THE MUSCLE METABOREFLEX DURING EXERCISE IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

by

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is characterised by deteriorating lung and airway function. Altered peripheral skeletal muscle properties, favouring glycolytic metabolism, are also well-documented in this population. Skeletal muscle properties such as those found in COPD patients may have significant effects on the magnitude of the muscle metaboreflex. **Hypotheses:** It was hypothesized that the muscle metaboreflex would be magnified in people with COPD compared to healthy controls, and that disease severity and exercise capacity would be correlated with the magnitude of the muscle metaboreflex. **Methods:** Eleven people with mild-to-severe COPD ($FEV_{1.0}$ = 56.3 ± 7.4% predicted) and 11 age- and gender- matched controls performed isometric handgrip exercise (IHG) for 2.5 minutes, at 35% MVC, followed by 2 minutes of post-exercise circulatory occlusion (PECO). Hemodynamic changes were measured throughout the protocol to assess the magnitude of the metaboreflex. Participants also performed a progressive cycle test to volitional exhaustion. **Results:** Heart rate, mean arterial pressure (MAP), leg blood flow and leg vascular resistance responses were similar between the COPD group and controls throughout IHG and PECO (%Δ from baseline) ($p > 0.05$). Heart rate was highest at minute 2.5 of IHG (COPD 18 ± 4%, control 18 ± 3%) and returned to baseline during PECO, while MAP peaked at minute 2.5 of IHG (COPD 29 ± 5%, control 30 ± 3%) and remained elevated throughout PECO (COPD 25 ± 3%, control 21 ± 2%). Total peripheral resistance rose more in the COPD group throughout the protocol and approached significance at minute 2 of PECO (COPD 39 ± 9, control 18 ± 4%, $p = 0.09$). Cardiac output remained significantly higher throughout IHG and PECO in the control group (IHG 2.5 min: COPD 0.08 ± 7, control 17 ± 4%, $p = 0.01$). There was no association between disease severity ($r = -0.22$, $p = 0.32$) or exercise capacity ($r = -0.02$, $p=0.92$) and the magnitude of the muscle metaboreflex. **Conclusions:** The muscle metaboreflex is preserved in

people with COPD. The mechanisms responsible remain unclear, however, unchanged upper limb skeletal muscle properties and desensitization of peripheral afferents to metabolites are plausible explanations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BMI = body mass index

COPD = chronic obstructive pulmonary disease

EFL = expiratory flow limitation

F_b = breathing frequency

$FEV_{1.0}$ = forced expiratory volume in 1 second

HF = high frequency band

HR = heart rate

HRV = heart rate variability

IHG = isometric handgrip exercise

LBF = leg blood flow

LF = low frequency band

LVR = leg vascular resistance

MAP = mean arterial pressure

MVC = maximal voluntary contraction

PECO = post-exercise circulatory occlusion

$PetCO_2$ = partial pressure of end-tidal carbon dioxide

Q = cardiac output

SaO_2 = arterial blood oxygen saturation

TPR = total peripheral resistance

VO_{2peak} = peak oxygen uptake

V_E = minute ventilation

V_T = tidal volume

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a progressive condition characterised by impaired lung and airway function. Eighty million people worldwide currently live with moderate-to-severe COPD, and the World Health Organization anticipates deaths from the disease will rise by almost 30% over the next 10 years (89). In 2005, 52,296 Canadians were diagnosed with COPD, costing the health care system approximately $5,178 per case (52). Pulmonary impairments account for 35% of deaths in this population, while cardiovascular disease and cancers account for another 48% (56). These statistics emphasize the need for effective smoking cessation and rehabilitation programs that work to improve quality of life and reduce the number of deaths caused by COPD and related co-morbidities. A more comprehensive understanding of the pathophysiology underlying this disease might contribute to improving the effectiveness of these rehabilitation programs.

Approximately 85% of COPD cases are caused by exposure to tobacco smoke, while 14% are caused by workplace irritants such as dust and fumes (89). A small number of cases, 1-2%, are caused by a genetic condition, alpha$_1$-antitrypsin deficiency, in which a deficiency in an elastase inhibitor causes elastic fibres of the lung to be broken down (41). Regardless of the cause, the symptoms experienced by people with COPD remain the same and include: fatigue, exertional dyspnea (breathlessness), excessive sputum production and chronic coughing (89). As COPD progresses, an individual's ability to perform low intensity exercise also declines, as is evidenced by lower maximal oxygen uptake values, and a stronger sensation of dyspnea at lower exercise intensities. This exercise intolerance often becomes the most pervasive symptom making activities of daily living (e.g. walking up stairs) challenging to perform (4).

Chronic obstructive pulmonary disease primarily affects the lungs and airways, thus, diagnosis and classification of disease severity is made based on pulmonary function; a forced

expiratory volume in 1 second ($FEV_{1.0}$) relative to forced vital capacity (FVC) of less than 70% post-bronchodilator is used to diagnose COPD, while disease severity is classified according to the percent predicted $FEV_{1.0}$ an individual achieves (55) (Table 1).

Table 1: Canadian Thoracic Society COPD classification. $FEV_{1.0}$ = forced expiratory volume in 1 second; % predicted = percent of predicted $FEV_{1.0}$ achieved according to age, gender and size adjusted normal values (9).

Classification	Criteria
Mild	$FEV_{1.0} \leq 80$ % predicted
Moderate	$FEV_{1.0}$ = 50-80 % predicted
Severe	$FEV_{1.0}$ = 30-50 % predicted
Very severe	$FEV_{1.0} \leq 30$ % predicted

Pulmonary Pathophysiology

There are two major subtypes of COPD, the first, chronic bronchitis, affects airways, while the second, emphysema, affects the lungs. Most people with COPD have a combination of the two (53). In the lungs, emphysema causes parenchymal damage leading to reductions in lung elastic recoil and increased alveolar dead space. In the airways, chronic bronchitis generates inflammation which causes airway remodelling and wall thickening (53). These altered airway properties, primarily in the peripheral airways, can contribute to exaggerated lumen diameter narrowing and consequently lead to expiratory flow limitation during dynamic exercise and even at rest in people with severe COPD (53; 58). Expiratory flow limitation, combined with reduced lung elastic recoil, limit the maximal expiratory flow rate an individual with COPD is able to achieve. During dynamic exercise, as ventilation increases to meet metabolic demands, ventilatory flow rates must also increase, making it difficult for people with COPD who have smaller maximal flow rates to empty their lungs sufficiently before commencing their next breath. Breathing at higher lung volumes, where there is less resistance

to flow, people with COPD are able to reduce their expiratory flow limitation (57; 59). Termed dynamic hyperinflation, this shift in the flow volume loop to higher lung volumes places the diaphragm in a position where the length-tension relationship is sub-optimal and the elastic workload on the respiratory muscles increases, ultimately leading to altered ventilatory mechanics and increased total work of breathing (12; 53).

Peripheral Skeletal Muscle Pathophysiology

While COPD is associated primarily with deteriorating lung and airway function, it is hypothesized that peripheral muscle changes also accompany the disease and contribute to exertional dyspnea and exercise intolerance (24; 25; 44-46; 87). Muscle biopsies taken from the vastus lateralis in people with COPD suggest oxidative enzyme activity and mitochondrial density is reduced and that fibre type distribution differs from that of healthy older adults. Specifically, the activity of 3-hydroxy-CoA dehydrogenase, citrate synthase and cytochrome oxidase, key enzymes of beta-oxidation, the citric acid cycle and the electron transport chain, respectively, are lower in people with COPD. Reduced concentrations of these enzymes is indicative of a reduced capacity of oxidative metabolism. An increased proportion of type-II glycolytic fibres and reduced proportion of type-I oxidative fibres are also well documented in the lower limbs of people with COPD. These changes are particularly noteworthy as they are opposite to the changes accompanying healthy ageing, where a greater proportion of type-I oxidative fibres is often present (25). Gosker et al. (24) measured hybrid type-I/IIA and type-IIA/IIB transition fibres in the vastus lateralis of people with COPD and healthy age-matched controls in an effort to explain the divergent fibre type distributions measured in these two populations**.** A larger proportion of transition fibres were present in the COPD group compared to controls suggesting COPD effects peripheral skeletal muscle properties and leads to fibre type conversion (type-I to type-I/IIA to type-IIA to type-IIA/IIB to type-IIB). These findings support

several other studies which have observed the presence of a greater proportion of glycolytic type-II fibres in people with COPD (24; 25; 71).

The phosphogen system relies on the breakdown of phosphocreatine to provide energy quickly at the onset of exercise and again during transitions to higher exercise intensities. Relative to healthy controls, people with COPD, rely more on the phosphogen system for energy provision and are slower to resynthesize phosphocreatine following a bout of sustained exercise (90). The consequence is an exaggerated accumulation of inorganic phosphates and lower intracellular pH in the working muscle of a person with COPD following high intensity exercise (90).

In contrast to the oxidative and phosphogen systems which might be compromised in people with COPD, the glycolytic system appears to be preserved. Concentrations of a number of key rate-limiting glycolytic enzymes such as phosphofructokinase, pyruvate dehydrogenase and lactate dehydrogenase are similar in people with COPD and healthy controls (24; 27; 46). Despite similar concentrations of these glycolytic enzymes, lactate production during exercise is exaggerated in people with COPD (46). Muscle biopsies were taken from the vastus lateralis of people with COPD and healthy controls during a stepwise exercise test on a cycle ergometer. Despite similar levels of lactate dehydrogenase and phosphofructokinase, the COPD group had a larger and "excessive" rise in blood lactate levels for a given exercise intensity compared to controls (46).

The effect reduced oxidative capacity, reduced phosphocreatine potential, and greater lactate production has on exercise tolerance in people with COPD is not well understood. However, significant associations between these skeletal muscle characteristics and reduced exercise tolerance have been made (25) including a positive correlation between citrate synthase concentrations and peak oxygen uptake (VO_{2peak}) ($r = 0.33$, $p = 0.006$) (45).

A number of mechanisms have been proposed to explain the changes in skeletal muscle properties highlighted above: 1) disuse, occurring as people with COPD avoid activities which elicit dyspnea, 2) malnutrition, the underlying cause remains unknown, but it is common in people with COPD and effects protein balance (17); 3) exposure to corticosteroid therapies, used to minimize pulmonary limitations (4) and; 4) chronic exposure to hypoxia and hypercapnea, resulting from the ventilation-perfusion mismatch and associated with impaired ventilatory mechanics (4). These mechanisms are not well understood, however, combined they contribute to changes in skeletal muscle properties which are hypothesized to contribute to the development of exercise intolerance in people with COPD.

The Muscle Metaboreflex

Three systems are thought to regulate the cardiovascular responses to voluntary exercise: central command, the arterial baroreflex and the pressor reflex (68; 81). The pressor reflex is comprised of two components: the mechanoreflex and the metaboreflex (or chemoreflex). While the mechanoreflex is elicited by mechanically sensitive group III afferents located peripherally in skeletal muscle, the muscle metaboreflex relies on chemically sensitive group IV afferents also located in skeletal muscle (67). A number of metabolites are involved in the initiation and control of the muscle metaboreflex during exercise including: lactate, hydrogen ions, potassium ions, analogues of ATP and interstitial phosphates (35; 51). Conceptually, the muscle metaboreflex aims to correct a mismatch between oxygen supply and demand and prevent excess metabolite accumulation in working tissues (42). There is some debate as to the effectiveness of the metaboreflex in restoring blood flow to working tissues (34). However, it is thought that to achieve homeostasis between blood supply and demand during exercise, initiation of the muscle metaboreflex, increases sympathetic nerve activity, and consequently, raises heart rate (HR), ventricular contractility, blood pressure and redistributes blood to active tissues away from

inactive tissues through vasoconstriction (68; 90). This response is termed "the pressor response".

To separate the effect the muscle metaboreflex has on the magnitude of the pressor response, independent from the effects induced by central command, the arterial baroreflex and the mechanoreflex, one can perform ischemic exercise (e.g. isometric handgrip exercise (IHG)), followed immediately by post-exercise circulatory occlusion (PECO) and cessation of handgripping. In this model, the by-products accumulated during IHG remain trapped in the arm during PECO (47). The build-up of metabolites from the IHG is sensed by group IV afferents prompting a rise HR, blood pressure and leg vascular resistance (LVR), while blood flow (LBF) to inactive tissues (in this case the legs) decreases. Increased cardiac output (Q) and ventricular contractility also occur as a part of the pressor response in healthy individuals (11).

The onset and magnitude of pressor response can be altered by the type and magnitude of metabolite accumulation. Using microdialysis of the vastus lateralis during quadriceps exercise in young healthy men, MacLean et al. (42) demonstrated that interstitial phosphates help initiate the pressor response, while muscle lactate concentrations establish the magnitude of the cardiovascular adjustments required. Fibre type distribution is a key determinant of skeletal muscle lactate concentrations and thus is relevant to the discussion of the muscle metaboreflex. Wilson et al. (88) applied chronic low-frequency stimulation to a rabbit's primarily glycolytic gastrocnemius muscle and elicited an increase in the proportion of type-I fibres and up-regulated oxidative enzyme activity. When the muscle metaboreflex was generated by the newly oxidative muscle, the magnitude of the pressor response was lower than the response elicited in the glycolytic control leg. Reliance on anaerobic metabolism, and specifically glycolytic type-II fibres for energy provision during exercise, is therefore a major determinant of the magnitude of the pressor response (as measured by changes in HR, blood pressure, blood flow and vascular resistance). In COPD, where production of lactate during exercise is "excessive" and oxidative

capacity is reduced (17), the metaboreflex may be magnified and exaggerating the cardiovascular response to exercise through redistribution of blood flow.

Insight into cardiovascular control in people COPD can be obtained from research examining the pressor response in heart failure patients. Altered skeletal muscle properties accompany the central hemodynamic changes that characterize heart failure. Not unlike COPD, people with heart failure exhibit a larger proportion of type-II fibres, less mitochondrial enzyme activity, rapid depletion of phosphocreatine during exercise and an overall reduced oxidative capacity (63). An exaggerated muscle metaboreflex response during handgrip exercise has been measured in heart failure (54). The similarities between the skeletal muscle properties of people with COPD and people with heart failure suggest an exaggerated pressor response, caused by skeletal muscle abnormalities, may also occur in COPD. In both populations these skeletal muscle decrements are negatively correlated with overall exercise capacity (26). To date, one study has examined the muscle metaboreflex response in people with COPD. Using IHG, Roseguini et al. (66) found HR and blood pressure responses were similar between people with COPD and healthy age-matched controls. They also found reduced calf blood flow during PECO in controls while the COPD group showed no change. These findings seem unexpected considering lactate accumulation is known to be greater in people with COPD and that lactate concentrations are partly responsible for determining the magnitude of the muscle metaboreflex (42; 83). The authors of this study did not examine the relationship between disease severity and exercise capacity on the magnitude of the pressor response. However, these are relevant relationships to consider, as reduced lung function is associated with the percentage of glycolytic type-II fibres and consequently reliance on anaerobic metabolism (25). Furthermore, exercise capacity, as a measure of exercise tolerance, if correlated with the magnitude of the muscle metaboreflex, may provide insight into the effect the pressor response has on people with COPD during whole body exercise.

OBJECTIVES & HYPOTHESES

Objectives

There were two objectives of this study: 1) To compare the muscle metaboreflex response in people with mild-to-severe COPD to that of healthy age- and gender- matched controls through measurement of heart rate, mean arterial pressure (MAP), cardiac output, total peripheral resistance (TPR), leg blood flow, and leg vascular resistance (LVR) during isometric handgrip exercise and post-exercise circulatory occlusion. 2) To assess the relationship between exercise capacity and disease severity and the magnitude of the muscle metaboreflex response.

Hypotheses

From this, two specific hypotheses were proposed and tested: 1) It was hypothesised that following isometric handgrip exercise, individuals with COPD, when compared with healthy age-matched controls, would demonstrate a greater magnitude muscle metaboreflex response, as indicated by a larger increase in heart rate, mean arterial pressure, leg vascular resistance and decrease in leg blood flow during post-exercise circulatory occlusion. 2) It was hypothesized that reduced exercise capacity and greater disease severity would be correlated with a larger muscle metaboreflex response.

METHODS

This study received ethical approval from the University of British Columbia clinical research ethics board and written informed consent was obtained from all participants. All testing was performed at the Vancouver General Hospital Pulmonary Function Laboratory and progressive exercise tests were supervised by a respirologist.

Participants

Eleven individuals diagnosed with mild-to-severe COPD (7 women) and 11 healthy age- and gender- matched controls participated in this study. Classification of the individuals with COPD was set according to the Canadian Thoracic Society criteria for % predicted $FEV_{1.0}$ (56) (Table 1). Individuals with COPD were clinically stable and had resting oxygen saturation (SaO_2) greater than 90% while breathing room air. Exclusion criteria for the COPD group included major underlying medical conditions such as heart failure, peripheral vascular disease, neuromuscular conditions, cancer and autonomic conditions. Participants took their medications as prescribed prior to testing (typical medications included bronchodilators, corticosteroids, β_2-sympathomimetics, theophylline and other respiratory medications). The healthy control group consisted of 11 age- and gender- matched participants (± 5 years) who demonstrated normal age-predicted respiratory function and were free of neurologic, immune and lower limb orthopaedic conditions as well as those conditions listed as exclusion for the COPD group.

Experimental Protocol

Upon arrival to the laboratory anthropometric measures and pulmonary function tests were performed. After these initial assessments, participants lay supine in a dark room for 10 minutes while heart rate variability (HRV) was assessed. Participants then resumed a seated

position before resting blood pressure was measured. Two maximal voluntary handgrip manoeuvres were performed by the right arm and held for 5 seconds to determine maximum grip strength (MVC). Two participants were left handed, however, grip strength was similar between hands and thus these participants were tested on the right side. Four minutes of resting HR, MAP, Q, TPR, LBF and LVR were collected and averaged to establish resting baseline values. These measures were taken continuously throughout the remainder of the protocol. Following this baseline period, participants performed IHG for 2.5 minutes at 35% MVC. During the IHG participants were instructed to avoid valsalva manoeuvres and breath holds and were provided with visual feedback on their gripping intensity as well as verbal encouragement. At 2.5 minutes the PECO cuff was inflated (DE Hokanson Inc, Bellevue, WA, USA). Participants continued IHG for another 10 seconds to ensure full occlusion had been attained before relaxing. The occlusion cuff remained inflated for 2 minutes after which it was deflated and the hemodynamic measures continued to be monitored at rest for another 4 minutes.

The variables of interest, HR, MAP, Q, TPR, LBF, LVR were measured continuously throughout the protocol and averaged over 1 minute intervals and compared to the baseline average obtained for each participant.

Following measurement of the muscle metaboreflex, exercise capacity was assessed using a symptom limited progressive cycle exercise test to volitional exhaustion.

Measurements and Procedures

Anthropometric Measures: Age, height, weight and body mass index (BMI) were collected for all participants. The COPD participants completed the Medical Research Council dyspnea scale (5) to rate their subjective feeling of breathlessness during daily activities as an indicator of their level of disability (Table 2).

Table 2: The Medical Research Council dyspnea scale used to assess functional capacity of people with COPD. Participants with COPD self-reported where they fell on the scale.

Grade	Description
1 (mild)	Not troubled by breathlessness except with strenuous exercise
2 (mild)	Troubled by shortness of breath when hurrying on the level or walking up a slight hill
3 (moderate)	Walks slower than contemporaries on the level because of breathlessness, or has to stop for breath when walking at own pace on the level
4 (moderate)	Stops for breath after about 100 m or after a few minutes on the level
5 (severe)	Too breathless to leave the house, or breathless when dressing or undressing

Pulmonary Function Testing: Pulmonary function was assessed using standard spirometry guidelines set by the American Thoracic Society (50). Participants performed 3 FVC manoeuvres on the spirometer (Vmax Series 2130 Spirometer, SensorMedics Corporation, California, USA) which interfaced with a computer running Vmax software. Measured parameters included FVC, $FEV_{1.0}$ and the ratio of $FEV_{1.0}$ to FVC ($FEV_{1.0}$/FVC). The highest recorded values were taken for each measure (9). These values were used to assess disease severity in the COPD group, and established normal respiratory function in the control group.

Heart Rate Variability: Heart rate variability was measured in order to provide insight into differences between the groups in tonic baseline autonomic function (61). Participants lay supine for 10 minutes in a dark room and were asked to match their breathing duty cycle to a metronome to maintain 12 breaths per minute to control for the effects of respiratory sinus arrhythmias (91). Heart rate variability was sampled and recorded at a rate of 1 kHz using 1-lead

electrocardiogram and an analog to digital converter (Powerlab/165P model ML795, ADInstrument Colorado springs, CO). Five minutes of stable continuous data was used in the assessment of HRV. Calculations were performed on normal R-R intervals. The square root of the mean of the square differences between NN intervals (rMSSD) was analyzed in the time domain. This measure reflects instantaneous HR and vagal tone. RMSSD is often used in clinical populations as it is more stable than other time domain indices. In the frequency domain analysis, low frequency (LF) (0.04 – 0.15 Hz) and high frequency (HF) (0.15-0.4 Hz) were calculated using Fast Fourier Transformation and reported in standardized units (nu). These variables describe oscillations of the HR signal based on differences in frequency and amplitude. The LF band is mediated by parasympathetic-sympathetic influences, while the HF band is thought to be mediated solely by parasympathetic components. LF/HF is the global measure of sympatho-vagal balance (1).

Muscle Metaboreflex: Heart rate and MAP were measured beat-by-beat using finger photoplethysmography on the non-exercising arm (left) (Finometer, FMS, Finapress Medical Systems BV, Arnhem, The Netherlands). The photoplethysmograph cuff was placed on middle digit on the middle phalynx. The blood pressure values attained were calibrated to the initial blood pressure measurement taken at rest (BPM-100, VSM Medtech Ltd., Vancouver, Canada). Cardiac output and TPR were derived from the photoplethysmograph based on a three-element Windkessel model of arterial input impedance.

Leg blood flow and LVR were assessed using a Doppler ultrasound (Sonos 5500, Philips Electronics, Andover MA) at the femoral artery. All measures were performed and analysed by the same investigator. Using an 11-3L linear transducer placed 2-3 cm distal to the inguinal ligament the time-averaged velocity mean was recorded. This landmark was used because it is easily accessible and is a site at which turbulent flow is minimized (64). Blood velocities (cm/s)

were calculated online approximately 6-9 times every minute and averaged over 1 minute intervals during offline analysis from the VHS recording. On-screen callipers were used to determine the two-dimensional femoral artery cross-sectional diameter following recovery to calculate femoral artery area (πr^2). Blood flow (L/min) was derived from the product of blood velocity and femoral artery area ((cm/s x cm^2 x 60)/1000). Leg vascular resistance was calculated as the quotient of LBF and MAP (MAP/LBF).

Exercise to Exhaustion: Participants performed cycle exercise on an electrically braked cycle ergometer (Ergoselect 100P, Ergoline, Lindenstrasse 5, Germany) to determine VO_{2peak}. Prior to commencing the exercise test, participants were equipped with a 12-lead electrocardiogram (Mac 5000 Resting ECG Analysis System, GE Systems Information Technologies, Wisconsin, USA). Participants then sat on the bike for 5 minutes while resting data was collected on ventilatory parameters. Each participant began their exercise test at either 5, 10, 15 or 20 watts and the workload increased by the same starting interval every minute until volitional exhaustion. Workload was chosen by the investigator to try to achieve a test lasting between 7 and 12 minutes. All but one COPD participant began at either 5 or 10 watts. Metabolic and ventilatory responses were collected breath-by-breath using open circuit spirometry (Vmax Series V6200 Autobox, SensorMedics Corporation, California, USA). At the end of each minute-long stage, participants were asked to rate their sensation of breathlessness and leg fatigue using the modified Borg scale (Table 3). Arterial blood oxygen saturation was determined using pulse oximetry (OXIMAX N-595 Pulse Oximeter, Nellcor Puritan Bennett Inc, California, USA). Peak HR, minute ventilation (V_E), breathing frequency (F_b), tidal volume (V_T) and partial pressure of end-tidal carbon dioxide ($PetCO_2$) were assessed breath-by-breath throughout exercise. Peak values were based on 30 second averages corresponding to VO_{2peak}. Upon

completion of the exercise test participants were asked whether they stopped exercise due to breathlessness, leg fatigue, or both their legs and breathing.

Table 3: The modified Borg scale used to assess breathlessness and leg fatigue each minute of the progressive exercise test to volitional exhaustion.

Scale	Severity
0	Nothing at all
0.5	Very, very slight (just noticeable)
1	Very slight
2	Slight
3	Moderate
4	Somewhat severe
5	Severe
6	
7	Very severe
8	
9	Very, very severe (almost maximal)
10	Maximal

Data Analysis and Statistics

Statistical software (SPSS 17, SPSS Inc, Chicago, Illinois, USA) was used to measure differences across groups by time. Independent samples t-tests were performed to identify differences in group characteristics. The variables of the pressor response, HR, MAP, Q, TPR, LBF, LVR were assessed using a 2x7 mixed model ANOVA procedure to examine the relationship between group (control and COPD) and time (Baseline: BL; Handgrip: H1, H2, H2.5; Occlusion: O1, O2; Recovery: R2) on these variables. All variables were examined using the percentage change from baseline values (%Δ) for each individual. A Tukey's HSD post-hoc analysis was used when a significant F-value was obtained to determine which means were significantly different from each other. In addition, a Pearson product-moment correlation was performed to assess the relationship between exercise capacity (VO_{2peak}) and disease severity (% predicted $FEV_{1.0}$) and the pressor response using the value attained at the second minute of

PECO (O2) for percent change in MAP from baseline (%ΔMAP). Statistical significance was satisfied at $p < 0.05$. Data are presented as mean ± standard error.

RESULTS

Participant Characteristics

Participant characteristics are presented as means ± SE for each group (COPD and control) and provided in table 4. Age, height, weight and BMI were not significantly different between groups ($p > 0.05$). The COPD group had moderate airflow obstruction with a mean % predicted $FEV_{1.0}$ of 56.3 ± 7.4%. Two participants were diagnosed by their physician as having COPD, however, spirometry indicated they only had mild obstruction (% predicted $FEV_{1.0}$ = 94% and 95% post-bronchodilator). When removed from the mean data, % predicted $FEV_{1.0}$ was 47.8 ± 5.8%, characterizing the remainder of the group as severe. The Medical Research Council dyspnea scores which provide prognostic information about the COPD group ranged from 1-4 on the 5-point scale with a mean score of 2.5. Characteristics for male and female participants are provided in table 5.

Table 4: Descriptive characteristics and resting pulmonary function. BMI = body mass index; MRC = Medical Research Council dyspnea scale; $FEV_{1.0}$ = forced expired volume in 1 second; FVC = forced vital capacity. * Significantly different at $p < 0.05$.

	COPD (n=11)	Controls (n=11)
Age (years)	66 ± 3	64 ± 4
Height (cm)	164.7 ± 3.3	167.5 ± 3.0
Weight (kg)	64.6 ± 4.6	68.2 ± 3.9
BMI (kg/m^2)	23.9 ± 1.7	24.2 ± 0.9
MRC dyspnea scale (1-5)	2.5 (1-4)	NA
$FEV_{1.0}$ (L)	1.4 ± 0.2	2.7 ± 0.2*
$FEV_{1.0}$ (% predicted)	56.3 ± 7.4	97.3 ± 3.0*
FVC (L)	2.9 ± 0.3	3.8 ± 0.3*
FVC (% predicted)	84.5 ± 4.7	107.1 ± 3.1*
$FEV_{1.0}$/FVC (%)	50.4 ± 5.1	70.9 ± 2.0*

Table 5: Descriptive characteristics and resting pulmonary function of men and women. BMI = body mass index; MRC = Medical Research Council dyspnea scale; $FEV_{1.0}$ = forced expired volume in 1 second; FVC = forced vital capacity.

	COPD Male (n=4)	COPD Female (n=7)	Control Male (n=4)	Control Female (n=7)
Age (years)	61 ± 6	69 ± 1	59 ± 6	66 ± 2
Height (cm)	177.6 ± 2.7	157.3 ± 1.3	177.4 ± 3.2	161.8 ± 2.6
Weight (kg)	70.7 ± 10.5	61.0 ± 4.4	81.8 ± 4.8	60.4 ± 2.2
BMI (kg/m^2)	22.4 ± 3.4	24.8 ± 2.0	26.0 ± 1.4	23.2 ± 1.1
MRC dyspnea scale (1-5)	2.8	2.3		
$FEV_{1.0}$ (L)	1.6 ± 0.4	1.3 ± 0.2	3.59 ± 0.2	2.2 ± 0.1
$FEV_{1.0}$ (% predicted)	44.0 ± 11.9	63.3 ± 8.9	97.0 ± 4.2	97.4 ± 4.4
FVC (L)	3.7 ± 0.2	2.4 ± 0.2	4.9 ± 0.3	3.2 ± 0.1
FVC (% predicted)	78.5 ± 0.6	87.9 ± 7.2	103.5 ± 3.9	109.1 ± 4.3
$FEV_{1.0}$/FVC (%)	44.0 ± 12.0	54.0 ± 4.5	73.5 ± 1.3	69.4 ± 3.1

Heart Rate Variability

Table 6 provides the group means for the variables measured to reflect HRV. One participant from the COPD group was eliminated due to excessive noise in the signal. The removed participant's age matched control was also eliminated as he had difficulty breathing to the metronome and had to breathe at his own rate. This participant's data was excluded in the analysis as variations in breathing patterns can modify the LF/HF relationship (22). In the time domain analysis, rMSSD was similar between groups ($p = 0.23$). In the frequency domain, LF, HF and LF/HF approached, but did not reach statistical significance ($p = 0.08$, 0.08 and 0.09 respectively), however, the COPD group demonstrated lower values for the LF band (COPD 35.1 ± 4.5, control 50.7 ± 6.9 nu), and larger values for the HF band (COPD 64.9 ± 4.6, control 49.3 ± 6.9 nu), reflecting a larger sympathetic contribution to the parasympathetic–sympathetic balance in people with COPD (1). Finally, a lower LF/HF ratio was measured in the COPD group (COPD 0.6 ± 0.1, control 1.8 ± 0.6), reflecting reduced HRV and elevated sympathetic activity which is associated in the literature with smoking, and increased morbidity and mortality (28).

Table 6: Heart rate variability parameters. rMSSD = square root of the mean of the squares of differences between adjacent NN intervals, LF= low frequency band, HF= high frequency band.

	COPD (n=11)	Controls (n=11)	p-value
rMSSD (ms)	49.6 ± 19.6	24.2 ± 4.6	0.23
LF (nu)	35.1 ± 4.6	50.7 ± 6.9	0.08
HF (nu)	64.9 ± 4.6	49.3 ± 6.9	0.08
LF/HF	0.6 ± 0.1	1.8 ± 0.6	0.09

Exercise to Exhaustion

Resting and peak exercise data are displayed in table 7. At rest, the COPD group had significantly higher V_E (COPD 13.0 ± 1.1, control 9.8 ± 0.7 L/min, p = 0.03) and F_b (COPD 18 ± 1, control 14 ± 1 breaths/min, p = 0.04). The COPD group reached a significantly lower peak work rate (COPD 64.5 ± 9.6, control 166.6 ± 21.2 W, p < 0.001) and VO_{2peak} (COPD 18.1 ± 1.6, control 32.4 ± 2.9 ml/kg/min, p < 0.001) than the control group. Exercise duration between the two groups was similar (COPD 8.2 ± 0.8, control 9.3 ± 0.9 min, p = 0.15) as each participant's workload increments were chosen prior to exercise to ensure an exercise duration between 7-12 minutes (i.e. 5, 10, 15, 20 watts). Peak HR was lower in the COPD group (COPD 126 ± 7, control 155 ± 4 bpm, p = 0.42). Peak V_E and V_T were also significantly lower in the COPD group (V_E: COPD 46.0 ± 5.9, control 78.5 ± 28.7 L/min, p = 0.006; V_T: COPD 1.37 ± 0.12, control 2.26 ± 0.20 L, p < 0.001). The participants were asked at the end of the exercise test why they stopped exercise (breathlessness, leg fatigue, or legs and breathing). In the COPD group, 7 answered breathlessness, 2 leg fatigue, and 2 both legs and breathing, while in the control group the responses were 5, 3 and 3 respectively.

Table 7: Rest and peak exercise values during the progressive cycle ergometry test to volitional exhaustion. HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; SaO_2 = arterial oxygen saturation; $PetCO_2$ = partial pressure of end-tidal carbon dioxide; V_E = minute ventilation; V_T = tidal volume; F_b = breathing frequency; VO_{2peak} = peak oxygen uptake; W = workload; RER = respiratory exchange ratio; Dyspnea = dyspnea score on modified Borg scale at peak exercise; Leg fatigue = score on modified Borg scale for leg fatigue at peak exercise

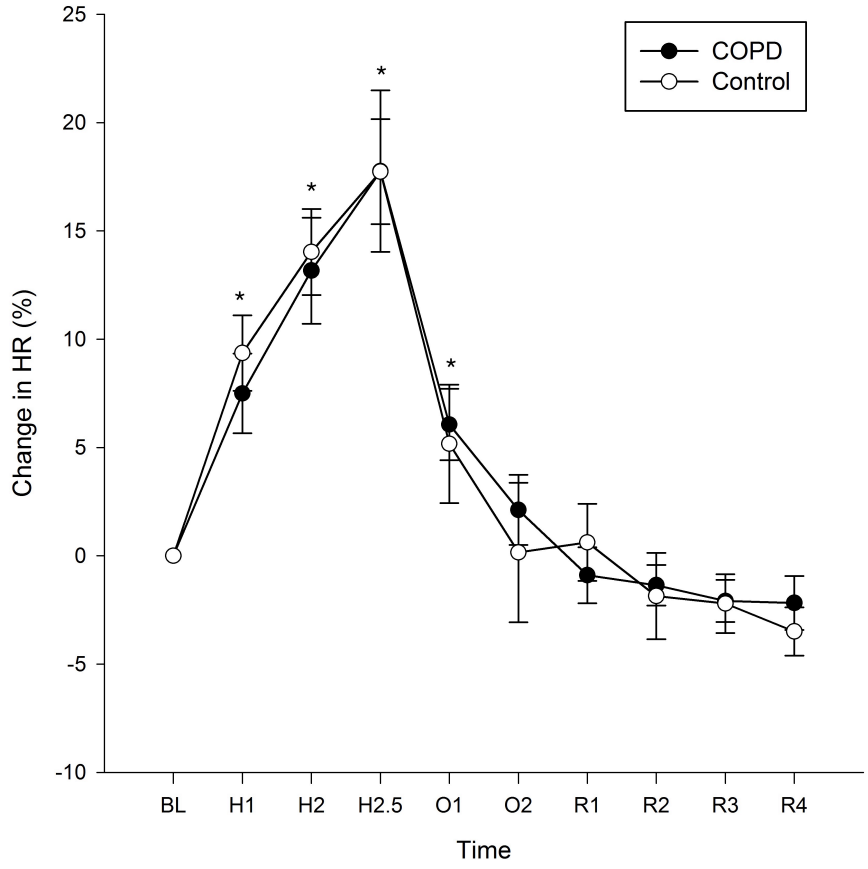
	COPD (n=11)	Controls (n=11)
Resting Values		
HR (bpm)	66 ± 3	63 ± 3
SBP (mmHg)	129.6 ± 4.4	121.2 ± 4.2
DBP (mmHg)	73.0 ± 2.3	72.1 ± 3.6
MAP (mmHg)	91.9 ± 2.5	88.5 ± 3.6
SaO_2 (%)	97.5 ± 0.3	98.8 ± 0.2
$PetCO_2$ (mmHg)	32.3 ± 1.3	35.8 ± 0.7*
V_E (L/min)	13.0 ± 1.1	9.8 ± 0.7*
V_T (L)	0.69 ± 0.04	0.78 ± 0.07
F_b (breaths/min)	18 ± 1	14 ± 1*
Peak Values		
VO_{2peak} (ml/kg/min)	18.1 ± 1.6	32.4 ± 2.9*
W_{peak} (watts)	64.5 ± 9.6	166.6 ± 21.2*
Exercise duration (min)	8.2 ± 0.8	9.3 ± 0.9
RER	1.09 ± 0.03	1.18 ± 0.02*
HR (bpm)	126 ± 7	155 ± 4*
SaO_2 (%)	94.0 ± 1.3	95.4 ± 0.9
V_E (L/min)	46.0 ± 5.9	78.5 ± 8.7*
V_T (L)	1.37 ± 0.12	2.26 ± 0.19*
F_b (breaths/min)	33 ± 2	36 ± 3
$PetCO_2$ (mmHg)	35.1 ± 1.9	37.6 ± 1.5
Dyspnea	8.9 ± 0.3	7.8 ± 0.7
Leg fatigue	7.7 ± 0.5	7.7 ± 0.6

Effect of IHG and PECO on the Pressor Response

Heart Rate: Figures 1-6 depict the group mean data for the percentage each participant changed from baseline for the variables measured during the IHG and PECO protocol. The ANOVA results for HR indicated a significant main effect of time [F (3.01*, 20) = 34.89, $p < 0.001$, partial eta-squared = 0.64]. Post-hoc analysis of time indicated %ΔHR was elevated relative to baseline at H1 ($p < 0.001$), H2 ($p < 0.001$), H2.5 ($p < 0.001$) and O1 ($p = 0.04$). Heart rate

returned to baseline at O2 (p = 1.0). The %ΔHR peaked at H2.5 in both groups (COPD 17 ± 4, control 18 ± 2%). No significant difference was found for the main effect of group [F (1, 20) = 0.002, p = 0.96, partial eta-squared < 0.001]. There was no interaction effect for group by time [F (3.01*, 20) = 0.25, p = 0.86, partial eta-squared = 0.01].

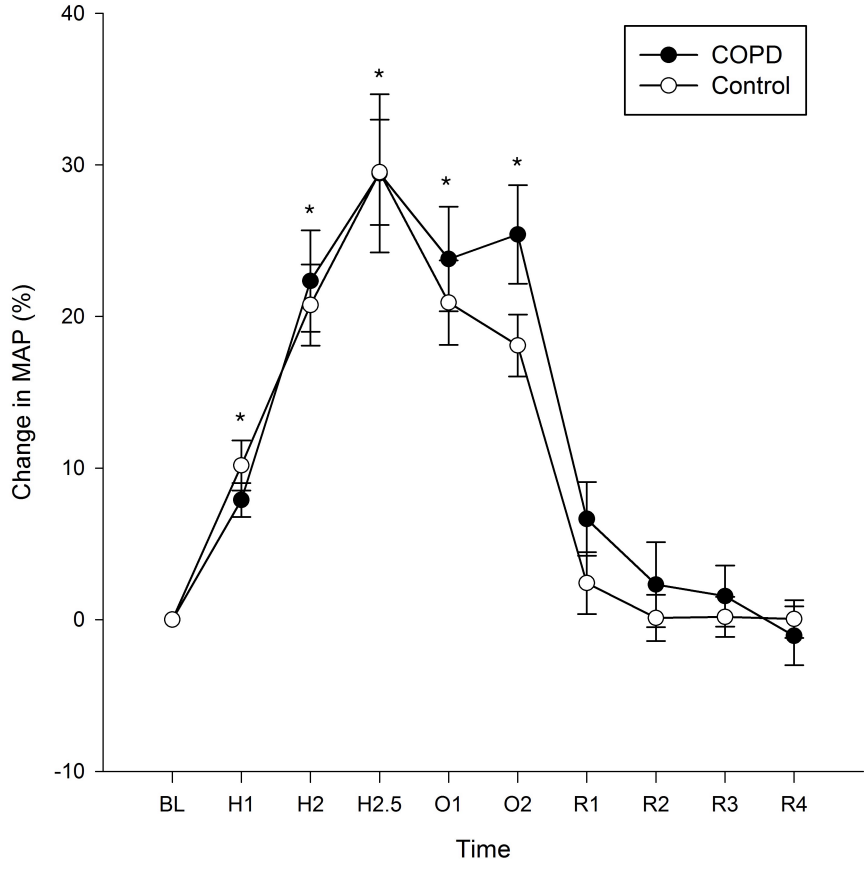
Figure 1: Changes in HR during IHG and PECO. Values represent the percent change from baseline.



Mean Arterial Pressure: Like HR, there was an effect of time on the magnitude of %ΔMAP in both groups [F (2.42*, 20) = 62.44, p < 0.001, partial eta-squared = 0.76]. All time points, with the exception of recovery, were significantly greater than baseline (p < 0.001). Unlike HR, %ΔMAP remained elevated above resting values at O2 (COPD 25 ± 3, control 18 ± 2%, p < 0.001). There was no main effect of group [F (1, 20) = .35, p = 0.56, partial eta-squared =

0.017]. Also, there was no interaction for group by time on the magnitude of the MAP response [F (2.42*, 20) = 1.07, p = 0.36, partial eta-squared = 0.05].

Figure 2: Changes in MAP during IHG and PECO. Values represent the percent change from baseline.



Leg Blood Flow and Leg Vascular Resistance: There was a significant main effect of time for %ΔLBF [F (2.47*, 20) = 8.58, p < 0.001, partial eta-squared = 0.3), which increased above baseline in both groups throughout IHG and PECO peaking at O1 (COPD 37 ± 16, control 51 ± 17%, p = 0.001). There was no main effect of group for %ΔLBF [F (1, 20) = 0.52, p = 0.5, partial eta-squared = 0.03]. Unlike LBF, there was no significant effect of time for %ΔLVR [F (3.02*, 20) = 0.54, p = 0.77], nor was there an effect of group [F (1, 20) = 1.20, p = 0.30]. There

was no interaction effect for either %ΔLBF [F (2.47*, 20) = 1.13, p = 0.34, partial eta-squared = 0.05] or %ΔLVR [F (3.03*, 20) = 1.96, p = 0.66, partial eta-squared = 0.09].

Figure 3: Changes in LBF during IHG and PECO. Values represent the percent change from baseline.

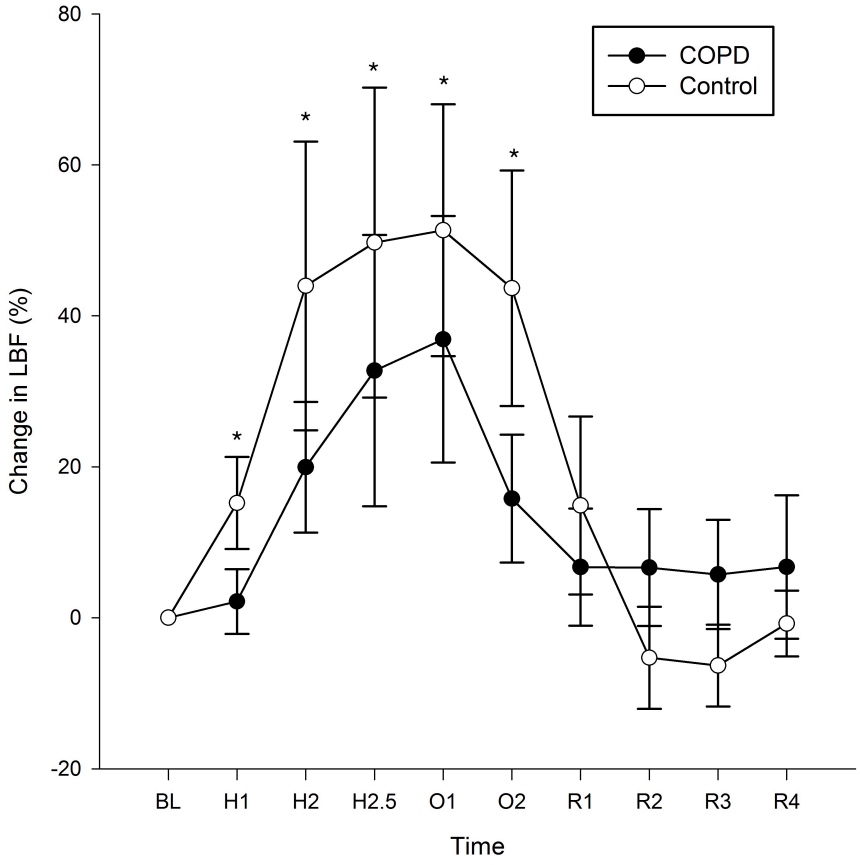
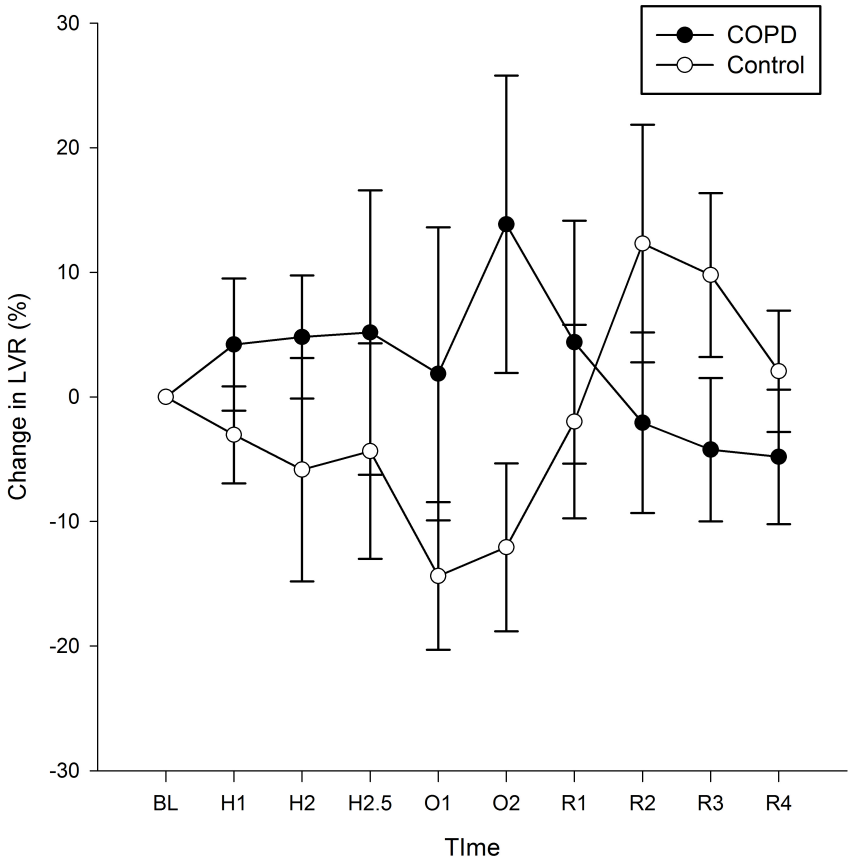


Figure 4: Changes in LVR during IHG and PECO. Values represent the percent change from baseline.



Cardiac Output and Total Peripheral Resistance: The main effect of time was statistically significant for %ΔQ [F (2.71*, 20) = 3.87, p = 0.02, partial eta-squared = 0.16]. The change from baseline was significant at H1 (p = 0.005), H2.5 (p = 0.04), O1 (p = 0.01) but not at H2 (p = 0.06) or O2 (p = 0.69). There was a significant main effect of group for %ΔQ [F (1, 20) = 4.79, p = 0.04, partial eta-squared = 0.19] with the largest difference between groups occurring at H2.5 (COPD 0.08 ± 6.9, control 16.6 ± 3.6 %) and O1 (COPD 1.1 ± 5.6, control 15.4 ± 2.4%). There was a main effect of time for %ΔTPR [F (1.83*, 20) = 9.5, p = 0.001, partial eta-squared = 0.32] where %ΔTPR was significantly different from baseline at H2 (p = 0.004), H2.5 (p = 0.01), O1 (p = 0.007), O2 (p < 0.001) before returning to baseline at R2 (p = 0.25). The main effect of group approached, but did not reach statistical significance for %ΔTPR [F (1, 20) = 3.13, p =

0.09, partial eta-squared = 0.14]. There was no interaction effect for %ΔQ [F (2.71*, 20) = 1.66, p = 0.19, partial eta-squared = 0.077], or %ΔTPR [F (1.83*, 20) = 2.07, p = 0.09, partial eta-squared = 0.14].

Figure 5: Changes in Q during IHG and PECO. Values represent the percent change from baseline.

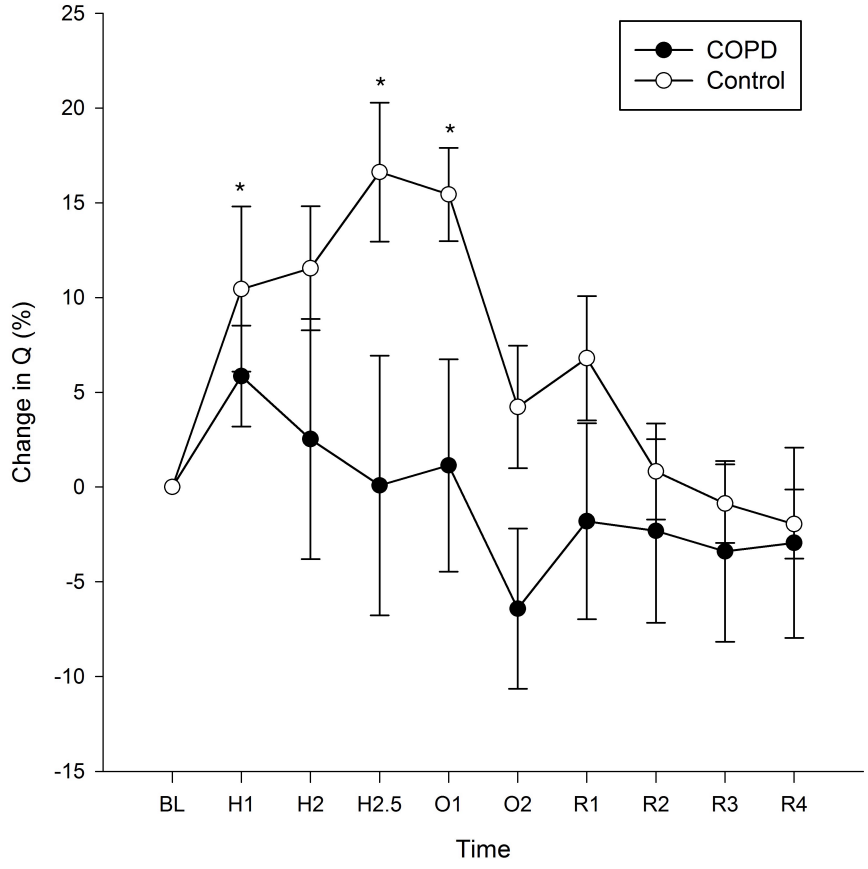
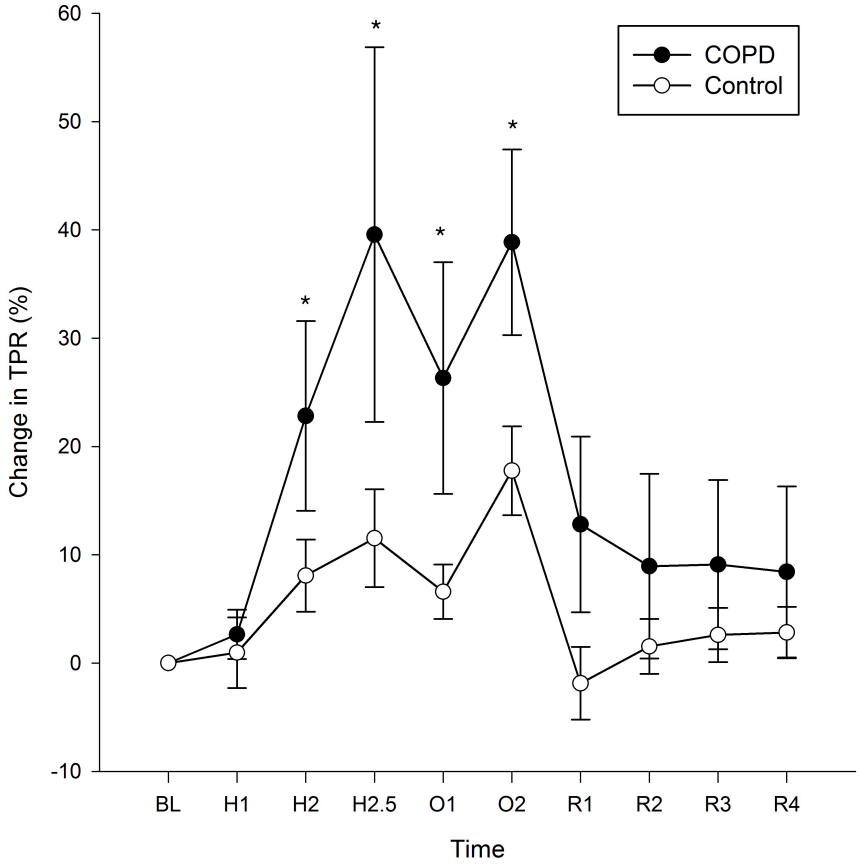


Figure 6: Changes in TPR during IHG and PECO. Values represent the percent change from baseline.



Effect of Exercise Capacity and Disease Severity on the Magnitude of the Muscle Metaboreflex

Figures 6 and 7 show the relationship between VO_{2peak} and % predicted $FEV_{1.0}$ and the %ΔMAP at O2, as an indicator of the magnitude of the metaboreflex response. A Pearson product-moment correlation revealed no significant relationship between %ΔMAP at O2 and VO_{2peak} ($r = -0.02$, $p = 0.92$). There was no statistically significant relationship between % predicted $FEV_{1.0}$ and %ΔMAP at O2 ($r = -0.22$, $p = 0.32$).

Figure 7: Relationship between disease severity and the muscle metaboreflex. Percent predicted $FEV_{1.0}$ and %ΔMAP at minute 2 of PECO.

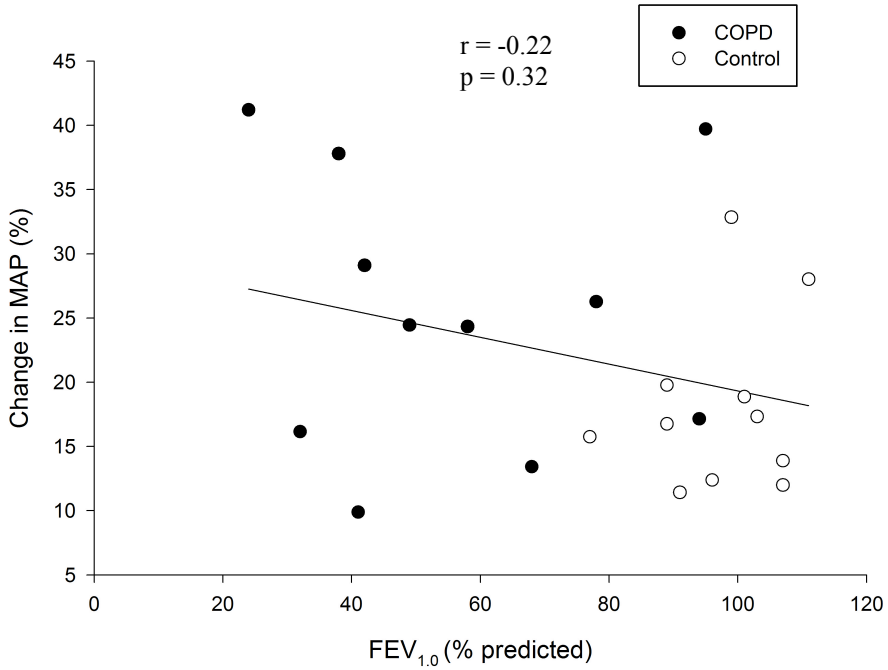
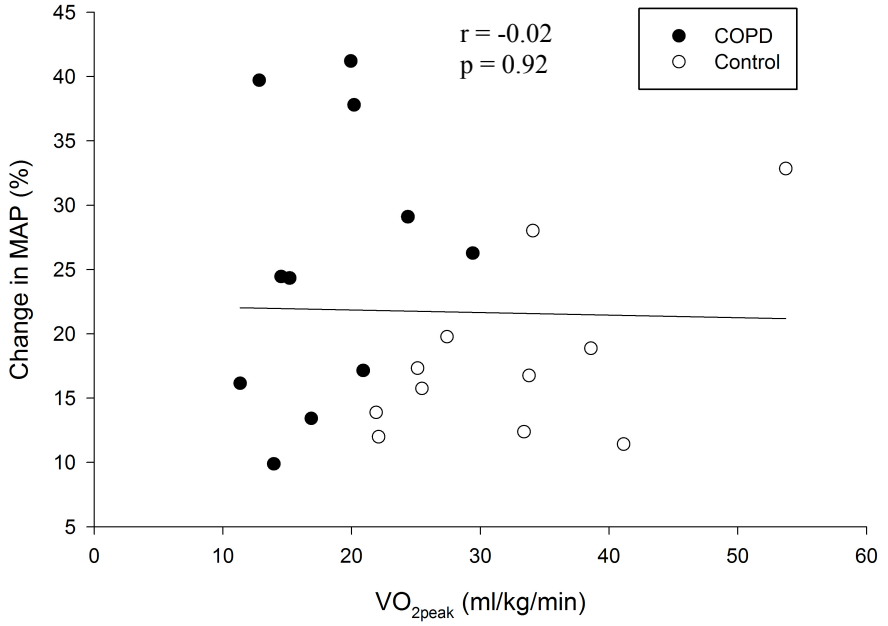


Figure 8: Relationship between exercise capacity and the muscle metaboreflex. Peak oxygen uptake and %ΔMAP at minute 2 of PECO.



DISCUSSION

Main Findings

The current study examined the cardiovascular responses to IHG and isolated the muscle metaboreflex during PECO in people with mild-to-severe COPD and healthy controls. The two main findings are: 1) People with COPD had similar HR, MAP, LBF and LVR responses throughout IHG and PECO compared to age- and gender- matched healthy individuals. 2) There was no association between exercise capacity (VO_{2peak}) or disease severity (% predicted $FEV_{1.0}$) and the magnitude of the MAP response during PECO. These findings suggest the exercise pressor reflex is unaltered in people with COPD. This study was designed with intention of testing the hypothesis that the muscle metaboreflex is exaggerated in people with COPD. The non-significant findings limit the ability to address the mechanisms responsible for the preserved muscle metaboreflex and thus only indirect explanations can be offered relating to: 1) the mechanisms involved in the preserved muscle metaboreflex and; 2) the factors which contributed to the disparity between our findings and previous work examining the muscle metaboreflex in people with COPD.

Mechanisms for Preserved Muscle Metaboreflex in COPD

Three mechanisms may explain why people with COPD demonstrated a preserved muscle metaboreflex response during exercise: 1) upper limb skeletal muscle properties were intact in people with COPD 2) desensitization of peripheral afferents to excess metabolite accumulation 3) elevated resting sympathetic tone reduced sympathetic responsiveness in the COPD group, while a secondary compensatory mechanism helped preserve the pressor response.

1) Preserved Upper Limb Skeletal Muscle Characteristics: The muscle metaboreflex is reliant on an accumulation of metabolic by-products during exercise to stimulate group IV afferents and initiate the pressor response. The metabolites involved in this process include: inorganic phosphates, potassium, lactate, and hydrogen ions (21; 32; 35; 42). It was hypothesized that altered skeletal muscle properties in the COPD group, which include impaired phosphogen kinetics, reduced oxidative capacity, and a shift in fibre type distribution towards glycolytic type-II fibres, would generate larger concentrations of these by-products during IHG and PECO, and consequently, produce a larger muscle metaboreflex response. Contrary to the hypothesis, changes in HR, MAP, LBF and LVR were similar in the COPD group and controls throughout IHG and PECO. Preserved upper limb skeletal muscle characteristics may explain these findings.

There are a number of studies indicating oxidative capacity is reduced, phosphocreatine potential is decreased, and lactate production is in excess in people with COPD (4; 6; 7; 13; 17; 24; 25; 27). However, the majority of these studies base their conclusions on biopsies taken from the vastus lateralis muscle. Few studies have examined the skeletal muscle characteristics in the upper limbs of people with COPD, likely because it is more challenging to obtain biopsies. Also, it has been thought that the major mechanisms inducing changes in skeletal muscle properties, such as hypoxemia, corticosteroid use, detraining, and malnutrition (4) would generate similar changes in the upper and lower limbs. However, a study performed by Gea et al. (23), which measured fibre type and enzyme activity in biopsy samples from the deltoid muscles of people with mild-to-severe COPD ($FEV_{1.0}$ = 51 ± 15% predicted) suggests otherwise. The COPD group, despite having significantly lower VO_{2peak} values to the control group (COPD 57 ± 20, control 85 ± 12% predicted, $p = 0.01$), maintained similar handgrip strength (COPD 77 ± 19 controls 87 ± 29% predicted), similar fibre type distribution, and comparable muscle fibre cross-sectional area ($p > 0.05$). These findings suggest upper limb skeletal muscle

characteristics are comparable between people with COPD and healthy age- matched controls. The preservation of upper limb skeletal muscle may reflect a smaller degree of detraining than seen in the lower limbs. Upper body activities utilise less muscle mass and generate a smaller ventilatory load than lower limb activities. Therefore, activities requiring the upper limbs generate less dyspnea and are not avoided as often by people with COPD compared to lower limb activities which use more muscle mass and elicit more dyspnea.

The demographic and disease characteristics (age, BMI, $FEV_{1.0}$, $FEV_{1.0}$/FVC) of the COPD group studied by Gea et al. were similar to those of the COPD group tested in the present study, and thus, it would be reasonable to assume some level of preserved upper limb strength and fibre type distribution also characterizes this COPD group. Fibre type is a an important determinant of the magnitude of the pressor response as glycolytic fibres, which rely more heavily on anaerobic metabolism than oxidative fibres, produce larger concentrations of the metabolites involved in the muscle metaboreflex (88). Described previously, chronic low-frequency stimulation applied to a primarily glycolytic muscle in the hindlimb of a rabbit induces metabolic changes favouring oxidative metabolism and generates a reduced pressor response, measured as the change in MAP from baseline in the control and experimental limb ($p = 0.008$). If the COPD group in our study had no decrements in upper limb skeletal muscle properties, then fibre type and metabolite accumulation would be similar between the COPD and control groups and would explain the preserved pressor response elicited by upper limb IHG and PECO in the COPD group.

2) Desensitization to Metabolite Accumulation*:* Returning to the study by Gea et al. (23), despite preserved upper limb strength and fibre type distribution, the participants with severe COPD ($FEV_{1.0} \leq 50\%$ predicted) had significantly greater concentrations of lactate dehydrogenase ($p = 0.001$) and citrate synthase ($p < 0.01$) at rest compared to controls. Up-

regulation of lactate dehydrogenase, in the presence of preserved upper limb fibre type distribution, suggests lactate and hydrogen ion concentrations elicited by IHG would be larger in individuals with more severe COPD, producing a magnified pressor response. However, no correlation between disease severity and the magnitude of the pressor response was found. Interestingly, similar findings have been seen in people with heart failure (HF) compared to healthy age- matched controls. Five minutes of rhythmic handgrip exercise produced larger concentrations of venous hydrogen ions (control 49.6 ± 1.0, HF 57.4 ± 1.3 nmol) and lactate (control 1.02 ± 0.2, HF 1.9 ± 0.2 mmol) in people with heart failure. Despite greater accumulation of these important metabolites, after 5 minutes of handgrip exercise (Post) the rise in HR (Rest: control 63 ± 3, HF 69 ± 4 bpm; Post: control 68 ± 2, HF: 75 ± 4 bpm) and MAP (Rest: control 100 ± 5, HF 80 ± 4 mmHg; Post: control 107 ± 5, HF: 90 ± 5 mmHg) was similar in people with heart failure and controls. As the changes in HR and MAP were smaller for a given level of metabolite accumulation, the authors propose that the metaboreflex response was attenuated. Desensitization of peripheral afferents to the metabolites responsible for determining the magnitude of the muscle metaboreflex, may explain why the pressor response was not magnified in the presence of a larger concentration of lactate. The rise in HR and MAP at the end of 5 minutes of handgrip exercise in the heart failure and control groups in Shoemaker's study parallel those attained by the COPD and control groups at the end of 2.5 minutes of IHG. Desensitization of peripheral afferents may also be a plausible explanation for the preserved muscle metaboreflex response measured in the COPD group, who like the heart failure group, was likely exposed to a greater accumulation of lactate than the controls.

3) Elevated Sympathetic Tone and Reduced Sympathetic Reactivity: Studies examining sympatho-vagal balance in people with COPD indicate overactivity of the sympathetic nervous system at rest (30) and reduced responsiveness of the autonomic nervous system to stimuli such

as a passive head up tilt manoeuvre (85). The mechanisms contributing to the chronic overstimulation and reduced reactivity of the sympathetic nervous system in people with COPD are not well defined. It has been speculated that these autonomic nervous system changes are the result of chronic exposure to hypoxemia, and consequently, modulation of peripheral chemoreceptor activation (30). Measurement of MSNA using microneurography at the peroneal nerve supports this hypothesis, showing people with COPD have higher resting MSNA activity than healthy age matched controls. When supplemental oxygen is provided, resting MSNA decreases in people with COPD but remains unchanged in controls. Other mechanisms which might be contributing to elevated resting sympathetic tone and reduced sympathetic reactivity include, sympatho-excitatory medications used by people with COPD such as theophylline and diuretics, and changes within arterial and cardiopulmonary baroreflexes. These mechanisms, in addition to others, are explained in detail by Heindl et al. (30).

Consistent with this literature showing impaired sympatho-vagal regulation, the COPD group tested had reduced resting HRV, elevated resting HF values, and lower LF values, which combined, is indicative of increased sympathetic tone (1). These findings may help explain the preserved pressor response in the COPD group. Specifically, the muscle metaboreflex relies on activation of the sympathetic nervous system to initiate the efferent arm of the pressor response. A study performed in people with COPD and healthy controls measured HRV at rest and again after a passive orthostatic challenge (passive head up tilt-test) (85). The COPD group, which was younger than those used in this study (55 ± 11 years) but had a similar level of disease severity ($FEV_{1.0}$ = 52 ± 8.3% predicted) and were normoxic, showed a reduced sympathetic response to the tilt test compared to the control group. The conclusion drawn from this study was that when people with COPD were faced with a challenge to the autonomic system, they had a smaller range within which to make sympathetic and parasympathetic adjustments because of their already elevated resting sympathetic tone. The preserved pressor response which occurred

in spite of reduced HRV and elevated sympathetic tone in the COPD group, supports the idea that a mechanism secondary to a rise in MSNA helped the COPD group maintain a similar pressor response to the healthy controls.

Dissociation between MSNA and vascular resistance during PECO has been suggested in young healthy individuals and may explain the preserved pressor response which occurred even in the presence of elevated resting sympathetic tone in the COPD group. Despite a continued rise in MSNA during IHG and PECO, Shoemaker et al. (77) observed a lowering of MAP during PECO. This reduction in MAP at the onset of PECO was associated with a decrease in femoral artery vascular resistance, which consequently, was hypothesized to be sensitive to changes in MAP separate from MSNA (which continued to rise throughout PECO). A similar drop in MAP at the end of IHG exercise was measured in both the COPD and control groups. If changes in MAP were, in part, responsible for modulating the vascular resistance response during PECO, then the similarity between the COPD and control groups in the MAP responses would be echoed in the LBF and LVR and may account for the preserved pressor response in the COPD group occurring despite elevated resting sympathetic tone.

Comparison to Current Literature

A previous study measuring the muscle metaboreflex in people with COPD and healthy controls concluded that the muscle metaboreflex is attenuated (66). Three factors may explain the disparity between these findings and those in the present work showing a preserved muscle metaboreflex: 1) interpretation of results 2) measurement techniques 3) heterogeneity of the population.

1) Interpretation of Results: A similar rise in HR and MAP was measured throughout IHG and PECO in people with COPD compared to controls in both the present study and the study by

Roseguini et al. (66). The blunted rise in calf vascular resistance measured in Roseguini's study was interpreted by the authors' as being indicative of an attenuated muscle metaboreflex in the COPD group. However, this interpretation fails to explain the maintained HR and MAP responses which we interpret as a measure of a preserved muscle metaboreflex. Similar discrepancies are present in the literature examining the muscle metaboreflex in people with heart failure. A review of the literature consistently shows similar rises in HR and MAP between heart failure patients and healthy controls when the muscle metaboreflex is being examined (10; 31; 37; 54; 63; 78; 82). Despite similar HR and MAP responses, these papers differ in their conclusions regarding the status of the muscle metaboreflex response. For example one study suggests it is preserved (78), two studies suggests it is augmented (54; 63), two studies suggest it is reduced (37; 81), and one suggests the blood pressure response is preserved with an altered hemodynamic response (10). Each of these studies measured the pressor response using different variables in addition to HR and MAP, such as MSNA, calf blood flow and femoral artery blood flow. Therefore, inconsistencies across findings may be a consequence of using varying measures to represent efferent activity and may depend on which measurement authors take to reflect the muscle metaboreflex (i.e. MAP or MSNA or blood flow). Regardless, the discrepancy between findings, both in the heart failure and now in the COPD literature, highlights the need for more comprehensive assessment of the muscle metaboreflex with measures not only of HR and MAP, but of MSNA concurrently with blood flow.

2) Measurement Technique: Venous occlusion plethysmography was employed by Roseguini et al. (66) to measure calf blood flow and determine calf vascular during IHG and PECO. Using this technique, calf blood flow is calculated by inflating an occlusion cuff around the upper thigh for cycles of 15 seconds. This occludes venous blood flow, while allowing arterial flow to

continue to enter the limb. A second cuff is inflated continuously around the ankle to prevent blood flow to the foot. A strain gauge plethysmograph is placed around the calf at its largest circumference and detects changes in blood flow to the limb by measuring the change in calf circumference, which increases as arterial blood continues to enter the limb and venous blood remains trapped (8). In this study Doppler ultrasound was used to measure blood velocity in the femoral artery and consequently derived, based on femoral artery diameter, femoral artery blood flow. Both venous occlusion plethysmography and Doppler ultrasound have been validated as techniques capable of measuring changes in blood flow and vascular resistance (8), but as they both quantify blood flow and vascular resistance in different parts of the leg and use different physiologic assumptions in making these measures, some of the disparity between findings may be explained.

3) Effect of participant characteristics: The discrepancy between blood flow and vascular resistance responses in this study compared to that of Roseguini et al. (66) may be attributed to differences in participant characteristics. The COPD participants tested by Roseguini et al. included 11 men characterized with severe airflow obstruction ($FEV_{1.0}$ = 35 ± 16% predicted), with mild desaturation at rest (SaO_2 = 94 ± 2%), and a low VO_{2peak} (15.8 ± 4.3 ml/kg/min). These participants therefore had more severe COPD than the participants in our study, who had mild-to-severe COPD ($FEV_{1.0}$ = 56.3 ± 7.4% predicted) and had normal resting SaO_2 (97.5 ± 0.3%) and slightly higher exercise capacity (18.1 ± 1.6 ml/kg/min). This difference in disease severity between studies may be relevant as % predicted $FEV_{1.0}$ is negatively correlated with the proportion of type-IIA fibres in skeletal muscle of people with COPD ($r = -0.21$, $p < 0.001$) (25). As discussed earlier, type-IIA fibres are primarily glycolytic and produce greater amounts of metabolic by-products such as lactate and hydrogen ions which are critical in determining the magnitude of the pressor response (42). Therefore, the heterogeneity of the COPD population in

our study (mild-to-severe) may have masked differences in the pressor response that were exaggerated in the study by Roseguini et al. who examined a more homogenous COPD population (severe) with a potentially greater proportion of type-IIA glycolytic fibres. These population differences may not account entirely for the disparity between our findings as we found no association between disease severity and the magnitude of the metaboreflex response.

Limitations

The present study has shown that the muscle metaboreflex response to handgrip exercise is preserved in people with COPD. These findings differ from previous work suggesting the muscle metaboreflex is attenuated in people with COPD (66). Measurement techniques and participant characteristics may account for some of these differences, however, more importantly, measurement of MSNA in addition to LBF, would provide a more comprehensive understanding of the hemodynamic response to exercise in people with COPD and would provide more information regarding the mechanism responsible for the preserved metaboreflex. Previous work by Seals (74) measured a correlation coefficient of $r = 0.67$ ($p < 0.001$) at 35% MVC for the relationship between MSNA and calf vascular resistance IHG in young healthy men. According to this correlation the use of HR, MAP, LBF and LVR as a method my which to assess the pressor response is valid. Measurement of blood lactate, blood pH and hydrogen ions would also provide more information about the mechanisms underlying the preserved muscle metaboreflex in COPD.

This study's findings are not generalizable to whole-body exercise as isometric handgrip exercise may only reflect activation of the muscle metaboreflex by the upper extremities. As mentioned, upper limb strength and fibre type distribution might be preserved in the upper limb of people with COPD and thus the metaboreflex response may differ if measured in the lower limbs where more marked changes in skeletal muscle properties have been measured.

Future research

An interesting finding in this study that is worth pursuing in future research relates to the Q and TPR responses measured during IHG and PECO in the COPD group. A significant difference in the Q response to IHG and PECO exercise occurred between the COPD group and healthy controls. The Q response in the control group showed a rise in Q throughout IHG which remained elevated until recovery, where the COPD group showed no change in Q throughout exercise remaining at or below baseline levels. This trend coincides with literature in heart failure patients showing they have reduced Q despite similar rises in blood pressure to healthy controls during IHG (10). Where the healthy control group attained their increased blood pressure by increasing Q, the heart failure group increased systemic vascular resistance to achieve this same rise in blood pressure. The rise in TPR we saw throughout exercise was not statistically significantly different between groups; however the COPD group did tend to have larger values but did not reach significance due to a large degree of between subject variability. Though direct comparisons cannot be made between people with heart failure and COPD as the hemodynamic characteristics of heart failure are different than those of COPD, these findings are worth pursuing further as they may contribute to our understanding of the hemodynamic responses to exercise in people with COPD.

Direct measurement of MSNA using microneurography at rest and during IHG and PECO would also be a valuable addition to the literature pertaining to the muscle metaboreflex in COPD. Concurrent measures of MAP, LBF and LVR with MSNA may also help clarify the mechanisms involved in the preservation of the muscle metaboreflex we measured in people with COPD.

Finally, characterizing the muscle metaboreflex response elicited by lower limb exercise, distinct from the response generated by upper limb exercise, may be valuable in understanding

the relationship between the pressor response and exercise intolerance, which is often brought on by whole body exercise rather than upper limb recruitment which is at play during handgrip exercise.

Conclusions

The results from this study indicate that the muscle metaboreflex is preserved in people with COPD and that exercise capacity and disease severity are not correlated with the magnitude of the muscle metaboreflex. While the mechanisms for this preserved pressor response remain unclear, retention of upper limb skeletal muscle characteristics and desensitization of peripheral afferents may be contributing factors. Also, increased resting sympathetic tone and reduced reactivity to sympathetic and parasympathetic stimuli associated with COPD suggests a compensatory mechanism helped the COPD group generate the preserved pressor response during IHG and PECO.

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APPENDIX A: REVIEW OF LITERATURE

Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive, irreversible condition affecting the respiratory system. The primary cause of this disease is smoking (85% of COPD population), while workplace irritants such as dust and fumes, and a genetic condition (alpha-1-anti-trypson deficiency) account for the remainder of COPD cases (14% and 1-2% respectively) (4; 55). Chronic obstructive pulmonary disease is characterized by impaired lung and airway function and presents as excess sputum production, chronic coughing, a sensation of breathlessness (dyspnea) upon exertion and repeated respiratory tract infections (55). Diagnoses and classification of this disease is based on pulmonary function. A ratio of forced expiratory volume in 1 second ($FEV_{1.0}$) relative to forced vital capacity (FVC) ($FEV_{1.0}$/FVC) of less than 0.7 post-bronchodilator determines the degree of obstruction. The percent of age and gender predicted $FEV_{1.0}$ achieved determines disease severity (55). Dyspnea is the primary complaint of COPD patients as low intensity exercise and activities requiring minimal exertion (such as activities of daily living) elicit this sensation (39; 58). The mechanism thought to generate the dyspnea associated with COPD is a reduction in the pulmonary system's ability to generate maximal expiratory flow rates due to pathology in the lungs, such as parenchymal damage, combined with inflammation and fibrosis in the airways (53). Dyspnea is therefore brought on by activities which increase ventilatory demands and consequently increase flow rates. Pharmacological interventions are used to minimize the effect flow limitation has on exercise capacity and daily living. However, evidence from biopsy studies suggest peripheral factors may also be contributing to the dyspnea experienced by people with COPD during low intensity activity (39; 56). Altered structural and functional characteristics, such as slower phosphogen kinetics, reduced mitochondrial enzyme activity and an increased proportion of type-II glycolytic

fibres, are present in the skeletal muscles of people with COPD (24; 25; 45; 46; 87). The relationship between these skeletal muscle characteristics and exercise intolerance may be related to activation of the muscle metaboreflex during exercise. The muscle metaboreflex is a reflex loop reliant on stimulation of group IV afferents located peripherally in skeletal muscle (3; 35). These afferents are sensitive to metabolites such as phosphates, potassium ions, and lactate and initiate a rise in muscle sympathetic nerve activity (MSNA). This elevated MSNA elicits a rise in heart rate, blood pressure and redistribution of blood flow away from inactive tissues towards working muscle. Conceptually, the muscle metaboreflex corrects a mismatch between blood supply and demand (21; 67). However, there is debate as to the reflex's effectiveness at restoring blood flow to working tissues (34). The cardiovascular changes associated with the muscle metaboreflex are termed the "pressor response". Skeletal muscle properties which favour glycolytic metabolism, such as those measured in people with COPD, produce larger concentrations of the by-products responsible for initiating and determining the magnitude of the muscle metaboreflex, and thus for a given exercise intensity produce larger increases in heart rate, blood pressure concurrently with reductions in blood flow in inactive tissues for a given exercise intensity (42; 88). Interestingly, heart failure, a disease that parallels COPD in that diminished capacity of a central organ (heart vs. lungs), is the primary characteristic of the disease. Like COPD, people with heart failure have altered skeletal muscle properties which accompany reduced muscle strength and exercise capacity (79). It is suggested that exercise intolerance in people with heart failure is caused both by the limited capacity of the heart to respond to the demands of exercise (centrally), and also by peripheral factors such as the altered skeletal muscle characteristics which are hypothesized to magnify the cardiovascular response to exercise through activation of the muscle metaboreflex (63).

It is reasonable to draw parallels between COPD and heart failure with regards to exercise intolerance and the contributing mechanisms. The muscle metaboreflex during exercise

must therefore also be explored in people with COPD as it may provide insight into the factors contributing to dyspnea and exercise intolerance which are so limiting for patients and often leads to reduced quality of life.

The purpose of this review is to describe the structural and functional changes occurring within the pulmonary system and skeletal muscles of individuals with COPD. The effect these central and peripheral changes have on the ventilatory and hemodynamic responses to exercise will be explored.

Pulmonary Physiology of COPD

Defining COPD:

Declining pulmonary function is the characteristic feature of COPD. Appropriately, spirometry is the primary diagnostic tool used to identify this disease and to determine disease severity. There are a number of underlying structural changes occurring within the lungs and airways which are accompanied by functional changes. Emphysema, present in most people with COPD, is characterized by parenchymal damage and consequently increases lung compliance and alveolar dead space causing a ventilation-perfusion mismatch (19; 53). In the airways, chronic bronchitis is associated with a chronically elevated inflammatory response leading to fibrosis and causing lumen diameter narrowing during expiration to an extent not seen in healthy individuals (53). The most problematic outcome of these structural and functional changes is the development of expiratory flow limitation and consequently altered breathing mechanics.

Pulmonary Function at Rest:

Expiratory flow limitation slows the rate of lung and airway emptying during expiration. At rest, when low flow rates are required, most individuals with COPD are able to breathe

without flow limitation. However, in moderate-to-severe COPD the consequences of expiratory flow limitation are present, as many patients have reduced resting SaO_2 and increased $PetCO_2$ (55). Pulmonary function at rest may also be indicative of overall exercise capacity. Diaz et al. (16) found that COPD patients who were flow limited at rest had greater end-expiratory lung volumes and poorer performance on a progressive exercise test (12 ± 0.5 ml/kg/min) than COPD patients with no expiratory flow limitation at rest (17 ± 0.9 ml/kg/min) ($p < 0.001$). Resting pulmonary function, specifically expiratory flow limitation, is an important predictor of the pulmonary response to exercise (16).

Pulmonary Function During Exercise:

As exercise intensity increases, so to do the demands placed on the pulmonary system. In an effort to match ventilation to the increasing metabolic demands associated with exercise, inspiratory and expiratory flow rates must also increase. Reduced lung elastic recoil, increased alveolar dead space and reduced lumen diameter limit the ability of a person with COPD to meet these increased flow rates (57; 59). To compensate, people with COPD will shorten the duration of their expiration, thus commencing their next inspiration before reaching their "normal" end-expiratory lung volume (57). This phenomenon is termed dynamic hyperinflation and results in air remaining trapped in the lungs at the end of expiration (19; 59). Breathing patterns and the degree of expiratory flow limitation determine the extent to which dynamic hyperinflation occurs and end-expiratory lung volume increases (39). In health, physical activity initiates a decrease in end-expiratory lung volumes and an increase in tidal volume (V_T) through recruitment of respiratory muscles. While respiratory muscle recruitment does occur in individuals with COPD, the increased breathing frequency accompanying exercise, combined with expiratory flow limitation, exaggerates dynamic hyperinflation and increases end-expiratory lung volume instead of V_T (58-60; 62). O'Donnell et al. (59) measured V_T at the end of an incremental cycle

exercise test and found that individuals with moderate COPD at peak exercise only reached a V_T of 1.10 ± 0.44 L compared to healthy subjects who reached 2.41 ± 1.04 L ($p < 0.0005$). This study demonstrates that individuals with COPD have a blunted ability to increase their V_T in response to the respiratory drive of exercise. Failure to raise V_T during exercise means these individuals must rely on changes in breathing frequency to meet ventilatory demands, and as a consequence, dynamic hyperinflation worsens (15; 39). In this same study, the correlation between peak V_T and peak oxygen uptake ($r = 0.682$, $p < 0.0005$) demonstrates that pulmonary function, as measured by the increase in V_T, is associated with exercise capacity.

The pulmonary pathophysiology of COPD is also associated with an increased work of breathing (12; 49; 62; 72). First, as COPD patients operate at higher lung volumes their inspiratory muscles must generate greater force to overcome the inward recoil of the lungs and chest wall. This prompts the recruitment of accessory inspiratory muscles to help overcome the increased resistance (19; 58). Second, elevated breathing frequency and dynamic hyperinflation during exercise increase the velocity at which muscle shortening must occur and lead to further muscle weakness and fatigue (58). Finally, at higher lung volumes, the diaphragm is placed at a disadvantage in two ways: the diaphragm becomes less dome-shaped and sits lower within the body which means with downward displacement of the diaphragm during inspiration, smaller than normal changes in intrathoracic pressure occur and inspiratory muscles must be recruited to compensate (12; 62). As well, the diaphragm experiences progressive shortening at higher lung volumes and thus, produces less than optimal force according to the length-tension relationship (49). A review by Dempsey et al. (14) suggests an increased work of breathing in healthy people exercising at very high intensities leads to competition for blood flow between respiratory and locomotor muscles. This may also occur in people with COPD as their increased work of breathing may reduce blood flow to peripheral skeletal muscles during exercise and contribute to exercise intolerance and early fatigue.

Levison and Cherniak (40) examined the work of breathing associated with pulmonary dysfunction by comparing the oxygen cost of breathing between COPD patients and healthy controls. During light exercise at the same ventilation (30 L/min), the COPD group's oxygen consumption (315 ml/min) was more than double that of the healthy group (140 ml/min). The oxygen consumption directed towards respiratory muscles was 10-15% of total VO_2 in health and upwards of 35-40% of total VO_2 in COPD (40). Clearly, COPD is associated with an increased oxygen cost of breathing.

Eves et al. (18) manipulated the work of breathing in COPD patients to assess the impact this variable had on exercise capacity. Ten patients with moderate COPD cycled at 60% of peak work until fatigue while inspiring one of four gas mixtures: air (21% oxygen, 79% nitrogen), hyperoxia (40% oxygen, 60% nitrogen), helium-oxide (21% oxygen, 70% helium), and helium-hyperoxia (40% oxygen, 60% helium). Exercise time while breathing the gas mixtures was longer than exercise performed in room air and was significantly longer when the helium-hyperoxia mixture was provided (air: 9.4 ± 5.2 min, hyperoxia: 17.8 ± 5.8 min, helium 16.7 ± 9.1 min, helium-hyperoxia 26.3 ± 10.6 min; $p = 0.0002$). Helium-hyperoxia reduced the work of breathing, and specifically inspiratory muscle work, and in doing so extended the amount of time participants could exercise before experiencing dyspnea and dynamic hyperinflation. Helium-oxide gas mixture as been shown to decrease airway resistance and lower expiratory flow limitation, while helium-hyperoxia helps not only to reduce airway resistance and flow limitation, but to decrease ventilatory demands (18). Interestingly, 7 of the 10 participants stopped exercising because of leg discomfort in the helium-hyperoxia trial, while in the room air trial 8 of 10 terminated exercise because of breathing discomfort.

There is a strong case implicating pulmonary impairments, specifically dynamic hyperinflation and elevated end-expiratory lung volumes with increased work of breathing and exercise intolerance in COPD (15; 39; 59). Strengthening this argument is evidence from a

study in which 61 of 104 COPD patients reported terminating a symptom limited incremental cycle test because of breathing discomfort, where only 18 reported leg discomfort as their reason for terminating the test (59).

Pulmonary Function: Not the Only Determinant of Exercise Capacity:

Ferrer et al. (20) demonstrated that, as an index of disease severity, spirometry alone (% predicted $FEV_{1.0}$) correlated poorly with health-related quality of life. As well, evidence from lung transplant and medication studies show that improvements in pulmonary function do not always correlate with improvements in exercise capacity (29; 38; 73).

Schwablmair et al. (73) examined exercise intolerance in COPD patients using a progressive cycle test pre- and post- either a single or double lung transplant. Despite the transplants ability to improve lung mechanics, gas exchange and capacity to meet the ventilatory demands imposed by exercise, patients continued to demonstrate VO_{2peak} values and peak workloads lower than predicted following the transplant. These findings support those of Lands et al. (38) who found that 18 months after receiving a lung transplant, work capacity and quadriceps strength continued to be limited. Both studies suggest abnormal peripheral skeletal muscle function is contributing to the exercise intolerance characteristic of COPD. It is important to recognize the limitations of these studies. Immunosuppressive medications such as cyclosporine A may be contributing to the limited work capacity following transplant through their effects on mitochondrial function and oxidative capacity (70).

In an effort to improve pulmonary function and decrease the impact of COPD on the ability to perform activities of daily living, medications including short and long acting bronchodilators and corticosteroids are used regularly. Following the administration of these medications COPD patients often see marked improvements in pulmonary function, specifically as a result of reduced expiratory flow limitation. However, some of these patients continue to

experience diminished exercise capacity and reduced ability to perform activities of daily living. Grove et al. (29) found that following administration of salmeterol, a long acting ß$_2$-agonist, pulmonary function improved, yet the COPD patients demonstrated no improvement in exercise tolerance. Similarly, Saey et al. (69) used magnetic stimulation of the femoral nerve to assess quadriceps fatigue following exercise. Two constant work rate cycle exercise tests to exhaustion were performed by individuals with moderate-to-severe COPD. Prior to the exercise bout either the bronchodilator ipratropium bromide (IB) or a placebo was administered. Twitch force was measured before beginning exercise and then again at 10 and 30 minutes following completion. Interestingly, while performing the placebo trial, half the COPD patients experienced a drop in quadriceps twitch force of more than 15%, indicating fatigue. During the bronchodilator trial, all COPD patients demonstrated improvements in $FEV_{1.0}$ (fatiguers: 11 ± 18%, non-fatiguers: 13 ± 18%). However, those who fatigued in the placebo trial showed no increase in endurance time in the bronchodilator trial (placebo: 394 ± 220 sec, IB: 400 ± 119 sec), while the COPD patients who did not fatigue in the placebo trial did improve endurance time (placebo: 249 ± 124 sec, IB: 479 ± 298 sec; $p < 0.05$). These findings strongly suggest that intrinsic skeletal muscle abnormalities are plaguing a significant number of COPD patients with direct implications on exercise performance.

Evidence for Skeletal Muscle Impairment

In COPD, skeletal muscle appears to exhibit metabolic and morphologic adaptations uncharacteristic of healthy age-matched individuals (4; 7; 24; 65). These findings support the hypothesis that skeletal muscle dysfunction is contributing to exercise intolerance, where dysfunction refers to a “disturbance, impairment, or abnormality of the functioning of an organ” (Dorland's Medical Dictionary2). This review, will not try and end the debate surrounding the underlying cause of skeletal muscle dysfunction and whether it is pathological in nature rather

than the outcome of disuse, but will instead provide an overview of the changes which have been noted in the lower limbs of people with COPD, and will briefly discuss the potential mechanisms leading to these changes.

Structural Changes:

Significant declines in peripheral skeletal muscle mass accompany COPD. Magnetic resonance imaging was used to determine the volume of the quadriceps, adductor and hamstring muscles in 20 individuals with COPD and 20 healthy older adults (48). Muscle volume was found to be statistically significantly lower in the COPD patients compared to the healthy controls for all three muscles imaged.

Other studies demonstrate that impaired muscle strength and endurance accompany this decline in muscle mass (43; 87). When comparing baseline quadriceps strength, after accounting for differences in quadriceps activation, COPD patients produced 72.9% of the force output achieved by healthy controls (43). In addition, exercising at an absolute workload for a fixed duration, COPD patients demonstrated significantly more contractile fatigue, measured as quadriceps twitch force, at 10, 30 and 60 minutes post-exercise than age-matched healthy controls ($p < 0.005$).

Among the most significant structural adaptations observed in the skeletal muscles of COPD patients is a shift in fibre-type distribution. Multiple studies have noted COPD patients exhibit a greater proportion of type-II, glycolytic, more quickly fatigued fibres and a reduced proportion of type-I, oxidative, fatigue resistant fibres compared to age-matched controls (24-26; 71; 87) These changes are atypical of healthy aging, which is often associated with a shift towards a greater proportion of type-I rather than type-II fibres (24; 71).

Gosker et al. (24) examined these changes in fibre type distribution in the vastus lateralis of 15 COPD patients and 15 healthy controls using muscle biopsies. Not only were the

proportion of type-I, type-IIA and type-IIB fibres assessed, but also the intermediate states of these fibres, labelled as type-I/IIA and type-IIA/IIB. A higher percentage of the hybrid fibres were found in COPD patients suggesting the shift in fibre type distribution is a gradual process starting from more oxidative fibres and transitioning to more glycolytic fibres (type-I to type-I/IIA to type-IIA to type-IIA/IIB to type-IIB). Chronic obstructive pulmonary disease patients also had a reduced proportion of type-I fibres (COPD: 16%, age-matched: 42%), which is consistent with previous literature and suggests the positive relationship between type-I fibres and typical healthy ageing is opposite in individuals with COPD.

A systematic review and meta-analysis of 19 studies was recently published by this same research group to examine the relationship between disease severity and fibre type distribution (25). The analysis demonstrated that individuals in the later stages of COPD exhibited a greater decrease in the proportion of type-I fibres and an elevated proportion of type-II fibres compared to those with less severe COPD. As well, there was an association between both $FEV_{1.0}$ and $FEV_{1.0}$/FVC and the proportion of type I fibres ($r = 0.56$, $p < 0.001$; $r = 0.57$; $p < 0.001$ respectively) demonstrating a relationship between skeletal muscle function and disease severity.

Metabolic Changes:

Enzymatic changes accompany the increased proportion of type-II fibres and reduced proportion of type-I fibres found in individuals with COPD. The study mentioned previously by Gosker et al. (24) which measured fibre type distribution in the vastus lateralis of individuals with COPD and healthy controls also examined their enzyme profiles. The COPD group had a similar proportion of type-IIA muscle fibres to the control group, but demonstrated reduced oxidative enzyme activity in these muscle fibres (cytochrome *c* oxidase (COX) ($p < 0.01$) and succinate dehydrogenase (SDH) ($p < 0.05$)). Recently, Green et al. (27) published a comprehensive study examining the metabolic pathways in individuals with moderate-to-severe

COPD and healthy controls. After measuring the maximal activity of 11 enzymes representative of oxidative and glycolytic pathways, the patterns of activity both within and between the metabolic pathways were compared. The group concluded that oxidative phosphorylation and beta-oxidation were suppressed relative to glycolysis in the COPD group. These findings are consistent with a number of studies (24; 33) noting reduced maximal activity of oxidative enzymes. Specifically, citrate synthase (COPD: 22.3 ± 7.3 μmol/min/g muscle, control: 29.5 ± 7.3 μmol/min/g muscle; $p < 0.0001$) and 3-hydroxy-CoA dehydrogenase (COPD: 5.1 ± 2.0 μmol/min/g muscle, control: 6.7 ± 1.9 μmol/min/g muscle; $p < 0.005$) (45). Interestingly, compiled, these enzyme studies fail to demonstrate consistent up-regulation of anaerobic enzymes such as hexokinase, phosphofructokinase and lactate dehydrogenase. However, arterial lactic acid measures taken during a step-wise cycle test showed a steeper increase throughout exercise than controls while no difference in glycolytic enzymes were noted (46). Consistent with this finding, is a study by Calvert et al. (6) which measured ammonia and lactate concentrations in people with COPD during both an incremental and a constant-work rate cycle exercise test. Ammonia is a surrogate measure of oxidative stress, as it is produced only at high intensity exercise in healthy individuals when ATP resynthesis cannot meet ATP demand. Excess ADP and AMP are broken down to increase phosphorylation potential and keep the adenylate kinase reactions occurring. While this process sustains the working tissue, it is only for a short period, as deamination of AMP is irreversible and reduces the ATP pool, increases muscle IMP and increases ammonia in the blood stream. The authors noted two distinct ammonia patterns in the COPD group. Half the group showed a rise in plasma ammonia levels similar to the controls but at lower work rates, while a portion of the COPD showed no increase in plasma ammonia concentrations at all. Both COPD subgroups had similar VO_{2peak} values and similar lactate increases during exercise that was lower than the healthy controls. No clear demographic differences distinguished these two groups and the authors offer no strong

hypotheses to explain this distinct difference in the COPD subgroups, only mentioning type-II fibres tend to produce greater ammonia levels and perhaps fatigue in the non-ammonia group was related to something other than skeletal muscle fatigue. This is not the first time subgroups have appeared within the COPD population (described below) and these findings may speak to the individuality of this disease on skeletal muscle responses to exercise.

The phosphocreatine (PCr) system provides an anaerobic energy source and may not be functioning optimally in people with COPD (76; 86; 90). Wuyam et al. (90) compared PCr utilisation and resynthesis in the calf muscles during contractions performed at 20, 35 and 50% MVC between individuals with COPD and healthy controls. Despite similar resting intracellular pH and P_i/PCr profiles (P_i/PCr as an index of PCr utilisation) at 50% MVC COPD patients demonstrated a greater P_i/PCr ratio and a lower intracellular pH (COPD: 6.65 ± 0.11, control: 7.06 ± 0.02; $p < 0.01$). As well, PCr resynthesis during recovery was slower in COPD patients. These findings, however, are not always consistent across studies (6).

Despite inconsistencies across the literature, there is still clear evidence pointing to altered lower limb skeletal muscle metabolic function in people with COPD. First, a greater proportion of glycolytic type-IIB fibres have been consistently documented, as has reduced oxidative enzyme activity indicating reduced oxidative potential. Second, the phosphogen system appears to be less efficient in its provision of energy during high intensity exercise. Third, lactate production is greater during exercise in COPD compared to controls in the absence of changes in the activity of its rate limiting enzyme lactate dehydrogenase. Finally, ammonia production is also in excess in a portion of people with COPD indicating oxidative stress is occurring at the level of the muscle during exercise. The variability in these study findings highlights the diversity of the COPD population while drawing attention to the potential of skeletal muscle changes to contribute to exercise intolerance.

Potential Causes of Skeletal Muscle Dysfunction

Deconditioning:

A number of mechanisms have been proposed to explain the morphologic and metabolic adaptations to skeletal muscle described above. In a critical review examining the potential mechanisms which lead to skeletal muscle dysfunction Wagner (86) hypothesizes that changes in skeletal muscle properties are related to long-term disuse as COPD patients avoid activities which generate dyspnea. Support for this hypothesis comes from a study by Serres et al. (75) which provided COPD patients with a physical activity questionnaire adapted for older adults. They found that this population not only had lower levels of physical activity than age-matched individuals ($p < 0.05$), but their activity scores were positively correlated with skeletal muscle endurance ($r = 0.60$, $p < 0.05$). However, deconditioning may not account entirely for the skeletal muscle changes associated with COPD (4; 90) Some of the other mechanisms hypothesized to explain this skeletal muscle dysfunction are described below.

Poor Blood Gases:

Amplification of physiologic dead space is a common outcome of dynamic hyperinflation and can generate a ventilation-perfusion mismatch. The mismatch results in impaired gas exchange indicated by elevated arterial carbon dioxide content and decreased arterial oxygen saturation. Even during sleep expiratory flow limitation causes some patients to experience episodes of desaturation lasting on average 100 minutes (71). These periods of sustained hypoxia and hypercapnea have been hypothesized to reduce phosphocreatine and glycogen concentrations, to decrease oxidative enzyme concentrations, and to contribute to fibre type redistribution (4; 76; 87). Satta et al. (71) measured pulmonary function and took biopsies from the vastus lateralis of 22 COPD patients and 10 healthy controls. Reduced diffusion

capacity across the lung, and a negative correlation between diffusion capacity and type-IIB fibre content was measured.

Malnutrition and Corticosteroids:

Malnutrition may also contribute to impaired skeletal muscle function by interfering with protein synthesis. The cause of malnutrition is not clear, however, it is an ongoing concern for many COPD patients. Corticosteroid therapies which are commonly used to address the pulmonary limitations in COPD, can cause muscle myopathy (7; 13; 76). Combined, these two factors may help to explain the significant amount of weight loss and muscle wasting prevalent in individuals with this disease.

Exercise Intolerance and Skeletal Muscle Dysfunction

It is apparent from the above discussion that skeletal muscle dysfunction is prevalent in COPD and may result from a combination of disuse, poor gas exchange, malnutrition and corticosteroids. The impact that this skeletal muscle dysfunction has on exercise capacity was explored by Killan et al. (36) who examined dyspnea and leg effort using the Borg scale during a maximal exercise test in 97 COPD patients. Contrary to O'Donnell (59), 26% of individuals with chronic airflow limitation reported dyspnea as being greater than leg discomfort, 43% reported leg effort was greater than dyspnea and 31% reported these to be equal. Skeletal muscle impairments are likely contributing to exercise intolerance.

Simon et al. (80) measured leg blood flow, leg VO_2 (VO_{2LEGS}) and whole body VO_2 (VO_{2TOT}) during exercise in 14 men with severe COPD during a symptom-limited incremental cycle test. Despite further increases in the workload and VO_{2TOT} during the incremental exercise, 6 subjects demonstrated a plateau in VO_{2LEGS}, leg blood flow and oxygen extraction. At submaximal exercise (30 W) the COPD patients who plateaued also demonstrated

significantly greater V_T ($p = 0.037$), minute ventilation ($p = 0.048$) and dyspnea ($p = 0.037$) and peaked at lower work rates than those who did not plateau (40 ± 13W vs. 51 ± 10W, $p = 0.043$). Not only was peripheral muscle blood flow impaired, but reduced oxygen extraction in the legs contributed to the reduced exercise capacity.

Central Pulmonary Versus Peripheral Skeletal Muscle

Debate surrounding central versus peripheral mechanisms in generating exercise intolerance in COPD patients is ongoing. Evidence for central pulmonary factors limiting exercise capacity comes from studies examining lung mechanics, gas exchange and work of breathing. Peripheral skeletal muscle as the limiting factor relates to changes in fibre type proportions and enzyme concentrations that decrease the muscle's ability to sustain aerobic activity, potentially triggering the muscle metaboreflex and limiting exercise capacity. The contribution of both central and peripheral factors in causing fatigue must therefore be considered.

The Muscle Metaboreflex and Isometric Handgrip Exercise

It is hypothesized that skeletal muscle dysfunction contributes to impaired exercise capacity in COPD through its impact on cardiovascular control. In health, an increase in MSNA accompanies the transition from rest to exercise, as does an increase in heart rate, blood pressure and peripheral vasoconstriction. Three mechanisms work together to regulate these changes. The first, "central command" initiates cardiovascular changes at the onset of voluntary exercise and is the primary mechanism responsible for the initial increase in heart rate (35; 84). The second mechanism, arterial baroreflex, aims to correct a mismatch between vascular conductance and cardiac output by controlling arterial vasoconstriction in skeletal muscles to regulate blood pressure. The baroreflex is activated early in exercise and continuously

throughout (68). The final mechanism, the pressor reflex, is comprised of the mechanoreflex and metaboreflex (or chemoreflex). The mechanoreflex is activated by mechanical deformation caused by muscle contraction or stretch. The muscle metaboreflex responds to a mismatch between oxygen supply and demand and does not play an active role in controlling cardiovascular activity until sufficient metabolite accumulation has occurred. Group IV afferents located in skeletal muscle act to initiate the efferent arm of the muscle metaboreflex, a rise in MSNA which produces the pressor response (21). The pressor response encompasses the rise in heart rate, blood pressure and vasoconstriction in inactive skeletal muscles seen during exercise (35). The pressor response attempts to eliminate the mismatch between oxygen supply and demand by increasing oxygenated blood flow to working skeletal muscles. However, not all studies suggest it is successful in doing so (34). The muscle metaboreflex relies on metabolite accumulation to not only initiate the pressor response, but also to control its magnitude. The type and intensity of exercise, as well as skeletal muscle characteristics, such as muscle fibre type and metabolism, can alter the onset and magnitude of the pressor response (21).

The specific metabolites initiating the efferent arm of the muscle metaboreflex are still unknown. However, lactic acid, bradykinins, phosphates and potassium ions are all speculated as being important metabolites in this reflex (81). MacLean et al. (42) used microdialysis probes inserted into the vastus lateralis muscle of 7 healthy men to assess the relationship between interstitial lactic acid, phosphate and potassium and the pressor response during static quadriceps exercise performed at 25% MVC intermittently for 5 minutes (20 seconds contracting followed by 5 seconds of relaxation). This study examined the time course of by-product accumulation and corresponding rise in MSNA during exercise. Their results indicate: 1) lactate influences the magnitude of the pressor response, but does not directly initiate the muscle metaboreflex 2) potassium concentrations, which increase rapidly at the onset of exercise and remain elevated following completion of exercise, contribute only to the initiation of the muscle

metaboreflex 3) interstitial phosphate, which rises and falls in conjunction with the pressor response during exercise and recovery, is the primary metabolite responsible for initiating and sustaining the pressor response. These findings have significant implications for understanding exercise intolerance in people with COPD as a greater proportion of type-II fibres, in impaired phosphogen kinetics combination with decreased oxidative enzyme activity, may make them more susceptible to greater levels of inorganic phosphates, lactate and hydrogen ion accumulation and intracellular acidosis during exercise (76).

The onset and magnitude of the pressor response is also affected by the type, duration and intensity of exercise. Isometric exercise followed by post-exercise circulatory occlusion is often the model chosen to assess the muscle metaboreflex. In this model, metabolites accumulated during ischemic work remain in the arm during occlusion at the same time the working muscle is allowed to relax. The contribution of central command and the mechanoreflex to the pressor response is therefore removed, and the metabolites that remain trapped in the arm during occlusion are responsible for the pressor response occurring during the occlusion period. The intensity at which these isometric contractions are performed is also relevant, as isometric handgrip exercise performed at 25 and 35% MVC elicits a greater increase in heart rate, blood pressure, MSNA and calf vascular resistance, than that elicited by 15% MVC (74).

Fibre type also influences the magnitude of the pressor response. Wilson et al. (88) applied 21 days of chronic stimulation to the tibial nerve of a rabbit's hindlimb, causing the primarily glycolytic gastrocnemius, to become primarily oxidative. Following this transformation, the converted gastrocnemius muscle initiated a smaller pressor response during sustained contraction (rest: 83 ± 3 mmHg, contraction: 91 ± 5 mmHg) than that achieved by the unchanged glycolytic muscle (rest: 84 ± 2 mmHg, contraction: 97.5 ± 5 mmHg), ($p = 0.008$). Interestingly, the unchanged glycolytic muscle was working at a lower percent of maximum than

the oxidative muscle, yet it elicited a greater magnitude pressor response than the oxidative muscle.

Based on our knowledge of COPD and the changes in skeletal muscle associated with this disease, it is reasonable to hypothesize that the muscle metaboreflex is altered. First, as mentioned previously, the metabolites which initiate and control the magnitude of the pressor response are more abundant in the skeletal muscles of COPD patients compared to healthy controls such as inorganic phosphates and lactate ions. Second, with reduced muscle mass and strength, COPD patients will be working at a higher percentage of their maximum while performing any isometric contraction (including those involved in activities of daily living); hence, a greater pressor response is expected. Third, a greater proportion of glycolytic fibres may also contribute to a magnified muscle metaboreflex response during exercise.

Little work has been done examining the relationship between exercise intolerance and the pressor response in COPD. To date, only one study has examined this relationship and the findings from this study differ from those hypothesized to occur. Roseguini et al. (66) compared changes in heart rate, blood pressure and calf vascular resistance in COPD patients and healthy controls following isometric handgrip exercise performed at 30% MVC for 3 minutes followed by circulatory occlusion. Surprisingly, the COPD group demonstrated a similar pressor response compared to healthy controls, with similar changes from baseline in heart rate and blood pressure in both groups. Calf vascular resistance, measured using strain gauge plethysmography, did differ between groups as it increased from baseline in the healthy group by 38% and increased by only 20% in the COPD group. These findings are unexpected considering the evidence pointing to the role skeletal muscle characteristics play in controlling the magnitude of the muscle metaboreflex. Furthermore, a similar study measuring the muscle metaboreflex was performed in heart failure patients who as mentioned previously, demonstrate similar levels of skeletal muscle dysfunction to COPD patients (26). After measuring heart rate, blood pressure

and MSNA throughout isometric handgrip exercise (30% MVC) and during post-exercise circulatory occlusion and recovery, Notarius et al. (54) found that despite similar baseline and exercise blood pressure values, heart rate in heart failure patients remained elevated during circulatory occlusion where in controls it returned to baseline ($p < 0.05$). MSNA followed this trend as well ($p < 0.05$). Obvious discrepancies exist between these 2 studies and further exploration of the relationship between COPD and the muscle metaboreflex is needed.

Summary

COPD is most commonly described as a disease of the lungs and airways. Though this emphasis on central pulmonary pathophysiology is warranted, evidence from studies examining skeletal muscle changes in the legs suggest peripheral factors are also at play in this disease. The effect central impairments have on exercise capacity is obvious, as it generates expiratory flow limitation and a ventilation-perfusion mismatch which, in turn, alter lung and diaphragm mechanics and the work of breathing during exercise and even at rest in people with severe COPD.

Detraining, altered blood gases, and malnutrition have been explored as potential mechanisms generating changes in the skeletal muscle properties of people with COPD. These changes include, reduced oxidative capacity, impaired phosphogen kinetics and a larger proportion of glycolytic fibres. Through activation of the muscle metaboreflex these changes in skeletal muscle properties may also be contributing, along with pulmonary impairments to the exercise intolerance associated with COPD.

APPENDIX B. INDIVIDUAL RAW DATA

Data presented in the appendix represent absolute values for each participant in the variables of interest. Participant ID's increase with increasing age in both the COPD and control groups.

Table 8: Individual subjects' anthropometric and pulmonary function characteristics

	ID	Age (years)	Sex 1=M 2=F	Weight (kg)	Height (cm)	BMI (kg/m^2)	$FEV_{1.0}$ (L)	$FEV_{1.0}$ (%predicted)	FVC (L)	FVC (%predicted)	FEV_1/FVC (%)
COPD	1	45	1	53.9	181.5	16.4	1.3	32	4.1	78	32
	2	61	1	86.5	183.0	25.8	1.6	42	3.8	77	43
	3	64	1	91.1	173.2	30.4	2.7	78	3.4	79	78
	4	64	2	47.1	160.6	18.3	1.3	58	2.4	81	56
	5	66	2	65.9	156.0	27.1	1.0	49	1.7	66	58
	6	66	2	47.9	158.5	19.1	0.8	38	2.5	91	33
	7	68	2	65.9	163.0	24.8	2.1	95	3.4	117	62
	8	71	2	71.0	155.3	29.5	1.8	94	2.5	101	71
	9	71	2	53.0	154.0	22.3	1.3	68	2.6	95	50
	10	74	1	51.4	172.7	17.2	0.8	24	3.3	80	23
	11	75	2	76.4	153.8	32.3	0.7	41	1.5	64	48
Controls	1	42	1	81.8	178.0	25.8	3.8	91	5.4	105	70
	2	60	2	69.0	160.0	27.0	2.5	107	3.3	110	77
	3	61	1	72.1	169.0	25.2	3.3	101	4.5	109	73
	4	62	1	94.9	178.3	29.9	3.3	89	4.3	92	76
	5	63	2	69.0	162.0	26.3	2.6	111	3.4	112	78
	6	64	2	56.4	163.0	21.2	2.3	99	3.0	97	79
	7	65	2	56.6	160.0	22.1	1.7	77	2.8	96	63
	8	68	2	57.1	174.3	18.8	2.4	89	3.6	104	65
	9	69	2	58.5	162.5	22.2	2.1	96	3.4	117	63
	10	69	1	78.5	184.3	23.1	4.0	107	5.3	108	75
	11	75	2	56.4	151.1	24.7	1.7	103	2.8	128	61

Table 11: Resting heart rate variability characteristics. The ID with a * indicates data was removed from average. rMSSD = square root of the mean of the squares of differences between adjacent NN intervals, LF= low frequency band, HF= high frequency band

	ID	LF (nu)	HF) (nu)	LF/HF	rMSSD (ms)
COPD	1	23.7	76.3	0.311	134.9
	2				
	3	43.7	56.3	0.776	11.1
	4	41.8	58.2	0.718	17.1
	5	26.1	73.9	0.354	12.9
	6	48.1	51.9	0.926	13.8
	7	51.5	48.5	1.062	9.6
	8	51.1	48.9	1.044	16.3
	9	14.5	85.5	0.170	29.0
	10	35.4	64.6	0.549	65.7
	11	14.7	85.3	0.173	185.9
Controls	1	45.9	54.1	0.847	39.5
	2	27.6	72.4	0.382	33.1
	3*	47.6	52.4	0.908	19.0
	4	46.5	53.5	0.867	18.4
	5	26.7	73.3	0.365	17.4
	6	74.3	25.7	2.896	13.7
	7	50.8	49.2	1.033	15.2
	8	34.4	65.6	0.523	52.6
	9	35.0	65.0	0.538	32.3
	10	79.1	20.9	3.795	13.9
	11	86.5	13.5	6.393	6.6

Table 12: Individual subject characteristics at rest. HR = heart rate; V_E = minute ventilation; V_T = tidal volume; F_b = breathing frequency; $PetCO_2$ = partial pressure of end-tidal carbon dioxide; SaO_2 = arterial oxygen saturation

	ID	HR (bpm)	V_E (L/min)	V_T (L)	F_b (bpm)	$PetCO_2$ (mmHg)	SaO_2 (%)
COPD	1	63	13.2	0.70	9	37.5	98
	2	48	15.6	0.93	17	31.2	97
	3	73	16.2	0.87	22	28.2	96
	4	63	12.5	0.55	22	27.5	98
	5	69	11.3	0.76	15	35.7	98
	6	74	11.3	0.66	17	27.8	97
	7	71	12.0	0.93	13	31.5	99
	8	67	8.3	0.53	16	30.8	99
	9	59	12.2	0.59	21	31.7	98
	10	83	21.5	0.50	20	31.5	97
	11	53	9.0	0.54	18	41.6	96
Controls	1	61	11.6	1.07	11	33.9	98
	2	60	9.3	0.92	11	34.4	100
	3	55	13.6	0.76	18	36.1	100
	4	57	13.7	0.75	19	37.2	98
	5	56	8.8	0.51	17	38.2	99
	6	79	7.8	0.92	9	38.2	99
	7	66	9.9	0.65	15	32.8	99
	8	54	7.3	0.49	15	37.1	
	9	58	10.7	0.82	13	33.2	99
	10	73	8.4	1.24	7	38.5	98
	11	70	7.0	0.49	16	34.0	98