar hypotheses (to chapter 4) was formulated, stating that vitamin C would augment the cerebrovascular response to hypoxia. The main findings were 1) "healthy" aging decreased the cerebrovascular response to acute hypoxia; 2) COPD did not have additional influence on the cerebrovascular response to acute hypoxia; 2) vitamin C did not appear to play a role in either the ventilatory or cerebrovascular responses to acute hypoxia.

Chapter 6 is a study to provide insight into the peripheral vascular regulation during isolated exercise (*i.e.*,10% MVC handgrip), and to assess the effect of a vitamin C intervention on endothelial function. The main findings were 1) moderate COPD patients have a preserved peripheral blood flow response to exercise, and exhibit similar vascular endothelial function (as assessed by FMD) to healthy older individuals; 2) although vitamin C improved endothelial function, this did not translate to greater improvements in exercise blood flow, in either COPD or controls. These findings do not suggest COPD patients have altered blood flow responses during exercise, as previously shown. Secondly, although there was a general improvement in endothelial function (suggesting a role of decreased NO), this appears to be age-related, rather than specific to COPD.

7.2 New Mechanistic Implications

Collectively, the studies described provide several new lines of evidence for the role of oxidative stress in moderate-COPD patients. In general terms, it appears that moderate COPD do exhibit cerebrovascular dysfunction, and to some extent, a reduced ventilatory

response to CO_2. Figure 13 (*page 173*) provides an overview of the main findings, and the proposed mechanistic link within this investigation. Cerebrovascular dysfunction may be related to endothelial dysfunction, and a reduction of NO. In chapter 2, COPD patients have reduced NOx, increased OS, and reduced cerebrovascular response to CO_2. Conversely, in chapter 4, COPD patients do not exhibit cerebrovascular dysfunction, nor respond to vitamin C. This may be related to the basal levels of oxidative stress. It has been found (unpublished observations, X. Waltz and S.E. Hartmann) that the latter cohort of patients (in chapter 5) in fact has similar levels of OS to controls (MDA, AOPP), and similar levels of NOx. This may explain why these COPD patients did not have a reduction in the cerebrovascular response, compared to controls. By reducing the oxidant burden (via vitamin C intervention), endothelial function is improved, however cerebrovascular responses to either CO_2 or hypoxia, are not. Interestingly, despite similar levels of OS between COPD and controls, the ventilatory sensitivity to CO_2 in COPD patients increases following vitamin C. This provides a new understanding in the ventilatory control in COPD, suggesting that the central chemoreceptors are redox-sensitive. In agreement with previous studies, this interaction does not occur during hypoxia (170).

7.3 Clinical implications

Although technically demanding, quantifying the cerebrovascular and ventilatory chemosensitivity has important clinical meaning. Firstly, a high cerebrovascular sensitivity to CO_2 is typically observed in health. The increase in CBF with hypercapnia is determined by the microvascular functionality of the pial vessels. Decreased cerebrovascular responses to CO_2 has been observed in disease, with poor clinical

outcomes. For example, impaired cerebrovascular reactivity to CO_2 is a predictor of cognitive decline in Alzheimer disease (201). Severely impaired cerebrovascular reactivity (<20% increase in MCA velocity) predicts the risk of stroke and transient ischemic attack risk in patients with carotid artery stenosis and occlusion (202). Similarly, the CBF-CO_2 reactivity has been found to predict stroke events in individuals with internal carotid occlusion (203). While the risk of stroke is increased following an exacerbation in COPD (178), it remains unknown whether this is related to further impairment of cerebrovascular reactivity to CO_2.

The primary role of the chemosensors are to regulate ventilation during fluctuations of Pa_{O_2} and Pa_{CO_2}. However, chemoreflexes are additionally involved in determining the sympathetic drive, and as such, have a large influence on neural circulatory control. Activation of the chemoreflex increases heart rate, blood pressure, ventilation and blood pressure. An enhanced central chemoreflex has been observed in heart failure patients (204). Furthermore, combined increased chemosensitivity to hypoxia and hypercapnia is a prognostic risk factor for mortality in heart failure (205). It has been suggested that enhanced chemosensitvity may promote a pathological cycle, eliciting autonomic imbalance, neurohumoral activation, abnormal ventilatory responses, and arrhythmias (205).

In our first study, we found a slightly lower HCVR ($P = 0.07$), and in Study #4 the HCVR was similar to age-matched controls. We also found that COPD patients did not have an increased peripheral chemoreceptor response. However, previous studies have found an increased drive to breathe at rest, and during hypercapnia, as determined by $P_{0.1}$ and EMGd. Despite our findings of a relatively well preserved HCVR in COPD patients, it remains possible that this is masked by mechanical limitations. At the present time, only

one study exists in the literature providing direct measures of sympathetic nerve activity (SNA), by microneurography. These findings (5) suggest increased SNA in respiratory failure. Unfortunately, it is unknown at what severity of the disease the presentation of increased SNA becomes evident.

Our finding that only COPD patients increased the HCVR following vitamin C infusion is interesting, particularly because hypercapnic ventilation was similar between COPD and age-matched controls. Indeed, a remaining question surrounding this result is ultimately focused on the impact to the patient. Increasing the HCVR in flow-limited patients will likely be followed by increased dyspnea (27).

7.4 Limitations

As with most human physiological studies, subject recruitment remains a barrier to developing large scale studies. Further, incorporating clinical subjects adds additional complications. One limitation resides within the lack of smoking history of our control group. All subjects were screened for co-morbidities, to exclude potential confounding variables. While scientifically, this represents a strength, it conversely may underestimate the actual pathophysiological challenges within these patients.

Noticeable variation existed between our COPD subjects, and the "severity" did not always best predict a physiological response. Although FEV_1 is an independent predictor of CVD (and in its own right, has some predictive value), it is also a crude measure of functional status in COPD patients. Additionally, there is likely great variation between our physiological outcomes, and the different phenotypes of COPD (*i.e.*, bronchitis vs.

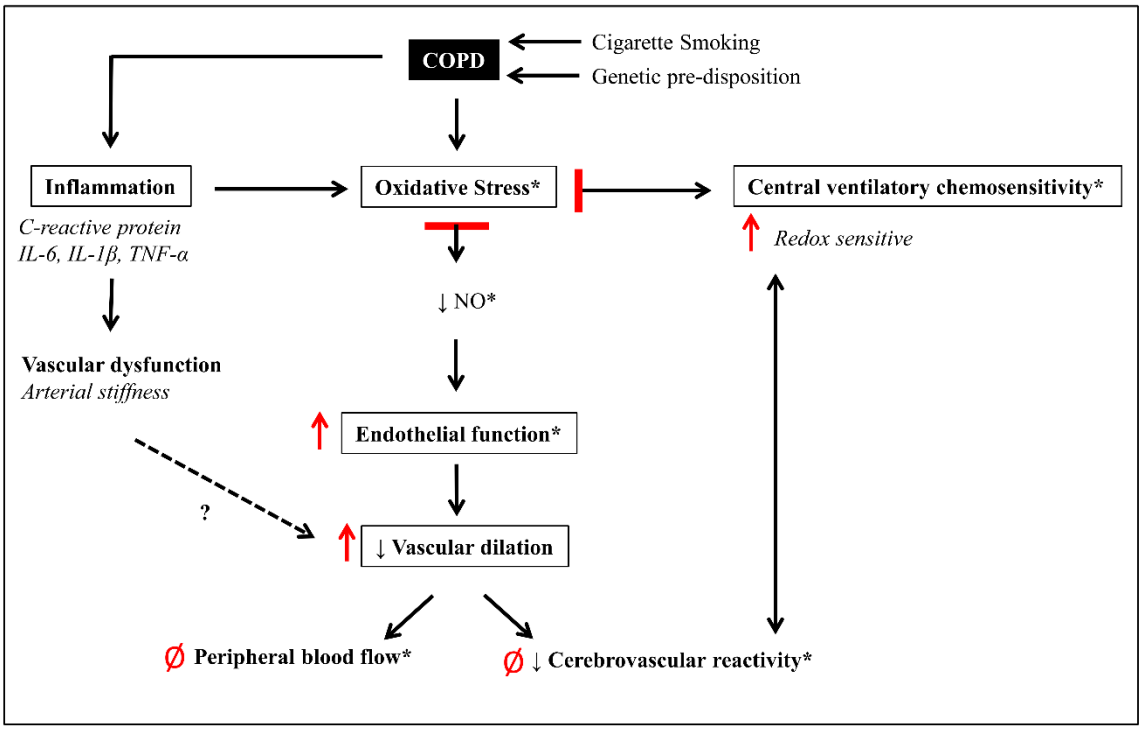
emphysema). Therefore, these two subtypes may have different cerebrovascular and ventilatory outcomes. This remains to be determined.

7.5 Future Directions

This collection of studies, and others, suggest some extent of vascular dysfunction in COPD patients. To date, very few studies have addressed this area. Additional pathways which have not received attention in the literature, include endothelial dysfunction in relation to endothelin-1, a vasoconstrictor, and the influence of chronic inflammation. Little is known regarding the systemic ET-1 response, and its effect in COPD patients. However, it has been suggested that patients that exhibit frequent desaturations have higher levels of ET-1 (206). This is an important observation that may explain the variability in the vascular responses (*i.e.*, desaturations vs. non-desaturations). Secondly, statin use has received notable attention for its effectiveness in reducing the inflammatory response in COPD patients, and reducing all-cause mortality (207, 208). This may additionally have added vascular health benefit.

7.6 Chapter Seven Figures and Tables

Figure 13. Main study findings: a mechanistic approach.



Footnote: The pathway linking oxidative stress and systemic disturbances in COPD. *Abbreviations*: * represent putative mechanisms, as supported by data from this thesis. Dashed lines represent possible interactions. Red text and symbols represent vitamin C intervention: *Arrows* represent direction of change, or *circle-dash* represent no change. Red bar represents the presumed site of action of the vitamin C intervention, on the target pathway(s).

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The citation is:

Hartmann, SE, Pialoux, V, Leigh, R, Poulin, MJ Decreased cerebrovascular response to CO2 in post-menopausal females with COPD: role of oxidative stress. Eur Respir J. 2012 40(6): 1354-61.

I have attached a permission request form, with this information included. Please notify me if you require additional information.

Sincerely,

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APPENDIX B: COEFFICIENT OF VARIATION AND RELIABILITY ANALYSIS

Table describing the coefficient of variation and intraclass correlations associated with repeated measurements of cerebrovascular and ventilatory responses to hypoxia and hypercapnia.

Reference	HCVR	HC_{CBF}	HVR	HX_{CBF}
Ainslie *et al.* (209)				
CV, %	7.9	-	17	-
Kolb *et al.* (210)				
CV, %	7.9	2.9	15.2	10.4
Spencer *et al.* (in preparation, 2015)				
CV, %	-	11.3	-	-
ICC	-	0.879	-	-
Beaudin *et al*. (doctoral thesis in preparation; 2015)				
CV, %	-	-	21	28
ICC	-	-	0.735	0.479

Footnote: CV: coefficient of variation; HC_{CBF}: hypercapnic cerebrovascular response; HCVR: hypercapnic ventilatory response; HVR: hypoxic ventilatory response; HX_{CBF}: hypoxic cerebrovascular response; ICC: intraclass correlation; Data in preparation: Spencer *et al*., 2015 (n=166) measures in healthy older adults (female 53%) 65±6 yrs. Two measurements were separated by six-months. Beaudin *et al*., 2015 (n=12) represents measures in healthy adults (33% female) aged 18-65 (mean: 50±10 yrs), two measurement were separated by ~19 days. Mean±SD.