The Effect of Pulmonary Rehabilitation on Carotid Chemoreceptor Activity and Sensitivity in Chronic Obstructive Pulmonary Disease

by

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Abstract

Chronic obstructive pulmonary disease (COPD) is associated with increased cardiovascular (CV) risk, which may result from heightened sympathetic nerve activity secondary to elevated carotid chemoreceptor (CC) activity/sensitivity. Our recent work indicates that CC activity/sensitivity is elevated in COPD compared to controls, which appears to contribute to increased CV risk resulting from tonic vasoconstriction. Exercise training has been shown to normalize CC activity/sensitivity in other populations characterized by elevated CC activity/sensitivity. COPD patients are commonly referred to pulmonary rehabilitation (PR), an exercise-based intervention shown to improve dyspnea, exercise tolerance, and quality of life. The purpose of this study was to determine whether PR can reduce CC activity/sensitivity in COPD.

A pre-post case-control study design was used; COPD patients on the PR waiting list were enrolled in the experimental group, while COPD patients not enrolled in PR were recruited as controls. CC activity/sensitivity, exercise tolerance, resting dyspnea, quality of life, arterial stiffness, and autonomic function were assessed before and after the intervention period. CC activity was determined by the reduction in minute ventilation ($\Delta\dot{V}_E$) while breathing transient hyperoxia (F_IO_2=1.0). CC sensitivity was evaluated by the increase in $\dot{V}_E$ relative to the drop in arterial saturation while breathing hypoxic gas ($\Delta\dot{V}_E/\Delta SpO_2$). Functional exercise tolerance was assessed by six-minute walk distance, the modified Medical Research Council questionnaire was used to determine resting dyspnea, and quality of life was assessed with the St. George's Respiratory Questionnaire. Arterial stiffness was assessed using carotid-radial pulse wave velocity while autonomic function was examined with heart-rate variability and baroreceptor sensitivity.

Data from 45 patients completing PR and 15 COPD controls were analyzed. Patients completing PR improved exercise tolerance ($p=0.01$), resting dyspnea ($p=0.03$), and health-related quality of life ($p=0.03$). There was no effect of PR on CC activity or CC sensitivity in stable COPD patients. When stratified by the degree of baseline CC activity, individuals with greater baseline CC activity significantly reduced CC activity following PR ($p<0.01$); however this improvement in CC activity occurred independently of changes in arterial stiffness or autonomic function.

These data indicate that, despite improving exercise tolerance, resting dyspnea, and health-related quality of life, PR does not appear to affect CC activity/sensitivity in COPD. PR may normalize CC activity in stable COPD patients, however this is independent of changes in indicators of CV risk. It is possible that the exercise training stimulus achieved during PR is insufficient to elicit changes in CC activity/sensitivity, or unlike what has been observed in experimental heart failure, exercise training may not be an appropriate intervention in reducing CC activity/sensitivity and the associated CV risk in stable COPD patients.

Preface

This thesis is an original work by Bradley William Byers. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Health Research Ethics Board Biomedical Panel, Project Name “The Impact of Pulmonary Rehabilitation on Cardiovascular Function in Chronic Obstructive Pulmonary Disease”, ID No. Pro00021962, August 2011.

Dedications

This thesis is dedicated to my parents, Donald and Marilyn Byers.

"I love you beyond paint, beyond melodies, beyond words. And I hope you will always feel that, even when I'm not around to tell you so." – Kiera Cass

"Chan ann leis a'chiad bhuille thuiteas a'chraobh" – It is not with the first stroke that the tree falls.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

BRS: Baroreceptor sensitivity

CC: Carotid chemoreceptors

CHF: Chronic heart failure

COPD: Chronic obstructive pulmonary disease

CPET: Cardiopulmonary exercise test

CV: Cardiovascular

FEV_1: Forced expiratory volume in the first second

GOLD: Global initiative for chronic obstructive lung disease

HRV: Heart rate variability

MMRC: Modified medical research council dyspnea questionnaire

MSNA: Muscle sympathetic nerve activity

PFT: Pulmonary function test

PR: Pulmonary rehabilitation

PWV: Pulse wave velocity

RMSSD: Square root of the mean of the sum of squares of differences between time intervals of adjacent sinus heart beats

SDNN: Standard deviation of the time interval between consecutive sinus heart beats

SGRQ: St. George's respiratory questionnaire

SNA: Sympathetic nerve activity

SpO_2: Estimated arterial oxygen saturation

$\dot{V}_E$: Minute ventilation

V_T/T_I: Mean inspiratory flow

6MWD: Six minute walk distance

6MWT: Six minute walk test

CHAPTER I: INTRODUCTION

1.1. Background

Chronic obstructive pulmonary disease (COPD) is a chronic lung disease primarily caused by smoking, and is characterized by impaired lung function, dyspnea, and physical inactivity.[1] Though originally considered a disease specific to the lungs, COPD has recently been associated with increased cardiovascular (CV) morbidity and mortality compared to the healthy population.[1] While the etiology of the increased CV risk in COPD remains undetermined, previous research suggests that the carotid chemoreceptors (CC) may contribute to this increased CV risk. The CC are located in the carotid bifurcation and are sensitive to reductions in the arterial partial pressure of O_2 (P_aO_2), hypercapnia, acidosis, reduced arterial blood flow, as well as changes in temperature and low glucose levels;[2] these receptors reflexively control ventilation ($\dot{V}_E$) to maintain resting blood gas levels. Research in chronic heart failure (CHF) demonstrates that the CC contribute to chronic elevations in sympathetic nerve activity (SNA).[3, 4] While acute elevations in SNA are initially beneficial in maintaining CV function, chronically-elevated SNA is associated with increased risk of CV morbidity and mortality.[5] Sympathetic activity is elevated in COPD patients,[6] and this may be secondary to heightened CC activity/sensitivity as individuals with COPD have an exaggerated heart rate[7] and ventilatory[8] response to hypoxia. Our recent findings demonstrate that non-hypoxemic, normocapnic mild/moderate COPD patients have increased CC activity/sensitivity as determined by an exaggerated ventilatory response to hyperoxia and hypoxia, respectively.[9]

One major CV risk factor is arterial stiffness, assessed by pulse wave velocity (PWV).[10] Both aortic[11-13] and peripheral[14] PWV are elevated in individuals with COPD compared to the healthy population, and increased aortic PWV is associated with greater risk of CV events and

mortality.[10, 15, 16] It is possible that this increased PWV in COPD is, in part, secondary to elevated SNA resulting from an increased CC activity/sensitivity, as CC inhibition with transient hyperoxia resulted in a significant reduction in peripheral PWV in COPD patients, but not healthy controls.[9] These findings suggest that interventions aimed at reducing CC activity/sensitivity may be beneficial in mediating the CV risk in COPD patients.

Additional indicators of autonomic dysfunction have been observed in COPD patients, including a reduced heart rate variability (HRV)[17] and a reduced baroreceptor sensitivity (BRS),[18] both of which are associated with increased risk of CV events.[19, 20] Low HRV and BRS indicate impaired parasympathetic autonomic control which may be a result of heightened CC activity/sensitivity. Supplemental O_2 has been shown to improve HRV[21] and BRS,[22] which the authors attribute to an increased vagal tone. It is possible that supplemental O_2 inhibits the CC, resulting in sympathetic withdrawal. Neurons from the CC and carotid baroreceptors are distributed in close proximity in the solitary and paramedian reticular nuclei in the medulla, and therefore interneuronal connections might facilitate interactions between these reflexes.[23] It is possible that this CC inhibition with supplemental O_2 resulted in reduced sympathetic inhibition of the vagal baroreceptor efferent activity. This relationship would then suggest a role of the CC in increasing CV risk in COPD, secondary to reductions in HRV and BRS.

Heightened CC activity/sensitivity may also potentiate dyspnea in COPD. Dyspnea is a major contributor to physical inactivity in COPD,[24] and is a better predictor of five year survival in COPD patients than other indicators such as lung function as assessed by forced expiratory volume in one second (FEV_1).[25] A positive relationship between CC activity/sensitivity and dyspnea has been suggested in previous literature;[26] one potential mechanism for the relationship between the CC and dyspnea is lung hyperinflation secondary to an increased ventilation,

resulting in neuromechanical uncoupling.[27] Therefore, reducing CC activity/sensitivity in COPD may be important in reducing dyspnea, thereby improving functional exercise tolerance and health-related quality of life in these patients.

Collectively, findings from previous research would suggest that the CC contribute to CV risk in COPD, and thus there is a need to determine appropriate interventions aimed at reducing CC activity/sensitivity in COPD. Exercise training is an important symptom-management strategy for many diseases, with multiple CV and autonomic benefits. Studies have shown that exercise training reduces CC activity/sensitivity and SNA in experimental CHF rabbits,[28] and reduces SNA in CHF patients.[5, 29] Exercise training in CHF has also been shown to significantly improve systemic vasodilation, $\dot{V}_E$, the slope relating $\dot{V}_E$ to CO_2 production, 24-hour HRV, as well as reduce norepinephrine spillover;[30] these data would suggest that exercise training is effective in improving sympathovagal balance in CHF patients. Additionally, exercise training improves BRS in patients recovering from myocardial infarction.[31] While the mechanism(s) underlying the improvements in sympathetic activity and autonomic function following exercise training in CHF and myocardial infarction are unknown, it is possible that these improvements are secondary to a reduction in CC activity/sensitivity. As both COPD and CHF are associated with an increased CC activity/sensitivity and SNA, similar benefits may be expected in both groups following exercise training. Consistent with this hypothesis, some studies have shown improvements in autonomic function and indicators of CV risk in COPD following aerobic exercise training programs.[32-34] It is possible that these improvements may be secondary to a reduction in chemosensitivity,[33] however this has not been previously determined. Improvements in CV risk[32-35] and exercise tolerance[36-39] have been noted in those with COPD following

exercise training and rehabilitation programs, however the mechanism(s) underlying these improvements require further investigation.

Pulmonary rehabilitation (PR) is an individually-tailored, exercise-based rehabilitation program, and is considered a crucial component of proper disease management in COPD. Pulmonary rehabilitation has been shown to be the most effective management strategy for improving dyspnea, health-related quality of life, and exercise tolerance in COPD.[40-42] Some studies have shown that PR reduces cardiac sympathetic activity as evaluated by HRV,[32] and improves BRS[33] in COPD patients, while some work has shown that aortic arterial stiffness is improved following PR.[34] It is possible that these improvements in CV risk following PR may be secondary to reduced SNA as a result of a reduction in CC activity/sensitivity, however there are currently no studies examining the effects of PR on CC activity/sensitivity in COPD. While it is documented that PR decreases resting and exertional dyspnea in COPD,[43] and exercise training reduces the ventilatory response to acute exercise,[36, 44] the underlying mechanism(s) for these improvements are unclear and no relationship between reductions in dyspnea and CC activity/sensitivity has been examined.

1.2. Purpose and Hypothesis

The purpose of this study was to examine the effects of PR on CC activity/sensitivity in stable COPD patients, as assessed by the ventilatory response to hyperoxia and hypoxia, respectively. Additionally, secondary indicators of sympathetic control, including PWV, HRV, and BRS were examined to observe if any changes in CC activity/sensitivity were accompanied by reductions in CV risk.

It was hypothesized that CC activity/sensitivity would be reduced in COPD patients following PR, while no changes would be observed in the control group. Additionally, it was

expected that indicators of CV risk would improve following PR but not in the control group. More specifically, it was predicted that peripheral PWV would be reduced in COPD patients following PR, whereas HRV and BRS would be increased following PR with no change seen in controls. Lastly, as literature has suggested that elevated CC activity/sensitivity may contribute to dyspnea, it was expected that resting dyspnea would be reduced in COPD following PR but not in controls, and this would be associated with a reduction in CC activity/sensitivity.

1.3. Delimitations

As PR is considered a standard of care for the management of COPD, randomization of individuals into the rehabilitation or control group is not ethical. For this reason, this study utilized a pre-post case-control study design, where individuals on the PR referral list were approached and recruited into the rehabilitation group, and individuals who were previous graduates of the PR program and currently self-managing their disease were recruited into the control group. All subjects were tested prior to and following 6-8 weeks of PR or time control. Participants consisted of both men and women, and were not matched for CV risk, age, or disease severity. Subjects were excluded if they had an exacerbation of their disease within the previous six months, or if they participated in PR within the last six months.

For the present study, the primary outcomes were CC activity and sensitivity, while the secondary variables included arterial stiffness, HRV, BRS, mean inspiratory flow, and resting dyspnea. To evaluate the effectiveness of the PR program, six minute walk distance, resting dyspnea, and health-related quality of life were examined, as these variables are commonly reported to improve following completion of PR.

The administration of 100% O_2 is a commonly-used method for assessing CC activity. Therefore, when examining CC activity, patients breathed hyperoxic gas (fraction of inspired

oxygen (F_IO_2)=1.0) delivered via an air-O_2 blender system. Hyperoxia inhibits the CC,[45] and the difference between baseline $\dot{V}_E$ and the lowest 10-second average $\dot{V}_E$ during acute hyperoxia (nadir) was taken as the index of basal CC activity;[46, 47] a greater reduction in $\dot{V}_E$ with hyperoxia represents higher CC activity. Additionally, as mean inspiratory flow (V_T/T_I) has been suggested to reflect central neural respiratory drive,[48] V_T/T_I was examined while the patient breathed normoxic gas as well as while they breathed 100% O_2 to assess inspiratory drive. It was hypothesized that if the change in V_T/T_I from normoxia to hyperoxia was reduced following PR compared to baseline, then this may further suggest a reduction in CC activity with PR. In all subjects, the hyperoxia trial was performed prior to the hypoxia trial to prevent hypoxia-induced CC sensitization.

Hypoxia is a potent CC stimulant and is used to evaluate CC sensitivity;[49-51] a widely-accepted method for assessing chemosensitivity is examining the slope of the ventilatory response to hypoxia. In the present study, a hypoxic gas mixture was administered by titrating nitrogen into the inspired gas until a target SpO_2 of 90% and 85% were reached, with each step reduction in arterial saturation being maintained for three minutes. Chemosensitivity was evaluated by the slope of the regression line relating average $\dot{V}_E$ determined at baseline, and the maximal 30-second average $\dot{V}_E$ at SpO_2 90%, and SpO_2 85% relative to arterial saturation (i.e. $\Delta\dot{V}_E/\Delta SpO_2$ slope).

To examine changes in peripheral vascular tone, peripheral PWV was measured at the carotid and radial arteries using applanation tonometry, and analyzed using the foot-to-foot method.[52] It was expected that a reduction in CC activity would be accompanied by a corresponding reduction in peripheral PWV as has been shown in previous work from our laboratory,[9] likely resulting from reduced tonic vasoconstriction. While central PWV has been

shown to independently predict CV events,[10, 15, 16] peripheral PWV was examined as a result of the increased smooth muscle control of the peripheral vasculature, thereby serving as a better indicator of sympathetic tone.

When assessing HRV, two variables were measured: 1) the standard deviation of the time interval between consecutive sinus heart beats (SDNN); and 2) the square root of the mean of the sum of squares of differences between time intervals of adjacent sinus heart beats (RMSSD). Furthermore, BRS was assessed by examining the change in the time interval between consecutive heart beats (RR interval) in response to spontaneous fluctuations in systolic blood pressure.[53] Both HRV and BRS were assessed during a standardized five minute period where the patient was resting motionless breathing normoxic gas, and ECG recording and blood pressure were stable and continuously measured.[54]

To assess functional exercise tolerance, all subjects completed the six minute walk test (6MWT) in a linear hallway, in accordance with the guidelines previously outlined by the American Thoracic Society.[55] The rehabilitation group performed three 6MWTs at the beginning and end of PR, and the best two of three 6MWTs were averaged to represent pre- and post-PR 6MWT values. The control group performed one 6MWT prior to and following the time control period.

Resting dyspnea was assessed with the Modified Medical Research Council (MMRC) dyspnea scale (scored 0-4). The MMRC is a reliable and effective measure of dyspnea in patients with COPD, and dyspnea ratings from this questionnaire has been shown to be associated with disease severity in COPD.[56] The St. George's Respiratory Questionnaire (SGRQ) was used to assess health-related quality of life in COPD, and has been shown to be correlated with MMRC dyspnea scores.[57]

1.4. Limitations

One limitation of this study is that only stable COPD patients who have not had an exacerbation of their condition within the previous six months, and who were able to attend both the PR program and the experimental testing days were recruited. Additionally, time controls consisted of COPD patients who completed the PR program a minimum of six months prior to participating in the study. It is therefore possible that asymptomatic, milder COPD patients were missed in this study. Additionally, those with more severe COPD and limited physical activity (ie. recent exacerbations/hospitalizations and orthopedic limitations) were also not included in the present study. The study may therefore not be representative of the general COPD population, and thus caution should be used when extrapolating the results of the present study.

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CHAPTER II: THE EFFECT OF PULMONARY REHABILITATION ON CAROTID CHEMORECEPTOR ACTIVITY AND SENSITIVITY IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

2.1. Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by progressive, partially-reversible airway obstruction, and while originally considered a disease specific to the lungs, COPD has recently been associated with increased cardiovascular (CV) morbidity and mortality compared to the healthy population.[1] Individuals with COPD are three times more likely to die of heart failure than smokers not diagnosed with COPD,[2] indicating that the elevated CV risk in COPD may be specific to the pathophysiology of the disease, and not simply smoking history. Autonomic dysfunction has been reported in COPD, which may contribute to the elevated CV risk observed in these patients; particularly, increased sympathetic nerve activity (SNA) has been reported in both hypoxemic and non-hypoxemic COPD patients.[3-7] While acute elevations in SNA are beneficial in maintaining CV function, chronically-elevated SNA is associated with increased risk of CV morbidity and mortality, with the degree of resting SNA being predictive of mortality and hospitalization in COPD.[7]

While the etiology of the increased SNA and CV risk in COPD remains undetermined, previous research suggests that the carotid chemoreceptors (CC) may contribute to this increased CV risk. Our laboratory has recently shown that non-hypoxemic, normocapnic mild/moderate COPD patients have increased CC activity/sensitivity compared to healthy age-matched controls as determined by an exaggerated ventilatory response to hyperoxia and hypoxia, respectively.[8] Additionally, research in chronic heart failure (CHF) demonstrates that the CC contribute to chronic elevations in SNA.[9, 10] In healthy males, arterial stiffness, assessed by pulse wave

velocity (PWV), correlated significantly with muscle SNA following adjustment for age and systolic blood pressure;[11] this is important as an elevated PWV is associated with greater risk of CV events and mortality.[12-14] Short-term sympathetic activation decreases arterial compliance in healthy individuals,[15] while CC inhibition with transient hyperoxia has been shown to significantly reduce PWV in COPD patients but not healthy controls.[8] Furthermore, reduced heart rate variability (HRV)[16] and baroreceptor sensitivity (BRS)[17] have been reported in COPD, which is associated with increased risk of CV events.[18, 19] Both HRV and BRS are improved in COPD with supplemental O_2 administration,[18, 19] which may suggest a role of the CC in autonomic dysfunction. Heightened CC activity/sensitivity may also potentiate dyspnea in COPD,[20, 21] which is a major contributor to physical inactivity in these patients.[22] Collectively, these findings suggest that the CC may play an important role in the elevated CV risk observed in COPD patients.

If the CC contribute to the increased CV risk in COPD, then interventions aimed at reducing CC activity/sensitivity in COPD require further examination. Exercise training is an important symptom-management strategy for many diseases, with multiple CV and autonomic benefits. Exercise training has been shown to reduce CC activity/sensitivity and SNA in experimental CHF rabbits.[23] In patients with CHF, exercise training has been shown to reduce SNA,[24, 25] as well as increase HRV and reduce norepinephrine spillover;[26] importantly all but two of these patients were on angiotensin converting enzyme (ACE) inhibitors. As ACE inhibitors affect parasympathetic tone,[27] these data indicate that the improvements in autonomic function following exercise training in CHF are additive to ACE inhibition.[26] Additionally, exercise training improves BRS in patients recovering from myocardial infarction.[28] Together, these findings would indicate an improvement in autonomic function with exercise training, and while

the mechanism(s) underlying these improvements in autonomic function are unknown, it is possible that exercise training results in decreased CC activity/sensitivity, resulting in an improved sympathovagal balance. As both COPD and CHF are associated with an increased CC activity/sensitivity and SNA, similar benefits may be expected in both groups with exercise training.

Research has shown improvements in autonomic function and indicators of CV risk[29-33] as well as exercise tolerance[34-37] in COPD following either aerobic exercise training or pulmonary rehabilitation (PR) programs. Pulmonary rehabilitation is an individually-tailored, exercise-based rehabilitation program, and is a crucial component of proper disease management in COPD. Pulmonary rehabilitation is the most effective management strategy for improving dyspnea, quality of life, and exercise tolerance in COPD.[38-40] The improvements in CV risk reported following PR may be secondary to a reduction in CC activity/sensitivity,[30] however no studies have examined the effects of PR on CC activity/sensitivity in COPD. Thus, the purpose of this study was to examine the effects of PR on CC activity/sensitivity in patients with stable COPD. It was hypothesized that PR would reduce CC activity/sensitivity compared to controls, as indicated by a reduced ventilatory response to hyperoxia and hypoxia, respectively. Additionally, as CC activity/sensitivity may potentiate dyspnea,[20, 21] arterial stiffness,[8] and autonomic dysfunction,[18, 19] it was expected that if a reduction in CC activity/sensitivity was observed following PR, then a reduction in resting dyspnea and PWV, as well as improvements in HRV and BRS would also be observed following PR.

2.2. Methods

The present study was approved by the University of Alberta Health Research Ethics Board (Biomedical Panel). Written informed consent was obtained by all subjects prior to undergoing any research procedures. Pulmonary rehabilitation has become a standard of care for individuals with chronic lung disease; therefore, the present study utilized a pre-post case-control study design, where COPD patients enrolled in PR were evaluated before and after a 6-8 week PR program, while stable COPD controls not participating in PR were tested prior to and following a 6-8 week time control period.

2.2.1. Rehabilitation Group

COPD patients enrolled in PR were recruited into the rehabilitation group. All subjects were recruited from the G.F. MacDonald Centre for Lung Health, at the Edmonton General Continuing Care Centre. COPD patients were selected using the American Thoracic Society criteria of irreversible post-bronchodilator airflow obstruction (FEV_1/FVC below the lower limit of normal predicted from height, age, sex).[41] Participants were excluded from enrollment in the study if they had a COPD exacerbation within the previous six months, or had participated in PR within the past six months. Subjects were not excluded from the study if they had stable CV disease, used prescribed vasoactive medications, had sleep apnea, or had a body mass index (BMI) $\geq$ 32.

2.2.2. Pulmonary Rehabilitation

The rehabilitation group attended the Breathe Easy PR program at the G.F. MacDonald Centre for Lung Health in the Edmonton General Continuing Care Centre, Edmonton, AB, Canada; this PR program has been previously described by Selzler *et al.* (2012).[42] Participants attended the PR program for either six weeks (three classes per week) or eight weeks (two classes per week). Each session consisted of two hours of group exercise and one hour of group education.

All exercise was supervised by respiratory or exercise therapists and followed PR guidelines for exercise training.[38] Group exercises included 20-40 minutes of aerobic exercise training (hallway and treadmill walking, and cycle/arm ergometry training), as well as light resistance training (free hand weights and resistive elastic tubing), flexibility, and breathing exercises. All participants were encouraged to perform aerobic exercise at an intensity of greater than 60% of maximal work rate,[43] or an exercising heart rate of 60-85% of the peak heart rate achieved during the pre-rehabilitation stress test. Ultimately, however, exercise prescription was highly individualized to each subject based on daily variations in their ability, symptoms, and exercise tolerance.

2.2.3. Control Group

Individuals with stable COPD who were not enrolled in PR were recruited into the control group, where they continued usual patient care for an equivalent 6-8 week time control period. Recruitment for the control group followed identical inclusion and exclusion criteria as outlined for the rehabilitation group.

2.2.4. Study Protocol

All participants completed three days of testing within a 2-3 month period. Prior to starting the PR program (rehabilitation group) or an equivalent time control period (control group), subjects came in for two testing days within a 30 day period. Following the intervention period, subjects returned to the laboratory for a third day of testing. For each testing day, participants came to the laboratory between 8:00 and 10:00AM, and were asked to refrain from caffeine, exercise, alcohol, and short-acting respiratory medications for at least six hours prior to testing.[44]

On the first day of testing, participants completed a brief medical history, a full pulmonary function test (PFT), as well as a cardiopulmonary exercise test (CPET). The PFT[45] and CPET[46] adhered to previously established guidelines. Subjects then returned to the

laboratory on a second day for assessment of CC activity/sensitivity, as well as other vascular and cardiorespiratory measures including arterial stiffness and autonomic function. Additionally, subjects completed questionnaires regarding dyspnea and health-related quality of life; control subjects also completed a six minute walk test (6MWT) at this time. Following the intervention period, participants returned to the laboratory for a third day of testing where CC function, vascular, and cardiorespiratory measures were reassessed and subjects completed the same questionnaires as before; control subjects also completed another 6MWT. Individuals in the rehabilitation group completed pre- and post-6MWTs during the PR program (see section 2.2.10 *Six Minute Walk Test*). For a schematic of the study outline, see Figure 2-1.

2.2.5. Instrumentation

On the second and third day of testing, participants came into the laboratory and completed the Modified Medical Research Council (MMRC) questionnaire to assess resting dyspnea, as well as the St. George's Respiratory Questionnaire (SGRQ) to examine health-related quality of life. Upon completing these questionnaires, participants lay supine on a bed and breathed through a mouthpiece with the nose occluded. Inspired gas was passed through a humidifier (HC 150; Fisher and Paykel Healthcare, Auckland, New Zealand) and delivered continuously using a flow-through system to prevent rebreathing of expired gas (flow 0.5-1.0 L/s). A pneumotachometer (3700 series; Hans Rudolph, Kansas City, MO) was placed just distal to the mouthpiece to determine ventilation. Expired CO_2 and O_2 (mmHg) was continuously measured (Analyzers 17630/17625; Vacumed, Ventura, CA) from a small sample port off of the mouthpiece. Arterial oxygen saturation (SpO_2) was estimated with pulse oximetry (N-595; Nellcor Oximax, Boulder, CO) using a sensor placed on the left index finger. Heart rate was recorded with a single-lead ECG (lead II, Dual Bio Amp; ADInstruments), and blood pressure was monitored using a finger photoplethysmograph placed on the left middle finger (Finometer model 2; Finapres,

Amsterdam, The Netherlands). Data were recorded and integrated through a data acquisition system and sampled at a rate of 1 k/s (Powerlab 16/30, ADInstruments, New South Wales, Australia) and analyzed offline using the associated software (LabChart 7.0, ADInstruments, New South Wales, Australia).

2.2.6. Arterial Stiffness

Following instrumentation, patients lay supine on the bed in a dark, temperature-controlled room for a minimum of 10 minutes to achieve a rested state. Upon stabilization of heart rate and blood pressure, resting arterial stiffness was assessed with carotid-radial PWV. Pulse waves were collected simultaneously using applanation tonometry (Mikro-tip Catheter Transducers model SPT-301, Millar Instruments, Inc., Houston, Texas) from the carotid and radial arteries. Pulse wave velocity was calculated as $PWV = D/\Delta t$, where D was the distance (m) between measuring sites, and Δt was the time difference (s) between pulse waves using the foot-to-foot method.[44] Distances were measured on the surface of the skin with a tape measure, beginning at the sternal notch and extending to the recording sites on the carotid and radial arteries; D was calculated as $D = radial\ (m) - carotid\ (m)$. Mean PWV was calculated as the average of at least 10 consecutive pulse waves in order to ensure that a complete respiratory cycle was covered.[44]

2.2.7. Autonomic Function

Following arterial stiffness measurements, a standardized five minute recording[47] was collected while the subject breathed normoxic gas to analyze HRV and BRS, measures of autonomic function. During this time, the patient lay motionless with their arms at their side so that ECG and blood pressure recordings were stable. Auto-calibration of the finometer was turned off to allow for the continuous measurement of beat-to-beat blood pressure.

Heart rate variability was evaluated according to the Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology, using the

standard deviation of the time interval between consecutive sinus heart beats (SDNN), as well as the square root of the mean of the sum of squares of differences between time intervals of adjacent sinus heart beats (RMSSD).[47] Calculation of SDNN (ms) and RMSSD (ms) was performed in LabChart 7.0 (LabChart 7.0, ADInstruments, New South Wales, Australia). Following detection of each heart beat (detected as the maximum of the QRS complex), the time interval between consecutive heart beats (RR interval) was transformed into a continuous digitized signal by resampling at a rate of 1/mean RR interval.[48] To remove the direct-current frequency component, the mean RR interval was subtracted from the resampled signal.

Baroreceptor sensitivity was evaluated using the sequencing method,[49] examining the change in RR interval (ms) relative to the change in systolic blood pressure (mmHg). Linear regression analysis of spontaneous sequences of three or more consecutive heart beats was performed, where changes in systolic blood pressure and the associated RR intervals had the same direction of change (up or down).[49] Sequences used for analysis included those in which blood pressure changed ≥1mmHg and RR interval changed ≥4ms. For each of these sequences, the regression coefficient between changes in systolic blood pressure and the same pulse beats (Lag 0) was calculated as an estimate of the BRS for that particular sequence. Total BRS was then estimated as the mean of the regression coefficients for each of the individual sequences analysed;[49] analysis of BRS was performed with a custom DosBox software (Version 31-03-93 of the BAROSLOPE program), following the analysis procedures outlined by Blaber *et al* (1995).[50]

2.2.8. Carotid Chemoreceptor Activity

Basal CC activity was evaluated by examining the transient reduction in resting ventilation in response to hyperoxia.[51, 52] Following the five minute HRV/BRS measurements, subjects breathed hyperoxic gas (F_IO_2=1.0) for one minute through a non-rebreathing system. The

difference between the baseline ventilation and the nadir (the lowest 10-second average ventilation achieved during the one minute of hyperoxia) was taken as the degree of basal CC activity.[53, 54] Transient hyperoxia was used in the present study, as longer term hyperoxia has been shown to act as a central stimulant.[55] Additionally, as mean inspiratory flow (V_T/T_I) has been suggested to reflect central neural respiratory drive,[56] V_T/T_I was measured during normoxia and transient hyperoxia to examine any changes in inspiratory drive which may be associated with changes in CC activity.

2.2.9. Carotid Chemoreceptor Sensitivity

Hypoxia is a potent CC stimulant, and is commonly used to evaluate CC sensitivity.[57, 58] Following a 10 minute recovery period after the hyperoxia trial, patients were reconnected to the breathing apparatus and breathed normoxic gas for five minutes through the non-rebreathe system to achieve a steady-state. Upon stabilization of heart rate and blood pressure, nitrogen was blended into the inspired gas to cause step reductions in SpO_2 to 90% and 85% for three minutes each. The average baseline ventilation and the maximal 30-second average increase in ventilation at 90% and 85% SpO_2 was recorded. Carotid chemosensitivity was evaluated by calculating the slope of the regression line relating the increase in ventilation ($\Delta\dot{V}_E$) from baseline relative to the reduction in arterial saturation (ΔSpO_2). For the present study, estimated arterial saturation (SpO_2) was targeted during hypoxia as opposed to end-tidal O_2 because of the uncertainty regarding estimation of arterial blood gas data using end-tidal values in patients with lung disease.

2.2.10. Six Minute Walk Test

All subjects in the rehabilitation group completed three 6MWTs at the beginning and end of the PR program. The best two of the three 6MWTs were averaged and recorded as their pre- and post-PR 6MWT distances; this was done to minimize any learning effect in this group. For the

control group, only one 6MWT was completed prior to and following the 6-8 week time control period, as all control subjects were previous graduates of the PR program and thus were familiar with the testing protocol. Control subjects performed the 6MWT immediately following the CC, vascular, and cardiorespiratory evaluation on testing days two and three. All subjects completed the 6MWT in accordance with the guidelines previously outlined by the American Thoracic Society.[59]

2.2.11. Statistical Analyses

For all inferential analyses, the probability of committing a Type I error was set at α=0.05. Between-group comparisons at baseline were made using unpaired student's t-tests where appropriate. A two-way repeated-measures ANOVA was used to examine the effect of time (pre vs. post; repeated factor) in the rehabilitation vs. control group (fixed factor). All statistical analyses were conducted using SPSS version 22 (IBM, Armonk, NY).

To further examine the impact of PR on CC activity/sensitivity, the rehabilitation group was stratified by their disease severity according to the Global Initiative for Obstructive Lung Disease (GOLD) guidelines (GOLD I/II – mild/moderate COPD; and GOLD III/IV – severe/very severe COPD),[60] as well as their degree of resting dyspnea (MMRC 0-2 – lower symptom burden; and MMRC 3-4 – higher symptom burden).[60] Statistical analyses were then completed as previously mentioned.

To examine whether baseline CC activity/sensitivity may affect the change in CC activity/sensitivity with PR, a Pearson product-moment correlation was conducted to examine the relationship between the degree of baseline CC activity/sensitivity and the magnitude of change in CC activity/sensitivity following PR. A significant relationship between baseline CC activity and the change in CC activity following PR was observed; the rehabilitation group was then categorized into quartiles based on the degree of baseline CC activity (Greater CC activity –

Upper quartile; Lower CC activity – Quartiles 2-4), and the two groups were compared as described above.

2.3. Results

2.3.1. The Effect of Pulmonary Rehabilitation on Carotid Chemoreceptor Activity and Sensitivity in Chronic Obstructive Pulmonary Disease

2.3.1.1. Subjects

Ninety-six stable COPD patients were initially recruited for this study. Out of these, 73 patients were enrolled in the rehabilitation group and were tested prior to PR. Data were obtained on 49 individuals who completed PR (33% attrition rate); the remaining 24 participants were excluded due to drop out (defined as attending less than 9/16 PR sessions) or they did not return for the post assessment. Data from 45 of the 49 patients completing PR (92%) were included in the data analysis; four subjects were excluded because of periodic breathing while on the mouthpiece. Twenty-eight of the 45 patients completing PR underwent CC sensitivity assessment (62%), whereas the remaining 17 patients were not cleared by a study physician for hypoxia administration. For the control group, 23 COPD patients not enrolled in PR were recruited and tested at baseline, with 16 tested again following a 6-8 week control period. Fifteen of the 16 control subjects (94%) were analyzed for this study; data from one subject was omitted as the subject had frequent coughs throughout the assessment, and variable ventilatory data. Of the 15 control subjects analyzed in this study, two subjects did not undergo CC sensitivity assessment due to reduced oxygen saturation and elevated blood pressure at rest.

Participant characteristics are presented in Table 2-1. At baseline, there were no between-group differences in age, BMI, or smoking history. However, the rehabilitation group had a greater degree of airway obstruction compared to the controls. Additionally, the degree of resting lung hyperinflation was greater in the rehabilitation group compared to the control group, and there was a significantly greater proportion of mild COPD patients in the control group compared to the rehabilitation group. Participants in the rehabilitation group had a significantly

greater self-reported resting dyspnea compared to controls, as assessed by the MMRC dyspnea questionnaire. Subject comorbidities as well as a list of drug classifications used are presented in Tables 2-2 and 2-3, respectively.

2.3.1.2. Effectiveness of the PR Program

Six minute walk distance (6MWD) data are presented in Figure 2-2a. There was a main effect of time ($p=0.01$) but no main effect of group ($p=0.08$) and no time-by-group interaction ($p=0.36$). These results indicate that both the rehabilitation group (Pre: 441 ± 80m; Post: 478 ± 110m) and control group (Pre: 396 ± 90m; Post: 414 ± 94m) increased 6MWD from baseline following the 6-8 week period. Of note, only the rehabilitation group demonstrated a clinically significant increase in 6MWD. When examining the effect of PR on resting dyspnea, there was a main effect of time ($p=0.03$) and group ($p<0.001$), but no time-by-group interaction ($p=0.77$), indicating that individuals in the rehabilitation group had a greater degree of resting dyspnea compared to controls, and that a reduction in dyspnea was observed in both the rehabilitation (Pre: 2.4 ± 0.8; Post: 2.2 ± 0.8) and the control group (Pre: 1.5 ± 0.9; Post: 1.1 ± 0.9) See Figure 2-2b.

There was a significant reduction in total SGRQ score in the rehabilitation group following PR (Pre: 42.4 ± 16.3; Post: 39.9 ± 17.9; $p=0.03$; Figure 2-2c). When the individual categories of the SGRQ were analyzed, there was no change in the Symptom score (Pre: 56.0 ± 20.7; Post: 56.1 ± 20.1; $p=0.93$) or Impact score (Pre: 28.8 ± 17.0; Post: 26.5 ± 18.4; $p=0.11$) following PR; however, a significant reduction in the Activity score was observed as a result of PR (Pre: 58.9 ± 18.8; Post: 54.5 ± 23.6; $p=0.02$).

2.3.1.3. Carotid Chemoreceptor Activity

The CC activity data is presented in Figure 2-3. There was no main effect of time (p=0.6) or group (p=0.54), and no time-by-group interaction (p=0.32). These findings indicate that there was no change in CC activity from baseline to post-PR in the rehabilitation group (Pre: -1.07 ± 1.55L/min; Post: -0.93 ± 1.46L/min), and no change in the control group following the equivalent time control (Pre: -1.04 ± 0.90L/min; Post: -1.39 ± 1.24L/min).

2.3.1.4. Carotid Chemoreceptor Sensitivity

The slope of the ventilatory response to hypoxia ($\Delta\dot{V}_E/\Delta SpO_2$) is presented in Figure 2-4. There was no main effect of time (p=0.92) or group (p=0.07), and no time-by-group interaction (p=0.69). These results indicate that there was no change in CC sensitivity from baseline to post-PR in the rehabilitation group (Pre: 0.14 ± 0.11L/min/SpO_2; Post: 0.13 ± 0.13L/min/SpO_2), and no change in the control group following the equivalent time control (Pre: 0.21 ± 0.11L/min/SpO_2; Post: 0.21 ± 0.12L/min/SpO_2).

2.3.1.5. Mean Inspiratory Flow

During normoxia, there was no main effect of time (p=0.98), however there was a main effect of group (p<0.01), and no time-by-group interaction (p=0.24). These results indicate that V_T/T_I measured during normoxia did not change from baseline in the rehabilitation group (Pre: 0.24 ± 0.08L/s; Post: 0.26 ± 0.12L/s) or the control group (Pre: 0.36 ± 0.13L/s; Post: 0.34 ± 0.22L/s); however V_T/T_I was significantly greater in controls compared to the rehabilitation group.

When examining V_T/T_I measured during hyperoxia, there was no main effect of time (p=0.66), but there was a main effect of group (p<0.01), and no time-by-group interaction (p=0.34). Similarly to the normoxia data, V_T/T_I measured during hyperoxia did not change from baseline in the rehabilitation group (Pre: 0.23 ± 0.08L/s; Post: 0.26 ± 0.13L/s) or the control

group (Pre: 0.36 ± 0.14L/s; Post: 0.35 ± 0.21L/s), but V_T/T_I was significantly greater in controls compared to the rehabilitation group.

Lastly, when examining the magnitude of the change in V_T/T_I from normoxia to hyperoxia ($\Delta V_T/T_I$), there was no main effect of time (p=0.26) or group (p=0.71), and there was no time-by-group interaction (p=0.67). These findings indicate that there was no change in $\Delta V_T/T_I$ in the rehabilitation group following PR (Pre: -0.004 ± 0.038L/s; Post: 0.002 ± 0.048L/s) and similarly no change in the control group (Pre: -0.001 ± 0.055L/s; Post: 0.009 ± 0.054L/s) following the equivalent time control period.

2.3.1.6. Arterial Stiffness

Arterial stiffness data is presented in Figure 2-5. There was no main effect of time (p=0.22), however there was a main effect of group (p=0.04) and a significant time-by-group interaction (p=0.01). These findings show that following the 6-8 week period, there was an increase in PWV in the control group from baseline (Pre: 9.33 ± 1.57m/s; Post: 10.17 ± 1.27m/s) with no change in the rehabilitation group (Pre: 8.99 ± 1.36m/s; Post: 8.69 ± 1.33m/s).

2.3.1.7. Heart Rate Variability

When examining the effect of PR on SDNN, there was no main effect of time (p=0.55) or group (p=0.07), and no time-by-group interaction (p=0.58). These findings show that there was no change in SDNN from baseline in either the rehabilitation group (Pre: 23.67 ± 16.15ms; Post: 26.57 ± 13.63ms) or the control group (Pre: 36.42 ± 30.00ms; Post: 36.56 ± 33.81ms) following the 6-8 week period. Similarly, for RMSSD there was no main effect of time (p=0.12) or group (p=0.11), and there was no time-by-group interaction (p=0.86). These data demonstrate that there was no change in RMSSD from baseline in the rehabilitation group following PR (Pre: 19.54 ±

16.52ms; Post: 24.18 ± 22.81ms) or in the control group following the equivalent time control period (Pre: 33.94 ± 38.98ms; Post: 37.66 ± 48.01ms).

2.3.1.8. Baroreceptor Sensitivity

There was no main effect of time (p=0.56), however there was a significant main effect for group (p=0.001) when examining the impact of PR on BRS, and no time-by-group interaction (p=0.72). Collectively, these findings indicate that there was no significant change in BRS from baseline in either the rehabilitation group (Pre: 9.36 ± 6.70ms/mmHg; Post: 10.30 ± 8.20ms/mmHg) or the control group (Pre: 19.14 ± 8.67ms/mmHg; Post: 19.36 ± 10.64ms/mmHg) following the 6-8 week intervention period. However, the control group had a significantly greater BRS compared to the rehabilitation group.

2.3.2. Effect of Disease Severity on the Carotid Chemoreceptor Activity and Sensitivity Response to Pulmonary Rehabilitation

2.3.2.1. Carotid Chemoreceptor Activity

When the rehabilitation group was stratified by disease severity, there was no main effect of time ($p=0.70$) or group ($p=0.77$), and no time-by-group interaction ($p=0.44$). These data indicate that there was no change in CC activity from baseline following PR in those with less severe COPD (Pre: -1.21 ± 1.57L/min; Post: -0.89 ± 1.24L/min) or those with more severe COPD (Pre: -0.89 ± 1.54L/min; Post: -0.99 ± 1.76L/min).

2.3.2.2. Carotid Chemoreceptor Sensitivity

Following stratification of the rehabilitation group by disease severity, there was no main effect of time ($p=0.62$) or group ($p=0.85$), and no time-by-group interaction ($p=0.27$), demonstrating that PR had no effect on CC sensitivity in those with less severe COPD (Pre: 0.16 ± 0.11L/min/SpO_2; Post: 0.11 ± 0.12L/min/SpO_2), or those with more severe COPD (Pre: 0.13 ± 0.11L/min/SpO_2; Post: 0.15 ± 0.14L/min/SpO_2).

2.3.3. Effect of Baseline Dyspnea on the Carotid Chemoreceptor Activity and Sensitivity Response to Pulmonary Rehabilitation

2.3.3.1. Carotid Chemoreceptor Activity

When stratifying for the degree of resting dyspnea, there was no main effect of time (p=0.77) or group (p=0.15), and similarly there was no time-by-group interaction (p=0.29). These data demonstrate that there was no change in CC activity from baseline following PR in those with less severe resting dyspnea (Pre: -1.40 ± 1.54L/min; Post: -1.03 ± 1.59L/min) or those with more severe resting dyspnea (Pre: -0.58 ± 1.45L/min; Post: -0.79 ± 1.29L/min).

2.3.3.2. Carotid Chemoreceptor Sensitivity

Following stratification of the rehabilitation group by degree of baseline dyspnea, there was no main effect of time (p=0.63) or group (p=0.55), and additionally there was no time-by-group interaction (p=0.33). These findings indicate that there was no change in CC sensitivity following PR in patients with less severe resting dyspnea (Pre: 0.12 ± 0.11L/min/SpO_2; Post: 0.13 ± 0.15L/min/SpO_2) or those with more severe resting dyspnea (Pre: 0.17 ± 0.10L/min/SpO_2; Post: 0.13 ± 0.12L/min/SpO_2).

2.3.4. Effect of Baseline Carotid Chemoreceptor Activity and Sensitivity on the Carotid Chemoreceptor Activity and Sensitivity Response to Pulmonary Rehabilitation

2.3.4.1. Carotid Chemoreceptor Activity

There was a significant correlation between baseline CC activity and the change in CC activity following PR (R^2=0.39, p<0.001; Figure 2-6). Thus, the rehabilitation group was stratified into two groups based on their degree of baseline CC activity (Lower CC activity: Quartiles 2-4 vs. Greater CC activity: Quartile 1). There were no between-group differences in age, BMI, or smoking history, and additionally there was no difference in lung function or self-reported resting dyspnea between those with lower baseline CC activity and those with greater baseline CC activity (see Table 2-4). There was a significant main effect of both time (p=0.04) and group (p<0.001), and there was a significant time-by-group interaction (p<0.01). These results indicate that those with greater baseline CC activity improved CC activity following PR (Pre: -3.03 ± 0.73L/min; Post: -1.55 ± 1.83L/min) whereas there was no change in CC activity in those with lower baseline CC activity (Pre: -0.44 ± 1.16L/min; Post: -0.73 ± 1.30L/min). See Figure 2-7.

See Table 2-5 for data on the effect of PR on 6MWD, MMRC, SGRQ, and PWV when stratified by baseline CC activity. There was a significant main effect of time (p=0.01), however there was no main group effect (p=0.92) or time-by-group interaction for 6MWD (p=0.27). This indicates that 6MWD was improved in both those with lower baseline CC activity as well as subjects with greater baseline CC activity following PR; however, only individuals with lower baseline CC activity achieved the minimal clinically important difference for an increase in 6MWD. For MMRC, there was no main effect of time (p=0.10) or group (p=0.20), and no time-by-group interaction (p=0.88) indicating that MMRC remained unchanged from baseline following PR in both groups. There was a significant main time effect (p=0.049) for SGRQ, however there was no main group effect (p=0.36) or time-by-group interaction (p=0.93); SGRQ

was reduced in both subjects with lower baseline CC activity, as well as those with greater baseline CC activity following PR. When examining PWV, there was no main effect of time ($p=0.07$) or group ($p=0.22$), and there was no interaction effect ($p=0.28$). This demonstrates that there was no change in PWV in either group following PR.

When examining the impact of PR on autonomic function in COPD when stratified by baseline CC activity, there was no main time ($p=0.31$) or group ($p=0.33$) effect for SDNN. Additionally, there was no time-by-group effect ($p=0.76$), demonstrating no change in SDNN from baseline in either group following PR. Similarly, there was no main effect of time ($p=0.07$) or group ($p=0.55$), and there was no time-by-group interaction for RMSSD ($p=0.43$). This indicates that RMSSD remained unchanged from baseline in both groups following PR. Lastly, when examining BRS there was no significant main effect of time ($p=0.37$) or group ($p=0.48$), and no time-by-group interaction ($p=0.89$), indicating no change in BRS in either group following PR. These data are presented in Table 2-6.

Mean inspiratory flow data when stratified by baseline CC activity is reported in Table 2-7. When examining V_T/T_I measured during normoxia, there was no significant main effect of time ($p=0.34$), however there was a main effect of group ($p=0.03$). There was no time-by-group interaction ($p=0.82$), indicating that there was no change in V_T/T_I measured during normoxia in either group following PR, however V_T/T_I was higher in those with greater baseline CC activity. There was no main effect of time ($p=0.09$) or group ($p=0.30$), and no time-by-group interaction ($p=0.37$) for V_T/T_I measured during hyperoxia. This demonstrates that the V_T/T_I measured during hyperoxia remained unchanged in both groups following PR. When examining the change in V_T/T_I from normoxia to hyperoxia ($\Delta V_T/T_I$) with PR, there was no significant main effect of time ($p=0.052$), however there was a significant main group effect ($p<0.01$) and a time-by-group

interaction (p=0.01). These findings indicate that there was a reduction in $\Delta V_T/T_I$ following PR in those with greater baseline CC activity but not those with lower baseline CC activity.

To examine the change in CC activity in each quartile individually, the CC activity response to PR was graphed for all quartiles. This can be seen in Figure 2-8.

2.3.4.2. Carotid Chemoreceptor Sensitivity

There was no significant correlation between baseline CC sensitivity and the change in CC sensitivity following PR (R^2=0.15, p=0.07; Figure 2-9). Therefore, no further subgroup analyses were conducted.

2.4. Discussion

This study was designed to examine the effects of a 6-8 week PR program on CC activity and sensitivity in clinically stable COPD patients. While PR resulted in significant improvements in functional capacity, dyspnea, and health-related quality of life, PR was not effective in reducing CC activity or sensitivity in stable COPD patients. While exercise training has been shown to improve arterial stiffness[31] and autonomic function in COPD,[29, 30] in the present study PR had no effect on arterial stiffness or autonomic function. Sub-group analysis found that patients with an elevated baseline CC activity significantly improved CC activity following PR, however this reduction in CC activity occurred independently of improvements in dyspnea or CV risk factors. Collectively these data suggest that PR is not effective in reducing CC activity/sensitivity in stable COPD patients.

2.4.1. The Effect of Pulmonary Rehabilitation on Carotid Chemoreceptor Activity and Sensitivity

The present study did not report a significant change in CC activity following PR, assessed by the reduction in ventilation following CC inhibition with 100% O_2. Additionally, there was no change in CC sensitivity following PR, as evaluated by the ventilatory response to hypoxia. When the rehabilitation group was stratified by disease severity, as well as the degree of resting dyspnea, there was no effect of PR on CC activity/sensitivity. While these findings contrast previous work indicating that exercise training is capable of reducing CC activity/sensitivity in rabbits with experimental CHF,[23] it is possible that a difference in methodology may explain the difference in findings between these two studies. In the study by Li *et al*. (2008),[23] CC activity was directly measured as the discharge frequency of the carotid sinus nerve, and CC sensitivity was assessed by plotting the change in carotid body discharge frequency and renal SNA in response to reductions in P_aO_2. As the methods for assessing CC activity/sensitivity in the

present study were less direct than those utilized in previous work, it is possible that the present study did not have the sensitivity to accurately evaluate changes in CC activity/sensitivity following PR. Alternatively, it is possible that the etiology of the elevated CC activity/sensitivity in experimental CHF is different from that observed in COPD, and therefore exercise training may not be an effective intervention for reducing CC activity/sensitivity in stable COPD patients.

Previous work by Coats *et al.* (1992) observed an improvement in peripheral vascular tone, ventilation, and autonomic function in CHF patients following exercise training.[26] Collectively these findings suggest that exercise training may be effective in reducing CC activity/sensitivity in CHF patients, however CC activity/sensitivity was not measured. The exercise program utilized in this previous study was of similar length and intensity, but greater frequency compared to the Breathe Easy PR program. Baseline autonomic function between the previous and present study cannot be compared, as Coats and colleagues recorded 24-hour HRV whereas the present study only recorded five minutes of HRV data. Therefore, whether the difference in findings between these two studies is a result of differences in baseline autonomic function cannot be determined. It is possible that the lower frequency of the Breathe Easy program (and therefore a reduced exercise training stimulus), or the different methodology used when assessing HRV may partly explain this difference in findings. As previous work suggests that elevated CC activity/sensitivity may contribute to increased CV risk in COPD,[8] other interventions aimed at reducing CC activity/sensitivity in COPD are needed.

2.4.2. The Effect of Baseline Carotid Chemoreceptor Activity/Sensitivity on the Carotid Chemoreceptor Activity/Sensitivity Response to Pulmonary Rehabilitation

In an attempt to examine if the degree of baseline CC activity/sensitivity determined the CC activity/sensitivity response to PR, a correlation was conducted between baseline CC activity/sensitivity and the change in CC activity/sensitivity following PR. While there was no

effect of baseline CC sensitivity on the CC sensitivity response to PR, a greater baseline CC activity was positively correlated with a greater reduction in CC activity following PR. To further examine this relationship, the rehabilitation group was stratified into quartiles based on the degree of baseline CC activity; individuals in the upper quartile significantly reduced CC activity following PR compared to those with lower baseline chemoreceptor activity. This would suggest that PR is effective in reducing CC activity in COPD patients with a greater baseline CC activity. However, these improvements in CC activity occurred independently of changes in resting dyspnea, arterial stiffness and autonomic function. This suggests that, while the ventilatory response to hyperoxia was reduced in these COPD patients following PR, there were no apparent reductions in indicators of SNA or CV risk. There was a change in $\Delta V_T/T_I$ following PR however; as V_T/T_I has been suggested to reflect central neural respiratory drive,[56] this suggests that there was a change in the physiological drive to breathe in those patients who significantly reduced CC activity following PR. When the CC activity response to PR is displayed for all quartiles (see Figure 2-8), both the upper and lower quartile appear to regress to the group mean following PR. Collectively, these results may suggest that the reduction in CC activity following PR in those with greater baseline CC activity is likely explained by regression to the mean. It is interesting to note, however, that when all quartiles are graphed separately, post-PR CC activity ranged from -0.51 to -1.37L/min. As these values are similar to those of healthy controls reported by Stickland *et al.* (2016) (-0.73 ± 0.78L/min),[8] these findings may indicate that PR may help normalize CC activity in COPD patients, with a reduction in CC activity in those patients with a higher baseline CC activity, and an increase in CC activity in those with a reduced baseline CC activity.

2.4.3. Resting Dyspnea

The improvement in resting dyspnea following PR occurred independently of any changes in CC activity/sensitivity following PR. This was an unexpected finding, as elevated CC activity/sensitivity is suggested to potentiate dyspnea in COPD patients.[20, 21] These data would suggest that the reduction in dyspnea with PR is driven by components other than chemoreception.

2.4.4. Arterial Stiffness

While previous work has shown a reduction in central PWV in COPD patients following PR,[33] as well as reductions in peripheral PWV following a cycling exercise training program in COPD patients,[31] there was no significant reduction in peripheral PWV following PR in the present study. As the CC have previously been shown to be related to PWV in COPD,[8] the lack of change in PWV following PR would be consistent with the lack of change in CC activity/sensitivity following PR. Baseline PWV in the present study was less than that reported in previous work, while there was no difference in patient disease severity between these studies.[31] As the duration and intensity of the cycling program utilized in the previous study was similar to the Breathe Easy PR program, the difference in findings between these two studies may suggest that the change in PWV following exercise training/rehabilitation in COPD is dependent upon the degree of baseline PWV. It is important to note, however, that in the present study there was a significant increase in peripheral PWV in the control group following the intervention period when compared to the rehabilitation group; this indicates that while PR did not reduce peripheral PWV, it was effective in attenuating the natural increase in PWV with disease progression (see Figure 2-5).

2.4.5. Autonomic Function

Previous studies have shown improvements in autonomic function following PR,[29, 30] however in the present study there was no effect of PR on autonomic function in COPD patients, as indicated by a lack of change in HRV and BRS. This lack of change in HRV and BRS following PR is consistent with our findings that CC activity/sensitivity remained unchanged following PR, as the CC have been shown to contribute to impaired BRS and reduced HRV.[63] Previous work did not examine changes in CC activity/sensitivity or SNA, and therefore it is difficult to explain the mechanism(s) underlying the improvements in autonomic function.[29, 30] Individuals in these previous studies had a greater degree of airway obstruction compared to the subjects in the present study; additionally, Borghi-Silva *et al*. (2009)[29] reported a lower baseline HRV, while Costes *et al*. (2004)[30] reported a lower baseline BRS in their sample of COPD patients compared to patients in the present study. This would suggest that the degree of baseline SNA was likely greater in these previous studies. As the PR[29] and aerobic training program[30] implemented in these previous studies were similar to the Breathe Easy PR program in duration and intensity, it is possible that the improvements in autonomic function following PR/aerobic training in COPD patients are dependent on the degree of baseline autonomic dysfunction.

2.4.6. Limitations and Considerations

Participants in the present study were all clinically stable and mobile COPD patients who were able to regularly attend PR and/or the testing sessions. While this is not indicative of all COPD patients, our sample is likely representative of the average COPD patient referred to PR. Therefore, generalization of these results should be limited to those COPD patients commonly referred to PR by their physician, and not the broader COPD population.

In the present study, both the rehabilitation and control groups improved 6MWD following the 6-8 week intervention period. While PR has been shown to improve 6MWD in

COPD patients, the increase in 6MWD in the control group was unexpected. It was assumed that, as control subjects were all previous graduates of the PR program, there would be no need for familiarization testing. However, a learning effect likely explains the small, yet significant, increase in 6MWD observed in the control group. This remains a limitation of the present study, and future research should include familiarization testing for all participants, regardless of previous experience.

It is possible that the exercise training stimulus achieved during PR may not have been sufficient to elicit changes in CC activity/sensitivity in our sample of COPD patients. In order to maximize training adaptations and physiologic benefits,[64] the Breathe Easy PR program encourages all participants to exercise at an intensity of greater than 60% of maximal work rate[43] or an exercising heart rate of 60-85% of the peak heart rate achieved during the pre-rehabilitation CPET. Ultimately, however, all exercises are individualized and subject to daily variations in patient abilities and symptoms. It is possible that this individualization resulted in an inconsistent exercise training stimulus across participants, as COPD patients are limited in their ability to exercise,[65, 66] and enhanced CC activity/sensitivity can further potentiate the ventilatory response to exercise.[61, 62] In the present study it was found that 75% of individuals completing PR met the American College of Sports Medicine guidelines for exercise training.[43] Therefore, it is possible that, while the majority of individuals achieved the PR exercise training guidelines, this training stimulus is insufficient to elicit changes in CC activity/sensitivity in stable COPD. It is important to note, however, that consistent with previous work[36, 37] and reviews,[38-40] individuals who participated in PR significantly increased 6MWD and health-related quality of life, and reduced resting dyspnea. This indicates that while PR does not affect CC activity/sensitivity in stable COPD, it has other important physiologic and psychosocial benefits.

Lastly, as the subjects in the present study were stable, normoxemic, and normocapnic, it is possible that the degree of CC activity/sensitivity may not have been severe enough to receive benefits from PR. In the present study, mean baseline CC sensitivity was less than that reported in CHF patients when assessed using the transient hypoxia test (Present study: 0.14 ± 0.11L/min/SpO_2; Ponikowski *et al.* (2001): 0.69 ± 0.50L/min/SpO_2),[67] and was also less than observed in our recent work in COPD which assessed CC sensitivity using the step hypoxia method (Present study: 0.14 ± 0.11L/min/SpO_2; Stickland *et al.* (2016): 0.24 ± 0.22L/min/SpO_2).[8] Additionally, baseline CC activity in our sample of COPD patients was less than what we have previously reported (Present study: -1.07 ± 1.55L/min; Stickland *et al.* (2016): -2.62 ± 1.90L/min).[8] While these previous studies did not examine the effects of exercise rehabilitation on CC activity/sensitivity, it is possible that the reduced CC activity/sensitivity observed in the present study may partly explain the lack of change in CC activity/sensitivity following PR.

2.4.7. Future Directions

Future studies should examine the effect of a longer duration rehabilitation program on CC activity/sensitivity in stable COPD, as the training stimulus may not have been sufficient in the present study to elicit changes in CC activity/sensitivity; additionally it would not be feasible to increase the intensity of the program as COPD patients experience ventilatory and musculoskeletal limitations to exercise. It is also possible that future research examining unstable COPD patients, or enrolling subjects who would not normally be referred to PR, may yield different results compared to those observed in the present study. If it is shown that longer duration exercise training programs are not effective in reducing CC activity/sensitivity in either stable or unstable COPD, then other interventions aimed at reducing CC activity/sensitivity in COPD require further examination.

2.4.8. Conclusion

This study examined the effects of a 6-8 week PR program on CC activity/sensitivity in patients with stable COPD. Pulmonary rehabilitation was found to not be effective in reducing CC activity/sensitivity in stable COPD patients. Individuals with a greater baseline CC activity significantly reduced CC activity following PR however this occurred independently of any reductions in resting dyspnea or markers of CV risk. This finding may be a result of a regression towards the mean, or the change in CC activity has no effect on CV risk in these patients. Alternate interventions beyond traditional PR require further investigation to reduce CC activity/sensitivity and CV risk in COPD.

Table 2-1. Participant characteristics. Data presented as Mean±SD.

	Rehabilitation	**Control**	**p**
Sample size (m/f)	45 (21/24)	15 (7/8)	
Age (years)	67±7	68±7	NS
BMI (kg/m^2)	26.99±4.86	29.59±7.71	NS
Smoking History (pack years)	37.29±17.23	37.62±30.49	NS
FEV_1 (%pred)	56.60±20.70	74.60±20.51	<0.01*
FEV_1/FVC	46.54±13.18	56.97±12.59	<0.01*
FEV_1/FVC (%pred)	60.83±16.20	75.59±15.69	<0.01*
TLC (%pred)	117.10±19.11	109.60±7.00	NS
RV (%pred)	147.52±13.95	113.96±46.82	0.01*
FRC (%pred)	134.45±32.64	117.07±36.68	NS
GOLD 1	6 (13%)	6 (40%)	0.03*
GOLD II	20 (44%)	8 (53%)	NS
GOLD III	14 (31%)	1 (7%)	0.06
GOLD IV	5 (11%)	0 (0%)	0.NS
MMRC Dyspnea Scale (0-4)	2.4±0.8	1.5±0.9	<0.01*

BMI, body mass index; FEV_1, forced expiratory volume in one second (post-bronchodilator); FVC, forced vital capacity (post-bronchodilator); TLC, total lung capacity; RV, residual volume; FRC, functional residual capacity; GOLD, global initiative for obstructive lung disease; MMRC: modified medical research council. * = significant difference between groups ($p<0.05$).

Table 2-2. Participant comorbidities. Data presented as frequency (percentage of total).

Comorbidities	**Rehabilitation (n=45)**	**Control (n=15)**
On Supplemental Oxygen	4 (9%)	0 (0%)
Coronary Artery Disease	5 (11%)	2 (13%)
Cerebrovascular Disease	1 (2%)	2 (13%)
Hypertension	24 (53%)	8 (53%)
Chronic Heart Failure	4 (9%)	2 (13%)
Valvular Heart Disease	3 (7%)	0 (0%)
Diabetes	5 (11%)	2 (13%)
Dyslipidemia	23 (51%)	5 (33%)
Musculoskeletal	23 (51%)	5 (33%)

Table 2-3. List of medications used by participants while participating in the study. Data presented as frequency (percentage of total)

Drug Class	**Rehabilitation (n=45)**	**Control (n=15)**
Long/Short Acting Bronchodilators[*]	41 (91%)	14 (93%)
Combination Inhalers[¥]	35 (78%)	11 (73%)
Anti-Hypertension Medications [§]	19 (42%)	7 (47%)
Cholesterol Controller Medication [ð]	17 (38%)	2 (13%)

[*] Includes $beta_2$ agonists as well as anticholinergics.
[¥] Includes combinations of $beta_2$ agonists and anticholinergics
[§] Includes diuretics, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and beta blockers.
[ð] Includes statins.

Table 2-4. Participant characteristics of the rehabilitation group, stratified by the degree of baseline CC activity. Greater CC activity was defined as having a CC activity in the upper quartile of subjects in the rehabilitation group. Lower CC activity was defined as having a CC activity in the lower three quartiles of subjects in the rehabilitation group. Data presented as Mean±SD.

	Greater CC Activity	**Lower CC Activity**	**p**
Sample size (m/f)	11 (6/5)	34 (15/19)	
Age (years)	67±7	67±7	NS
BMI (kg/m^2)	28.46±4.71	26.51±4.88	NS
Smoking History (pack years)	42.27±17.03	35.68±17.23	NS
FEV_1 (%pred)	62.73±15.41	54.61±21.97	NS
FEV_1/FVC	49.91±9.58	45.45±14.10	NS
FEV_1/FVC (%pred)	64.55±12.11	59.41±17.49	NS
TLC (%pred)	117.46±21.00	116.98±18.79	NS
RV (%pred)	143.46±42.64	148.84±39.62	NS
FRC (%pred)	132.27±31.56	135.18±33.43	NS
MMRC Dyspnea Scale (0-4)	2.1±0.8	2.5±0.8	NS

BMI, body mass index; FEV_1, forced expiratory volume in one second (post-bronchodilator); FVC, forced vital capacity (post-bronchodilator); TLC, total lung capacity; RV, residual volume; FRC, functional residual capacity; MMRC: modified medical research council dyspnea scale.

Table 2-5. The effect of baseline CC activity on the change in six minute walk distance, resting dyspnea, health related quality of life, and arterial stiffness following 6-8 weeks of pulmonary rehabilitation. Greater CC activity was defined as having a CC activity in the upper quartile of subjects in the rehabilitation group. Lower CC activity was defined as having a CC activity in the lower three quartiles of subjects in the rehabilitation group. Data presented as Mean±SD.

	Greater CC Activity			**Lower CC Activity**		
	n	**Pre PR**	**Post PR**	**n**	**Pre PR**	**Post PR**
6MWD (m)	11	453±58	470±113	34	437±86	480±110*
MMRC	9	2.1±0.8	1.9±0.8	30	2.5±0.8	2.2±0.9
SGRQ (Total)	11	38.5±16.0	35.7±17.8	34	43.7±16.5	41.3±17.9*
PWV (m/s)	8	8.73±1.62	8.01±1.59	28	9.06±1.30	8.88±1.21

6MWD, six minute walk distance; MMRC, modified medical research council dyspnea scale; SGRQ, St. George's respiratory questionnaire; PWV, pulse wave velocity. No between-group differences were reported for 6MWD, MMRC, SGRQ, or PWV at baseline. * = significant difference from pre within group ($p<0.05$).

Table 2-6. The effect of baseline CC activity on the autonomic function response to 6-8 weeks of pulmonary rehabilitation. Greater CC activity was defined as having a CC activity in the upper quartile of subjects in the rehabilitation group. Lower CC activity was defined as having a CC activity in the lower three quartiles of subjects in the rehabilitation group. Data presented as Mean±SD.

	Greater CC Activity			**Lower CC Activity**		
	n	**Pre PR**	**Post PR**	**n**	**Pre PR**	**Post PR**
SDNN (ms)	9	26.39±10.07	30.80±17.17	27	22.77±17.79	25.16±12.29
RMSSD (ms)	9	20.85±12.29	29.26±33.30	27	19.10±17.89	22.49±18.63
BRS (ms/mmHg)	7	10.92±8.09	12.11±10.36	25	8.92±6.38	9.79±7.67

SDNN, standard deviation of the time interval between consecutive sinus heart beats; RMSSD, square root of the mean of the sum of squares of differences between time intervals of adjacent sinus heart beats; BRS, baroreflex sensitivity. No between-group differences were reported for SDNN, RMSSD, or BRS at baseline.

Table 2-7. The effect of baseline CC activity on the mean inspiratory flow response to 6-8 weeks of pulmonary rehabilitation. Greater CC activity was defined as having a CC activity in the upper quartile of subjects in the rehabilitation group. Lower CC activity was defined as having a CC activity in the lower three quartiles of subjects in the rehabilitation group. Data presented as Mean±SD.

	Greater CC Activity (n=11)		**Lower CC Activity (n=34)**	
	Pre PR	**Post PR**	**Pre PR**	**Post PR**
V_T/T_I (L/s) (normoxia)	0.29±0.09	0.30±0.11	0.22±0.07	0.24±0.12
V_T/T_I (L/s) (hyperoxia)	0.24±0.09	0.30±0.11	0.23±0.08	0.25±0.13
$\Delta V_T/T_I$ (L/s)	-0.044±0.032	-0.005±0.058*	0.010±0.030[¥]	0.004±0.045

V_T/T_I, mean inspiratory flow. $\Delta V_T/T_I$ is defined as the change in V_T/T_I from normoxia to hyperoxia. * = significant difference from pre within group ($p<0.05$). [¥] = significant difference between groups ($p<0.01$).

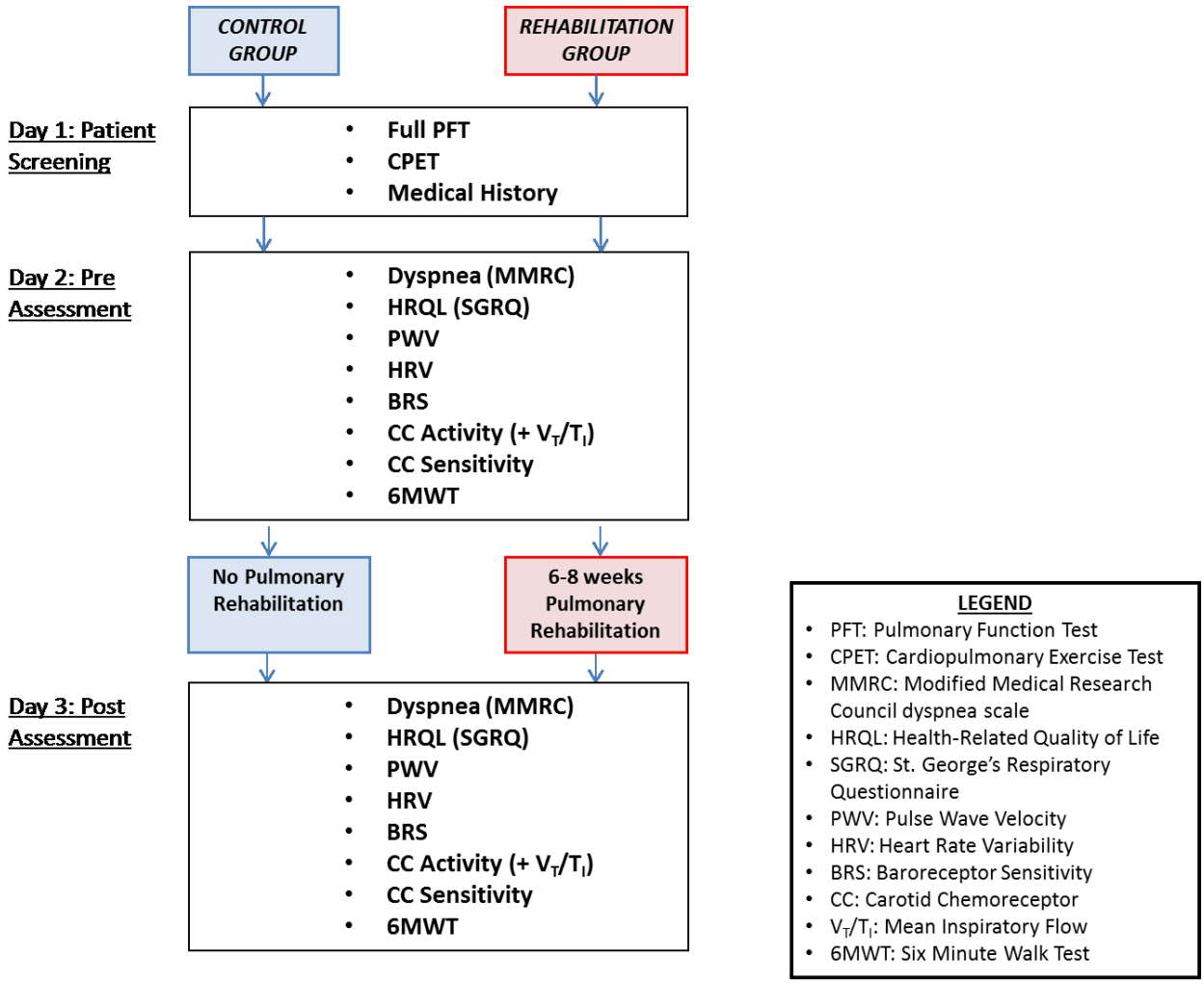

Figure 2-1. Study outline schematic.

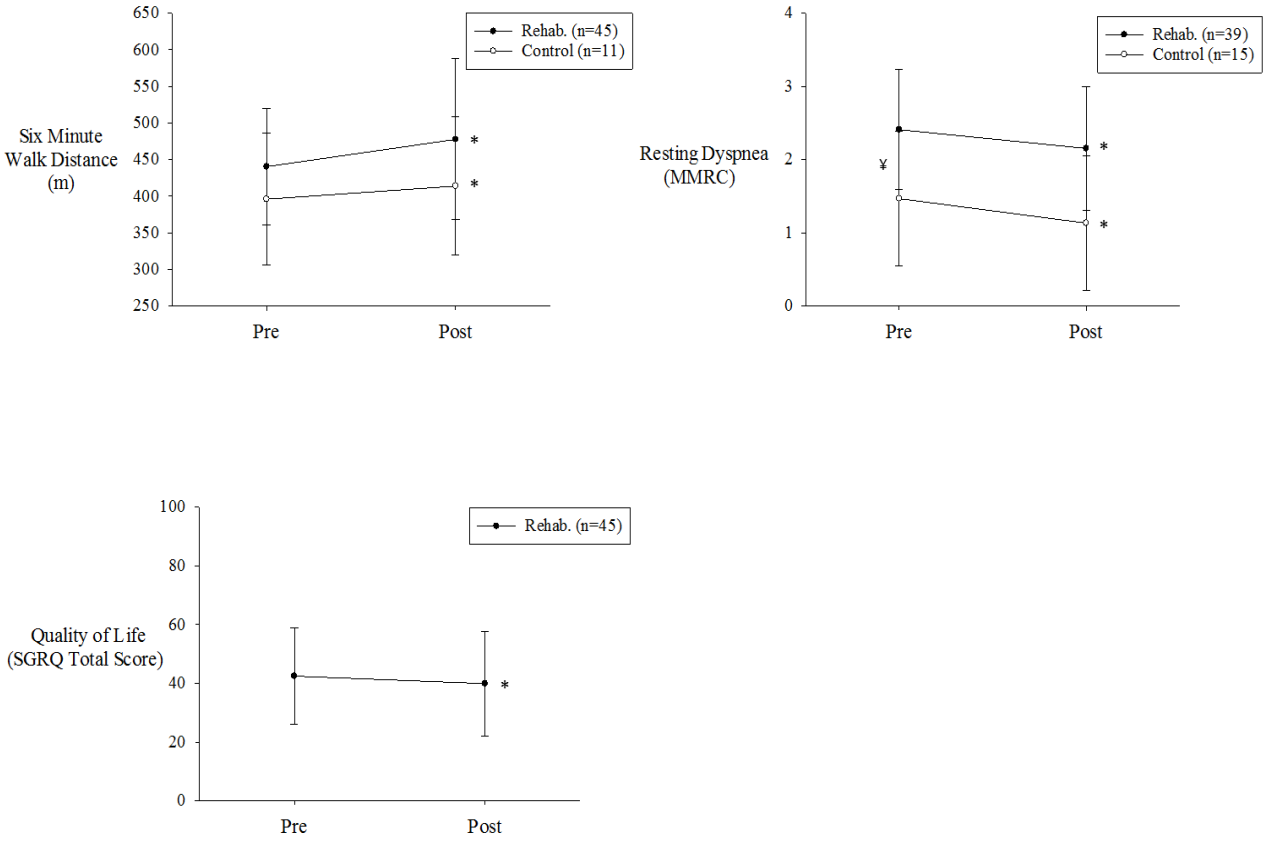

Figure 2-2. Mean (±SD) (A) six minute walk distance, (B) resting dyspnea, and (C) quality of life in COPD patients prior to and following 6-8 weeks of pulmonary rehabilitation (Rehab.) or an equivalent time control period (Control). * = significant difference from pre within group ($p<0.05$). ¥ = significant difference between groups ($p<0.001$).

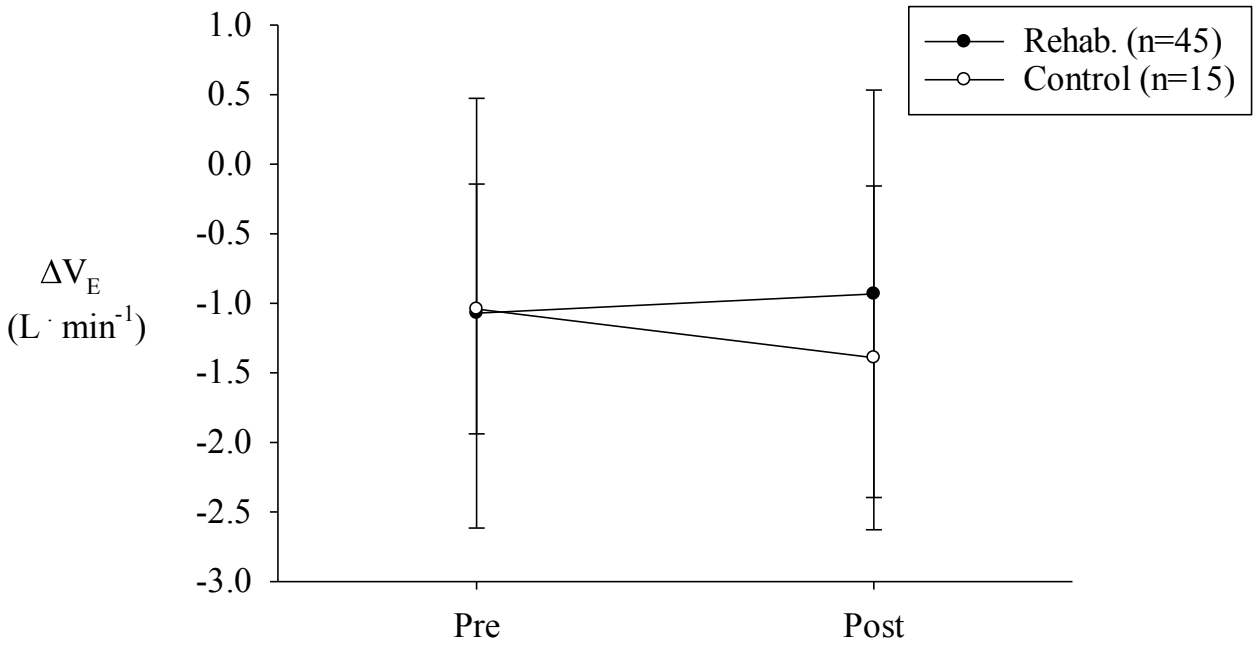

Figure 2-3. Mean (±SD) transient reduction in minute ventilation in response to breathing 100% oxygen (CC activity) in COPD patients prior to and following 6-8 weeks of pulmonary rehabilitation (Rehab.) or an equivalent time control period (Control).

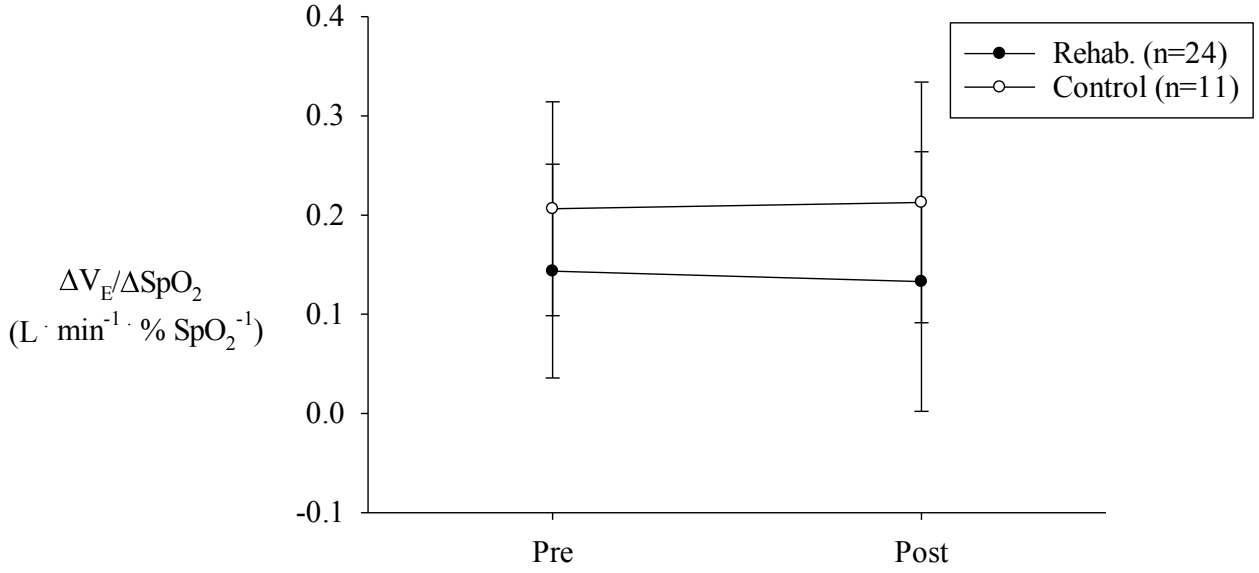

Figure 2-4. Mean (±SD) ventilatory response to incremental step reductions in SpO_2 ($\Delta \dot{V}_E/\Delta SpO_2$ slope; CC sensitivity) in COPD patients. CC sensitivity was assessed using step hypoxia, where an SpO_2 of 90% and 85% were targeted for three minutes each; the slope of the linear regression line relating the increase in ventilation to the reduction in SpO_2 was measured prior to and following 6-8 weeks of pulmonary rehabilitation (Rehab.) or an equivalent time control period (Control).

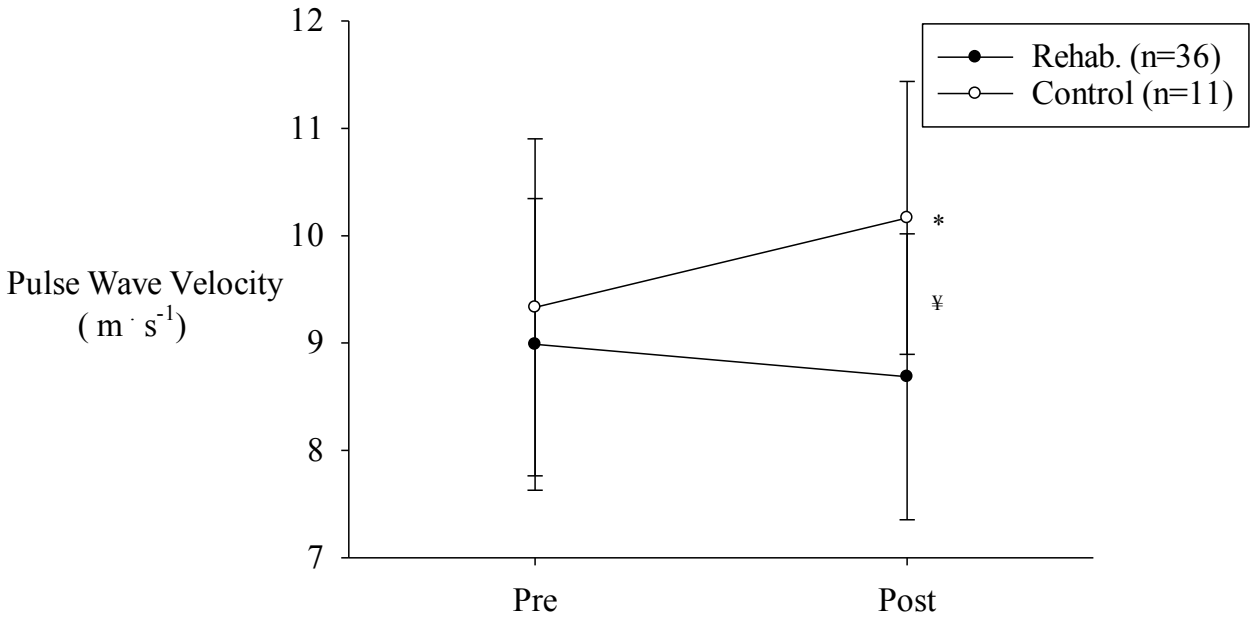

Figure 2-5. Mean (±SD) peripheral pulse wave velocity in COPD patients prior to and following 6-8 weeks of pulmonary rehabilitation (Rehab.) or an equivalent time control period (Control). * = significant difference from pre within group ($p<0.05$). ¥ = significant time-by-group interaction ($p<0.05$).

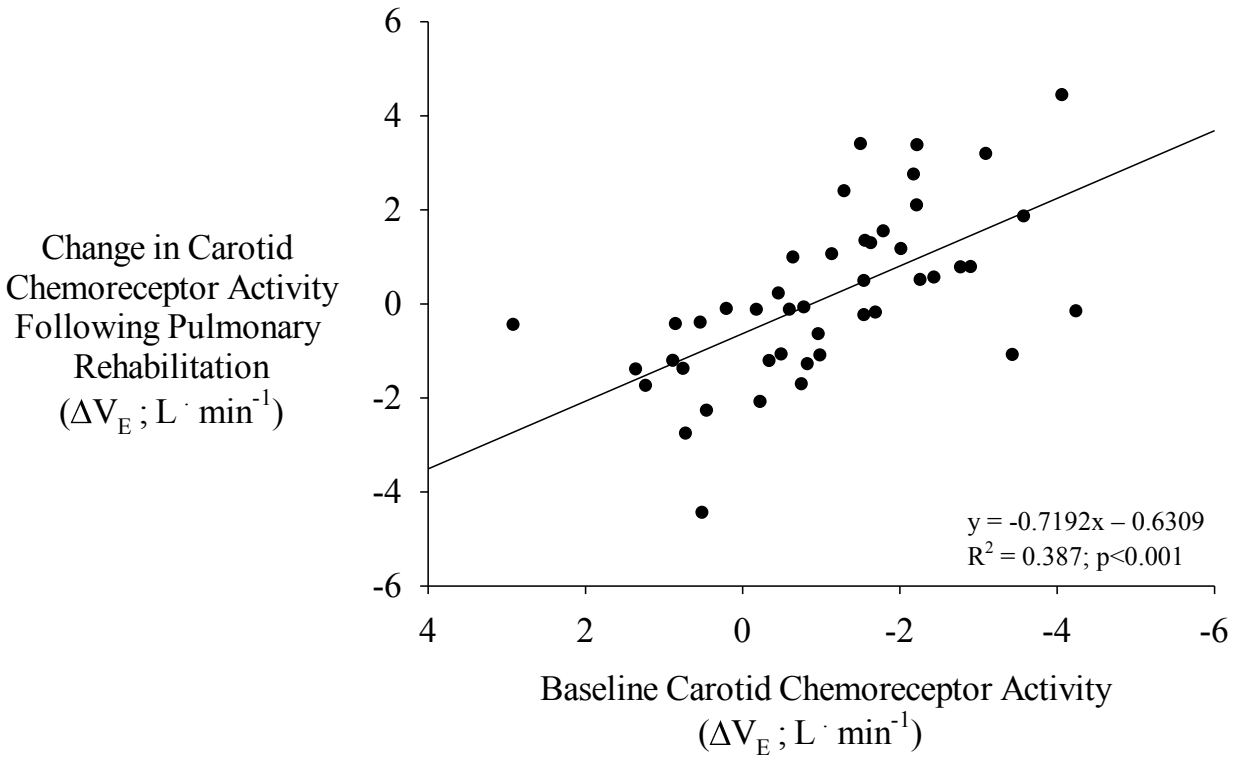

Figure 2-6. Pearson's correlation relating baseline CC activity with the change in CC activity following 6-8 weeks of pulmonary rehabilitation. n = 45. NOTE: moving upwards along the y-axis is associated with a greater improvement in CC activity following PR; moving rightwards along the x-axis is associated with a greater baseline CC activity.

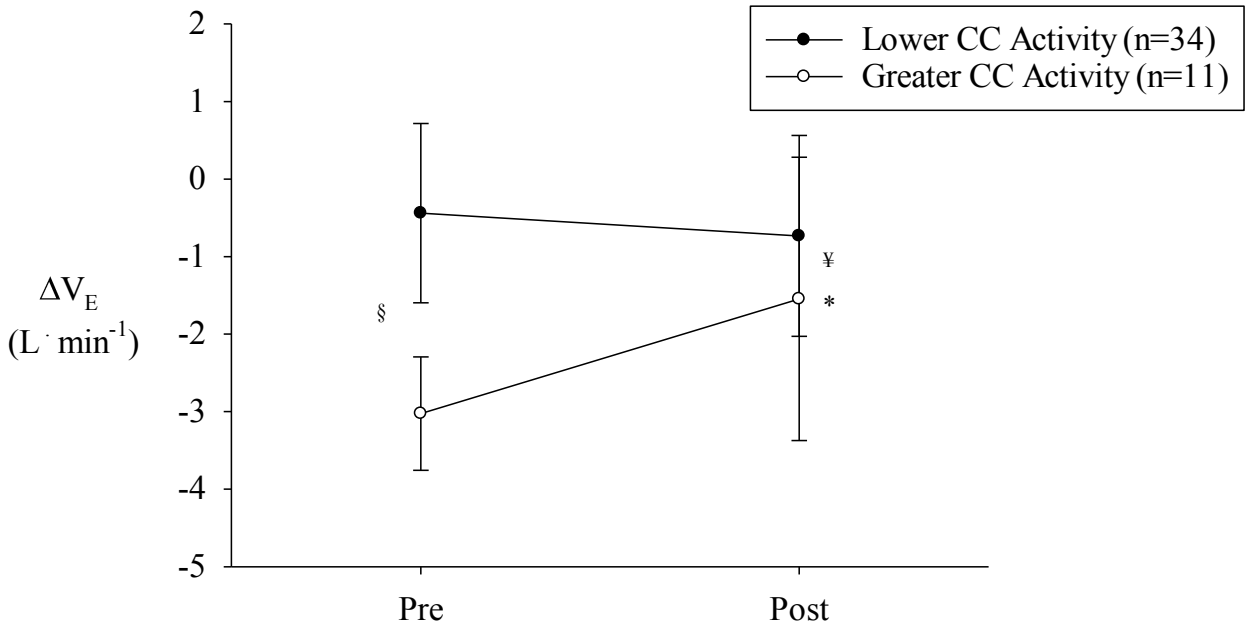

Figure 2-7. Mean (±SD) transient reduction in minute ventilation in response to breathing 100% oxygen (CC activity) prior to and following 6-8 weeks of pulmonary rehabilitation. Subjects completing pulmonary rehabilitation were stratified by their degree of baseline CC activity. Greater CC activity was defined as having a CC activity in the upper quartile of subjects in the rehabilitation group. Lower CC activity was defined as having a CC activity in the lower three quartiles of subjects in the rehabilitation group. * = significant difference from pre within group ($p<0.05$). § = significant difference between groups ($p<0.001$). $^{¥}$ = significant time-by-group interaction ($p<0.01$).

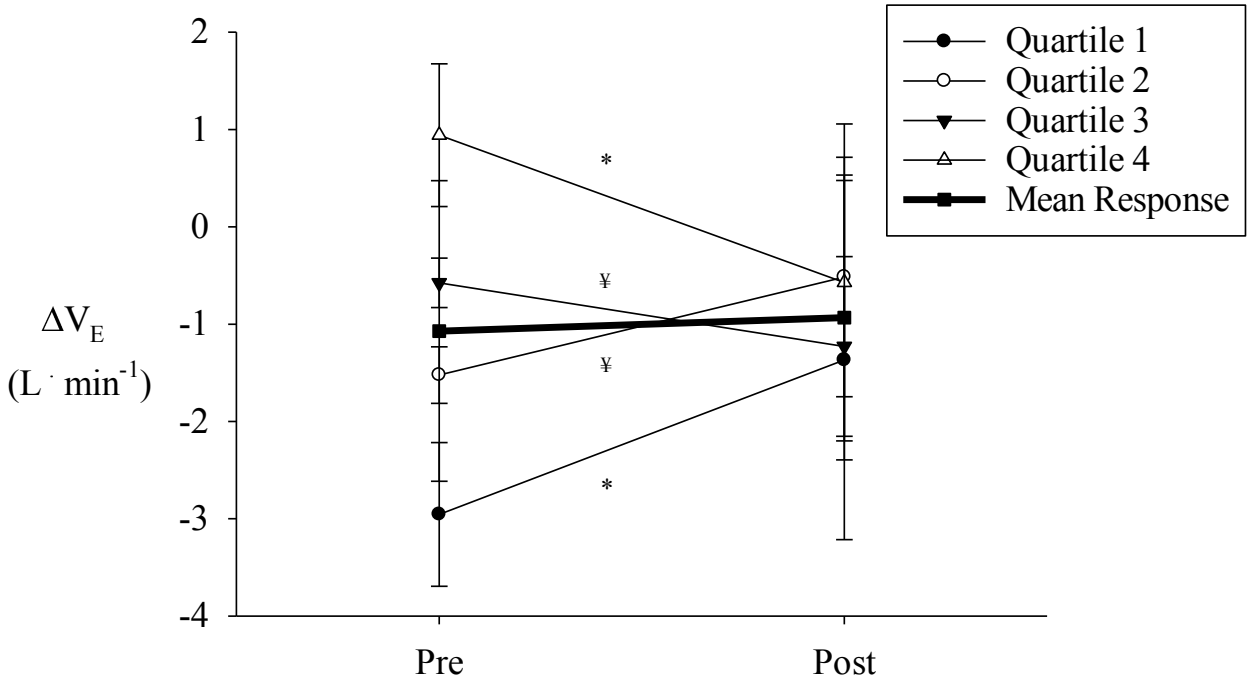

Figure 2-8. Mean (±SD) transient reduction in minute ventilation in response to breathing 100% oxygen (CC activity) prior to and following 6-8 weeks of pulmonary rehabilitation. Subjects completing pulmonary rehabilitation were stratified into quartiles based on their degree of baseline CC activity. * = significant difference from pre within group ($p<0.05$). ¥ = significant difference from pre within group ($p<0.01$).

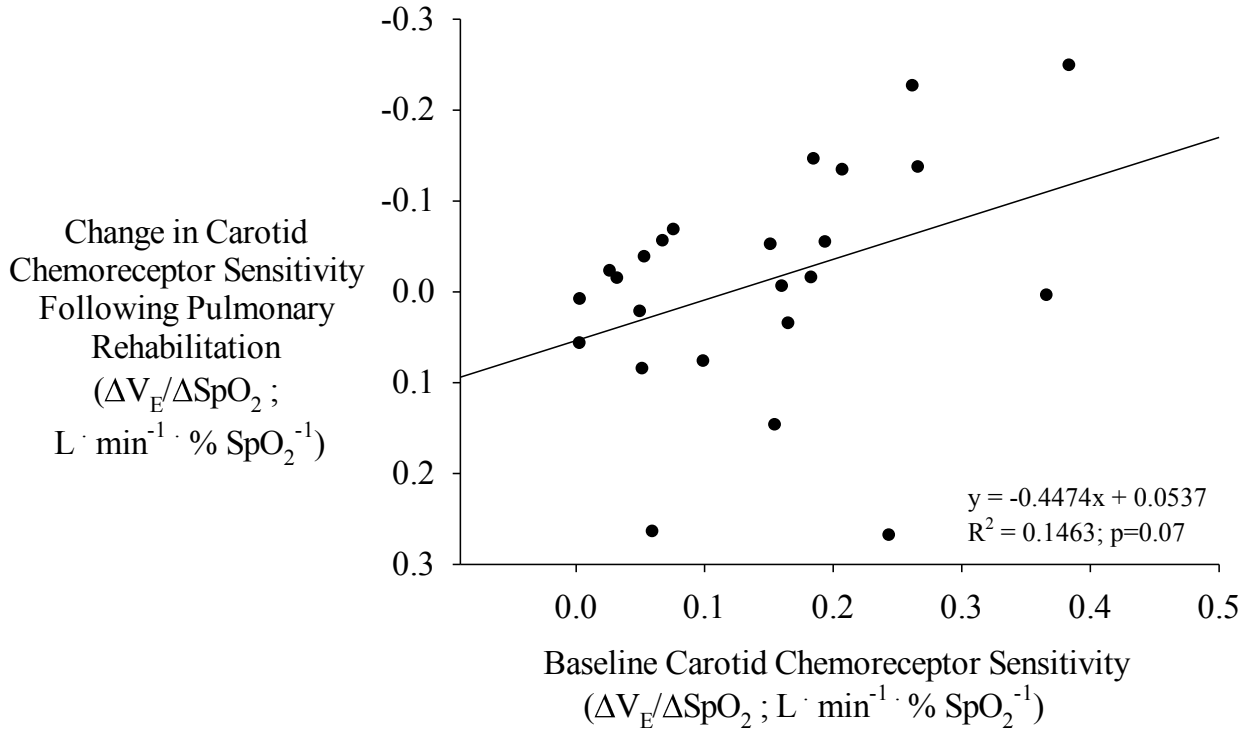

Figure 2-9. Pearson's correlation relating baseline CC sensitivity with the change in CC sensitivity following 6-8 weeks of pulmonary rehabilitation. n = 24. NOTE: moving upwards along the y-axis is associated with a greater reduction in CC sensitivity following PR; moving rightwards along the x-axis is associated with a greater baseline CC sensitivity.

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CHAPTER III: GENERAL DISCUSSION AND COMMENTARY

3.1. The Importance of Elevated Carotid Chemoreceptor Activity and Sensitivity in Chronic Obstructive Pulmonary Disease

The CC are of particular importance in COPD, as they likely contribute to elevated CV risk. Activation of the CC results in an increase in sympathetic outflow,[1-3] and chronically this is associated with an increased risk of CV morbidity and mortality.[4] As it has been reported that individuals with COPD have a chronically-elevated SNA when compared to healthy controls,[5-7] and that the degree of SNA is predictive of mortality and hospitalization in COPD,[7] it is important to study the elevated sympathoexcitation in COPD. Reductions in SNA may mitigate the increased CV risk in COPD, and additionally reduce the considerable healthcare costs associated with COPD hospitalizations. While the etiology of this increased SNA in COPD is unknown, it may be secondary to elevated CC activity/sensitivity as stimulation of the CC results in sympathetically-mediated vasoconstriction.[8, 9] This finding would suggest a relationship between the CC, SNA, and elevated CV risk. Importantly, our laboratory has recently reported that COPD patients have an elevated CC activity/sensitivity compared to healthy controls,[10] and CC inhibition results in a significant reduction in blood pressure and PWV in COPD patients but not in healthy controls.[10] These findings suggest that the CC likely play a role in the elevated CV risk observed in COPD patients compared to healthy controls, and therefore reducing this elevated CC activity/sensitivity may be effective in decreasing CV risk in COPD patients.

Previous research has shown that autonomic function is impaired in COPD patients,[11, 12] and this is associated with increased risk of CV events.[13, 14] While the mechanism(s) underlying this autonomic dysfunction is unclear, it is possible that this may be partly explained by an elevated CC activity/sensitivity. Neurons from the CC and carotid baroreceptors are distributed in close proximity in the solitary and paramedian reticular nuclei in the medulla, and therefore

interneuronal connections might facilitate interactions between these reflexes; it has been proposed that activation of the peripheral chemoreflex is associated with an inhibition of the baroreflex.[15] As the sympathetic and parasympathetic nervous systems are in close interaction, it is not unreasonable to suggest that an over-activation of the CC results in a sympathetically-mediated inhibition of the parasympathetic baroreflex arc. This would result in a diminished parasympathetic inhibition of sympathetic drive, resulting in a further propagation of sympathetic activity. One study found that activation of the baroreflex via pharmacologically increasing blood pressure inhibited the sympathetic response to hypoxia,[16] supporting the complex interaction between baroreceptors and the CC in the regulation of SNA in normal humans. This suggested relationship between the CC and autonomic dysfunction is further supported by previous research which has shown that supplemental O_2 administration results in an improvement in autonomic function in COPD patients.[13, 14] It is likely that supplemental O_2 inhibits the CC, resulting in a reduced sympathetically-mediated inhibition of the parasympathetic baroreflex arc. This increase in parasympathetic control of the heart would lead to an improved sympathovagal balance, and likely reduced risk of mortality.[17] The CC may therefore be an important contributor to autonomic dysfunction and CV risk in COPD.

Dyspnea is a major contributor to physical inactivity in COPD,[18] and physical inactivity has many negative consequences in COPD, including increased hospitalization and mortality.[19] It has been suggested that an elevated CC activity/sensitivity may play a role in the marked dyspnea experienced by COPD patients.[20] As recent studies have shown that supplemental O_2 results in a reduction in resting and exertional dyspnea, and that these changes were independent of lung volume[21] and correlated with changes in breathing frequency,[22] it is possible that these improvements are secondary to CC inhibition. Chemoreceptor inhibition would result in a

reduced afferent signal to the central respiratory centers via the carotid sinus nerve, thereby reducing ventilation primarily through a reduction in breathing frequency. This decrease in breathing frequency would likely result in reduced work of breathing, which is suggested to contribute to the sensation of breathlessness.[23, 24] Additionally, this reduction in ventilation would result in an improved neuromechanical coupling, which is suggested to contribute to dyspnea in COPD.[25] These findings would suggest a relationship between elevated CC activity/sensitivity, dyspnea, physical inactivity, and increased CV risk in COPD.

Together these findings suggest that the CC may play an important role in the elevated CV risk observed in COPD patients, and therefore these receptors are a potentially important target of future interventions aimed at improving health outcomes in COPD. Reductions in CC activity/sensitivity in COPD are likely to result in reductions in CV risk, and therefore COPD hospitalizations and the associated economic costs. Additionally, by reducing CC activity/sensitivity, it is possible that sensations of breathlessness and physical activity may improve. The present study is important as it is the first study to examine the effect of PR on CC activity/sensitivity in COPD.

3.2. The Effectiveness of Exercise Training in Chronic Obstructive Pulmonary Disease

In the present study it was found that while PR had no effect on CC activity/sensitivity in stable COPD patients, there were other important physiologic and psychosocial benefits including an improvement in 6MWD, health-related quality of life, and resting dyspnea. This is important, as an improvement in 6MWD and resting dyspnea should improve an individual's ability to perform activities of daily living as well as their independence, and the change in 6MWD following PR has been shown to be significantly associated with age- and comorbidity-adjusted survival in COPD.[26] Additionally, the improvement of health-related quality of life in COPD

patients is an important psychosocial outcome, and previous research has shown a significant correlation between SGRQ scores and mortality in COPD.[27]

Importantly, the present study observed that while there was no improvement in PWV following PR, there was a significant increase in PWV in the control group when compared to the rehabilitation group. There was no significant difference in baseline PWV between groups, and this finding is unlikely to be a result of a change in sympathetic tone as there was no change in CC activity/sensitivity or autonomic function following PR. This would indicate that PR is effective in reducing the natural deterioration of PWV associated with disease progression, however the mechanism(s) for this are unknown.

It is clear from the findings of the present study, then, that while there was no effect of PR on CC activity/sensitivity in stable COPD patients, there are multiple other important benefits received from PR. As a result, exercise training and rehabilitation should continue to be prescribed as part of the disease management process for COPD, and should not be disregarded because of the lack of effect on CC activity/sensitivity. Additionally, it should be noted that this is the first study to examine the effect of PR on CC activity/sensitivity in stable COPD. It is possible that future research may find an effect of exercise training or rehabilitation on CC activity/sensitivity in COPD, and therefore exercise training/rehabilitation should not be omitted as an effective intervention in targeting the CC as a result of this one study.

3.3. Limitations and Considerations

3.3.1. Six Minute Walk Test

In the present study, there was a significant increase in 6MWD in both the rehabilitation and control groups following the intervention period. It was speculated that perhaps four control subjects were responsible for the statistically significant increase in 6MWD observed in the control group following the intervention period. These subjects improved 6MWD by 40m, 26m,

25m, and 27m respectively. To examine this, these four subjects were temporarily removed from the analysis. While the mean improvement in 6MWD in the control group following the intervention period was reduced to 11m, this increase was still statistically significant, supporting our conclusion that these improvements were a result of a learning effect. Therefore future studies should include familiarization testing for all participants, regardless of previous experience with the procedure. While this result is not desirable, it has negligible impact on the findings of the present study regarding the effect of PR on CC activity/sensitivity.

3.3.2. Generalizability of Findings

Participants in the present study were all clinically stable and mobile COPD patients who were able to regularly attend PR and/or the testing sessions. While this is not indicative of the average COPD patient, our sample is likely representative of the typical COPD patient referred to PR. It is possible that the observed findings would be different if we examined the impact of PR on CC activity/sensitivity in asymptomatic COPD patients, patients who recently had an exacerbation of COPD, or in individuals with greatly reduced physical activity as a result of their disease severity. Therefore, generalization of these results should be limited to those COPD patients commonly referred to PR by their physician, and not the broader COPD population.

3.3.3. Exercise Training Stimulus During Pulmonary Rehabilitation

It is possible that the exercise training stimulus achieved during PR may not have been sufficient to elicit changes in CC activity/sensitivity in our sample of COPD patients. In order to maximize training adaptations and physiologic benefits,[28] the Breathe Easy PR program encourages all participants to exercise at an intensity of greater than 60% of maximal work rate[29] or an exercising heart rate of 60-85% of the peak heart rate achieved during the pre-rehabilitation CPET. Ultimately, however, all exercises are individualized and subject to daily variations in patient abilities and symptoms. This individualization may have resulted in an inconsistent

exercise training stimulus across participants, as COPD patients are limited in their ability to exercise as a result of a ventilatory limitation[30, 31] and/or peripheral muscular dysfunction.[59] While we found that 75% of our participants achieved the American College of Sports Medicine guidelines for exercise training,[29] it is still possible that the exercise training stimulus required to alter CC activity/sensitivity in COPD is greater than the majority of individuals achieved in the Breathe Easy PR program. This potential inconsistency in the exercise training stimulus may then explain, in part, the lack of change in CC activity/sensitivity observed following PR. As it would not be feasible to increase the exercise intensity in COPD patients as a result of functional limitation, increasing the volume of exercise performed by increasing the length of the PR program may result in a sufficient training load to elicit significant changes in CC activity/sensitivity. Therefore future studies should examine the impact of a longer duration PR program on CC activity/sensitivity in COPD patients.

3.3.4. Degree of Baseline Carotid Chemoreceptor Activity and Sensitivity

As the subjects in the present study were stable, normoxemic, and normocapnic, it is possible that the degree of CC activity/sensitivity may not have been severe enough to receive benefits from PR. Baseline CC sensitivity in the present study was less than our recent work in COPD which assessed CC sensitivity using the step hypoxia method (Present study: 0.14 ± 0.11L/min/SpO_2; Stickland *et al.* (2016): 0.24 ± 0.22L/min/SpO_2); additionally, baseline CC activity in our sample of COPD patients was also less than what we have previously reported (Present study: -1.07 ± 1.55L/min; Stickland *et al.* (2016): -2.62 ± 1.90L/min).[10] It is possible that the difference in CC activity/sensitivity observed in the present study may be a result of our previous work excluding patients with CV comorbidities, or individuals using vasoactive medications. However the presence of CV comorbidities in the present study would be predicted to be associated with a greater CC activity/sensitivity than what was previously reported. As

vasoactive medications such as β-blockers and angiotensin II receptor blockers can suppress the CC and/or ventilation,[32-35] it is possible that the use of these medications by subjects in the present study explains the difference in CC activity/sensitivity between these two studies. Additionally, Stickland and colleagues assessed CC activity using a 15-second average nadir, and CC sensitivity using the final minute average $\dot{V}_E$ at SpO_2 90% and 85%; the present study used a 10-second average nadir to assess CC activity, and the maximal 30-second average change in $\dot{V}_E$ at SpO_2 90% and 85% when assessing CC sensitivity. Therefore this difference in analysis may also partly explain the difference in CC activity/sensitivity between studies.

Observed mean baseline CC sensitivity in our study was substantially less than that reported in CHF patients when assessed using the transient hypoxia test (Present study: 0.14 ± 0.11L/min/SpO_2; Ponikowski *et al.* (2001): 0.69 ± 0.50L/min/SpO_2).[36] It is possible that the use of step hypoxia in the present study explains a portion of the difference in CC sensitivity compared to the findings of Ponikowski *et al.* (2001),[36] as transient hypoxia can result in large changes in ventilation (particularly when inspiring 5-8 breaths of pure nitrogen) and does not represent steady-state ventilation. However this is unlikely to be the only explanation for the difference in CC sensitivity between studies. It was hypothesized that the difference in findings between these two studies could be the result of COPD patients being ventilatory limited, therefore resulting in a lower $\dot{V}_E$ and calculated CC sensitivity. However this was not supported by the present data, as the subjects in this study were able to achieve a mean exercising $\dot{V}_E$ of ~50L/min, and the average $\dot{V}_E$ achieved during hypoxia was ~10L/min. Ponikowski and colleagues excluded patients who were receiving treatment with β-blockers, which may partly explain the reduced CC sensitivity observed in the present study; however this is unlikely to fully explain the nearly five-fold difference in CC sensitivity between the two studies. The potential

remaining explanation(s) for this difference in CC sensitivity are unknown, but may be related to the difference in pathophysiology between CHF and COPD. While neither Ponikowski *et al.* (2001)[36] or Stickland *et al.* (2016)[10] examined the effects of exercise rehabilitation on CC activity/sensitivity, it is possible that the lower baseline CC activity/sensitivity observed in the present study may partly explain the lack of change in CC activity/sensitivity following PR.

3.3.5. Autonomic Function During Hyperoxia and Hypoxia

In the present study we were unable to examine changes in autonomic function from normoxia with hyperoxia and hypoxia. A standardized five minute recording is required to assess short-term HRV,[37] and in the present study we assessed BRS during the same five minute period. We administered hyperoxia in one minute intervals, as prolonged hyperoxia has been shown to result in hyperventilation,[38] which is presumed to be secondary to free radical production. Additionally, during the hypoxic trial, each reduction in SpO_2 was targeted for three minutes, similar to Howard and Robbins (1994),[39] who maintained step reductions in SpO_2 for two minutes at each stage. As a result, we were unable to analyze HRV or BRS during hyperoxia or hypoxia as the sampling window was too small. It is possible that the autonomic function response to acute hyperoxia/hypoxia may have changed following PR, indicating a potential change in sympathovagal balance. However, as there was no change in autonomic function during normoxia following PR, and similarly the ventilatory response to hyperoxia and hypoxia remained unchanged following PR, it is doubtful that autonomic function measured during hyperoxia and hypoxia would have changed following PR. As a result, this information would have been unlikely to alter the conclusions of the present study. Furthermore, the purpose of this study was to examine the effects of PR on CC activity/sensitivity, and not autonomic function. Therefore, changes in autonomic function during hyperoxia and hypoxia, conditions which do not represent typical physiological states, is of minor importance in the present study.

3.4. Future Research Direction

As the exercise training stimulus may not have been sufficient in the present study to elicit changes in CC activity/sensitivity, and individuals with COPD are limited in their ability to exercise, future studies should examine the impact of longer duration programs on CC activity/sensitivity in COPD patients. Pulmonary rehabilitation promotes the individualization of exercise training to accommodate for daily variations in symptoms as well as individual differences in ventilatory and musculoskeletal limitations to exercise. In theory, all individuals enrolled in PR should be exercising near the greatest intensity that is possible for them; it is therefore not feasible to design a program in which the exercise training intensity is standardized among patients, as this intensity may be too great for some patients and not enough for others. It is possible that by extending the length of the program from 6-8 weeks up to four or five months, thereby increasing the exercise training load, this may elicit significant changes in CC activity/sensitivity. Through increasing the length of the PR program, this would likely allow for an increased physical conditioning so that individuals may be able to exercise at greater intensities in the future. However, it must be recognized that the ability to increase the duration of the PR program is dependent upon the resources available, and this may prove to be a limiting factor for future research.

Examining a more heterogeneous sample of COPD patients may yield different results compared to those observed in the present study. While speculative, it may be possible that unstable COPD patients, or individuals with a greatly reduced physical activity or lung function as a result of their disease, could have a greater CC activity/sensitivity upon entering PR; these patients may therefore have the most potential for improvements in CC activity/sensitivity as a result of PR. This may not result in different findings from the present study, as we observed that the improvement in CC activity in individuals with greater baseline CC activity was a result of

regression to the mean. Furthermore, in the present study there was no difference in baseline CC activity/sensitivity when subjects were stratified based on their GOLD status or degree of resting dyspnea. However, it is possible that other factors associated with disease severity and physical deconditioning may play a role in the CC activity/sensitivity response to PR, and not just the baseline CC activity/sensitivity alone. Furthermore, as the present study only examined stable and mobile COPD patients, future research into the broader COPD population is required to produce more generalizable findings.

Both COPD and CHF have been shown to have an elevated CC activity/sensitivity, however previous research on the effects of exercise training on CC activity/sensitivity in CHF has only been completed in animal models. A previous study examining the effects of exercise training in clinical CHF patients found that those patients completing an eight week exercise training program significantly reduced systemic vascular resistance, $\dot{V}_E$ and the slope relating $\dot{V}_E$ to CO_2 production. Furthermore, these CHF patients significantly improved autonomic function, as indicated by HRV and norepinephrine spillover.[40] Collectively these findings would suggest that an eight week exercise training program may be effective in reducing CC activity/sensitivity in CHF patients, however CC activity/sensitivity was not assessed in this study. Therefore, future research may also be conducted to examine the effects of cardiac rehabilitation on CC activity/sensitivity in clinical CHF patients. It would then be possible to see if either the results are similar to what has been observed in the present study, or what has been previously shown in animal models of CHF.[41] One could assess CC activity/sensitivity in these CHF patients using the ventilatory response to hyperoxia and hypoxia, thus allowing comparisons between these subjects and individuals in the present study. If there is no change in CC activity/sensitivity in CHF patients following cardiac rehabilitation, then it may be that exercise training is not

effective in reducing CC activity/sensitivity in clinical populations. However, if there is a significant reduction in CC activity/sensitivity following cardiac rehabilitation in CHF patients, one could then examine if the difference between these studies is a result of a difference in baseline CC activity/sensitivity, or intensity and duration of the rehabilitation programs. If there is no difference in baseline CC activity/sensitivity between these studies, and if the rehabilitation programs are similar in intensity and duration, then this may allow one to suggest that there is a pathophysiological difference in the elevated CC activity/sensitivity between COPD and CHF. If the etiology of the elevated CC activity/sensitivity in COPD differs from that in CHF, this would then necessitate the need to investigate alternate interventions aimed at reducing CC activity/sensitivity in COPD as a means of mitigating the elevated CV risk observed in COPD patients.

3.5. Commentary

Throughout my Master's degree, I have obtained valuable skills in patient rapport and recruitment, running cardiopulmonary exercise tests and pulmonary function tests on clinical populations, the safe administration of medical-grade gases to patients, as well as the collection and analysis of various recorded physiological data. I have gained an increased understanding of clinical physiology, as well as pulmonary rehabilitation. Lastly, in my Master's program I have learned to compile and report data concisely in a comprehensive report.

When working with older clinical populations, it is important to clearly communicate with all participants so that they understand the purpose of the study in a way that is relatable and meaningful to them. Additionally, it was difficult to organize for the participants to attend three testing sessions at either the University of Alberta or G.F. MacDonald Centre for Lung Health, and proper communication was required to ensure that participants were able to attend each

session. It was important to reassure all participants that their safety and comfort was my top priority, and that I was there at their convenience. Throughout my Master's program I have developed a superior patient rapport, and this has been stated by many of my participants on multiple occasions. I am confident that this improvement in interpersonal interaction will aid me positively in my future endeavours. Additionally, working with a clinical population has increased my appreciation for clinical research, as I had multiple participants drop out of the study as a result of illness, or they were unable to continue with the time commitment – this is something that is typically less common when working with younger, apparently-healthy individuals.

In my undergraduate degree, I obtained an introductory education on the basics of cardiopulmonary exercise testing and pulmonary function testing, however this information was presented in reference to healthy young athletes, and we did not acquire hands-on practice in performing these techniques. During my Master's degree, in addition to gaining the technical knowledge of performing these assessments, I also gained a better understanding of the values obtained from these tests. Not only did I improve my ability to interpret the reports generated from the CPETs and PFTs, but I was able to see how these values compared to those of an average young healthy individual, and gain perspective on the different pulmonary and musculoskeletal limitations that individuals with COPD encounter.

When examining CC sensitivity, I would administer a nitrogen-gas mixture through an air-O_2 blender system to cause step reductions in SpO_2. Improper administration of these gases can be dangerous, especially when dealing with individuals with lung disease. Therefore, an understanding of the technique and of the live recording of data was crucial to ensure that these gases were being properly administered in a way safe for the participant. Additionally, it was

important to be able to interpret end-tidal gas values to ensure that the gas was being administered effectively. Recalling one subject, upon administration of hypoxic gas, I observed the raw data and saw that end-tidal values of O_2 and CO_2 were not approaching the values typically observed with hypoxia. Upon further inspection I had found that the seal around the mouthpiece had momentarily come loose, resulting in a leak of expired gases. I was then able to fix this immediately and resume the testing following a short break.

Data from the present study was recorded using different tools, including digital data acquisition programs as well as hand written results. Additionally multiple data analysis programs were used, including Lab Chart and Dos. It was therefore important to learn how to neatly organize the data so that it could be compiled into a single worksheet for further statistical analysis. In addition to gaining proficiency in the use of these various data collection and analysis programs, I also had to develop my skills in using statistical analysis programs including SPSS and SigmaPlot, programs that I had no experience with prior to my Master's degree.

While completing my Master's degree, I had the opportunity to get involved with the Breathe Easy PR program at the G.F. MacDonald Centre for Lung Health. During this time, I developed a greater understanding of the structure and purpose of the PR program. I was able to participate in the educational seminars to observe the information provided to these patients regarding disease awareness and self-management. Additionally, I was able to help the respiratory and exercise therapists in the daily administration and supervision of patient exercises. It was a very humbling experience to see first-hand what it is like for these patients to exercise, and the difficulties they encounter. I feel very fortunate to have had the opportunity to talk with many of these individuals and gain an enhanced understanding of their experiences with COPD and the impact of their disease on their lives. I was interested to learn more about the mindset of

this demographic upon entering the PR program, and how this may have changed upon program completion. It was a privilege to be able to watch these patients grow stronger and more confident throughout the short 6-8 week period. This was personally the most rewarding component of my Master's program.

In summary, the ability to interact with and recruit participants, administer various physiological procedures, and analyze and interpret the data from these procedures have all been crucial components which have aided the completion of the present study. The training I have received in the laboratory during my Master's program, in addition to the support from my supervisory committee, has been paramount in developing my understanding of designing a research study, collecting and analyzing the data, and compiling the results in a comprehensive report. Additionally, the ability to communicate with my participants and spend time at the Center for Lung Health has provided me with superior interpersonal skills that I will carry with me in my future endeavours.

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APPENDIX A: LITERATURE REVIEW

Chronic obstructive pulmonary disease (COPD) is a respiratory disease primarily caused by smoking and characterized by progressive, partially-reversible airway obstruction. COPD patients experience marked resting dyspnea, and are less physically active than the general population, resulting in reduced exercise tolerance.[1] Approximately 4.3% of the Canadian population was *reported* as having COPD in 2013, however these numbers are likely an underrepresentation of the actual incidence rate of COPD in Canada.[2] COPD mortality rates have been steadily increasing, and COPD is currently the third leading cause of death globally, following ischemic heart disease and stroke.[3] COPD is also the primary cause of hospital admissions in Canada;[4] this is significant, as COPD is estimated to cost Canada approximately $1.5 billion in healthcare costs annually.[5] It is clear then that COPD poses a current and growing threat to the health of Canadians, highlighting the necessity for evaluating effective means of mitigating its health impacts.

A.1. Increased Cardiovascular Risk in Chronic Obstructive Pulmonary Disease

While COPD is typically regarded as a disease specific to the lungs, it is also associated with an increased risk of developing cardiovascular (CV) disease. Importantly, the most prevalent causes of mortality in COPD are not pulmonary exacerbations, but CV events including ischemic heart disease and stroke.[6, 7] One report examining mortality of patients with obstructive airway disease stated that a CV event was the primary cause of death in approximately 50% of patients.[8] The diagnosis of COPD increases the risk of CV disease more than two-fold,[9] and this increased CV risk is independent of traditional risk factors, including smoking.[10] Individuals with COPD are 2-3 times more likely to die of heart failure than smokers who have not been diagnosed with COPD, suggesting that there is some variable specific to COPD, and not smoking history itself,

which may be responsible for this increased CV risk. However, the etiology of the increased CV risk in COPD is currently uncertain and requires further investigation.

The degree of CV risk in COPD is associated with disease severity, which may suggest that the increased CV risk in COPD is specific to the pathology of COPD. The risk of ischemic heart disease, stroke, and sudden cardiac death in COPD patients increases with modest reductions in forced expiratory volumes in one second (FEV_1).[6] The Baltimore Longitudinal Study of Ageing[11] found that those with the most rapid decline in FEV_1 over a follow-up of 16 years were 3-5 times more likely to die from CV causes than those with slower reductions in FEV_1. Furthermore, a 10% decrease in FEV_1 is associated with a 30% increase in risk of death as a result of CV events.[12-14] While the association between reduced lung function and increased CV risk in COPD is not well understood, it is postulated that arterial stiffness may be elevated in those with poorer lung function,[15] and this elevated arterial stiffness may result in the increased CV risk in COPD compared to healthy controls.

Aortic arterial pulse wave velocity (PWV), an indicator of central arterial stiffness, is a clinically significant predictor of CV risk. Central arterial stiffness has been shown to predict CV risk in both health[16-18] and disease,[16, 19, 20] independent of traditional risk factors.[21, 22] Furthermore, arterial stiffness is elevated in stable COPD compared to healthy controls,[22-24] and is an independent predictor of all-cause and CV mortality in stable COPD.[25] The relationship between COPD and arterial stiffness is not entirely known, however one potential mechanism is tonic vasoconstriction secondary to an increased sympathetic nerve activity (SNA).

A.2. Potential Contributors to Increased Cardiovascular Risk in Chronic Obstructive Pulmonary Disease

A.2.1. Sympathetic Nerve Activity

One contributor to the increased CV risk in COPD is an increased SNA. COPD patients have been shown to have greater resting SNA compared to healthy controls as assessed through microneurography.[26] Elevated muscle SNA (MSNA) is associated with negative prognosis in COPD, with the degree of resting SNA being predictive of mortality and hospitalization in COPD patients;[27] however, the relationship between elevated SNA and CV risk in COPD is complex.

While acute elevations in SNA are beneficial in maintaining cardiac function, chronically-elevated SNA is associated with CV deterioration,[28] including peripheral vasoconstriction. Not surprisingly, a positive association has been observed between potentiated SNA and a reduction in arterial compliance,[29] and peripheral vasoconstriction resulting from chronic elevations in SNA is associated with greater arterial stiffness.[30, 31] In healthy males, PWV correlated significantly with MSNA following adjustment for age and systolic blood pressure.[31] Furthermore, short-term sympathetic activation decreases arterial compliance in healthy individuals.[29] Collectively these findings indicate a positive association between arterial stiffness and SNA, thereby providing a likely explanation for the increased CV risk observed in COPD, as has been previously suggested by other researchers.[32] However, elevations in SNA and the associated CV consequences in COPD have not been fully studied. While the pathophysiology of the potentiated SNA in COPD is not clearly understood, it is possible that an increased chemoreceptor activity/sensitivity may contribute to the elevated SNA observed in COPD patients compared to controls.

A.2.2. Enhanced Carotid Chemoreceptor Activity and Sensitivity

The carotid chemoreceptors (CC) are peripheral chemoreceptors located in the carotid bodies at the bifurcation of the common carotid arteries. These receptors are sensitive to changes in the arterial partial pressure of oxygen (P_aO_2), arterial partial pressure of carbon dioxide (P_aCO_2), H^+ concentration, and perfusion.[33] When the CC are stimulated, signals are sent along the carotid sinus nerve towards respiratory centers in the brainstem via the glossopharyngeal nerve; this ultimately leads to an increase in minute ventilation ($\dot{V}_E$).[34] Typically, when P_aO_2 falls below 60mmHg, afferent carotid sinus nerve firing increases, resulting in an increased $\dot{V}_E$ – this hyperventilation aims to reduce arterial PCO_2 and raise alveolar, and thus arterial, PO_2 to baseline levels, thereby maintaining oxygenation. Importantly, when the CC are stimulated they also elicit an increase in SNA,[35-37] while CC denervation practically eliminates the sympathetic[35] as well as ventilatory[38] response to hypoxia. This elevation in SNA is physiologically important, as pharmacological stimulation of the CC results in vasoconstriction to skeletal[39] as well as coronary[40] vasculature, and this vasoconstriction can be blocked with sympathetic blockade,[39, 40] indicating that CC stimulation causes vasoconstriction through increases in SNA. Additionally, inhibition of the CC with transient hyperoxia results in a significant reduction in PWV in COPD patients but not controls, which is thought to be secondary to a reduction in sympathoexcitation.[41] Furthermore, CC stimulation decreases the inotropic state of the left ventricle.[42] These findings indicate that in addition to influencing ventilation, CC stimulation increases SNA which can have important CV consequences.

The CC are tonically active at rest, and the degree of this activity can be indirectly determined by the reduction in $\dot{V}_E$ with breathing transient hyperoxia. Administration of 100% O_2 is known to rapidly inhibit the CC[43] and therefore is an appropriate measure of resting CC activity (see section B.3.2.3.4 for methodology).[44] As CC activity is associated with SNA,

greater CC activity likely results in an elevated resting SNA. Siński *et al*. (2012)[45] found that deactivation of the CC with 100% O_2 reduced MSNA in males with essential hypertension but not in normotensive controls, suggesting that tonic chemoreflex activation may be cause for the high sympathetic activity in these patients. Comparatively, CC sensitivity is evaluated by the increase in $\dot{V}_E$ relative to the drop in arterial saturation while breathing hypoxic gas (i.e. $\Delta\dot{V}_E/\Delta SpO_2$ slope)[36, 37, 46] (see section B.3.2.3.5); increased CC sensitivity has been found to be predictive of mortality in clinical populations.[47] Collectively, augmented CC activity/sensitivity can increase SNA which contributes to the development of CV disease. Therefore, the CC are a potential target for interventions aimed at reducing CV risk in populations characterized by heightened SNA.

A.2.2.1. Potentiation of the Carotid Chemoreceptors in Chronic Heart Failure and Hypertension

Potentiated CC activity/sensitivity has been noted in chronic heart failure (CHF) models. Rabbits with experimental CHF are shown to have an enhanced CC activity and sensitivity compared to sham rabbits[48] as indicated by a greater baseline carotid sinus nerve discharge frequency, and an exaggerated carotid sinus nerve discharge frequency response to hypoxia, respectively. Additionally, humans with CHF are characterized by a greater resting SNA, and the degree of SNA at rest parallels the impairment in cardiac performance.[49] Augmented CC activity/sensitivity likely contributes to this increased SNA, as 100% O_2 administration in CHF patients with elevated peripheral chemosensitivity resulted in a significant reduction in MSNA;[50] however these same findings were not found in CHF patients with normal peripheral chemosensitivity, suggesting that other factors may also contribute to the elevated SNA in CHF, such as reduced baroreceptor sensitivity. Importantly, both the level of resting SNA[49, 51] and

degree of CC sensitivity[47] (indicated by the $\Delta\dot{V}_E/\Delta SpO_2$ slope) is predictive of mortality in CHF.[47, 52, 53]

Both work in animals and humans suggest a contribution of the CC to heightened SNA in CHF. Inhibition of the CC results in a reduction in sympathetic vasoconstrictor outflow in dogs with experimental CHF but not in healthy dogs,[54] suggesting that the CC contribute to the increased SNA and vasoconstrictor outflow observed in CHF. Additionally CC denervation in experimental CHF results in a reduction in CC activity/sensitivity, SNA, and arrhythmia incidence, while increasing cardiac function[55] and survival.[56] In CHF patients, CC inhibition with either low-dose dopamine or hyperoxia reduced ventilation, and this result was not observed in healthy controls.[57] Low-dose dopamine administration also resulted in improvements in cardiac output and stroke volume in CHF patients at rest[57] which is likely secondary to reduced total peripheral resistance. This finding would indicate an important contribution of the CC to vascular control, likely through sympathetically-mediated peripheral vasoconstriction. Collectively, these findings suggest that CC inhibition improves autonomic function in CHF animals and humans.

Borderline hypertensive subjects also have an exaggerated chemosensitivity as assessed by the sympathetic nervous response to hypoxia.[58] In untreated patients with systemic arterial hypertension, CC activation may contribute to an increased SNA; in these same patients, CC deactivation with acute hyperoxia results in a normalization of SNA - this reduction in SNA is shown to be accompanied by a decrease in heart rate. Importantly, following normalization of SNA with acute hyperoxia, there was little difference in the magnitude of resting SNA between hypertensive and control patients. The normalization of SNA with acute hyperoxia would suggest that tonic chemoreflex activity is likely responsible for a portion of the sympathetic over-

activity observed in hypertensive patients. It is possible that, similar to CHF and hypertension, an increased CC activity leads to sympathoexcitation in COPD, resulting in important CV consequences.

A.2.2.2. Chronic Obstructive Pulmonary Disease and the Carotid Chemoreceptors

Similar to CHF, SNA is augmented in individuals with COPD, and this may play a role in the potentiated CV risk in COPD. While the etiology of this increased SNA is unknown, one potential explanation may be an elevated CC activity/sensitivity. Heindl *et al.* (2001)[59] reported that hypoxemic COPD patients have elevated MSNA compared to normoxic COPD. Interestingly, when supplemental O_2 was given to hypoxemic COPD patients, MSNA was reduced but still remained elevated in comparison to healthy controls.[59] Oxygen supplementation is known to inhibit the CC, which may explain the reduction in MSNA. It is possible, then, that the increased SNA in COPD is secondary to elevated CC activity/sensitivity. Furthermore, COPD patients typically have an exaggerated heart rate[60] and ventilatory[61] response to hypoxia; these increased responses to hypoxia are likely the result of enhanced CC sensitivity in COPD, as CC denervation virtually abolishes the sympathetic[35] and ventilatory[38] responses to hypoxia. Potentiated CC sensitivity in COPD has clinical importance, as both the ventilatory response to hypoxia, as well as the ventilatory response to exercise, are associated with increased mortality in other diseases.[47, 52, 53] However, CC activity/sensitivity has not been well studied in COPD.

More recently, work from our laboratory has shown that COPD patients have an exaggerated basal CC activity, as CC inhibition with transient hyperoxia resulted in a greater reduction in resting $\dot{V}_E$ in COPD compared to healthy controls.[41] Furthermore, arterial stiffness and blood pressure were reduced in COPD patients following short-term administration of 100% O_2; similar findings were not observed in healthy controls, indicating a potential contribution of the CC to elevated CV risk in COPD. Additionally COPD patients had an exaggerated CC

sensitivity compared to healthy controls, determined by the ventilatory response to hypoxia. This increased CC activity/sensitivity would explain the elevated MSNA, arterial stiffness, and consequently CV risk, observed in COPD.

Individuals with CHF and COPD often share a similar history of smoking and physical inactivity, and therefore the mechanism(s) responsible for the sympathoexcitation observed in both conditions may be similar. As a result, COPD patients may have increased MSNA secondary to heightened CC activity, as has been demonstrated in CHF, and this potentiated CC activity may be irrespective of blood gas status as both normoxemic and hypoxemic COPD patients have evidence of increased SNA compared to healthy controls.[26, 27, 59, 62, 63] Collectively these findings indicate that CC activity/sensitivity is elevated in COPD compared to healthy controls, and this is likely partly responsible for the elevated CV risk observed in COPD, resulting from an increased arterial stiffness and impaired autonomic function.

A.2.2.3. Dyspnea

Individuals with COPD are characterized by potentiated resting dyspnea.[64] Dyspnea is a significant consequence of COPD, and is likely a major contributor to the decreased physical activity and quality of life observed in COPD patients.[65] It has been reported that the categorization of COPD patients on the basis of dyspnea severity indicated by the Medical Research Council dyspnea scale was a stronger predictor of 5-year mortality than lung function as evaluated by FEV_1.[66] As dyspnea has been shown to be associated with patient mortality, the etiology of dyspnea in COPD, and potential management strategies, requires further examination.

A relationship between dyspnea and CC activity/sensitivity has been proposed in the literature. An increased sympathetic activation is associated with elevated breathing frequency,[67] which may increase dyspnea as a result of lung hyperinflation. This would then result in

neuromechanical uncoupling, which is suggested to be a contributor to dyspnea in COPD.[68] As CC activation is associated with an increase in SNA as well as ventilation, it is possible that the CC contribute to the elevated resting and exertional dyspnea observed in COPD patients. When hyperoxia was administered to individuals with COPD at rest, there was a significant reduction in dyspnea (as indicated by a reduction in modified Borg rating), but no significant change in resting lung volume or ventilation.[69] Somfay *et al* (2001)[70] reported that in mildly hypoxemic, severe COPD patients, exertional dyspnea was attenuated while breathing supplemental O_2; importantly, this reduction in dyspnea correlated with reductions in respiratory frequency. Potentiated CC activity/sensitivity may therefore be a contributor to the increased resting and exertional dyspnea observed in COPD. Interventions aimed at reducing CC activity/sensitivity, then, become important in managing dyspnea and its associated effects in COPD.

A.2.2.4. Health Related Quality of Life

The St. George's Respiratory Questionnaire (SGRQ) is commonly used to examine health-related quality of life in COPD patients. This questionnaire is a valid measure of health in COPD, and is highly repeatable and sensitive;[71] furthermore, the SGRQ is strongly correlated with the medical research council questionnaire ($r^2 = 0.50$).[71] Individuals with moderate to severe COPD were reported as having a clinically significant (≥ 4 units) improvement in all domains of the SGRQ following pulmonary rehabilitation.[72]

A.2.2.5. Autonomic Dysfunction

Autonomic dysfunction has been identified in COPD, and is multifactorial in nature. Disturbance in autonomic reflexes are risk factors for cardiac morbidity and mortality.[73, 74] Patients with COPD have a lower heart rate variability (HRV) at rest compared to healthy controls.[75] HRV is said to provide a quantitative assessment of activity of the cardiac autonomic nervous system;[76] a more efficient cardiac autonomic control mechanism is indicated by a greater HRV,[77] and

decreased HRV is associated with increased risk of CV events in healthy individuals, even following correction for known risk factors.[78] Scalvini *et al*. (1998)[62] reported that HRV was normalized in COPD patients following administration of supplemental O_2 which the authors suggest may be a result of increased vagal tone, as vagal tone has previously been shown to improve in COPD following O_2 administration.[63] It may be possible that there is sympathetic withdrawal during supplemental O_2 administration, as 100% O_2 is known to inhibit the CC; inhibition of the CC would reduce SNA, improving vagal tone and thereby autonomic function. Perhaps, then, heightened CC activity/sensitivity plays a role in the exaggerated autonomic dysfunction observed in COPD patients compared to healthy controls.

Baroreceptor sensitivity (BRS) is a measure of the baroreceptor-heart rate reflex, and is an established indicator of autonomic control of the CV system.[79] Quantification of BRS involves examination of the relationship between systolic blood pressure values (mmHg) and the length of the next RR interval (the time interval between consecutive heart beats – msec).[80] A greater BRS represents a greater slowing of heart rate for a given rise in systolic blood pressure, indicating an improvement in the autonomic control of the heart. In individuals with hypertension, BRS is positively correlated with HRV.[81] Individuals with COPD have a reduced BRS,[82] which could partly explain the elevated CV risk in this population; however the reason(s) for the attenuated BRS in COPD compared to the healthy population is unclear. As neurons from the CC and the carotid baroreceptors are distributed in close proximity in the solitary and paramedian reticular nuclei in the medulla, interneuronal connections might facilitate interactions between these reflexes;[83] it is possible that an elevated CC activity/sensitivity may attenuate the vagal efferent activity of the baroreceptors, resulting in autonomic dysfunction. Bartels *et al.* (2000)[63] noted a 17% increase in BRS following O_2 administration in COPD

patients, and this degree of increase is within the range shown to improve outcomes in cardiac patients.[84] It is possible that the observed increase in BRS is secondary to CC inhibition, thereby further signifying the clinical importance of augmented CC activity/sensitivity in COPD.

As supplemental O_2 is effective in improving autonomic function, and O_2 administration is known to inhibit the CC, it is possible that the improvement in autonomic function following supplemental O_2 is secondary to CC inhibition. However, when seeking to manage autonomic dysfunction through reducing CC activity/sensitivity, other treatments are required as long term supplemental O_2 therapy for these patients would be costly and has been shown to predict mortality in patients with severe emphysema.[85] For this reason, alternate interventions are gaining increasing popularity as potential therapies with long term benefits.

A.3. Disease Management Strategies in Populations Characterized by Potentiated Carotid Chemoreceptor Activity/Sensitivity

A.3.1. Exercise Training and Rehabilitation

Exercise training has many CV benefits, and is now recognized as an important medical therapy for a variety of chronic diseases. Exercise training appears to be associated with reductions in arterial stiffness in both animals[86] and healthy humans.[87] Similar results have been found in coronary artery disease, where 12 weeks of endurance training resulted in a 4% decrease in the augmentation index (a measure of the enhancement of central aortic pressure) as well as an eight millisecond increase in reflected pulse wave transit time;[88] this suggests an overall improvement of the systemic arterial stiffness in individuals with coronary artery disease upon completion of an endurance training program. In stable COPD, a four week cycling program significantly reduced peripheral PWV by 10% in the trained group compared to controls;[89] this decrease in PWV was related to changes in peak aerobic capacity. Importantly, this change in PWV has been shown to be comparable to changes as a result of classic CV medications.[90] Taken together, these

findings suggest that exercise training may reduce arterial stiffness, and therefore CV risk, in COPD patients. However, the mechanism(s) through which exercise training decreases arterial stiffness in COPD is not fully understood.

Exercise training has also been shown to have beneficial effects on CC activity/sensitivity as well as SNA in CHF. Aerobic exercise training in experimental CHF rabbits results in normalization of CC activity/sensitivity, and resultantly a reduction in resting SNA.[91] This would suggest that exercise is an effective intervention in normalizing CC activity/sensitivity as well as SNA in CHF. Due to the similarities between CHF and COPD, exercise training may be capable of reducing CC activity/sensitivity in COPD as has been observed in CHF. A reduction in CC activity/sensitivity would reduce SNA, thus providing an explanation for the reduction in PWV observed in COPD following aerobic exercise training.

Improvements in autonomic function have also been observed following exercise training programs. Exercise training is shown to reduce autonomic dysfunction at rest in CHF,[92] however the mechanism(s) responsible for this improvement are unknown. Furthermore, exercise training reduced peripheral vascular resistance, $\dot{V}_E$, the slope relating $\dot{V}_E$ to CO_2 production, whole-body norepinephrine spillover, and increased 24-hour HRV in CHF, suggesting a decrease in sympathetic control.[93] If exercise training is effective in reducing CC activity/sensitivity, this would explain the improvements in ventilation and sympathovagal balance observed in CHF following exercise training. Additionally, exercise training improves BRS in healthy subjects,[94] and exercise-induced improvements in BRS are positively associated with survival.[95] It is possible then, that an exercise-based program implemented in COPD may result in improvements in HRV and BRS, indicating improved autonomic function.

Exercise-based rehabilitation programs have been reported to have a positive impact on decreasing CV risk in CHF. O'Connor *et al.* (2009)[96] have shown that in individuals with CHF, exercise-based rehabilitation programs are effective in reducing all-cause mortality or hospitalization, and CV mortality or heart failure hospitalization following adjustment for highly prognostic predictors. These programs are a fundamental part of the disease management strategy for CHF; this might suggest that exercise-based rehabilitation programs may be suitable in the management of CV complications in COPD as well. While some studies have shown improvements in CV risk in COPD patients following exercise training, the mechanism(s) underlying these improvements are poorly understood.

A.3.2. Pulmonary Rehabilitation

Pulmonary rehabilitation (PR) is a 6-12 week exercise-based rehabilitation program, and is a critical component of proper disease management in COPD. The definition of PR was updated in 2013 by the American Thoracic Society as "*a comprehensive intervention based on a thorough patient assessment followed by patient-tailored therapies, which include, but are not limited to, exercise training, education, and behavior change, designed to improve the physical and psychological condition of people with chronic respiratory disease and to promote the long-term adherence of health-enhancing behaviors".*[97] Pulmonary rehabilitation is the most effective management strategy for improving dyspnea, quality of life, and exercise tolerance in COPD,[98-101] and PR also reduces the risk of future hospitalizations and healthcare costs.[102] While PR is shown to be effective in improving health outcomes in COPD, there is little understanding regarding the physiological adaptations responsible for the improvements in health outcomes in COPD as a result of PR. Additionally, how PR impacts the CV consequences of COPD is presently unknown. Reductions in CV risk as a result of PR have been identified in stable COPD compared to healthy non-exercising controls. Gale *et al.* (2011)[103] reported a decrease in aortic

PWV following a seven week PR program in patients with stable COPD, however these findings are not consistent across the literature. It is possible that any reductions in PWV observed following PR may be a result of reduced sympathoexcitation secondary to decreased CC activity/sensitivity, however no associations have been made in previous literature.

Reductions in the ventilatory response to exercise have been observed in COPD patients following PR,[104-106] and this has been primarily associated with a decreased lactic acid production as a result of skeletal muscle conditioning with exercise training.[105, 107] Unpublished findings from our laboratory suggest that COPD patients exhibit a reduced ventilatory response to oxygen consumption ($\dot{V}_E/VO_2$) and carbon dioxide production ($\dot{V}_E/VCO_2$) following PR; these ventilatory responses are measured before the onset of lactic acidosis, suggesting that the changes in ventilatory drive during exercise in COPD following PR may be a result of improvements in chemosensitivity, and not muscular conditioning. If these findings are a result of reduced chemosensitivity, this would then support the theory that reductions in dyspnea in COPD patients following PR may be secondary to reductions in CC activity/sensitivity. Additionally, an attenuation in CC activity/sensitivity would explain the reductions in arterial stiffness observed in COPD following PR.[89] Currently, the relationship between PR and CC activity/sensitivity in COPD has not been examined.

A.4. Summary

Individuals with COPD have an increased CV risk compared to controls which is associated with disease severity. Potential mechanisms for this increased CV risk include increased arterial stiffness and impaired autonomic function secondary to greater basal SNA. Increased CC activity/sensitivity may contribute to the increased SNA observed in COPD. Previous studies suggest that the CC contribute to an increased SNA, and therefore the enhanced SNA in COPD

may be secondary to elevated CC activity/sensitivity. Data from our laboratory indicates an enhanced CC activity/sensitivity in COPD, and a positive correlation between the CC and PWV; a reduction in PWV was observed in COPD with supplemental O_2, which suggests that the reduction in arterial stiffness is secondary to a decrease in SNA resulting from CC inhibition. Additionally, supplemental O_2 has been shown to improve autonomic function and dyspnea in COPD. Collectively these findings would suggest that the CC contribute to elevated CV risk in COPD, and this CV risk may be decreased by reducing CC activity/sensitivity.

In stable COPD, aerobic exercise training through PR improves central arterial stiffness and autonomic function however the mechanism(s) for these improvements are unknown. In experimental CHF aerobic exercise training normalizes CC activity/sensitivity and SNA; however, whether PR improves CC activity/sensitivity in COPD has not been examined previously. Therefore, the potential effect(s) of PR on CC activity/sensitivity in COPD requires further examination.

A.5. Purpose and Hypothesis

The purpose of this study was to examine the effects of PR on CC activity/sensitivity in patients with stable COPD. It was hypothesized that PR would reduce CC activity/sensitivity compared to controls, as indicated by a reduced ventilatory response to hyperoxia and hypoxia, respectively. Additionally a reduction in peripheral PWV was expected following PR, and this reduction in PWV would be associated with a reduction in CC activity/sensitivity. As improvements in indicators of autonomic function have been observed in other clinical conditions following exercise training, it was expected that autonomic function would also be improved as a result of PR, as indicated by an increased HRV and BRS. It was also expected that

the reduction in CC activity/sensitivity observed following PR would be related to an improvement in resting dyspnea following PR.

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APPENDIX B: DETAILED METHODS

B.1. Pulmonary Rehabilitation

A typical PR program is 6-12 weeks in length, and incorporates aerobic and strength training, stretching, breathing retraining, and education to optimize patient symptom management.[1] The "Breathe Easy" program is a 6-8 week PR program, located within the G.F. MacDonald Centre for Lung Health at the Edmonton General Continuing Care Centre, Edmonton, AB, Canada; this program provides multi-disciplinary PR to stable ambulatory patients with confirmed COPD, and this program is designed on best-practice evidence-based guidelines.[2] Major differences between PR and exercise training is the individualization of exercise prescription, as well as educational sessions in an effort to promote lifestyle changes. The one hour educational sessions focused on patient self-management and were presented by a multi-disciplinary team of respiratory therapists, physical therapists, pharmacists, dieticians, kinesiologists, and health psychologists.[3] Topics for these seminars included: basic lung disease pathophysiology, exercise training, respiratory medications, nutrition, travel/home care, and oxygen therapy.

B.2. Study Overview

B.2.1. Testing Day One: Patient Screening and Initial Assessment

B.2.1.1. Pulmonary Function Test

A complete pulmonary function test (PFT) was performed to assess lung function and included: spirometry (forced vital capacity [FVC] and forced expiratory volume in one second [FEV_1]); lung volumes (vital capacity [VC], residual volume [RV] and total lung capacity [TLC]); lung diffusion capacity (D_{LCO}); and airway reversibility.[4] The PFT was conducted in accordance with the American Thoracic Society guidelines (CareFusion, Yorba Linda, CA, USA).[5-7]

B.2.1.2. Cardiopulmonary Exercise Test

A graded, physician-supervised cardiopulmonary exercise test (CPET) to maximal exertion was completed on a treadmill (TMX425C, Full Vision Inc., Newton, Kansas, USA) prior to and following PR or equivalent time-delay to assess exercise tolerance. The CPET was individualized to each subject; the selected exercise protocol was chosen to achieve maximal patient exertion within 8-10 minutes of exercise. Breath-by-breath measurements (minute ventilation, $\dot{V}_E$; oxygen consumption, VO_2; carbon dioxide production, VCO_2; and end-tidal partial pressure of O_2 and CO_2, $P_{ET}O_2$ and $P_{ET}CO_2$ respectively) were collected through a mouthpiece connected to the Vmax metabolic cart (CareFusion, Yorba Linda, CA, USA). Subjects were monitored by 12-lead ECG (Cardiosoft; SensorMedics, Yorba Linda, CA). Blood pressure and estimated arterial saturation (SpO_2) (N-595; Nellcor Oximax, Boulder, CO) were also monitored continuously during exercise. The peak oxygen consumption value was recorded as VO_{2peak}. Maximal patient exertion was confirmed using the following criteria: 1) a plateau in VO_2 despite increasing workload; 2) a respiratory exchange ratio ≥ 1.1; 3) a heart rate $\geq 90\%$ of predicted maximum; 4) a score of $\geq 9/10$ on the Modified Borg scale; or 5) evidence of a respiratory limitation to exercise.[8]

B.2.2. Testing Days Two and Three: Chemoreceptor, Vascular, and Cardiorespiratory Evaluation

B.2.2.1. Resting Dyspnea

Individuals completed the Modified Medical Research Council (MMRC) dyspnea scale to assess resting dyspnea. The MMRC dyspnea scale consists of five statements (scored 0-4) which best explain an individual's current perceived breathlessness; 0 represents no dyspnea and 4 indicates nearly complete incapacity as a result of dyspnea. A score ≥ 2 indicates patients with more symptoms (ie. some activity limitations due to breathlessness during daily life).[9] This

questionnaire has been shown to be a reliable and effective measure of dyspnea in patients with COPD, and dyspnea ratings from this questionnaire have been shown to be associated with COPD severity and mortality.[10] A copy of this questionnaire can be found in *Appendix C*, Figure C-1.

B.2.2.2. Health-Related Quality of Life

To assess health-related quality of life, subjects completed the St. George's Respiratory Questionnaire (SGRQ). The SGRQ contains three sections: Symptoms (items concerned with patient symptomatology); Activity (items regarding physical activities that cause or are limited by dyspnea); and Impacts (the impact of disease on the patient, including employment and activities of daily living).[11] Each section is scored individually, ranging from 0-100%, and the entire questionnaire is scored 0-100%, with 0% indicating no impairment in quality of life, and 100% indicating complete impairment. This questionnaire is a valid measure of impaired health in COPD, and is highly repeatable and sensitive.[11] Additionally the SGRQ has been shown to be strongly correlated with the MMRC ($R^2 = 0.50$).[11]

B.2.2.3. Carotid Chemoreceptor, Vascular, and Cardiorespiratory Evaluation

A schematic of the chemoreceptor, vascular, and cardiorespiratory evaluation can be found in *Appendix C*, Figure C-2.

B.2.2.3.1. Arterial Stiffness

As a secondary outcome, peripheral pulse wave velocity (PWV) was assessed in all subjects. Pulse wave velocity is a marker of arterial stiffness, and a non-invasive assessment of CV risk; aortic PWV has been shown to independently predict CV events and mortality.[12] While not shown to be predictive of CV risk in health and disease, peripheral PWV may also be used as a marker of CV risk. The peripheral vasculature is more muscular than the central, elastic arteries, and therefore peripheral PWV may be a more effective indicator of sympathetic control on

vascular tone than central PWV. The effect of sympathetic control on the peripheral vasculature has been indicated by a reduction in radial artery compliance under physiological pressure and flow conditions in healthy subjects during the cold pressor test.[13]

B.2.2.3.2. Autonomic Function

When assessing HRV, two variables were examined: 1) the standard deviation of the time interval between consecutive sinus heart beats (SDNN), which reflects all the cyclic components responsible for variability in the period of recording;[14] and 2) the square root of the mean of the sum of squares of differences between time intervals of adjacent sinus heart beats (RMSSD), which estimates high frequency variations in heart rate.[15] These methods for assessing HRV have been recommended by the Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology in 1996 as valid measures of HRV.[14] While the ratio of low frequency to high frequency power was originally indicated as a measure of sympathetic control, recent research suggests that low frequency power is not an index of cardiac sympathetic tone;[16] therefore this ratio was not examined when assessing HRV in the present study.

B.2.2.3.3. Carotid Chemoreceptor Activity

Basal CC activity was assessed by examining the reduction in resting minute ventilation ($\Delta\dot{V}_E$) in response to transient hyperoxia; this technique is widely accepted in the literature as an effective measure of resting CC activity.[17, 18] Transient hyperoxia was used instead of prolonged hyperoxia, as five minutes of 100% O_2 has been shown to significantly increase $\dot{V}_E$ from a normoxic baseline.[19] Prolonged hyperoxia results in the production of reactive oxygen species, and it is speculated that the hyperventilatory response to prolonged hyperoxia is a result of the brainstem's sensitivity to reactive oxygen species.[20] Chemoreceptor activity was quantified by measuring the difference between the baseline $\dot{V}_E$ and the lowest 10-second average $\dot{V}_E$ (nadir)

achieved during hyperoxia, with a larger $\Delta\dot{V}_E$ indicating greater basal CC activity. In normoxic conditions, transient hyperoxia has been shown to reduce $\dot{V}_E$ by 10% after a delay of 10 seconds.[18] Additionally, following the administration of 100% O_2 for one minute, Sladek *et al.* (1993)[21] calculated $\Delta\dot{V}_E$ using 10-second averages of $\dot{V}_E$. For these reasons, a 10-second average was used when assessing the reduction in $\dot{V}_E$ with transient hyperoxia; furthermore, longer time averages may under-estimate the potential reduction in $\dot{V}_E$ with hyperoxia.

Mean inspiratory flow (V_T/T_I) was measured prior to and during the administration of hyperoxia as an indicator of inspiratory drive. As V_T/T_I has been suggested to reflect central neural respiratory drive,[22] it was suspected that this may be related to changes in CC activity; if the change in V_T/T_I from normoxia to hyperoxia was reduced following PR compared to baseline, then this may suggest an attenuation in CC activity. Analysis of V_T/T_I was performed in LabChart 7.0, using a custom macro which measured the ratio of tidal volume (V_T) and inspiratory time (T_I) during two minutes of normoxic baseline and the following one minute of hyperoxia.

B.2.2.3.4. Carotid Chemoreceptor Sensitivity

Hypoxia is a potent stimulant of the CC, and is commonly used to evaluate CC sensitivity.[23, 24] Following 10 minutes of recovery from the hyperoxia trial, and five minutes of breathing normoxic gas through the breathing apparatus, nitrogen was blended into the inspired gas mixture to cause step reductions in SpO_2 to 90% and 85% for three minutes each; this protocol is a modification of previous techniques used to assess the acute hypoxic ventilatory response.[25] For analysis, the three minutes of SpO_2 90% and 85% were divided into 12 15-second averages, and the greatest consecutive 30-second average $\dot{V}_E$ at the SpO_2 of interest was reported. Previous work by Howard and Robbins (1994)[26] used the final 30-second average $\dot{V}_E$ from two minute stages of incremental step hypoxia. As the present study utilized three minute stages, and the

ventilatory response to hypoxia can be biphasic, the maximal 30-second average $\dot{V}_E$ was used instead of the final 30-second average $\dot{V}_E$. The 30-second average $\dot{V}_E$ at SpO_2 90% and 85%, as well as the two minute average $\dot{V}_E$ prior to the administration of hypoxia was plotted and a regression line relating the increase in $\dot{V}_E$ to the corresponding reduction in SpO_2 was obtained. The individual slope of this regression line ($\Delta\dot{V}_E/\Delta SpO_2$ slope) was used to evaluate CC sensitivity for each participant, with a steeper slope indicating a greater CC sensitivity. The hypoxic trial was performed following hyperoxia so as to eliminate any hypoxia-induced sensitization of the CC.

While the ventilatory response to transient hypoxia has been shown to predict mortality,[27] COPD patients may be flow limited, and transient hypoxia can result in large increases in ventilation, especially as the number of breaths of pure nitrogen increases. This may encroach on the individual's breathing reserve, and as a result the ventilatory response to hypoxia may be blunted secondary to the flow limitation; transient hypoxia may therefore not accurately determine the degree of chemosensitivity in individuals with COPD. As a secondary concern, transient hypoxia consists of 2-8 breaths of pure nitrogen, and therefore does not allow for the ventilatory response to fully develop.[28] When performing the transient hypoxia maneuver, the subject does not achieve a steady state ventilation (defined as when the metabolic production of CO_2 is matched by the CO_2 expired) as the hypoxic stimulus is only present for 2-8 breaths. Additionally, the data is more susceptible to spontaneous variations in ventilation as only a few breaths are measured, making the data more difficult to confidently interpret. For these reasons, the present study utilized the incremental step hypoxia technique to assess CC sensitivity in COPD.

B.3. Statistical Analyses

B.3.1. Sample Size Calculation

No previous research has shown a quantifiable change in CC activity or sensitivity as a result of exercise training or rehabilitation. Therefore, prediction of an expected clinically or statistically significant change in these variables is difficult. Based on pilot data examining the change in CC activity for n=6 participants completing PR and n=6 controls, a two way repeated measures ANOVA yielded η^2=0.057 which corresponded to an effect size of 0.25. The correlation between repeated measures was r=0.91, however, a more conservative correlation of r=0.7 was used for calculating estimated sample size. Using α=0.05 and a power of 0.80, a total sample size of 22 subjects was calculated (11 experimental, 11 controls). Typically a ~25% drop-out rate is observed with PR, thus it was required that at least an additional 6 subjects were recruited to account for this attrition rate. Therefore for the present study, a minimum of 14 experimental COPD patients undergoing PR and 14 non-rehabilitation COPD controls were required for recruitment. Similarly, these calculations were completed using CC sensitivity as the variable of interest. With η^2=0.102, an effect size of 0.34, and a conservative r=0.7, it was determined that n=7 experimental and n=7 controls would be necessary. Therefore for the present study, we aimed to recruit n=14 COPD patients enrolled in PR and n=14 COPD controls, with a minimum of n=11 subjects analyzed in each group. Upon study completion, data was collected and analyzed on n=45 COPD patients completing PR and n=15 COPD controls.

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APPENDIX C: SUPPLEMENTAL FIGURES

Scale	
mMRC Grade 0	I only get breathless with strenuous exercise.
mMRC Grade 1	I get short of breath when hurrying on the level or walking up a slight hill.
mMRC Grade 2	I walk slower than people of the same age on the level because of breathlessness, or I have to stop for breath when walking on my own pace on the level.
mMRC Grade 3	I stop for breath after walking about 100 meters or after a few minutes on the level.
mMRC Grade 4	I am too breathless to leave the house or I am breathless when dressing or undressing.

Figure C-1. The modified medical research council dyspnea questionnaire.

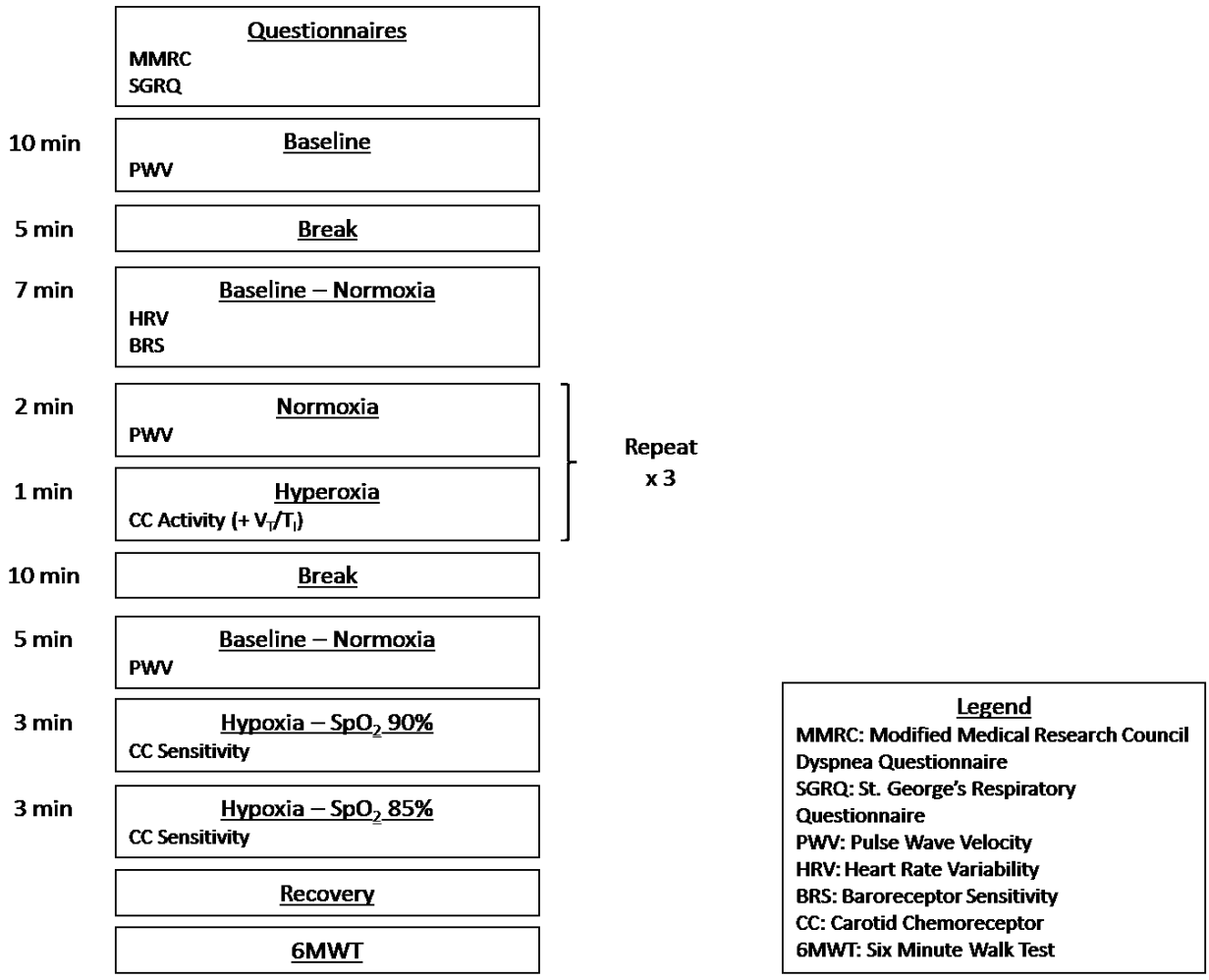

Figure C-2. Chemoreceptor and cardiorespiratory assessment outline schematic (Day 2 and 3).

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