THE EFFECTS OF AEROBIC EXERCISE TRAINING ON PERIPHERAL VASCULAR STRUCTURE AND FUNCTION AND INFLAMMATION IN PATIENTS WITH CHRONIC OBSTRUTIVE PULMONARY DISEASE

by

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Abstract

Background: COPD is associated with chronic systemic inflammation that has been linked to an increased risk of atherosclerosis, ischemic heart disease, and stroke. Endothelial dysfunction, vascular remodeling and arterial stiffness are early processes in the pathogenesis of atherosclerosis and are predictive of future cardiovascular events. Aerobic exercise training can reduce the risk of cardiovascular disease in patients with other chronic conditions. However, little is known about the benefits of exercise for improving vascular health in patients with COPD.

Methods: Ten non-smoking patients with COPD (mean age=69±9yr, FEV1%pred=67±3%) and six healthy controls matched for age, sex, BMI and activity level underwent pulmonary function and cardiopulmonary exercise testing. Endothelial function was assessed by flow-mediated dilation (FMD) in response to reactive hyperemia in the brachial artery, and carotid artery intima-medial thickness (IMT) was measured by ultrasound. Central and peripheral pulse wave velocity (PWV), carotid compliance and beta stiffness index were determined by applanation tonometry and ultrasound. A venous blood sample was collected to measure systemic inflammation. Following completion of baseline testing, aerobic exercise was performed on lower and upper extremity cycle ergometers, 3x/week for 8 weeks. All baseline measurements were repeated upon completion of the exercise training.

Results: An improvement in peak O_2-consumption (VO_{2peak}, 18.0±4.6 to 19.8±3.6 ml/kg/min, $p<0.01$) and peripheral PWV (7.6±2.5 to 6.8±1.8 m/sec, $p=0.03$) occurred with training in patients with COPD. However, there was no significant change in measures of FMD, central arterial stiffness, carotid IMT or biomarkers of systemic

inflammation, (p>0.05). There was also an improvement in VO_{2peak} (19.8±2.6 to 26.0±4.1 ml/kg/min, p=0.03) in controls, with no other significant changes following training.

Conclusion: This pilot study demonstrated that 8-weeks of aerobic training improved peak aerobic power and peripheral arterial stiffness but had little effect on other established markers of cardiovascular disease risk in patients with COPD. These findings suggest that a typical 8-week pulmonary rehabilitation program may not be long enough to greatly improve vascular function or structure or reduce the systemic inflammation associated with increased cardiovascular disease risk in patients with COPD. Larger randomized, controlled studies are now needed to confirm these preliminary findings.

Preface

Chapters 2 is based on work conducted at the University of British Columbia (Okanagan campus) and at the Kelowna General Hospital by Ms. Jinelle Gelinas, Dr. Neil Eves, Dr. Nia Lewis, and Dr. Douglass Rolf. The study idea was conceived by Ms. Gelinas and Dr. Neil Eves. Ms. Gelinas was responsible for recruitment of all study participants, conducting all testing sessions and exercise training sessions, data analysis and interpretation, and writing and editing of the thesis. Dr. Neil Eves was responsible for overseeing all aspects of the study, he supervised exercise testing and contributed to data interpretation and editing of the thesis. Dr. Nia Lewis assisted with vascular data collection and analysis. Dr. Douglass Rolf assisted with patient recruitment and was responsible for ensuring the clinical management of patients during the study. Ethics approval was obtained from both the Interior Health Ethics Board and the University of British Columbia (CREB number H11-02770). Certificates obtained are presented in Appendix B.

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List of Abbreviations

COPD	Chronic obstructive pulmonary disease
A1AT	Alpha 1 -antitrypsin
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
TLC	Total lung capacity
FRC	Functional residual capacity
RV	Residual volume
CD8+	Cytotoxic T cell
LTB_4	Leukotriene B_4
PGE_2	Prostaglandin E_2
IL-6	Interleukin-6
IL-8	Interleukin-8
TNF-α	Tumor necrosis factor- alpha
IL-10	Interleukin-10
IL-1	Interleukin-1
TGF-B	Transforming growth factor-B
ICAM-1	Intracellular adhesion molecule-1
VCAM-1	Vascular cell adhesion molecule-1
CRP	C-reactive protein
LDL	Low density lipoproteins
eNOS	Endothelial derived nitric oxide synthase
NO	Nitric oxide
ET-1	Endothelin-1
PWV	Pulse wave velocity
AIx	Augmentation index
Ca^{2+}	Calcium ion
VSMC	Vascular smooth muscle cells
EDHF	Endothelial derived hyperpolarizing factor
ATP	Adenosine tri-phosphate
GTN	Glycerol trinitrate
FMD	Flow mediated dilation
IMT	Intima-media thickness
AGE	Advanced glycation end products
RAGE	Advanced glycation end product receptor
ROS	Reactive oxygen species
O^{2-}	Superoxide anion
H_2O_2	Hydrogen peroxide
BH_4	Tetrahydrobiopterin

ACh   Acetylcholine
HRT   Hormone replacement therapy
L-NMMA N^G-monomethyl-L-arginine
VO_2 Maximal oxygen consumption
CT   Computer tomography

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that I am truly grateful.

Chapter 1: Review of Literature

1.1 The Prevalence and Burden of COPD

Chronic obstructive pulmonary disease (COPD) is a progressive yet treatable respiratory disease. It is characterized by partially reversible, expiratory airflow limitation and increased levels of inflammation both within the lungs and the systemic circulation.[1,2] Cigarette smoke is the primary cause of COPD, however long term exposure to particulate matter, such as dust, air pollution, pulmonary irritants, and chemical fumes are also contributable causes.[1]

The term COPD is a global term which encompasses the combination of chronic and obstructive bronchitis, characterized by hypresectretion of mucous and narrowing or closure of small airways, and emphysema, defined as destruction of the lung parenchyma and an enlargement of alveolar spaces.[2] Patients with COPD present with a combination of these conditions, however the extent to which each pathology contributes to the disease state will vary considerably between individuals. The ultimate consequence of COPD is increased airway resistance and expiratory airflow limitation due to 1) a loss of lung elasticity resulting in an increase in lung compliance and/or 2) increased airway inflammation and bronchial mucous hypersecretion.

COPD is an increasing global health concern and is expected to become an increasing burden to the rising costs of Canadian healthcare.[3] According to recent statistics, 8.2% of Canadian adults ≥40 years old have COPD.[4] This is accompanied by an estimated combined direct and indirect cost of ~$4.1 billion on the Canadian healthcare system.[3,4] Since the progression of airflow obstruction associated with COPD is slow and may go undiagnosed for many years, the prevalence of COPD in Canadians

may be vastly underestimated miscalculating the predicted economic burden. It has been projected that by 2020 COPD will become the 5th most prevalent disease [5,6] and by 2030 will become the 4th leading cause of mortality. [7] These statistics clearly illustrate that COPD will be of critical concern for at least the next 20 years and likely considerably longer. The systemic inflammation associated with COPD has been linked to a number of secondary cardiovascular co-morbidities such as atherosclerosis, ischemic heart disease and stroke. [8,9] In fact, the risk of dying from a cardiovascular or cerebrovascular incidence it three to five times higher in COPD patients compared to age and sex matched controls, [10,11] thus cardiovascular co-morbidities potentiate the morbidity of COPD leading to increased mortality and healthcare costs.

Reasons for this progressive increase in the prevalence of COPD include increased tobacco use and the exposure to smoke from the combustion of biofuels and coal for heating and cooking in poorly ventilated areas, [12,13] as well as the rising age of the baby boomer generation who commonly present with a history of smoking. Knowledge and understanding of the physiological parameters associated with COPD is critical in developing long-term treatments and prevention options to reduced the projected burden of COPD as well as its management in the future population.

1.2 Definition and Diagnosis of COPD

Although long-term exposure to particulate matter, primarily cigarette smoke, is the primary cause for the development of COPD genetics also play an imperative role. COPD results from a gene-environment interaction, as not all patients who have the same smoking history will develop COPD. [14] Risk factors such as occupational role, socioeconomic status, life expectancy, and lifestyle, coupled with a genetic predisposition

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increase the risk of developing COPD. The exact roles that genetics and environmental risk factors play in COPD initiation and prevalence have yet to be elucidated.

Emphysema is characterized by the loss of alveolar spaces resulting from destruction of the surrounding capillary beds and lung parenchyma. In addition, small airways and to a lesser extent, larger airways may be narrowed due to thinned and atrophied walls. This results in a reduced number of airway generations, compromised airway patency, and decreased elastic recoil of the lungs. Emphysema may present in different patterns ranging from centriacinar (destruction limited to the central part of the lobule, while peripheral alveolar ducts and alveoli may remain intact), to panacinar (distension and destruction to the whole lobule). In cases of severe emphysema it may be difficult to distinguish varying types of emphysema, which may coexist in one lung.

Patients who have an alpha 1-antitrypsin (A1AT) deficiency will frequently develop severe panacinar emphysema often without a cough or smoking history, as a result of reduced inhibition of neutrophil elastase production.[15,16] Less than 2% of all reported cases of COPD occur due to an A1AT deficiency[16] and replacement therapies are now available for those patients.

Chronic bronchitis is characterized by excessive mucous production, sufficient to cause an increase in sputum production for at least 3 months in two consecutive years.[17] Hypertrophy of mucous glands in the large bronchi leads to excessive mucous production in the airways and occlusion of the smaller bronchi. In addition, chronic inflammation of the small airways leads to narrowing of the airway lumen as a result of cellular infiltration and edema of the walls. The presence of granulation tissue may form and hypertrophy of bronchial smooth muscle may also reduce airway lumen size.

Patients with COPD exhibit expiratory airflow limitation and increased lung volumes, which may be evaluated though spirometry and body plethysmography. According to the Canadian Thoracic Society, [18] the classifications of obstruction severity are based on the percent predicted forced expiratory volume in one second (FEV_1) values only determined if the post-bronchodilator FEV_1/ forced vital capacity (FVC) <0.7. Total lung capacity (TLC), functional residual capacity (FRC), residual volume (RV) and expiration time, are typically increased in COPD patients. Excessive mucous in the lumen and thickening of the airway wall caused by inflammatory changes, leads to a reduced airway lumen size and airflow. The reduced elastic recoil of the lungs as a loss of elastic tissue, alveolar/parenchyma destruction, and loss of radial traction increases airway resistance. This affects the smaller elastic recoil pressure of the lung whereby reducing the driving pressure of forced expiration, and allowing the airways to collapse sooner during expiration. As a consequence, FVC is reduced due to premature airway closure during expiration resulting in an abnormally high residual lung volume. An RV and FRC of >140% of predicted is indicative of gas trapping and static hyperinflation repetitively within the lungs as is found in COPD. [17]

1.3 Pathogenesis of COPD

The pathogenesis of COPD has been largely studied. A number of studies have linked the structural changes resulting from repeated injury and repair that occur as a consequence of COPD to abnormal and chronic inflammation in the airways, alveoli and pulmonary vasculature. [19,20] These inflammatory changes occur in response to inhaled particulate matter and are associated with the severity of airflow obstruction, exacerbation frequency and smoking history. [13,21,22] A normal inflammatory response occurs in all smokers as a

consequence of cigarette smoke, however there is an enhanced and adaptive immune response in the lungs of smokers who develop COPD. In smokers with a susceptible lung, airway inflammation continues even after smoking cessation, sometimes for many years before COPD develops. [23,24] COPD is unique compared to other chronic conditions as the inflammatory condition continues despite the removal of the stimulus (cigarette smoke). The mechanisms responsible for this enhanced immune response remains to be unknown, however oxidants present in cigarette smoke cause infiltration of macrophages, neutrophils and CD8+ T-cells, which release pro-inflammatory mediators into the lungs and initiate the inflammatory cascade in an effort to continue to repair the damage within the airways caused by cigarette smoke. [25-27]

The inflammatory response provoked within the airways and lungs is largely facilitated by alveolar macrophages. In patients with COPD there is a marked increase in alveolar macrophage number and activity in response to cigarette smoke within the lungs when compared to normal smokers, which has also been correlated to disease severity. [27,28] This increased number of macrophages has been attributed to the increased proliferation of monocytes from the circulation in response to monocyte-selective chemokine production from epithelial cells cause by smoke extract. [26]

Alveolar macrophages contribute to the inflammatory response through the signaling and secretion of inflammatory mediators. Lipid mediators such as leukotriene's (primarily LTB_4) and prostaglandins (PGE_2) are generated by macrophages, and enhance the recruitment of neutrophils to areas of damage in the lungs, aid in the release of proteases, which assist the breakdown of tissues as well as the development of fibrosis. [29,30] Chemokines and cytokines, such as interleukin-6 & 8 (IL-6, IL-8) and tumor necrosis

factor- alpha (TNF-α) are also secreted by macrophages and in turn stimulate the surround cells to produce and release pro-inflammatory factors sustaining the inflammatory process. [22,31] Induced sputum samples and bronchoalveolar lavage samples from patients with stable COPD show increased baseline levels of IL-6, IL-8 and TNF-α compared to normal smokers and non-smokers. [31,32] In addition, TNF-α appears to be a key mediator in the infiltration of neutrophils into the lungs and the subsequent release of elastase, which is an important mediator of tissue damage and airway remodeling. [33] From an anti-inflammatory perspective, concentrations of inhibitory cytokines such as IL-10, IL-1 and transforming growth factor (TGF)-B are also released from alveolar macrophages. Baseline levels of IL-10, a potent anti-inflammatory mediator, were shown to be reduced in the sputum of patients with asthma and COPD, [34] however more research is needed on the specific roles theses anti-inflammatory mediators play in offsetting the inflammatory state present in COPD.

1.3.1 From Lungs to Systemic Inflammation

Patients with COPD have higher circulating levels of pro-inflammatory markers compared to healthy individuals or ex-smokers without COPD. [35,36] The exact mechanisms linking chronic lung inflammation to systemic inflammation are not well understood. However, it is thought that there is a 'spill-over' effect of inflammatory mediators from the lungs into the systemic circulation, causing systemic inflammation. Animal studies have shown that lung inflammation induced by ambient air pollution particles stimulates leukocyte and platelet production from the bone marrow, and is correlated to the amount of particles phagocytosed by alveolar macrophages with in the lungs. [37,38] The proliferation and release of leukocytes and monocytes from the bone

marrow is further enhanced by increased concentrations of IL-6, IL-8 and TNF- α from alveolar macrophages. [39]

An interesting relationship exists between systemic inflammation caused by pollutant particles in the lungs and the progression of atherosclerosis. A brisk systemic inflammatory response has been associated with the progression of atherosclerosis when Watanabe Heritable Hyperlipidemic rabbits were exposed to ambient air particles. [40] This progression was directly proportional to the concentration of alveolar macrophages that contained the particulate matter. [40] It was also found that up-regulation of intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) which are important for the recruitment of leukocytes into the atherosclerotic plaques, occurred on the endothelium overlying the plaques. [41] Together, these studies suggest that inflammation occurring in the lungs resulting from air pollutants can induce a state of systemic inflammation which could further the progression of existing diseases, such as atherosclerosis. These studies provided insight into the possible link between inflammation within the lungs of COPD patients and the high risk of cardiovascular disease in this population.

1.4 Cardiovascular Disease and COPD

Cardiovascular disease is the leading cause of mortality and the primary reason for hospitalization in COPD. [42] In a longitudinal healthcare data base study in which over 11,000 COPD patients were followed for three years, a three to fourfold increase in mortality from cardiovascular disease was observed. [43] In The Lung Health Study a 10% reduction in lung function was associated with a ~30% increase in the risk of death from cardiovascular disease in patients with COPD. [44] Moreover, population studies have

demonstrated that COPD patients are at an increased risk of congestive heart failure, acute myocardial infarction, arrhythmia, and stroke compared to age and sex-matched control. [10] These and other studies have shown that reduced FEV_1, independent of established cardiovascular risk factors such as cigarette smoke, total cholesterol, and hypertension, is an important factor in predicting cardiovascular mortality. [44-47] Collectively these finding suggests that a reduction in lung function is a risk factor for the development of cardiovascular disease and places COPD patients at the high risk of having a cardiovascular event.

1.4.1 Systemic Inflammation and Atherosclerosis

Atherosclerosis is the hallmark of cardiovascular disease. The pathogenesis of atherosclerosis is complex and multifactorial in nature. Increased levels of systemic inflammation, which have been linked to ischemic heart disease, stroke and myocardial infarction [8,48] are pivotal to plaque formation and the progression of atherosclerosis. Atherosclerosis starts with injury to the vascular endothelium, in which circulating inflammatory mediators (such as C-reactive protein (CRP), IL-6 & IL-8) and reactive oxidative species (ROS) increase the permeability of the vascular wall and the expression of surface adhesion molecules. [49] Low density lipoproteins (LDL), and leukocytes infiltrate the vascular wall through the help of monocytes, macrophages and vascular smooth muscle cells and promote the development of foam cells and the formation of fibro-fatty lesions. [49] This results in vessel wall fibrosis and calcification, producing a plaque with a fibrous cap and lipid-rich core.

There is growing evidence to support a causal role of specific cytokines, which are elevated in COPD in the initiation, development and rupture of atherosclerotic

plaques. C-reactive protein is an acute phase protein and a recognized biomarker of increased cardiovascular risk and mortality in the general population and in COPD.[50,51] Circulating levels of CRP are increased in patients with COPD and continue to increase with disease severity.[52] A CRP level greater than 3mg/L, is related to increased hospitalization and mortality outcomes in individuals with COPD, even when adjusted for age, sex, FEV_1% predicted, tobacco consumption, and ischemic heart disease.[53] CRP has also been found to promote the uptake of LDLs by macrophages, which contribute to the formation of fibrous plaques.[54] Studies have also found that CRP inhibits the expression of endothelial derived nitric oxide synthase (eNOS), the enzyme responsible for nitric oxide (NO) production (a powerful vasodilator), while stimulating the production of the vasoconstrictor, endothelin-1 (ET-1).[9,54]

IL-6, IL-8 and TNF- α, which stimulate the production of CRP following vascular damage, have been implicated in plaque formation and thus the pathogenesis of atherosclerosis.[49,54] These cytokines play a role by increasing chemokine expression, adhesion molecules, neutrophil, platelets and fibrinogen concentrations thus creating a pro-coagulant state on the vascular endothelium.[49] Il-6, in particular has been shown to reduce NO release from the vasculature, limiting the bioavailability of NO and resulting in a reduction in endothelial dependent vasodilation.[55] There are very few reports that have shown that increased levels of proinflammatory cytokines in COPD are counterbalanced by the upregulation of anti-inflammatory mediators such as IL-10, and therefore the role of anti-inflammaory mediators in the develpoment of artherosclerosis in COPD remains unclear.

COPD is an inflammatory disease characterized by chronic systemic inflammation. Many of the inflammatory mediators which are chronically elevated in COPD have been demonstrated to be involved in the development of atherosclerosis. Vascular dysfunction and increased arterial stiffess occur in the early atherosclerosis process as a consequence of vascular injury and repair due to systemic inflammation and are predictive of future cardiovascular events. [56,57] Patients with COPD are at a greater risk of developing vascular dysfunction and increased arterial stiffness as a consequence of elevated levels of systemic inflammation. It is therefore imperative to study the associations between systemic inflammation and the vasculature as a potential mechanism for increased cardiovascular disease in this population.

1.5 Healthy Vascular Function

1.5.1 Structure and Function of Arteries

Arteries are composed of three distinct layers. The luminal surface or tunica intima consists of a single layer of specialized endothelial cells. The endothelial layer is important in the modulation of vascular tone through the release of vasoactive agents in response to the mechanical or parallel force of blood flow against the luminal wall. [58] The tunic media, or middle layer, contains variable amounts of elastin lamellae with imbedded collagen fibers and smooth muscle cells circumferentially arranged which act as an elastic reservoir to distribute stress evenly throughout the vessel wall to lessen tensional forces. [59] The longitudinal, smooth muscle layer controls the distribution of blood flow via vasoconstriction or vasodilation initiated by vasoactive agents and neural modulation. The outer most layer, the tunic adventitia, consists of a dense collagenous

extracellular matrix, which aids in the prevention of vascular injury resulting from extreme high pressures.

1.5.2 Arterial Stiffness

Arterial stiffness, referring to the rigidity of the arterial wall has become increasingly used in the clinical assessment for predicting prognosis and the risk of cardiovascular events as it plays a primary role in numerous diseases such as atherosclerosis, left ventricular hypertrophy and failure, myocardial infarction and stroke. [60-62] Arterial stiffening is heterogeneous and non-uniform throughout the arterial tree. [63] Large-conduit arteries contain a high ratio of elastic to collagen properties making them highly distensible. This allows arteries such as the aorta and the common carotid artery to act as a buffering system to pulsatile flow by preventing large swings in intra-arterial pressure during the cardiac cycle. This ability to compensate changes in volume for a given change in pressure is termed compliance, and is inversely related to stiffness. Alternatively, peripheral arteries are more muscular and less elastic and are highly modulated by local and regional vasoactive factors, the vascular endothelium and the sympathetic nervous system making them more resistive and less distensible. [63]

As a pulse travels down the arterial tree it becomes amplified. The propagation of the pulse wave from more elastic proximal arteries to stiffer distal arteries and the branching and bifurcations within the vascular tree, produce sites where the pressure wave can be reflected. [64] These reflections return in the opposite direction and amplify the forward pressure signal. This is knows as the amplification phenomenon, which is demonstrated by a lower pulse wave velocity (PWV) in the aorta, which gradually increases as it travels distally through the peripheral arteries. [65] Increased aortic or central

arterial stiffness as determined by PWV, is a predictor of cardiovascular events as a 1m/s increase is associated with a 15% increased risk of cardiovascular and all-cause mortality. [66]

Pulse pressure, the difference between systolic and diastolic pressures (SBP-DBP), is used as a clinical index of the pulsatile load on the aorta during systole. Conceptually, pulse pressure is proportional to stroke volume, and inversely related to the arterial compliance of the aorta. Pulse pressure is used as a predictor of future cardiovascular events, as a 10mmHg increase in pulse pressure is associated with a 20% increased risk of cardiovascular morbidity and mortality. [67,68] In healthy aging and in a variety of disease processes, arteries stiffen due to structural degeneration and may result in minimization of the stiffness gradient from central to peripheral arteries and a subsequent rise in pulse pressure, leading to an increase in cardiovascular risk. [65,69]

1.5.2.1 Measuring Arterial Stiffness

Regional arterial stiffness is determined by PWV, by capturing pressure waveforms by placing transducers on the carotid, radial and femoral arteries. Velocity is estimated by dividing the distance travelled between transducers by the time it takes for the pulse wave to travel between each site. The velocity of the pulse is related to the stiffness of an arterial segment between measurement sites, and thus the greater arterial stiffness the faster the PWV. Central PWV, which is calculated by using the carotid and femoral arteries simultaneously, is more strongly correlated with cardiovascular outcomes than peripheral PWV, which uses the carotid and radial measurements. A central PWV ≥ 11.8 m/sec, is associated with a 48% increased risk of first major cardiovascular disease event including myocardial infarction, unstable angina, heart failure and/ or stroke. [70]

The augmentation index (AIx) is an indirect global measure of arterial stiffness and is defined as the difference between the second (reflected wave) and first (forward wave) systolic peaks of the arterial waveform expressed as a percentage of pulse pressure.[65] The AIx has shown independent predictive value for coronary artery disease,[71] however it does not dissociate between varying regions of stiffness throughout the arterial tree and is dependent upon PWV, height, heart rate, and changes in peripheral vascular smooth muscle cell tone.[65] Therefore, AIx can be misleading as stiffening of the central arteries such as that which occurs with aging, cannot be dissociated from peripheral arterial stiffening.

Local arterial stiffness or carotid compliance, is determined as the absolute change in diameter for a given change in pressure[65] and is obtained by concurrent measurements of carotid PWV and carotid diameter via ultrasound. Carotid distensibility takes into account the initial dimensions of the artery and is considered the relative change for a given pressure.[65] In turn, an equation that provides an index of arterial compliance independent of distending pressures is the β stiffness index, which has been shown to increase with age and degree of vessel disease in myocardial infarction.[72]

The risk of cardiovascular disease morbidity and mortality is greatly increased in patients with COPD. Increases in arterial stiffness precede atherosclerosis and are predictive of future cardiovascular events. Further exploring the link between arterial stiffness and COPD will be important in understanding the mechanisms linking COPD and cardiovascular disease and exploring means to reduce the cardiovascular risk in this population.

1.5.3 Endothelial Function

The vascular endothelium is a single layer of cells that serves as a complex and integrative interface between the tunica intima and laminar blood flow. The ability of the vasculature to dilate is important in the regulation of blood pressure and protection against vascular injury. The parallel force of blood flow against the vessel wall, known as shear stress, is an important mechanism of vascular vasodilation. Vasodilation in response to increased shear stress is modulated through mechanotransduction and the initiation of signaling cascades established by the cytoskeleton and other structural components of the endothelium. [73] Displacement of glycoproteins within the extracellular matrix initiates downstream intracellular signaling; causing calcium channels to open allowing an influx of calcium ions (Ca^{2+}) into the endothelial cells. [73] This causes subsequent activation of the enzyme, endothelial nitric oxide synthase (eNOS) and the conversion of the amino acid, L-arginine into nitric oxide (NO). [74] Nitric oxide diffuses across the extracellular space, and activates the soluble form of guanylyl cyclase, causing voltage gated calcium channels to open on the surface of vascular smooth muscle cells (VSMC) of the tunic media. [74] The intracellular calcium content is lowered via the efflux of Ca^{2+} out of the VSMC and the result is smooth muscle relaxation. [74] In addition to NO, the endothelium also releases other mediators of vasodilation such as prostaglandins, endothelial derived hyperpolarizing factor (EDHF) and adenosine tri-phosphate (ATP) and mediators of vasoconstriction such as ET-1, and thromboxane. [75]

Nitric oxide is one of the most studied endothelial molecules as it is a potent vasodilator and posessess many anti-atherogenic properties. NO has been shown to inhibit activation of adhesion molecules and cytokines related to the inflammatory

process, prevent platelet aggregation and proliferation of VSMC, and inhibit ET-1 production. [76,77] Insufficient NO bioavailability reduces the anti-inflammatory and antithrombotic effects of the endothelium increasing the likelihood of vascular injury and the development of atherosclerosis. [76, 77]

1.5.3.1 Measuring Endothelial Function

The gold standard assessment of endothelial function is venous occlusion plethysmography, which employees the use of cuffs and strain gauges positioned around the forearms and wrists in order to occlude blood flow to the hands and prevent venous return back to the heart while allowing arterial inflow. Changes in forearm volume results in corresponding changes in strain gauge length from which total forearm blood flow can be calculated. [78] This highly reproducible and accurate technique permits the detailed study of infused receptor agonist and antagonist agents as well as the administration of subsystemic doses of intra-arterial drugs. [78]

As technologies improved, less invasive and costly techniques to assess endothelial function in the upper extremities have become more available. Ultrasonography allows a simultaneous measurement of vessel diameter, blood flow velocity and calculation of shear stress, all of which are important factors in determining arterial vasodilatory response as a measure of endothelial function. [79] Flow mediated dilation (FMD) or endothelium-dependent dilation is performed by inflating a cuff around the forearm to supra-systolic pressures, whereby inducing an ischemic response in the forearm. Ultrasound is used to image a section of a peripheral artery (mainly the brachial artery) proximal to the cuff. This initiates a reactive hyperemic response upon cuff release in which shear stress targeting the endothelium is increased. [79] The change in

vessel diameter relative to shear stress is calculated pre and post occlusion, and is thought to be primarily NO mediated when the cuff is placed distally to the ultrasound probe. [80]

The assessment of endothelium- independent dilation is performed using sublingual administration of glycerol trinitrate (GTN) as it acts as an NO donor and therefore targets the VSMCs directly to initiate vasodilation. Arterial blood flow velocity, and diameter are measured pre and post administration. [79] When used along side with endothelial-dependent dilation, the endothelium's full capacity to release vasodilatory factors and the smooth muscles ability to respond and cause vasodilation can be determined.

Improvements in vascular function are associated with a reduction in cardiovascular morbidity and mortality. A 1% increase in FMD has been shown to correlated with a 13% reduction in cardiovascular risk. [81] Since the risk of cardiovascular morbidity and mortality is increased in COPD, understanding the association between COPD and FMD as well as methods to improve vascular function are important to reducing the burden of cardiovascular disease in this population.

1.5.4 Carotid Intima-Medial Thickness

Carotid intima-media thickness (IMT) is defined as the distance between the lumen intima interface and the media-adventitia interface. This increased wall thickness is often compensated for by an increase in lumen diameter, and therefore measuring the intima-media thickness may be a more accurate representation rather than just the lumen diameter alone. [82] The exact process leading to intimal thickening remains unknown, however changes in carotid IMT may represent subclinical vascular disease as

associations between IMT and atherosclerotic changes are visualized in the coronary arteries during angiography. [83,84]

Contemporary ultrasound techniques and software analysis now allow for non-invasive measures of IMT. Large clinical trials have established that carotid IMT is associated with increased risk for adverse cerebral events (i.e. stroke), cardiac events (i.e. myocardial infarction) and peripheral vascular disease (i.e. hypertension). [85-87] A change as small as a 0.1mm increase in carotid artery IMT has been associated with an increase in age- and sex-adjusted relative risk of 18% for stroke and 15% for myocardial infarction. [88]

COPD is a systemic inflammatory disease in which the risk of developing cardiovascular disease is drastically increased. Since carotid IMT proves to be a valuable marker of cardiovascular disease and predictive of cardiovascular events, it may provide important insight into the relationship between the progression of cardiovascular disease and associated risk in the COPD population.

1.6 Vascular Structure and Function with Aging

1.6.1 Arterial Stiffness and Aging

Cross-sectional studies have found a 40-50% difference in large elastic artery stiffness and compliance between ages ~25 and 75yrs in healthy adults without clinical disease or major coronary risk factors. [89,90] Systolic blood pressure increases with age even in individuals considered normotensive. [91] Diastolic blood pressure increases until the sixth decade of life and then commonly declines in healthy individuals due to arterial remodeling and stiffening throughout the arterial tree. [69] Arterial stiffening reduces the compliance and distensibility of the vessel wall, which causes a faster propagation of the

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forward pulse wave velocity. The resultant reflected waves are superimposed much sooner on the forward wave leading to an increase in systolic blood pressure and a reduction in diastolic blood pressure. Increased systolic blood pressure can further accelerate arterial stiffening and remodeling, where as a reduction in diastolic pressure reduces coronary blood flow, which may increase the risk of a cardiac event (i.e. myocardial infarction). [92]

Stiffening occurs due to the fraying of elastin fibers, increased collagen synthesis and increased calcification of the arterial wall. [60] An age related increase in advanced glycation end products (AGEs), and the increased binding of AGEs to their receptor (RAGE) located on numerous tissues including endothelial cells, results in inflammation, increased vascular permeability, and reduced NO availability, which has been associated with increased arterial stiffening highlighted by increased PWV in the elderly. [93] This reduction in NO increases vascular tone in the smaller arteries, increasing total peripheral resistance and leads to structural and functional changes upstream in larger arteries, such as increased stiffness, and elevated blood pressure (in particular pulse pressure). Ultimately, normal aging results in arterial remodeling and a concomitant increase in PWV and pulse pressure, which increases the risk of a cardiovascular event.

1.6.2 Vascular Function and Aging

Age associated changes to the vascular endothelium are broad and multifactorial in nature. Aging is associated with chronic low-grade inflammation, due to a pro-inflammatory shift in the vascular gene expression profile independent of other cardiovascular risk factors. [94,95] This shift is reflected in increased plasma concentrations of inflammatory markers (CRP), the up regulation of cytokines and chemokines (IL-6,

IL-8), and increased expression of adhesion molecules at the level of the endothelium. [94,96,97] The inflammatory process is largely mediated by reactive oxygen species (ROS), as a number of immune cells utilize ROS to exert their inflammatory effect. [94,98] Endothelial function is modulated by the delicate balance of endothelium derived vasodilators, particularly NO, and ROS. Age related increase in oxidative stress, due to unchanged or reduced antioxidant defenses leads to endothelial dysfunction primarily through the inactivation and scavenging of NO. [99-101]

An inverse relationship has been reported between brachial artery FMD and circulating biomarkers of oxidative stress in healthy older adults. [102,103] Upon administration of an antioxidant therapy (i.e. Vitamin C) brachial artery FMD was restored in older adults, illustrating oxidative imbalance plays a major role in endothelial dysfunction with age. [100,102] More specifically, endothelial dysfunction in the elderly may also be attributed to an excess production of the free radical superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2) [101,104,105] through the reduction in the regulating cofactor Tetrahydrobiopterin (BH_4)[104,107,108] Endothelial dysfunction assessed by FMD improved in sedentary older adults upon BH_4 administration, [106,107] further illustrating that mechanisms that reduce oxidative stress are important in restoring vascular function in older adults.

1.6.3 Aging and Arterial Thickness

Intima-medial wall thickness of the carotid artery increases with age in healthy older adults and is an independent predictor of cardiovascular disease. [86,108] Age associated increases in carotid IMT are due to thickening of the intimal and medial layer of the artery and remodeling may be attributed to hemodynamic changes related to local arterial

blood pressure. In a study of 129 healthy normotensive men aged 18-77, brachial blood pressure remained unchanged but carotid systolic blood pressure increased progressively with age with an associated ~50% increase in carotid IMT in the older compared to the younger men. [109] This study illustrates that carotid IMT increases with age in healthy men in the absence of elevations in peripheral systolic blood pressure, and that increasing carotid systolic blood pressure is significantly related to increases in carotid IMT. [109] One postulation for this increase is the elevated pro-atherogenic endothelial cell phenotypes which occurs in response to elevations in local distending pressure. [110] These phenotypes have been associated with reduced eNOS expression and higher levels of ROS, thus increasing the likelihood of vascular injury and atherosclerosis. [110] However, more research is needed to clarify the role of specific patterns of blood pressure as stimuli for arterial wall remodeling.

Interventions targeted to reduce these age related changes in vascular structure and function are especially important for COPD patients. Since COPD is a systemic inflammatory disease these age related vascular changes could be accelerated and may provide a possible link to the increased risk of cardiovascular disease. Exercise is one intervention which has been studied to improve the age associated changes in the vasculature.

1.7 The Benefits of Exercise Training Vascular Structure and Function with Aging
1.7.1 Aging, Arterial Stiffness and Exercise

The effects of age related arterial stiffening may be attenuated by habitual exercise as endurance trained older adults have reduced central PWV, augmentation index, systolic and pulse pressures compared to sedentary peers. [89,111,112] These reductions were also seen

in healthy endurance-trained older women compared to postmenopausal and premenopausal sedentary counterparts. [112,113] A progressive 3-month aerobic exercise intervention, consisting of walking 25-45mins, 3-6 days/ week at 60-75% of heart rate max resulted in a 25% improvement in arterial compliance and a 20% reduction in beta stiffness in a cohort of 20 sedentary middle aged men. [89] When comparing sedentary (no regular physical activity), recreationally active (light to moderate exercise ≥ 3 times per week), and endurance trained (vigorous aerobic-endurance exercise ≥5 times per week and active in local road running races) young, middle aged and older men, the difference in central arterial compliance was approximately half as great in the endurance trained (25%) men than in sedentary older men (45%) compared to the younger groups. [89] However, central arterial compliance was 20-35% higher in the endurance trained middle-aged and older men compared to their sedentary and recreationally active peers. [89]

In another 3 month aerobic exercise study, in which post-menopausal women who were taking hormone replacement therapy (HRT) were asked to walk at a moderate intensity (65-80% of heart rate max) for 40-45min/day, 4-5days per week, carotid artery compliance increased by 40%. [90] These studies indicate that despite an age related increase in arterial stiffness, aerobic exercise training results in improvements in arterial stiffness even in middle-aged and older adults.

The mechanisms underlying these improvements in arterial stiffness with exercise have yet to be elucidated. Reduced arterial stiffness may be attributed to the reversal or maintenance of age-related structural changes to the arterial wall in the setting of prolonged exercise training, however there is limited data to support this postulation. Utilizing animal models, these changes in arterial stiffness have not been found to be

dependent on alterations in collagen and elastin distribution, [114] although functional adaptation through modifications of gene expression associated with local vasodilator signaling have been found in the aorta of endurance trained rats. [115] Reduced oxidative stress associated with moderate aerobic training may provide another mechanism to increased arterial compliance as carotid artery compliance was improved in sedentary subjects when administered ascorbic acid (vitamin C), but had no effect on endurance trained postmenopausal women. [116]

1.7.2 Aging, Endothelial Function and Exercise

Numerous studies have been conducted and reviewed to understand the effects of exercise on endothelial function. The exercise induced increases in shear stress, as a result of increased cardiac output, blood pressure and pulse pressure are thought to be important in stimulating the endothelial adaptations and improvements in endothelial function observed as local and systemic effects. [117-119] It has been strongly suggested that subject groups consisting of older adults, or those with cardiovascular risk factors and impaired endothelial function are more amiable to improvements in NO function as a result of exercise training than healthy subjects. [102,120,121] Forearm blood flow in response to intra-arterial infusion of acetylcholine (ACh) was 25% lower in middle aged and older sedentary men compared to younger sedentary counterparts, however there was no difference in the vasodilatory response amongst endurance trained older men verse endurance trained younger men. [121] Moreover, in a group of 13 middle-older aged men forearm blood flow in response to ACh increased 30% following a progressive 3 month aerobic exercise intervention consisting of walking 30-45mins, 3-6 days per week at 60-75% of heart rate max. [121] Following this improvement there was no significant difference

in forearm blood flow for the exercise intervention group compared to young, middle aged and older endurance trained men. [121] Similarly, when comparing young sedentary (no physical activity), older sedentary and older habitually aerobically trained men (vigorous aerobic-endurance exercise, > 3 times per week), a 50% reduction in FMD was observed in older sedentary verses younger sedentary, however FMD was similar in the older trained vs. younger trained groups. [102] These studies show that habitual aerobic exercise as well as training interventions can offset endothelial dysfunction in middle-older aged healthy men and improve the vasodilatory capacity to that of younger trained men.

When comparing the effects of aerobic exercise in older post-menopausal women (non-HRT), a single bout of aerobic exercise performed on a treadmill for 45 minutes at 60% of heart rate max was shown to nearly double the FMD in postmenopausal but not premenopausal women. [122] This finding is important as there is a reduction in NO synthesis and bioavailability and consequent reduction in FMD, due to the cessation of endogenous estrogen production following menopause. [122] These observations suggest that exercise may be an important non-pharmacological intervention to reducing cardiovascular risk in postmenopausal women. However, following an 8-week aerobic intervention of brisk walking (6 days/week, 50mins/day at 70-75% of maximal heart rate), brachial artery FMD did not change in postmenopausal women (non-HRT), while there was a 50% improvement in FMD in middle-older aged men. [123] These findings indicate that postmenopausal women not on HRT, may be less responsive to habitual aerobic exercise than middle-older aged men, however more studies need to be conducted

to conclude the role specific exercise prescription plays in improving vascular function in postmenopausal women.

Mechanisms for improved endothelial function in older adults who perform regular aerobic exercise include enhanced NO bioavailability through a reduction in oxidative stress preceded by the preservation of tetrahydrobiopterin (BH_4) bioactivity with exercise. [102,106] Upon administration of BH_4, brachial artery FMD was increased by 45% in older sedentary men, but did not affect FMD in the younger sedentary or older trained groups. [102] Middle-aged and older adults who habitually exercise have a higher total oxyradical scavenging capacity when compared to age-matched sedentary controls. [103] This increase in antioxidant capacity is related to enhanced endothelial dependent dilation. [103]In elderly athletes, vitamin C administration did not change vasodilation to ACh, however in elderly sedentary subjects vitamin C increased vasodilation and restored the inhibitory affects of N^G-monomethyl-L-arginine (L-NMMA), a nitric oxide-synthase inhibitor. [124] Also, eNOS expression may be increased follow exercise, leading to increased NO release and bioavailability. Upon examining the effects of 4 weeks of aerobic exercise (3x/day, 10mins row ergometer, 10mins cycle ergometer) on ACh responses in the left internal mammary artery of patients with stable coronary artery disease, exercise training increase FMD responses by 150% and upon tissue sampling revealed significantly higher eNOS protein expression compared to untrained controls. [120]

1.7.3 Aging, Arterial Wall Thickness and Exercise

Studies examining the effects of endurance training on carotid IMT have observed little evidence to support that exercise training alters arterial wall thickness. In a study of 137 endurance trained (vigorous endurance activity >5 times/week) and sedentary men (no

physical activity), no significant difference was found in carotid IMT.[125] Similarly, in a cross sectional study in which the carotid IMT of 150, 20-40 year old men and women without cardiovascular disease was correlated to endurance exercise (3+ hours per week of vigorous aerobic exercise for a minimum of 6 months), recreational exercise (3+ hours of general sports per week) and sedentary lifestyle (<3hours of sport or general exercise per week for less than 6 months), no significant correlation was found between carotid IMT and fitness level.[126] Following a progressive 3 months endurance training program consisting of walking/jogging 25-45mins/day, 3-6 days/ week at 60-75% of maximal heart rate, in 18 middle-older aged sedentary men, carotid IMT, IMT/lumen ratio and carotid systolic blood pressure did not change following the exercise intervention.[125] These studies suggest that habitual exercise may not be a powerful enough stimulus to prevent or reduce the age-associated elevation in carotid distending pressure and arterial wall thickening.

When comparing postmenopausal sedentary (regular exercise <2 day/week) and endurance trained (regular aerobic exercise, 60mins/day, 5 days/week for at least 5 years) women, no difference in carotid IMT was found between groups.[108] However, endurance trained women on hormone replacement therapy, showed a 21% reduction in femoral IMT compared to sedentary women not on HRT. This reduction was non-significant when compared to sedentary women on HRT or endurance trained non-HRT women.[108] These findings suggest that endurance training may be a non-pharmacological alternative in postmenopausal women to offset peripheral but not central arterial wall thickening. It was also found that femoral artery IMT was reduced and lumen diameter was increased following 12-weeks of daily walking in middle and older aged men in the absence of

changes in cardiovascular risk factors. [127] These changes in peripheral artery IMT may reflect expansive arterial remodeling to normalize wall stress in response to increased arterial blood flow evoked by aerobic exercise, however they do not reflect changes in carotid IMT, which is associated with cardiovascular risk.

1.7.4 Exercise Prescriptions and Vascular Structure and Function

As previously described, aerobic exercise training at light to moderate loads is associated with improvements in vascular structure and function in older adults, however different modalities or intensities of exercise may impact the magnitude of vascular adaptation. A study conducted compared high intensity interval training (4-6, 30sec Wingate tests 3day/week) to endurance training (40-60mins of cycling at 60% VO2peak 5days/week) in 20 young, healthy males and females over a total duration of 6 weeks. [128] Popliteal artery distensibility was improved in both training groups, however carotid IMT and carotid distensibility remained unaltered. [128] Another study which examined the effects of moderate (cycling at 40% heart rate reserve, 3-5 time/week) vs. vigorous (cycling at 70% heart rate reserve, 3-5 times/week) intensity aerobic training in 17 post-menopausal women (non-HRT) over 12 weeks, found a significant reduction in β stiffness index in both the moderate and vigorous groups. [129] However the magnitude of change did not differ between the groups. [129] Furthermore, a study performed in 65 medicated hypertensive patients (23 male, 42 female) examined the effects of 16 weeks of interval (treadmill exercise alternating between 80% heart rate reserve for 1min and 50% for 2mins, for a total of 40mins, 3 times/week) vs. continuous (treadmill exercise at 60% heart rate reserve, for 40mins, 3 times/week) aerobic training on central PWV. [130] It was found that interval training resulted in a significant reduction ~6% in central PWV

compared to continuous exercise or a control group. [130]Although inconclusive, these studies suggest that aerobic exercise training improves some measures of arterial stiffness particularly in older adults, however more studies need to be done to determine the implications of different exercise protocols on different measures of arterial stiffness.

Vascular function and the effect of varying exercise protocols has been explored although mainly in younger cohorts. In 9 young (~26 yrs), healthy males, following 3 months of high intensity training consisting of 4 one hour running session per week at 70-80% of VO_{2max}, endothelial dependent function was reduced 32-35% in response to ACh infusion, while endothelial independent function remained unaltered. [131] It was also found the circulating levels of measured antioxidants (uric acid, SH-groups, a-tocopherol, b-carotene, retinol) were reduced [131] leading to the postulation that high intensity training may lead to reduced antioxidant capacity and reductions in endothelial function. Another study performed by Goto et al., [132] examined the effects of low (25% VO_{2max}), moderate (50%VO_{2max}) and high (75%VO_{2max}) intensity training over 12 weeks in 26 healthy young (~25yrs) men. Training was performed on a cycle ergometer for 30minutes, 5-7 times/week. Moderate intensity exercise was shown to augment forearm blood flow in response to ACh, whereas mild and high intensities did not show significant differences following training. [132] The improvement in the moderate group occurred in the absence of changes in oxidative stress, however high intensity exercise was associated with increased concentrations of oxidative stress markers. [132] These studies suggest that low intensity exercise (25% VO_{2max}) may not be a great enough stimulus to improve endothelial function, where as high intensities associated with increased oxidative stress may prevent, or have a negative effect on the vascular adaptations to exercise.

These studies propose that moderate intensity and interval exercise protocols may affect measures of arterial stiffness where as continuous moderate intensities of aerobic exercise are associated with improvements in endothelial function as a result of unaltered or improved oxidative stress. However, more studies need to be conducted to further explore the relationship between exercise intensities and protocols on vascular function.

1.8 Vascular Structure and Function in COPD

Both pulmonary and systemic inflammation are known risk factors for secondary co-morbidities of COPD. [8,50,133] The presence of acute-phase proteins, oxidative stress, and immune responses are markedly increased in the peripheral circulation of COPD patients compared to smokers who have not developed the disease. [35,36] It is known that the progression of atherosclerosis is amplified in response to increased systemic inflammatory mediators, enhanced production of procoagulant factors and increased oxidative stress, which places COPD patients at a greater risk of developing atherosclerosis and having a cardiovascular incident. [134,135] Endothelial dysfunction and increased arterial stiffness occur in the early process of atherosclerosis and are predictive of cardiovascular risk in both healthy and chronic disease populations. [56,57] To date, few studies have examined the link between alterations in vascular structure and function, and inflammation in COPD.

Arterial stiffness has been shown to be elevated in COPD patients compared to ex smokers, and healthy controls. Patients with COPD have increased central PWV, AIx, and β stiffness index compared to healthy and smoking-status matched controls. [136-140] Increased central and peripheral PWV has been shown to correlate to severity of airflow obstruction (FEV_1 % predicted), and in particular the severity of emphysema as assessed

by computed tomography (CT). [136,138,139] Mills and colleagues reported an increased AIx in COPD compared to controls. [140] This increase in arterial stiffness was found in both COPD patients with and without cardiovascular comorbidities and correlated with serum CRP levels. [140] Sabit et al., reported a positive correlation between central PWV and IL-6[137] however no significant relationship was found between central or peripheral PWV to CRP levels in COPD patients. [138,139] A study performed by Janner et al., found a relationship between the AIx and severity of COPD but only in younger men (<60 years) with moderate to severe COPD, suggesting that perhaps the AIx in not a suitable measurement of arterial stiffness in older COPD cohorts and is not applicable throughout the entire range of disease severity. [141] This finding may help to explain the discrepancy between correlations of the AIx and PWV to levels of CRP. Taken as a whole, these studies indicate that arterial stiffness is increased in patients with COPD and may be related to certain markers of systemic inflammation.

It has been reported that both endothelium dependent and independent vasodilation is significantly impaired in stable patients with COPD and the impairment is related to severity of airflow obstruction. [142-144] Barr et al., observed a linear association of endothelial dysfunction as measured by FMD with both FEV_1 and CT percentage of emphysema in former smokers across the range of normal lung function to moderately severe COPD. [142] They reported that a 1 standard deviation decrease in FMD was associated with a 1.32L decrement in FEV_1 and a 2.6% increase in CT percentage of emphysema. [142] In another study performed by Moro et al., which measured FMD as well as endothelial independent dilation with glyceryl trinitrate (GTN) in COPD vs. controls, a significant reduction in FMD (5.4 ± 3.1% vs. 8.9 ± 2.1%) and to a lesser extent GTN, was

reported in COPD. [144] A linear relationship was also observed between FMD and FEV_1/FVC in the COPD patients suggesting that endothelial dysfunction is related to airflow obstruction and disease severity [144] Eickhoff and colleagues, reported that FMD was reduced in COPD compared to smoking and non-smoking controls, however GTN in COPD was only impaired when compared to nonsmokers. [143] FEV_1 % predicted, CRP and leukocyte count were independent predictors of FMD, however IL-6 was not. [143] These studies suggest that endothelial dependent and independent dilation are reduced in COPD and the reduction is related to disease severity and circulating markers of systemic inflammation such as CRP. On the contrary, one study performed by Maclay et al., assessed endothelial dependent and independent dilation with the gold standard forearm plethysmography and found no significant difference between COPD patients and smoking status-matched controls. [138] Despite their findings they postulate that the effects of chronic smoking or aging may dominate any effects of COPD on resistance vessel vasomotor function as they have previously demonstrated vascular dysfunction in smokers. [138]

Vascular function and arterial stiffness appear to be altered in COPD. It is known that inflammatory mediators play an important role in regulating cardiovascular function and have a direct influence on the vasculature. Therapies that reduce systemic inflammation such as aerobic exercise, could have a significant effect on the development of atherosclerosis and the speed and progression of vascular dysfunction, whereby reducing cardiovascular risk. These measures and therapies need to be greatly explored to better understand vascular function/dysfunction in patients with COPD.

1.9 Exercise and Vascular Structure and Function in COPD

Although exercise is a known anti-atherogenic therapy and has been linked to improvements in blood lipids, decreased systemic inflammation, increased anti-inflammatory cytokines, reduced oxidative stress, and enhanced endothelial function [117,145,146] the exact relationship between the effects of exercise on the vasculature and how exercise may affect primary and secondary manifestations of COPD has yet to be elucidated. Two studies to date have examined the effects of exercise on vascular structure in COPD. A study conducted by Gale et al., [147] examined the effects of a pulmonary rehabilitation program on central and peripheral PWV, AIx and IL-6 in thirty-two COPD patients. Pulmonary rehabilitation consisted of a combination of generic aerobic and strength training performed at 60-70% VO_{2peak} as well as educational sessions 3 times per week for a total of 7 weeks. [147] Compared to a control group, COPD patients had greater central PWV and IL-6 levels at baseline, and following pulmonary rehab had a significant reduction in central PWV. Changes in peripheral PWV, AIx and IL-6 levels were non-significant [147] In a more structured exercise study, the effects of 4 weeks of lower body cycling (5 day/week for 18-30mins/ session at 38-65% of peak work rate) on peripheral PWV and CRP was studied in ten COPD patients. [148] A 10% reduction in peripheral PWV was observed with no differences in CRP levels following exercise training. [148] These studies suggest that arterial stiffness may be reduced following aerobic exercise training however, no study has investigated the effects of structured exercise training on central arterial stiffness, which is more preferentially associated with cardiovascular disease. [70,149] Furthermore, no study has investigated changes in endothelial function, carotid IMT or the relationship to systemic inflammation following

an exercise training intervention in COPD. Therefore, the objective of this study was to examine the effects of a structured exercise training program on vascular structure, function and inflammation in COPD patients.

1.10 Primary Aim

The primary aim of the study was to determine the change in endothelial depended vasodilation following an aerobic exercise training intervention in patients with COPD.

1.11 Primary Hypothesis

It was hypothesized that eight weeks of aerobic exercise training would improve endothelial dependent dilation as measured by brachial artery flow mediated dilation to reactive hyperemia in patients with COPD.

1.12 Secondary Aim

The secondary aim was to determine the effects of aerobic exercise training on endothelial independent dilation, arterial stiffness and biomarkers of systemic inflammation in patients with COPD.

1.13 Secondary Hypothesis

It was hypothesized that eight weeks of aerobic exercise training would have no effect on endothelial independent dilation, but would improve arterial stiffness and reduce systemic inflammatory biomarkers involved in the development of atherosclerosis, and that systemic inflammation and exercise capacity would be associated with changes in vascular function.

Chapter 2: The Effects of Aerobic Exercise Training on Peripheral Vascular Function and Systemic Inflammation in Patients with Chronic Obstructive Pulmonary Disease

2.1 Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive, treatable respiratory disease characterized by partially reversible, expiratory airflow limitation. [18,150] Cigarette smoke is the primary cause of COPD, however long-term exposure to any toxic particulate matter can act as a contributable cause. [1] An enhanced immune response resulting in inflammation occurs within the lungs of COPD patients, and airway inflammation continues for many years even after smoking cessation. [23,24] Animal studies have indicated that inflammation within the lungs results in an increase in circulating inflammatory mediators. [37,38] This 'spill-over' effect of lung inflammation is believed to be the primary cause of the chronic systemic inflammation in patients with COPD, which manifests as a number of secondary comorbidities such as cardiovascular disease. [151] The risk of cardiovascular disease is three to five times higher in patients with COPD and is the leading cause of mortality and the primary reason for hospitalization. [11,42] Population based studies have indicated that reduced FEV_1, independent of other established risk factors such as cigarette smoke, total cholesterol, and hypertension, is an important risk factor in cardiovascular disease mortality in this population. [44-47]

Many of the inflammatory mediators that are elevated in COPD (i.e. CRP, IL-6, IL-8 and TNF-α) are pivotal to plaque formation and the progression of atherosclerosis, and have been linked to ischemic heart disease, stroke and myocardial infarction. [8,48] CRP has been found to promote the uptake of LDLs by macrophages, which contributes to the formation of fibrous plaques. [54] It has also been found to inhibit the expression of eNOS, the enzyme responsible for NO production and vasodilation, while stimulating the

production of the vasoconstrictor, ET-1. [9,54] IL-6, IL-8 and TNF- α, contribute to the pathogenesis of atherosclerosis by stimulating the production of CRP following vascular damage and increasing chemokine expression, adhesion molecules, neutrophil, platelets and fibrinogen concentrations at the site of vascular injury thus creating a pro-coagulant state on the vascular endothelium. [49] Il-6, in particular has been shown to reduce NO release from the vasculature, limiting the bioavailability of NO and resulting in a reduction in endothelial dependent vasodilation. [55] Even though there is a marked increase in a number of pro-inflammatory mediators, it would appear that anti-inflammatory mediators such as IL-10 are reduced greatly influencing the pro/anti-inflammatory balance.

The chronically elevated levels of systemic inflammation in COPD have a direct influence on the vasculature [9,54,55] and have been implicated in the development and progression of atherosclerosis. [49] Endothelial dysfunction and increased arterial stiffness occur in the early process of atherosclerosis and are predictive of cardiovascular risk. [56,57] Arterial stiffness, and to a lesser extent vascular function appear to be altered in COPD patients, [136-140,142-144] and therefore may provide a link between cardiovascular disease and COPD.

Exercise is a known anti-atherogenic therapy and can improve blood lipids, decrease systemic inflammation, increase anti-inflammatory cytokines, reduce oxidative stress, and enhance endothelial function. [117,145,146] Aerobic exercise is well known to reduce the risk of cardiovascular disease in other chronic conditions, and is most likely to be attributed to the anti-inflammatory benefits of exercise and improvements in vascular structure and function. In healthy individuals and in those with coronary artery disease, as

little as 4 weeks of aerobic exercise training has been shown to increase NO bioavailability and improve endothelial function primarily through an increase in shear stress stimulus due to increased cardiac output. [117,152-155] It is also thought that a reduction in reactive oxygen species (ROS) and an increased anti-oxidant effect with moderate to heavy exercise training contributes to improved NO bioavailability and vasodilatory capacity. [103,106,131,132]

In healthy aged individuals who partake in habitual aerobic exercise and in moderate intensity aerobic exercise interventions, arterial stiffness has been show to be reduced with improvements in endothelial dependent vasodilation. [89,90,112,113,121] Modifications of gene expression associated with local vasodilator signaling and reduced inflammation and oxidative stress associated with moderate aerobic training may provide possible mechanisms to these vascular improvements related with exercise. [116] However, no study to date has examined the effects of exercise training as a therapy for modulating the risk of cardiovascular disease (i.e. vascular structure and function) and reducing systemic inflammation in patients with COPD.

The purpose of this study was to examine the effects of 8-weeks of aerobic exercise training on vascular structure and function and systemic inflammation in patients with COPD. The primary hypothesis of the study was that aerobic exercise training would improve endothelial function as measured by flow-mediated dilation of the brachial artery. Secondary hypothesis included that aerobic exercise training would decrease central and peripheral arterial stiffness and reduce systemic inflammation in patients with COPD, with no effect on endothelial independent dilation and carotid IMT.

We also hypothesized that reductions in systemic inflammatory markers would be correlated with improvements in endothelial function and reductions in arterial stiffness.

2.2 Methodology

2.2.1 Patients and Participants

Stable patients (> 3 months exacerbation free) with >10 pack year smoking history, between the ages of 40-80yrs with physician confirmed mild to very severe COPD were recruited for the study. Patients were recruited from a database of individuals who had previously graduated from the Central Okanagan Respiratory Rehabilitation program, offices of respirologists and the pulmonary function laboratory at Kelowna General Hospital (KGH). Potential participants were contacted via a standard letter of initial contact inviting them to participate in the study. Interested participants contacted the study coordinator who provided additional information and answered any questions or concerns regarding the study. Individuals still interested following this initial conversation were then scheduled for study screening (Visit 1).

Due to the lack of information regarding some of the vascular and systemic inflammatory measurements made in the current study and how they change with exercise in individuals free from disease, healthy age, sex, BMI and activity matched controls were recruited as a comparison group. Healthy controls were recruited through advertisements at the local University and through local church groups. Participants were excluded from the study if they were < 40 years and >80 years of age, currently smoking, on supplemental oxygen, diabetic, had a BMI $\geq 35 kg/m^2$, presented with cardiovascular contraindications to exercise or had a previous history of major cardiovascular disease (i.e. heart attack or stroke). Institutional and hospital clinical research ethics boards

approved this study and written informed consent was obtained from all participants prior to entry into the study.

2.2.2 Study Design

This was a single-armed efficacy study to investigate whether individually prescribed aerobic exercise training could improve vascular structure and function and reduce local and regional arterial stiffness and systemic inflammation in patients with COPD. An additional aim of this pilot study was to generate sufficient data to accurately power a phase 2 randomized controlled trial examining the specific benefits of exercise training for reducing cardiovascular disease risk in patients with COPD.

All participants conducted two pre-visits and a venous blood sample prior to starting the exercise training intervention, as described in Figure 2.1. Pre-visit one was a screening session at KGH and included a pulmonary function test to determine disease severity and a cardiopulmonary exercise test (CPET) to: 1) screen for any cardiovascular contraindications to exercise, 2) determine peak oxygen consumption (VO_{2peak}), and 3) determine maximum workload in order to individually prescribe relative exercise intensities. Pre-visit two consisted of a comprehensive vascular assessment, which was performed at the integrative Clinical Cardiopulmonary Physiology (iCCP) Laboratory at the UBC Okanagan campus. Endothelial function was assessed by flow -mediated dilation in response to reactive hyperemia in the brachial artery and endothelial independent function was assessed with a sublingual spray of glycerol trinitrate. Local and regional arterial stiffness, distensibility and compliance as well as carotid artery intimal-media thickness were also determined via applanation tonometry and ultrasound. Within 24 hours of the vascular assessment, a 12-14 hour fasting venous blood sample

was collected and analyzed for blood lipids, hematology and biomarkers of systemic inflammation.

Upon completion of all baseline testing, participants performed individually prescribed, supervised, aerobic exercise training three times per week for a total of 8 weeks at the iCCP Laboratory. All exercise was performed on cycle ergometers (95R Achieve & Integrity series, Life Fitness, Schiller Park, IL) and upper extremity ergometers (Rehab ergometer 881E, Monark, Langley, WA). As this was an efficacy study, patients were allowed to make up any missed session. If a participant felt like they were going to have an exacerbation, they were instructed to see their Doctor and take antibiotics and/or corticosteroids if they had a current prescription. The participant was asked to refrain from training until they felt good enough to return for at least one week or had finished their medication. Upon return, the participant performed a light intensity shorter duration exercise session and was slowly progressed back to where they had stopped. Upon completion of 24 sessions, all baseline assessments (apart from lung volumes and diffusion capacity) were repeated to determine the effects of the exercise training intervention on all vascular and systemic inflammatory mediators.
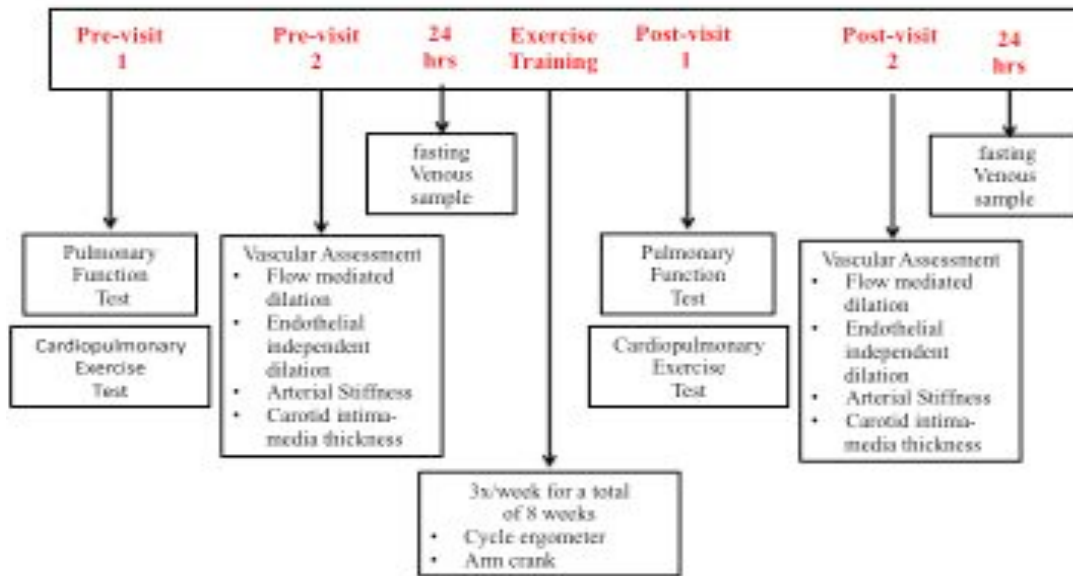
Figure 2.1- Experimental Design

2.2.3 Aerobic Exercise Intervention

The exercise training intervention consisted of 24 aerobic exercise sessions performed over 8-weeks. As previously mentioned, participants were screened (via CPET) before commencing exercise training and as such were considered safe to exercise in a laboratory setting as prior screening of patients with COPD has been shown to result in minimal risks independent of whether exercise training is performed in a hospital, community or home based setting. [156] A trained exercise specialist with experience exercising patients with respiratory disease supervised each session in order to critically maximize adherence in terms of both attendance and exercise prescription. All exercise sessions included a 5-min warm-up and 5-min cool down on a cycle ergometer at the beginning and end of each training session, respectively. On average, exercise-training sessions were approximately 35-65mins in duration.

Heart rate (PC3, Sigma, St. Charles, IL), oxyhemoglobin saturation (N-65 Oximax, Nellcore, Mansfield, MA), manual blood pressure, dyspnea and leg fatigue (scale 0-10; Borg, 1982) were monitored and recorded at rest, at 5 and 10min and then every subsequent 10mins of exercise as well into recovery. As high intensity exercise could have adverse effects on vascular function in patients who are already inflamed due to increased oxidative stress, a moderate intensity longer duration exercise prescription was used to maximize shear stress on the vascular wall. A progression to higher intensity training was believed to be appropriate following the initial 4 weeks in order to continue progressively increasing the shear stress stimulus to enhance vascular adaptation after patients had become accustomed to a moderate to heavy training volume.

Weeks 1-4 focused on building an aerobic base, by increasing training volume primarily through increasing the duration of training of moderate intensity exercise. This was achieved by starting at 40-50%Wmax for 20-25mins and building to 70-75%Wmax at 45mins over the four weeks. Weeks 5-8 focused on initiating training adaptations through higher intensity interval training (80-90%Wmax for 3mins; 40-45%Wmax for 3mins for a total of 5-6 sets), while maintaining and progressing training volume with two longer duration continuous workouts. Exercise sessions were stratified and followed a hard, light and moderate day training order, as depicted in Table 2.1.

Table 2.1- Exercise Prescriptions for 8 Weeks (24 sessions) of Aerobic Exercise Training in COPD Patients and Healthy Controls

	Visit 1	Visit 2	Visit 3
Week 1	LB: 50%Wmax @ 20mins UB: 10-15W @ 5-10mins	LB: 50%Wmax @ 20-25mins UB: 10-15W @5-10mins	LB: 50-55%Wmax @ 20-25mins UB: 10-15W @ 5-10mins
Week 2	LB: 50%Wmax @ 20-30mins UB: 10-15W @ 10mins	LB: 55%Wmax @25-30mins UB: 10-15W @10mins	LB: 55-60%Wmax @ 20-30mins UB: 10-15W @ 10mins
Week 3	LB: 55%Wmax @ 30-40mins UB: 15-20W @ 10-15mins	LB: 55%Wmax @ 30-40mins UB: 15-20W @ 10-15mins	LB: 60%Wmax @ 20-35mins UB: 15-20W @ 10-15mins
Week 4	LB: 55%Wmax @ 30-45mins UB: 15-20W @ 15min	LB: 55%Wmax @ 30-45mins UB: 15-20W @ 15mins	LB: 50-60%Wmax @ 30-40mins UB: 15-20W @ 15mins
Week 5	LB: 80-85%Wmax @ 3mins 30-40%Wmax @ 3mins Total 5 reps = 30mins UB: 15-20W @ 15-20mins	LB: 50-60%Wmax @ 45mins UB: 15-20W @ 15-20mins	LB: 55-65%Wmax @ 35-40mins UB: 15-20W @ 15-20mins
Week 6	LB: 80-90%Wmax @ 3mins 30-40%Wmax @ 3mins Total 5-6 reps= 30-36mins UB: 20W @ 15-20mins	LB: 55-60%Wmax @ 45mins UB: 20W @ 15-20mins	LB: 65-70%Wmax @ 35-40mins UB: 20W @ 15-20mins
Week 7	LB: 85-90%Wmax @ 3mins 30-40%Wmax @ 3mins Total 6 reps= 36mins UB: 20W @ 20mins	LB: 55-65%Wmax @ 45mins UB: 20W @ 20mins	LB: 65-75%Wmax @ 35-40mins UB: 20W @ 20mins
Week 8	LB: 75-80%Wmax @ 30-35mins UB: 20W @ 20-25mins	LB: 60-70%Wmax @ 45mins UB: 20W @ 20-25mins	LB: 70-75%Wmax @ 35-40mins UB: 20W @ 20-25mins

Abbreviations: LB, lower body exercise on a cycle ergometer; UB, upper body exercise on an upper extremity ergometer.

The exercise progression was increased on an individual bases to try and optimize the volume of exercise for each patient. Progression in training load occurred if the patient completed the previous session without stopping, and reported symptoms of dyspnea and leg fatigue <3 on the Borg scale throughout the session. Patients who could not complete the prescribed duration of exercise were allowed to stop but were encouraged to continue once their symptoms had resided. As patients got fitter and started working at higher exercise intensities for longer duration subjects were administered supplemental oxygen if they started to desaturate during exercise to <88%. Oxygen was titrated just enough to maintain SpO2 at around 90%.

2.2.4 Specific Measurements

2.2.4.1 Pulmonary Function Testing

All participants performed a pulmonary function test (PFT) administered by a registered respiratory therapist at KGH. Participants performed routine spirometry, single-breath diffusion capacity for carbon monoxide (DLCO), and constant-volume body plethysmography for the determination of lung volumes (6200 Autobox; SensorMedics, Yorba Linda CA). All measurements were obtained in the sitting position according to American Thoracic Society guidelines [157-159] and established reference equations were used to determine predicted values for spirometry, [160] lung volumes [161] and diffusing capacity. [162]

2.2.4.2 Cardiopulmonary Exercise Testing

Each participant performed a physician-supervised incremental CPET to symptom limitation. All incremental tests were performed on an electrically braked cycle ergometer (Ergoline 800S, SensorMedics, Yorba Linda, CA) and expired gases were

collected and continuously analyzed to determine VO_{2peak} using a metabolic measurement system (Sensormedics Vmax 29C, SensorMedics, Yorba Linda, CA). VO_{2peak} was considered the highest 30-s VO_2 reading obtained during the test. The exercise testing protocol was performed according to ATS recommendations. [163]

After stable resting metabolic values were achieved, participants began cycling unloaded for two minutes before the load was increased by 10 $W^.min^{-1}$ until symptom-limitation. During each test, oxyhemoglobin saturation and heart rate were continuously monitored using pulse oximetry (Radical 7, Maximo, Irvine, CA) and ECG monitoring (CardioSoftTM, GE Healthcare, Waukesha, WI), respectively. Blood pressure was measured and recorded every two minutes by manual sphygmomanometer and ratings of exertional symptoms (dyspnea and leg fatigue) were also assessed using the modified Borg scale. [164]

To assess changes in end-expiratory lung volumes, patients performed a minimum of two inspiratory capacity (IC) maneuvers at rest and during every two minutes of exercise. This technique assumes that total lung capacity (TLC) does not change with exercise [165] such that end-expiratory lung volume = TLC-IC which has previously been shown to be reliable technique for measuring end-expiratory lung volumes during exercise in individuals with COPD. [166,167] To ensure IC maneuvers were performed accurately during exercise, tidal breathing was continuously displayed on the monitor of the metabolic measurement system (Sensormedics Vmax 29C, SensorMedics, Yorba Linda, CA). At the end of a normal expiration the patient was asked to breathe in without warning and to give an additional effort on top of a maximal inspiration. [167] Dynamic hyperinflation was considered to occur when inspiratory capacity was reduced

by >0.2L. A maximal effort in the incremental exercise test was defined as the achievement of one or more of the following: 1) ventilatory limitation (defined as a ventilatory reserve of <11L or a VEmax/MVV of >0.85), 2) a maximal heart rate of 90% of predicted, 3) a peak and plateau in oxygen consumption was observed, and 4) RER >1.15. [163]

2.2.4.3 Endothelial Function – Dependent and Independent Vasodilation

Endothelial function was assessed by flow mediated endothelial dependent vasodilation (FMD) in response to reactive hyperemia in the brachial artery. Subjects fasted and refrained from caffeine for a minimum of 4 hours and did not perform exercise for ≥24 hours prior to testing. Patients were permitted to drink water and take prescription medications as normal. Preparation for each test was identical between pre and post measurements for each subject. Upon arrival to the laboratory, the patient laid in the supine position in a dimly lit room at standard room temperature 15 minutes before measurements were made. [79] The ultrasound probe was oriented over the brachial artery of the right arm and positioned at an angle of 60 degrees to obtain a clear arterial blood velocity signal with no interference from adjacent venous blood flow. Angles of insonation greater than 60 degree are associated with an exponential increase in error when velocity assessment if used for shear rate calculation. [79] All FMD measurements were made by a highly trained and experienced post-doctoral fellow with over 6 years of experience. All measurements were made above the antecubital fossa, proximal to the blood pressure cuff using a 10-Mhz multifrequency linear array probe and a high-resolution ultrasound machine (T3000L; Terason, Burlington, MA).

Duplex ultrasound for concurrent acquisition of high-resolution B-mode and pulse-wave Doppler velocity signal was used to measure brachial artery diameter, time to peak blood flow and to simultaneously quantify shear stress during the FMD response. [75,168] In duplex ultrasound, signals for both the Doppler frequency shift and arterial diameter are detected by the same transducer, but have competing requirements for data acquisition. However, modern duplex ultrasound systems incorporate a narrower Doppler beam of 20-30 degrees to ensure that measurable Doppler shifts are achievable at an approximate angle of 60 degrees while maintaining optimal B-mode imaging. [75] Reactive hyperaemia (FMD) was induced by inflation of a blood pressure cuff around the forearm to ~220mmHg and maintained for 5 minutes. This cuff-position has been shown to be almost solely nitric oxide (NO) dependent and is the current recommendation by recently published guidelines. [75,80] Brachial artery diameter and blood flow velocity were continuously recorded 1 minute before cuff inflation, 30 seconds prior to deflation and 3 minutes following cuff deflation. The standard error of measurement was 0.30% with a coefficient of variation of 3.6%.

After 30 minutes of rest, which allowed arterial diameter to return to baseline, another 3 minutes of baseline recordings were made before a sublingual 400-µg spray dose of nitroglycerine or glyceryl trinitrate (GTN) was administered. Images were recorded for a further 10 minutes to measure endothelial independent vasodilation of the brachial artery. [117] Each subject was screened for a history of headaches or migraines, anemia, glaucoma, kidney disease, liver disease, overactive thyroid and medications, as these may result in an adverse interaction with GTN. Only those patients with no history received GTN. Repeat assessment of endothelial dependent dilation pre and post exercise

training were performed at the same time of day to account for any diurnal variation in FMD response.[169] Post-exercise vascular assessment was performed within 24-36 hours of completion of the last training session in order to obtain measurements during the period of greatest potential vascular adaptation.

2.2.4.4 Arterial Stiffness and Carotid Intima-Media Thickness Measurement

Central and peripheral arterial stiffness, as determined by measuring carotid-femoral and carotid-radial pulse wave velocity (PWV) respectively, were assessed non-invasively using a hand held-tonometer (SPT-301 Millar Instruments, Houston, TX). Carotid and femoral artery waveforms were recorded simultaneously in the supine position with the tonometers applied directly to the skin over the area of greatest pulsation on the right side of the participant. With concurrent electrocardiography to obtain R-R intervals, twenty consecutive reproducible beats as determined by two technicians through consistent pressure waveform (shape and relative magnitude), were collected simultaneously at both sites. Radial waveforms were also recorded in the supine position on the right side. Pulse distance was measured using an anthropometric measuring tape. Central pulse distance was determined as the distance of carotid artery site to the sternal notch subtracted from the distance of sternal notch to femoral artery site. Peripheral pulse distance was calculated by subtracting the distance of carotid artery site to the sternal notch from the distance of sternal notch to the radial artery site with the arm abducted 90 degrees. This technique has been validated as the most optimal non-invasive technique when compared to aortic PWV measured invasively using cardiac catheterization.[170] PWV was then determined as $D/\Delta t$, where D is the distance between measurement sites and Δt is the pulse transit time. Δt was defined as t_2 (pulse arrival time at distal site) - t_1 (pulse arrival

time at proximal site). Pulse arrival time was determined by online analysis in which the ECG trace was used to determine the time at the R-spike and systolic upstroke, also known as the foot of the waveform. A band pass filter was applied to filter out the lower and higher frequencies (<5Hz, >30Hz), which allowed the foot of the waveform to be identified as the minimum values of the filtered signal. [171] The standard error of measurement was 0.22 m/s with a coefficient of variation of 2.9% for central PWV and 2.5% for peripheral PWV.

Local arterial stiffness was assessed at the carotid artery to approximate central artery stiffness. Carotid artery blood pressure waveforms were obtained using the hand-held tonometer positioned over the area of greatest pulsation on the right carotid artery. Concurrent carotid images were obtained using B-mode ultrasound collected at a minimum of 10 frames/sec (T3000L; Terason, Burlington, MA), positioned longitudinally over the left carotid artery ~2 cm proximal to the bifurcation of the external and internal carotid arteries. Continuous blood pressure measurement (Finapres Medical Systems, Biomedical Instruments) and electrocardiograph were recorded for simultaneous recordings of brachial blood pressure and R-R intervals. Ten consecutive carotid and brachial waveforms were collected and analyzed using commercially available software (LabChart v7, ADInstruments, Austin, Texas) with 10 complete simultaneous cardiac cycles collected with the ultrasound machine. Continuous blood pressure was calibrated to manual blood pressure. If a discrepancy existed between the two measures an average of the manual blood pressure pre and post collection was used to manually calibrate the finometer throughout the test. Lumen diameters in maximum systole and minimum diastole were measured from the far wall of the lumen and intima

to the near wall interface of the adventitia and media [89] and were analyzed using edge-detection software as described in section 2.2.4.5. Carotid arterial compliance, distensibility and ß-stiffness index (an index of arterial compliance adjusted for distending pressure) were calculated as described by Tanaka et al. [89] (Equation 2.1).

Carotid IMT was measured in the supine position with a slight hyperextension of the neck and at a 45 degree lateral flextion away from the side being scanned. Imaged were acquired on the participants left carotid artery using an 8 MHz high frequency linear array transducer (Vivid-q, GE, Fairfield, CT, USA). Images were taken of the far wall, 1 cm proximal to the carotid bulb. The IMT at end diastole (1 frame prior to the R-interval) of 10 successive beats was averaged in the longitudinal plane in the lateral and medial positions.

Equation 2.1- Equations for Determining Arterial Stiffness Measures

$$Compliance = \frac{\Delta CSA}{PP} = \frac{\Pi r^2 - \Pi r^2}{PP} = \frac{\Pi\left(\frac{d\,max}{2}\right)^2 - \Pi\left(\frac{d\,min}{2}\right)^2}{PP}$$

$$Distensibility = \frac{\Pi\left(\frac{d\,max}{2}\right)^2 - \Pi\left(\frac{d\,min}{2}\right)^2}{\Pi\left(\frac{d\,min}{2}\right)^2 \times PP}$$

$$\beta = \frac{\ln\left(\frac{SBP}{DBP}\right)}{\left(\frac{d\,max - d\,min}{d\,min}\right)}$$

2.2.4.5 Analysis of Vascular Ultrasound Images

Post-test analysis of the brachial artery FMD was performed using specialized custom-designed 'gold-standard' edge detection and wall tracking software (Courtesy of Prof. Daniel Green, University of Western Australia), which has been shown to be independent of investigator bias. [172] The reproducibility of the FMD using this semi-automated

software possesses a coefficient of variation of 6.7-10.9% and has been validated against Perspex phantom arteries with the mean resolving power of the software estimated to be 8.3microm. [172] All ultrasound video images were converted to DICOM files, which were then used for analysis. Automated calibration of both the diameter and velocity traces was performed by highlighting a region of interest (ROI) on both the B-mode image and Doppler strip scales, respectively. An ROI was drawn around the entire Doppler trace, which automatically tracked the velocity trace in real time at 30Hz. Arterial diameter was determined by drawing a second ROI capturing both walls of the artery of the B-mode image. Within the ROI of the diameter image, a pixel-density algorithm automatically identified the angle-corrected near and far-wall lines for every pixel column for diameter assessment. The algorithm identified the edge of the artery by determining the point where the pixel intensity changed most rapidly using a Rake routine by scanning from bottom to top (and vice versa) on both the near and fall wall. The ROI selection was chosen manually and chosen based on clarity of the image throughout the entire image capture, which contained between 200-300 diameter measures per frame, and occurred at 30 frames per second. Diameter measures were then synced with velocity measures at 30 Hz to calculate blood flow and shear rate. Erroneous data was manually identified and deleted from both the B-mode and Doppler trace. This was only completed when it was clear that the software was not picking up the wall of the artery by confirming on the B-mode image the diversion of the generated line from the arterial wall. Subjects were removed from analysis if this occurred for significant portions of the test.

The time to peak diameter (in seconds) was calculated from the point of cuff deflation to maximum diameter. Calculation of FMD and time to peak were therefore

observer-independent and based on standardized algorithms applied to the data, which had undergone automated edge-detection and wall tracking. Post-deflation shear rate stimulus responsible for the endothelium-dependent FMD was acquired from simultaneously acquired velocity and diameter measurement at 30 Hz.[173] Shear rate area under the curve was calculated using the Reiman Sum Technique and calculated shear up to time of peak dilation. Shear rate (an estimate of shear stress without velocity) was calculated as 4 x peak velocity divided by average vessel diameter,[174] and was determined automatically by the analysis software.

The carotid IMT was determined by semi automated contour detection software (GE Healthcare, Echopac Dimensions, Version 110.1.2) of the intima and medial layers in order to calculate carotid IMT on a user defined ROI along the posterior vessel wall. An ROI of approximately 150 data points was used for each sample, and determination of the near and fall wall were determined by identifying changes in pixel intensity (described above). Maximum and minimum diameters for the carotid artery were calculated as described above for brachial artery diameters. Diameters from each frame collected over 10 cardiac cycles were exported to an excel document, where 10 maximum and 10 minimum diameters were selected and averaged.

2.2.4.6 Systemic Markers of Cardiovascular Disease Risk and Biomarkers of Inflammation

Following at least 12 hours of fasting, a venous blood sample was taken from the anticubital vein for the measurement of traditional cardiovascular risk factors by Valley Medical Laboratory Services in Kelowna (i.e. low and high density lipoproteins, total cholesterol triglycerides fasting blood glucose levels and CRP). Additional samples were also centrifuged and the serum was stored at -70°C for later analysis of specific

biomarkers that have been linked to the development and progression of atherosclerosis namely IL-6, IL-8, IL-10, and TNF-alpha.

Systemic inflammatory mediators were measured in serum using Luminex multiplex bead-based technology (Applied Cytometry Systems, UK) and a multiplex human kit from Millipore (Massachusets, USA). In brief, the serum matrix provided with each kit was used to generate a standard curve for each analyte. Serum samples and standards (50μl per well) were added to 50μl of the bead mixture and incubated overnight at 4°C with agitation. Wells were then washed 3 times with buffer and incubated with the detection antibodies for 1-hour, at room temperature with agitation. The beads were then washed again 3 times and incubated for 30-min at room temperature with phycoerythrin. Finally, the beads were washed and resuspended in 75 ul of sheath fluid before being analyzed using StarStation V.2.3 software (Applied Cytometry Systems, UK).

2.2.5 Study Endpoints

The primary outcome of this study was the change in endothelial dependent dilation as determined by FMD % peak in COPD patients following 8 weeks of aerobic exercise training. Since COPD is a systemic inflammatory condition, which is associated with vascular dysfunction and aerobic exercise is known to improve vasodilator capacity we felt that FMD % peak would be improved in COPD patients following an 8 weeks exercise intervention. Secondary outcome variables included measures of endothelial independent dilation (% change with GTN), arterial stiffness (central PWV, peripheral PWV, β stiffness index, carotid artery compliance and distensibility), and arterial structure (carotid IMT). Additional secondary outcomes measured included pro-

inflammatory biomarkers (CRP,IL-6, IL-8 and TNF-α) and anti-inflammatory mediators (IL-10).

2.2.6 Power Calculation and Statistical Analysis

No study to date has examined the effect of eight weeks of exercise training on FMD in patients with COPD. As such, the aim of this study was to generate pilot data to appropriately power a future randomized controlled trial assessing the benefits of exercise for reducing the cardiovascular risk of COPD. With a standard deviation of 1.0% and a probability of a type 1 error of 0.05, a sample size of 10 would be able to detect a clinically relevant change in FMD% peak of 1% with a power of 0.84. Subsequently, 10 subjects in this pilot study were considered appropriate to initially evaluate whether FMD could be changed in COPD patients with aerobic exercise.

All statistical analysis performed on descriptive statistics utilized means ± standard deviations for parametric data while median and interquartile ranges were used for nonparametric data. A Shapiro-Wilk Tests was used to determine distribution normalcy for each variable. For parametric data, dependent t-tests were performed to evaluate the difference between all pre and post variables following exercise training in patients with COPD and as well as controls. This was performed as our primary analysis in order to examine the effects of exercise training on vascular function in COPD. Nonparametric data was evaluated using Mann-Whitney Rank Sum test. Secondary exploratory analysis consisted of using an independent t-tests to compare the change scores of COPD and control groups to assess any difference between groups following exercise training. This analysis was performed instead of a repeated-measures ANOVA in an effort reduce the chance of a type 2 error, and to maintain statistical power with a

small sample size. The alpha level was set at a p of 0.05. To examine any relationships which may exist between disease severity, fitness level or the volume of exercise training performed and changes in outcome measures, Pearsons correlations were performed between FEV_1 % predicted, VO_{2peak} relative and total training volume to changes in all variables of vascular structure and function and biomarkers of systemic inflammation.

2.3 Results

2.3.1 Patients

All participants were recruited between February 2012 and April 2013 and the study flow is presented in Figure 2.2. A total of 663 respiratory disease patients were informed of the study. 640 letters of initial contact were sent to individuals who had previously graduated from the Central Okanagan Respiratory Rehabilitation program. From the 640 letters, 453 (71%) of subjects did not respond to the letter of initial contact, 97 (15%) had moved and 16 (3%) were deceased. An additional 23 potentially eligible subjects were also recruited from offices of respirologists, the pulmonary function laboratory and respiratory rehabilitation at KGH. In total 97 potentially eligible patients were interested in performing the study of which 14/97 (15%) met study criteria. The remaining 83 patients were excluded due to known cardiovascular disease (n=8), diabetes (n=4), currently smoking (n=5), >80 years of age (n=5), did not have COPD (n=4) could not commit (n=39), and for other reasons (n=18). Out of the 14 eligible subjects, 4 were excluded due to either cardiovascular contraindications to exercise (n=3) or for receiving a hip replacement during the time of entering the study (n=1). As such, a total of 10 (71%) eligible COPD subjects participated in the study. Controls of similar age, sex and BMI were also recruited for the local community. Control subjects were recruited by either

word of mouth from other participants or from a recruitment e-mail sent out to UBC faculty. A total of 6 controls were recruited and all 6 met the inclusion criteria and participated in the study. Two adverse events occurred during exercise testing as testing was stopped due to complex ectopy. In these circumstances patients were sent for further cardiac follow up. No adverse events occurred during exercise training.

2.3.2 Adherence to the Exercise Program

An adherence of 98% was achieved in the COPD patients to exercise training where as an adherence of 96% was achieved in the control group. Adherence rate was not statistically different between groups.

2.3.3 Subject Characteristics and Pulmonary Function Test

Subject characteristics and pulmonary function test are presented in Table 2.2. There were no significant differences in age, and body mass index (BMI) between the COPD and control groups and each group consisted of an even number of males to females. Compared to controls, the COPD patients had a significant reduction in FEV_1 (3.3 ± 0.6 L vs. 1.6 ± 0.5 L, p<0.001), FVC (4.4 ± 0.8 L vs. 3.5 ± 0.7 L, p= 0.02), FEV_1/FVC (75 ± 4 % vs. 49 ± 15 %, p<0.001), D_{LCO} (22.4 ± 4.6 ml.mmHg.$^{-1}$min vs. 15.0 ± 4.1 ml.mmHg.$^{-1}$min, p<0.01), and also had a higher RV/TLC (31 ± 5 % vs. 45 ± 8%, p<0.01). These findings indicate classic airway obstruction and expiratory airflow limitation associated with COPD.

Figure 2.2- Patient Flow

2.3.4 Effects of Exercise Training on Vascular Structure and Function in Patients with COPD

All measures of endothelial dependent and independent dilation, arterial stiffness and carotid IMT following 8 weeks of exercise training in patients with COPD are presented in Table 2.3, Figures 2.3, 2.4 and 2.5, panels A and C. There were no significant changes in baseline diameter, peak diameter, peak dilation, average shear rate, shear rate area under the curve (SRAUC) or time to peak dilation when comparing flow mediated dilation pre and post exercise training ($p > 0.05$). Endothelial independent dilation was only measured in four individuals. There were no significant changes in baseline diameter, peak diameter, GTN peak, or time to peak dilation when comparing pre and post exercise training ($p > 0.05$). Peripheral PWV was significantly reduced following exercise training (7.6 ± 2.5 m/sec vs. 6.8 ± 1.8 m/sec, $p = 0.03$), however, no significant differences were found in central PWV, carotid pulse pressure, carotid compliance, carotid distensibility, and β stiffness index or carotid IMT following exercise training ($p > 0.05$).

2.3.5 Effects of Exercise Training on Blood Lipids, Hematology and Biomarkers of Inflammation in Patients with COPD

All blood lipids, hematology and biomarkers of inflammation pre and post exercise training in COPD patients is presented in Table 2.4. There were no significant changes from pre to post training in blood lipids, hematology or biomarkers of systemic inflammation in patients with COPD ($p > 0.05$).

Table 2.2- Subject Characteristics and Pulmonary Function Test

Variable	Control (n=6)	COPD (n=10)	P
Age (yrs)	62 ± 4	69 ± 9	0.09
Male:Female	3:3	5:5	-
BMI (kg.m^{-2})	25.6 (24.8, 29.3)	25.9 (24.2, 27.4)	0.7
FEV_1 (L)	3.3 ± 0.6	1.6 ± 0.5	<0.01
FEV_1 (% pred)	113 ± 13	67 ± 23	<0.01
FVC (L)	4.4 ± 0.8	3.5 ± 0.7	0.02
FVC (% pred)	118 (114, 122)	101 (96, 111)	0.01
FEV_1/FVC (%)	75 ± 3.8	49 ± 14.7	<0.01
TLC (L)	6.5 ± 0.8	6.3 ± 1.6	0.8
TLC (% pred)	109 ± 8	108 ± 18	0.88
RV (L)	2.0 ± 0.2	2.9 ± 1.2	0.08
RV (% pred)	93 ± 14	131 ± 52	0.1
RV/TLC (%)	31 ± 5	45 ± 8	<0.01
FRC (L)	3.1 ± 0.2	4.1 ± 1.4	0.12
FRC (% pred)	96 ± 10	126 ± 35	0.06
D_{LCO} (ml.mmHg.$^{-1}$min)	22.4 ± 4.6	15.0 ± 4.1	<0.01
D_{LCO} (% pred)	91 ± 8	66 ± 16	<0.01
D_{LCO}/V_A (ml.mmHg.$^{-1}$min)	3.9 ± 0.3	3.5 ± 0.9	0.3
D_{LCO}/V_A (%pred)	91 ± 8	83 ± 22	0.41
Medications, n			
Short-acting β_2-adrenoceptor agonist	0	5	
Long-acting β_2-adrenoreceptor agonist	0	0	
Anticholinergic	0	6	
Combined Therapy*	0	8	
Corticosteroids	0	3	
Statins	0	2	
Diuretics	3	4	
ACE inhibitors	0	1	
Angiotensin 2 inhibitors	1	2	
Direct renin inhibitor	0	1	
Antiarrhythmic	0	1	
Proton pump inhibitor	0	2	
Other	3	5	

Normally distributed data presented as mean ± SD, non-normally distributed variables reported as median [interquartile range (IRQ)]. *Abbreviations*: BMI, body mass index; FEV_1, forced expiratory volume in 1 sec; FVC, forced vital capacity; TLC, total lung capacity; RV, residual volume; FRC, functional residual capacity; D_{LCO}, diffusion capacity for carbon monoxide; D_{LCO}/V_A diffusion capacity corrected for alveolar volume. *=combination therapy of fluticasone proprionate and salmeterol.

Table 2.3- Changes in Vascular Structure and Function Following 8 Weeks of Exercise Training in Patients with COPD

Variable	Pre Training	Post Training	Change Score	P
Flow Mediated Dilation (FMD) (n=10)				
Baseline Diameter (cm)	0.46 ± 0.06	0.47 ± 0.10	0.01 ± 0.10	0.67
Peak Diameter (cm)	0.48 ± 0.06	0.50 ± 0.11	0.02 ± 0.10	0.60
FMD peak (%)	5.0 ± 2.0	5.6 ± 2.5	0.6 ± 2.1	0.40
Average Shear rate (1/s)	253 ± 170	252 ± 134	-0.3 ± 137.0	1.00
SRAUC (ttp)	14589 ± 13045	15765 ± 7790	1176 ± 12434	0.77
Time to peak (s)	51 ± 24	68 ± 21	15 ± 38	0.18
Endothelial Independent Dilation (n=4)				
Baseline Diameter (cm)	0.5 ± 0.1	0.6 ± 0.1	0.04 ± 0.10	0.29
Peak diameter (cm)	0.6 ± 0.1	0.6 ± 0.1	0.04 ± 0.04	0.17
GTN Peak (%)	15 ± 2	14 ± 3	-0.7 ± 4.0	0.76
Time to Peak (s)	452 ± 28	446 ± 32	-5 ± 53	0.86
Arterial Stiffness (n=10)				
Central PWV (m/sec)	10.9 ± 1.5	11.4 ± 3.3	0.5 ± 2.3	0.54
Peripheral PWV (m/sec)	7.6 ± 2.5	6.8 ± 1.8	-0.8 ± 1.0	0.03
Carotid Pulse pressure (mmHg)	34.1 ± 7.8	37.9 ± 8.5	3.8 ± 9.8	0.25
Carotid Compliance (mm^2/mmHg)	0.10 ± 0.06	0.10 ± 0.80	-0.006 ± 0.100	0.72
Carotid Distensibility (mm/mmHg)	0.004 ± 0.002	0.004 ± 0.003	0.0001 ± 0.0020	0.85
β Stiffness Index	7.0 (4.7, 8.3)	7.0 (5.7, 8.4)	2.8 ± 8.0	0.28
Carotid Intima-Media Thickness (n=10)				
C-IMT Average (mm)	0.78 ± 0.13	0.74 ± 0.12	-0.03 ± 0.08	0.26
C-IMT Maximum (mm)	0.87 ± 0.15	0.84 ± 0.13	-0.03 ± 0.09	0.27
C-IMT Minimum (mm)	0.64 ± 0.11	0.63 ± 0.11	-0.008 ± 0.080	0.75

Normally distributed data presented as mean ± SD, non-normally distributed variables reported as median (interquartile range [IQR]). *Abbreviations:* SRAUC, shear rate area under the curve; PWV, pulse wave velocity.

Table 2.4- Changes in Blood Lipids, Hemtaology, and Biomarkers of Systemic
Inflammation Following 8 Weeks of Exercise Training in Patients with COPD

Variable	Pre Training	Post Training	Change Scores	*P*
Blood Lipids				
Fasting Blood Glucose (mmol/L)	5.2 ± 0.6	5.2 ± 0.4	-0.01 ± 0.40	0.94
Cholesterol (mmol/L)	5.6 ± 1.5	5.5 ± 1.3	-0.08 ± 0.40	0.56
Triglycerides (mmol/L)	1.4 ± 1.0	1.3 ± 0.5	-0.09 ± 0.80	0.72
HDL (mmol/L)	1.7 ± 0.4	1.7 ± 0.4	-0.06 ± 0.30	0.44
LDL (mmol/L)	3.2 ± 1.2	3.2 ± 1.1	0.03 ± 0.30	0.77
Risk Ratio	3.3 ± 1.0	3.4 ± 0.8	0.04 ± 0.60	0.83
Hematology				
Hemoglobin (g/dL)	14.6 ± 11.1	14.2 ± 12.0	-3.4 ± 6.0	0.11
White Blood Cells (10^9/L)	6.0 ± 2.4	5.9 ± 2.3	-0.1 ± 1.2	0.74
Platelets (10^9/L)	198 ± 74	236 ± 58	38 ± 65	0.10
Red Blood Cells (10^{12}/L)	4.6 ± 0.5	4.5 ± 0.5	-0.06 ± 0.20	0.21
Hematocrit (L)	0.40 ± 0.03	0.40 ± 0.03	-0.005 ± 0.010	0.29
Neutrophils (10^9/L)	3.5 ± 1.6	3.4 ± 1.3	-0.1 ± 0.9	0.72
Lymphotcytes (10^9/L)	1.7 ± 0.7	1.7 ± 0.7	0.0 ± 0.2	1.00
Monocytes (10^9/L)	0.5 ± 0.2	0.6 ± 0.3	0.05 ± 0.20	0.45
Eosinophils (10^9/L)	0.1 (0.1,0.2)	0.1 (0.1,0.3)	-0.07 ± 0.30	0.69
Basophils (10^9/L)	0	0	0.01 ± 0.03	-
Biomarkers of Systemic Inflammation				
C-Reactive Protein (mg/L)	2.2 (1.1,3.3)	1.9 (1.5,2.9)	-0.2 ± 3.5	0.78
IL-6 (pg/ml)	0.9 (0.6,3.9)	0.6 (0.5,3.2)	-0.2 ±0.9	0.26
IL-8 (pg/ml)	15.9 ± 9.0	12.6 ± 6.4	-3.5 ± 3.9	0.36
TNF-α (pg/ml)	13.7 ± 7.3	12.7 ± 7.3	-1.1 ±1.9	0.77
IL-10 (pg/ml)	2.2 ± 1.7	2.0 ± 0.9	-0.2 ± 1.6	0.77

Normally distributed data presented as mean ± SD, non-normally distributed variables reported as
median (interquartile range [IQR]). *Abbreviations* HDL, high density lipoprotein; LDL, low
density lipoprotein; IL-6, interleukin- 6; IL-8, interleukin-8; TNF- α, tumor transcription factor
αlpha; IL-10, interleukin-10.

2.3.6 The Effects of Exercise Training on Cardiorespiratory Responses to Exercise in Patients with COPD

All cardiopulmonary responses to exercise following 8 weeks of aerobic exercise training in COPD are presented in Table 2.5. No significant changes were observed in BMI, or resting HR, SBP, SpO_2, IC, dyspnea and leg fatigue, from pre to post exercise training (p>0.05). However, resting DBP was significantly reduced (79 ± 8 mmHg vs. 73 ± 8 mmHg, p=0.05).

At maximal exercise, there was a significant increase in relative (18.0 ± 4.6 ml/kg/min to 19.8 ± 3.6 ml/kg/min, p<0.01) and absolute VO_{2peak} (1.3 ±0.4 L/min vs. 1.5 ± 0.3 L/min, p<0.01). A significant reduction was observed in peak SpO_2 (94 ± 3 % vs. 91 ± 5 %, p<0.01) and peak IC (2.0 ± 0.5 L vs. 1.8 ± 0.5 L, p=0.02) following exercise training. Respiratory exchange ratio (1.07 ± 0.1 vs. 1.00 ± 0.1, p=0.05), peak dyspnea (6 ± 2 Borg units vs. 4 ± 1 Borg units, p<0.01), and peak leg fatigue (7 ± 2 Borg units vs. 4 ± 1 Borg units, p<0.01) were also significantly reduced, while peak SBP was increased (175 ± 8 mmHg vs. 190 ± 11 mmHg, p<0.01). There were no significant differences in all other cardiopulmonary variables following exercise training (Table 2.5).

Table 2.5- Changes in Cardiopulmonary Responses to Exercise Following 8 Weeks of Exercise Training in Patients with COPD

Variables	Pre Training	Post Training	Change Scores	*P*
Resting Values				
BMI (kg.m^{-2})	26 ± 3	27 ± 3	0.4 ± 1.5	0.46
Heart rate (beats.min^{-1})	90 ± 11	87 ± 13	-3 ± 14	0.54
Systolic blood pressure (mmHg)	130 ± 8	130 ± 7	0.3 ± 12.0	0.93
Diastolic blood pressure (mmHg)	79 ± 8	73 ± 8	-6 ± 8	0.05
SpO_2 (%)	97 ± 1	96 ± 1	-1 ± 2	0.07
Inspiratory capacity (L)	2.3 ± 0.4	2.2 ± 0.5	-0.2 ± 0.4	0.13
Dyspnea (Borg units)	0	0 ± 1	0.0 ± 0.4	0.08
Leg Fatigue (Borg units)	0	0	0 ± 0.5	-
Peak Exercise Responses				
Heart rate (bpm)	135 ± 16	137 ± 20	2 ± 13	0.59
Systolic blood pressure (mmHg)	175 ± 8	190 ± 11	15 ± 8	<0.01
Diastolic blood pressure (mmHg)	80 ± 9	73 ± 12	-7 ± 13	0.11
SpO_2 (%)	94 ± 3	91 ± 5	-3 ± 3	<0.01
ΔSpO_2 (%)	-4 ± 2	-5 ± 3	-2 ± 3	0.09
VO_{2peak} relative (ml.kg.min^{-1})	18.0 ± 4.6	19.8 ± 3.6	1.8 ± 1.2	<0.01
VO_{2peak} relative (% pred)	81 ± 23	89 ± 23	8 ± 6	<0.01
VO_{2peak} absolute (L.min^{-1})	1.3 (1.1,1.4)	1.4 (1.3,1.5)	0.2 ± 0.1	0.01
VO_{2peak} absolute (% pred)	74 ± 19	82 ± 18	8 ± 7	<0.01
Watts	80 (65, 90)	80 (70, 90)	4 ± 23	0.83
Watts (% pred)	71 ± 23	79 ± 40	8 ± 23	0.29
Minute ventilation (L.min^{-1})	50.8 ± 13.5	51.9 ± 15.3	1.1 ± 5.6	0.55
Minute ventilation (% pred)	91 ± 18	92 ± 15	1.0 ± 9.9	0.77
Tidal volume (L)	1.5 ± 0.3	1.5 ± 0.3	-0.03 ± 0.20	0.58
Tidal Volume (% pred)	100 ± 20	101 ± 18	1 ± 16	0.80
Respiratory rate (breaths.min^{-1})	31 ± 6	34 ± 4	2 ± 6	0.28
V_D/V_T (L)	0.20 ± 0.07	0.20 ± 0.06	0.002 ± 0.030	0.82
V_D/V_T (% pred)	112 ± 37	112 ± 32	-0.2 ± 16	0.97
Inspiratory capacity (L)	2.3 ± 0.5	1.8 ± 0.4	-0.2 ± 0.3	0.03
Δ Inspiratory capacity (L)	0.3 ± 0.4	0.3 ± 0.4	0.01 ± 0.40	0.93
End tidal CO_2 (mmHg)	36 ± 6	36 ± 8	0.2 ± 4.0	0.87
End tidal O_2 (mmHg)	106 ± 6	105 ± 9	-1.5 ± 5.0	0.36

Variables	Pre Training	Post Training	Change Scores	P
Respiratory exchange ratio	1.07 ± 0.05	1.03 ± 0.06	-0.04 ± 0.07	0.15
O_2 Pulse ($LO_2.beat^{-1}$)	9.9 ± 2.4	10.9 ± 2.2	1.0 ± 1.7	0.10
Dyspnea (Borg units)	6 ± 2	4 ± 1	-2 ± 2	<0.01
Leg Fatigue (Borg units)	7 ± 2	4 ± 1	-3 ± 2	<0.01

Normally distributed data presented as mean ± SD, non-normally distributed variables reported as median (interquartile range [IQR]). *Abbreviations*: SpO2, oxyhemaglobin saturation; ΔSpO_2, change in oxyhemaglobin saturation max to rest; Δ Inspiratory capacity , change in inspiratory capacity. V_D/V_T, ratio of dead space to tidal volume.

2.3.7 The Effects of Exercise Training on Vascular Structure and Function in COPD Patients Versus Healthy Controls

The effects of exercise training on vascular structure and function in COPD patients compared to healthy controls can be found in Table 2.6, and Figures 2.3, 2.4, and 2.5, panel A. Baseline central PWV was significantly higher in the COPD compared to the controls (10.9 ± 1.5 vs. 8.9 ± 2.1 m/sec, p=0.05). No significant differences were observed between COPD and controls for all other measurements of vascular structure and function at baseline. There were no significant changes in flow mediated dilation, arterial stiffness or carotid IMT in the controls following exercise training. Also, no significant differences were found between COPD and control for the change in any dependent variable from pre to post training.

2.3.8 Effects of Exercise Training on Blood Lipids, Hematology and Biomarkers of Inflammation in Patients with COPD Versus Healthy Controls

No significant differences were observed between COPD and controls for baseline blood lipids, hematology and biomarkers of systemic inflammation. There were also no significant changes in any variables in the controls following exercise. Additionally, no differences were observed between COPD and control for changes in any hematological markers with exercise training (p>0.05; Table 2.7).

Table 2.6- Changes in Vascular Structure and Function Following 8 Weeks of Exercise Training in COPD Patients Versus Healthy Controls

Variable	Control			COPD			Diff Between Groups	
	Baseline	8 weeks	P	Baseline	8 weeks	P	Mean Change	P
Flow Mediate Dilation								
Baseline Diameter (cm)	0.45 ± 0.10	0.4 5± 0.10	0.90	0.46 ± 0.06	0.47 ± 0.10	0.67	0.0	0.77
Peak Diameter (cm)	0.46 ± 0.10	0.47 ± 0.10	0.44	0.48 ± 0.06	0.50 ± 0.11	0.60	0.0	0.86
Peak Dilation (%)	4.4 ± 2.4	5.7 ± 2.6	0.30	5.0 ± 2.0	5.6 ± 2.5	0.40	0.7	0.60
Average Shear Rate (1/s)	334 ± 169	351 ± 100	0.74	253 ± 170	252 ± 134	1.00	17.6	0.80
SRAUC (ttp)	17030 ± 8122	18616 ± 4437	0.76	14589 ± 13045	15765 ± 7790	0.77	408.8	0.95
Time to Peak Dilation (s)	57 ± 33	58 ± 26	0.97	51 ± 24	68 ± 21	0.18	-14.3	0.52
Arterial Stiffness								
Central PWV (m/s)	8.9 ± 2.1	8.4 ± 1.5	0.57	10.9 ± 1.5	11.4 ± 3.3	0.54	-1.0	0.42
Peripheral PWV (m/s)	7.6 ± 2.2	6.0 ± 2.1	0.24	7.6 ± 2.5	6.8 ± 1.8	0.03	-0.8	0.44
Carotid Pulse Pressure (mmHg)	38.3 ± 7.6	34.5 ± 10.0	0.55	34.1 ± 7.8	37.9 ± 8.5	0.25	-7.7	0.23
Carotid Compliance (mm²/mmHg)	0.09 ± 0.05	0.10 ± 0.09	0.47	0.10 ± 0.06	0.1 ± 0.8	0.72	0.0	0.40
Carotid Distensibility (mm/mmHg)	0.003 ± 0.001	0.003 ± 0.002	0.35	0.004 ± 0.002	0.004 ± 0.003	0.85	0.0	0.64
B Stiffness Index	9.1 ± 3.4	8.6 ± 3.4	0.61	7.6 ± 4.6	10.4 ± 12.0	0.28	-3.3	0.35
Carotid Intima Media Thickness								
IMT Average (mm)	0.67 ± 0.19	0.70 ± 0.12	0.45	0.77 ± 0.13	0.74 ± 0.12	0.26	0.1	0.19
IMT Maximum (mm)	0.74 ± 0.23	0.77 ± 0.15	0.50	0.87 ± 0.15	0.84 ± 0.13	0.27	0.1	0.21
IMT Minimum (mm)	0.59 ± 0.15	0.62 ± 0.11	0.36	0.6 4± 0.11	0.63 ± 0.11	0.75	0.0	0.35

Normally distributed data presented as mean ± SD, non-normally distributed variables reported as median (interquartile range [IQR]). *Abbreviations:* SRAUC, shear rate area under the curve; PWV, pulse wave velocity. Mean change is presented as controls – COPD.
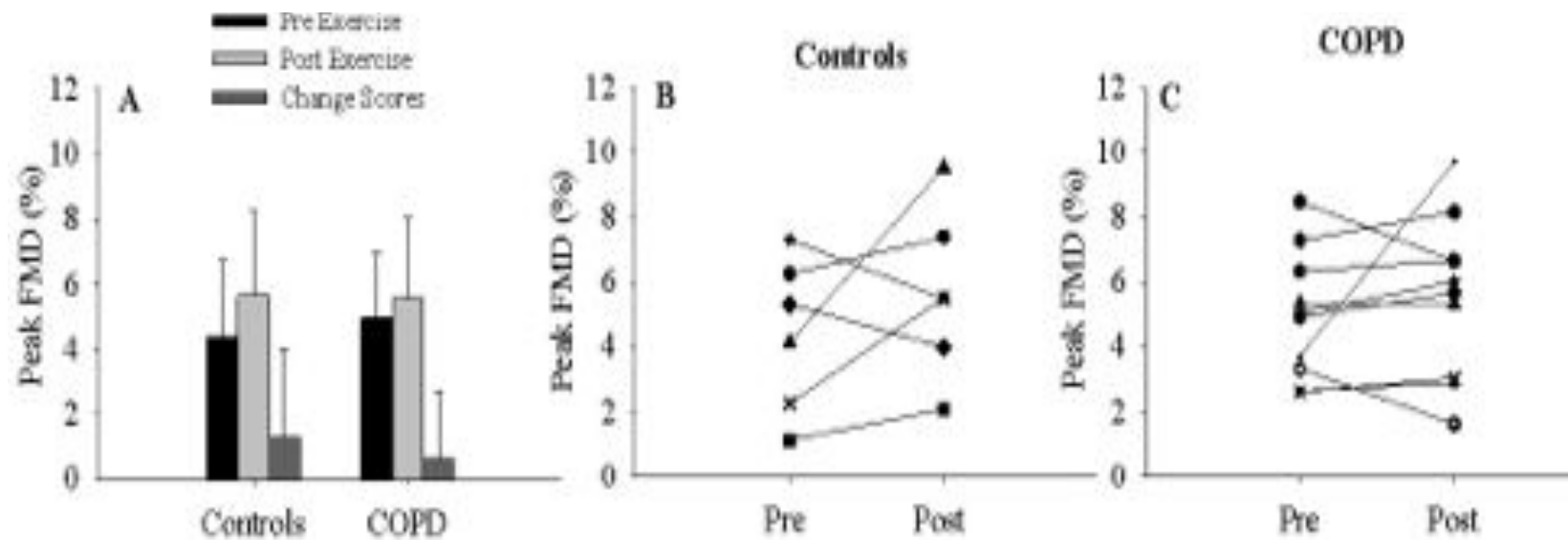
Figure 2.3- Changes in peak flow mediated dilation (FMD) following 8 weeks of exercise training in COPD and healthy controls. (A) There were no changes in COPD or healthy controls following exercise training. (B) Individual changes in peak flow mediated dilation pre to post exercise training in healthy controls. (C) Individual changes in peak flow mediated dilation pre to post exercise training in COPD.
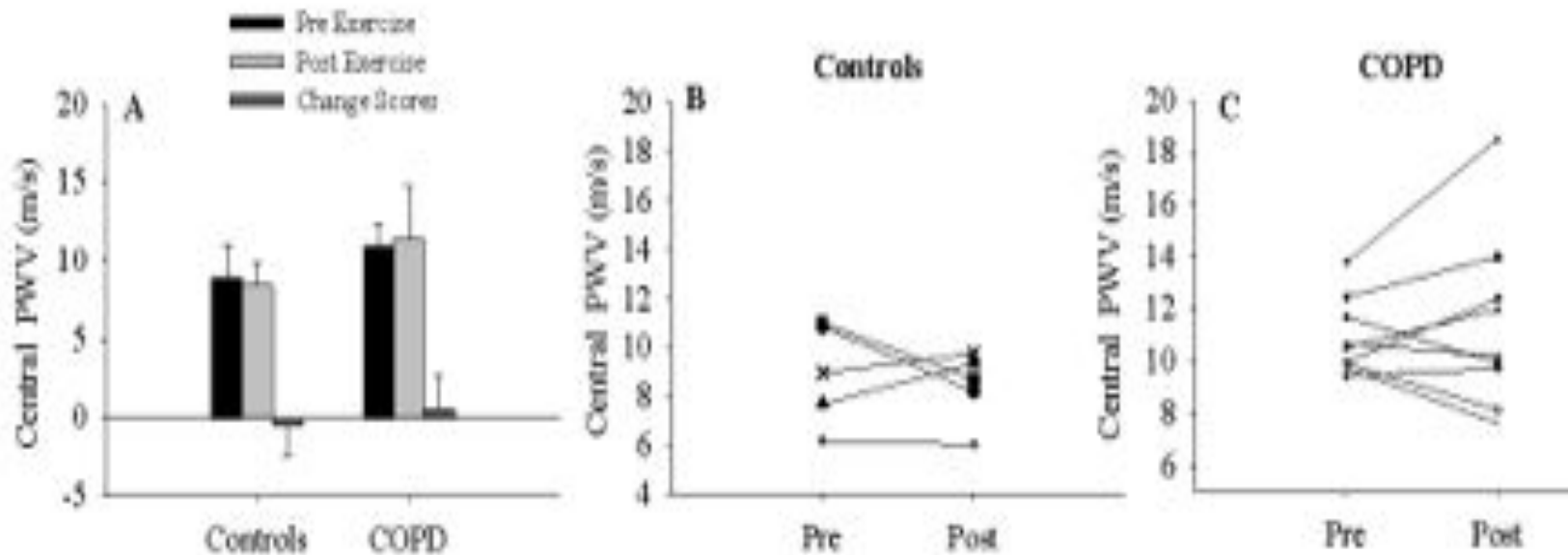
Figure 2.4- Changes in central pulse wave velocity (PWV) following 8 weeks of exercise training in COPD and healthy controls. (A) There were no changes in COPD or healthy controls following exercise training. (B) Individual changes in central pulse wave velocity pre to post exercise training in healthy controls. (C) Individual changes in central pulse wave velocity pre to post exercise training in COPD.
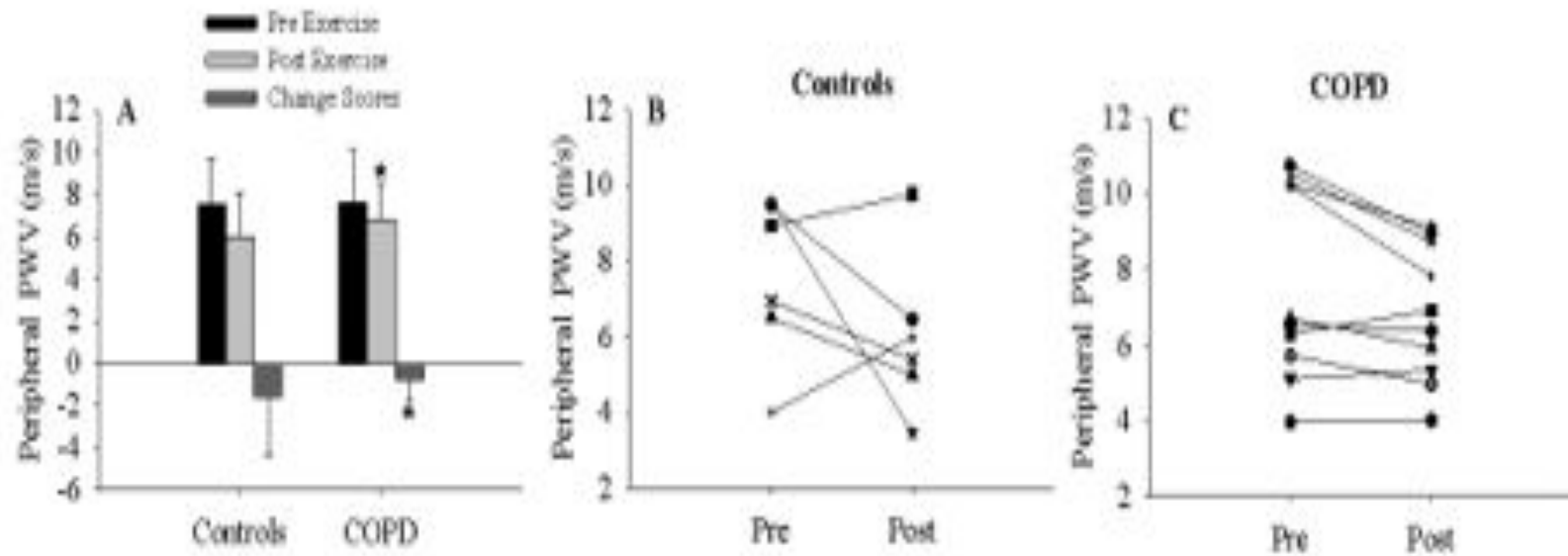
Figure 2.5- Changes in peripheral pulse wave velocity (PWV) following 8 weeks of exercise training in COPD and healthy controls. (A) There was a significant reduction in peripheral PWV in COPD, with no change in the healthy controls following exercise training. (B) Individual changes in peripheral pulse wave velocity pre to post exercise training in healthy controls. (C) Individual changes in peripheral pulse wave velocity pre to post exercise training in COPD.

Table 2.7- Changes in Blood Lipids, Hematology, and Biomarkers of Systemic Inflammation Following 8 Weeks of Exercise Training in COPD Patients Versus Healthy Controls

Variable	Control			COPD			Diff Between Groups	
	Baseline	8 weeks	P	Baseline	8 weeks	P	Mean Change	P
Blood Lipids								
Fasting Blood Glucose (mmol/L)	5.5 ± 0.5	5.5 ± 0.6	0.88	5.2 ± 0.6	5.2 ± 0.4	0.94	0.0	0.86
Cholesterol (mmol/L)	5.8 ± 1.4	6.1 ± 2.0	0.40	5.6 ± 1.5	5.5 ± 1.3	0.56	0.4	0.22
Triglycerides (mmol/L)	1.3 ± 0.7	1.2 ± 0.2	0.64	1.4 ± 1.0	1.3 ± 0.5	0.72	0.0	0.94
HDL (mmol/L)	1.5 ± 0.3	1.7 ± 0.5	0.25	1.7 ± 0.4	1.7 ± 0.4	0.44	0.2	0.14
LDL (mmol/L)	3.7 ± 1.2	4.0 ± 1.5	0.33	3.2 ± 1.2	3.2 ± 1.1	0.77	0.2	0.35
Risk Ratio	3.9 ± 0.6	3.7 ± 0.3	0.37	3.3 ± 1.0	3.4 ± 0.8	0.83	-0.3	0.39
Hematology								
Hemoglobin (g/dL)	14.3 ± 15.0	14.3 ± 9.4	0.88	14.6 ± 11.1	14.2 ± 12.0	0.11	2.9	0.41
Hematocrit (L)	0.40 ± 0.03	0.40 ± 0.02	0.08	0.40 ± 0.03	0.40 ± 0.03	0.29	0.0	0.34
White Blood Cells (10^9/L)	5.7 ± 1.5	5.8 ± 1.6	0.78	6.0 ± 2.4	5.9 ± 2.3	0.74	0.2	0.68
Neutrophils (10^9L)	3.2 ± 1.2	3.3 ± 1.2	0.72	3.5 ± 1.6	3.4 ± 1.3	0.72	0.2	0.64
Lymphocytes (10^9L)	1.9 ± 0.4	1.9 ± 0.4	0.80	1.7 ± 0.7	1.7 ± 0.7	1.00	-0.1	0.77
Monocytes (10^9L)	0.4 ± 0.1	0.4 ± 0.1	0.47	0.5 ± 0.2	0.6 ± 0.3	0.45	0.0	0.85
Eosinophils (10^9L)	0.1 ± 0.1	0.2 ± 0.2	0.36	0.3 ± 0.4	0.2 ± 0.2	0.69	0.1	0.38
Systemic Inflammation								
C-Reactive Protein (mg/L)	1.5 ± 0.7	2.2 ± 1.7	0.33	2.9 ± 3.1	2.7 ± 2.7	0.78	0.9	0.58
IL-6 (pg/ml)	0.8 (0.6, 1.5)	0.8 (0.4, 1.5)	0.60	0.9 (0.6, 3.9)	0.6 (0.5, 3.2)	0.26	-0.1	0.84
IL-8 (pg/ml)	16.3 ± 4.3	15.7 ± 3.8	0.78	15.9 ± 9.0	12.6 ± 6.4	0.36	-2.7	0.16
TNF-α (pg/ml)	19.1 ± 7.8	19.8 ± 8.2	0.77	13.7 ± 7.3	12.7 ± 7.3	0.77	-1.7	0.58
IL-10 (pg/ml)	2.8 ± 2.6	1.6 ± 1.1	0.31	2.2 ± 1.7	2.0 ± 0.9	0.77	1.0	0.22

Normally distributed data presented as mean ± SD, non-normally distributed variables reported as median (interquartile range [IQR]). *Abbreviations:* HDL, high density lipoprotein; LDL, low density lipoprotein; IL-6, interleukin- 6; IL-8, interleukin-8; TNF- α, tumor transcription factor α; IL-10, interleukin-10.

2.3.9 The Effects of Exercise Training on Cardiorespiratory Responses to Exercise in Patients with COPD versus Healthy Controls

2.3.9.1 Comparison of Pre-Training Variables in COPD versus Healthy Controls

All cardiorespiratory responses to exercise in patients with COPD versus healthy controls can be found in Table 2.8. Baseline resting diastolic blood pressure (87 ± 7 mmHg, vs. 79 ± 8 mmHg, p=0.04) and resting inspiratory capacity (3.0 ± 0.6L vs. 2.3 ± 0.4L, p=0.02) were lower in COPD compared to controls. At peak exercise, the COPD patients had a lower heart rate max (153 ± 8 beats.min^{-1} vs. 135 ± 16 beats.min^{-1}, p=0.03), systolic blood pressure max (204 ± 43 mmHg vs. 175 ± 8mmHg, p=0.02), S_pO_{2max} (97 ± 2% vs 94 ± 3%, p=0.05), tidal volume (2.3 ± 0.7L vs 1.5 ± 0.3L, p<0.01) maximum inspiratory capacity (3.4 ± 0.9L vs 2.5 ± 0.5L, p<0.01) and respiratory exchange ratio (1.18 ± 0.14 vs 1.07 ± 0.05, p=0.03) compared to the controls. The COPD patients also had a significantly higher minute ventilation % predicted (55 ± 10% vs. 91 ± 18%, p=0.01) and V_D/V_T (0.1 ± 0.04 L vs. 0.2 ± 0.07 L, p=0.01) , V_D/V_T % predicted (61 ± 26% vs. 112 ± 37%, p=0.01) and change in S_pO_2 (-1 ± 2% vs. -4 ± 2%, p=0.04) compared to controls.

2.3.9.2 Effects of Exercise Training in Healthy Controls

There were no significant differences in resting values in the control group pre vs. post exercise training. During peak exercise, there was a significant increase in VO_{2peak} relative (19.8 ± 2.6 ml/kg/min vs. 26.0 ± 4.1ml/kg/min, p=0.03), VO_{2peak} absolute (1.53 ± 0.39 L vs. 2.00 ± 0.48 L, p=0.02), peak power output (102 ± 23 vs. 128 ± 22, p<0.01), minute ventilation (59.5 ± 6 L/min vs. 70.9 ± 9.8 L/min, p=0.03) and O_2 Pulse (10.7 ± 2.8 L O_2/beat vs. 13.4 ± 2.9 LO_2/beat, p=0.01).

2.3.9.3 Differences Between COPD and Healthy Controls

There were no significant differences in change scores between COPD patients and the control group. At peak exercise, leg fatigue was significantly different (3 Borg units, p=0.03), and there was a significant reduction in SBP in the COPD patients compared to the controls (-12.4 mmHg, p=0.01). There was also a significant reduction peak SpO_2 in the COPD versus controls (3.4%, p=0.02).

2.3.10 Regressions

2.3.10.1 The Relationship Between Baseline Variables in COPD

A significant relationship was found between baseline carotid IMT and baseline central PWV (r=0.17, p=0.02), as well as baseline relative VO_{2peak} and baseline IL-10 (r=0.76,p=0.02). No significant relationship was observed between FEV_1 % predicted and baseline FMD % peak (r=0.31, p=0.37). All other relationships were non-significant.

2.3.10.2 The Relationship Between Baseline and Change Scores in COPD Following Exercise

There was a significant relationship between baseline relative VO_{2peak} to both change in relative VO_{2peak} following exercise training (r=0.86, p<0.01), and total training volume (r=0.700, p=0.02). All other variables were non-significant.

2.3.10.3 The Relationship Between Change Scores in COPD Following Exercise

There was a significant relationship between the change in peripheral PWV and change in FMD% peak following exercise (r=0.76, p=0.01) as well as between change in relative VO_{2peak} and total training volume (r=0.8, p<0.01). All other variables were non-significant.

2.3.10.4 The Relationship Between Baseline Variables in COPD and Healthy Controls

When combining the control and COPD patient's baseline values, a significant relationship was also observed between baseline FMD% peak baseline and peripheral PWV (r=0.57, p=0.03). A significant relationship was also found between baseline relative VO_{2peak} and the total training volume performed in the study (r=0.75, p<0.01). All other variables were non-significant.

2.3.10.5 The Relationship Between Change Scores Between COPD and Healthy Controls Following Exercise

A significant relationship was found between the change in IL-10 and change in β stiffness index (r=0.72, p=0.02) and change in IL-6 and change in central PWV (r=0.58, p=0.04) following exercise training. All other variables were non-significant.

Table 2.8- Changes in Cardiopulmonary Responses to Exercise Following 8 Weeks of Exercise Training in COPD Patients Versus Healthy Controls

Variable	Control			COPD			Diff Between Groups	
	Baseline	8 weeks	P	Baseline	8 weeks	P	Mean Change	P
Resting Values								
BMI (kg.m^{-2})	27 ± 4	27 ± 4	0.75	26 ± 3	27 ± 3	0.46	-0.4	0.50
Heart rate (beats.min^{-1})	88 ± 12	77 ± 8	0.07	90 ± 11	87 ± 13	0.54	-7.5	0.27
Systolic blood pressure (mmHg)	137 ± 13	129 ± 19	0.06	130 ± 8	130 ± 7	0.93	-8.3	0.16
Diastolic blood pressure (mmHg)	87 ± 7	85 ± 12	0.46	79 ± 8	73 ± 8	0.05	3.2	0.48
SpO$_2$ (%)	98 ± 1	98 ± 1	0.44	97 ± 1	96 ± 1	0.07	0.4	0.64
Inspiratory Capacity (L)	3.0 ± 0.6	3.2 ± 0.9	0.50	2.3 ± 0.4	2.2 ± 0.5	0.13	0.3	0.13
Dyspnea (Borg units)	0	0	-	0	0 ± 1	0.08	-0.2	0.32
Leg Fatigue (Borg units)	0.5 ± 0.5	0	0.08	0	0	1.00	-0.5	0.07
Peak Exercise Responses								
Heart rate (beats.min^{-1})	153 ± 8	153 ± 11	0.89	135 ± 16	137 ± 20	0.59	-6.3	0.35
Systolic blood pressure (mmHg)	204 ± 34	207 ± 27	0.51	175 ± 8	190 ± 11	<0.01	-12.4	0.01
Diastolic blood pressure (mmHg)	87 ± 8	83 ± 14	0.38	80 ± 9	73 ± 12	0.11	4.2	0.49
SpO$_2$ (%)	97 ± 2	98 ± 1	0.70	94 ± 3	91 ± 5	0.01	3.4	0.02
Δ SpO$_2$ (%)	-1 ± 2	-1 ± 1	0.58	-4 ± 2	-5 ± 3	0.09	1.8	0.12
VO$_{2peak}$ relative (ml.kg.min^{-1})	19.8 ± 2.6	26.0 ± 4.1	0.03	18.0 ± 4.6	19.8 ± 3.6	<0.01	4.5	<0.01
VO$_{2peak}$ relative (% pred)	84 ± 11	109 ± 18	0.02	81 ± 23	89 ± 23	<0.01	16.7	<0.01
VO$_{2peak}$ absolute (L.min^{-1})	1.53 ±0.39	2.00 ± 0.48	0.02	1.33 ± 0.36	1.47 ± 0.30	0.01	0.3	<0.01
Watts	102 ± 23	128 ± 22	<0.01	83 ± 26	88 ± 31	0.83	22.1	0.05
Watts (% pred)	79 ± 16	101 ± 23	<0.01	71 ± 23	79 ± 40	0.29	14.2	0.21
Minute ventilation (L.min^{1})	59.5 ± 6.0	70.9 ± 9.8	0.03	50.8 ± 13.5	51.9 ± 15.3	0.55	10.3	0.01
Minute ventilation (%pred)	55 ± 10	63 ± 15	0.05	91 ± 18	92 ± 15	0.77	7.2	0.17

Table 2.8 Continued

Variable	Control			COPD			Diff Between Groups	
Tidal volume (L)	Baseline	8 weeks	P	Baseline	8 weeks	P	Mean Change	P
V_D/V_T (L)	2.3 ± 0.7	2.4 ± 0.4	0.70	1.5 ± 0.3	1.5 ± 0.3	0.58	0.1	0.49
V_D/V_T (% pred)	0.10 ± 0.04	0.10 ± 0.02	0.48	0.20 ± 0.07	0.20 ± 0.06	0.82	0.0	0.39
Inspiratory capacity (L)	61 ± 26	52 ± 12	0.39	112 ± 37	112 ± 32	0.97	-9.6	0.36
Δ Inspiratory capacity (L)	3.4 ± 0.9	3.4 ± 0.5	0.55	2.3 ± 0.5	1.8 ± 0.4	0.03	0.1	0.48
End tidal CO_2 (mmHg)	-0.4 ± 0.5	-0.2 ± 0.5	0.62	0.3 ± 0.4	0.3 ± 0.4	0.93	0.2	0.60
End tidal O_2 (mmHg)	36 ± 4	37 ± 4	0.74	36 ± 6	36 ± 8	0.87	0.2	0.91
Respiratory exchange ratio	107 ± 7	108 ± 7	0.83	106 ± 6	105 ± 9	0.36	2.0	0.45
O_2 Pulse ($LO_2.beat^{-1}$)	1.18 ± 0.14	1.15 ± 0.14	0.08	1.07 ± 0.05	1.03 ± 0.06	0.15	0.0	0.98
Dyspnea (Borg units)	10.7 ± 2.8	13.4 ± 2.9	0.01	9.9 ± 2.4	10.9 ± 2.2	0.10	1.7	0.07
Leg Fatigue (Borg units)	5 ± 2	4 ± 1	0.33	6 ± 2	4 ± 1	<0.01	1.3	0.13
	5 ± 2	5 ± 2	0.26	7 ± 2	4 ± 1	<0.01	2.0	0.03

Normally distributed data presented as mean ± SD, non-normally distributed variables reported as median (interquartile range [IQR]). *Abbreviations*: SpO2, oxyhemaglobin saturation; ΔSpO2, change in oxyhemaglobin saturation max to rest; Δ Inspiratory capacity, change in inspiratory capacity. V_D/V_T, ratio of dead space to tidal volume. Mean change is presented as controls – COPD.

2.4 Discussion

This is the first study to comprehensively examine the effects of aerobic exercise training on vascular structure and function in patients with COPD. Contrary to our primary hypothesis, endothelial dependent vasodilation was not altered following 8-weeks of aerobic exercise. Our secondary hypothesis was partially supported as peripheral PWV was reduced following exercise training while endothelial independent dilation remained unaltered. However, all other markers of arterial stiffness (central PWV, β stiffness index, carotid IMT) and all biomarkers of systemic inflammation were not significantly changed with exercise training. These results indicate that 8 weeks of aerobic exercise training may not be of sufficient length or intensity to elicit beneficial changes in vascular structure and function or reduce systemic inflammation in patients with COPD.

2.4.1 The Effect of 8-weeks of Aerobic Exercise Training on Changes in Vascular Function in Patients with COPD

In the present study there were no significant changes observed in endothelial dependent or independent vasodilation following 8 weeks of aerobic training. No previous study has investigated whether exercise of any kind can influence endothelial function in patients with COPD. However, in healthy older adults habitual exercise and aerobic training interventions of 8-12 weeks in duration have been shown to improve vasodilator function. [102,121,122] In a group of middle to older aged men who participated in a 12-week moderate intensity (60-75% heart rate max) exercise training program, forearm blood flow response to ACh was increased by 30%. [121] Furthermore, others have reported that tonic NO production is enhanced in older men who regularly perform aerobic exercise, [124] and FMD is improved 50% in previously sedentary older men who participated in an 8-week

moderate intensity walking intervention. [123] These studies have shown that vascular function is improved on a global scale in older adults who partake in aerobic exercise as the vasodilator response to the infusion of the NO agonist ACh, as well as to the shear stress stimulus elicited by FMD is maintained or improved.

Our findings suggest that 8 weeks of structured aerobic exercise training may not be a strong enough stimulus to elicit a change in endothelium dependent and independent mediated vasodilation in COPD. Additionally, only 2 individuals had a clinically relevant improvement in FMD of 1% or greater which has been associated with a 13% reduction in cardiovascular risk (Figure 2.3, panels A and C). [81] Baseline diameter did not change following exercise indicating that we were also unable to induce structural adaptations to normalize shear stress in response to repetitive exposure to increased flow. The exact time course of vascular remodeling is yet to be elucidated and is thought to be dependent upon the size and location of the vessel as well as the stimulus involved. [145,175] The present data would suggest that either exercise cannot improve endothelial function in patients with COPD or that 8-weeks is not long enough in duration to elicit structural adaptations in this patient population. However, it is also cannot be ruled out that adaptations that occurred during the first month of training may have been negated by the high intensity training performed in the 2nd month. Varying training stimuli such as high intensity interval training versus moderate intensity steady state have been shown to elicit different responses in vascular function primarily mediated through changes in oxidative/anti-oxidative capacities. [131,132] COPD is associated with increased resting levels of ROS and reduced anti-oxidant capacity [55] and high intensity exercise may have

acutely exacerbated this problem, resulting in a less than optimal milieu for vascular adaptation to occur.

Studies that have examined the effects of exercise training in other chronic conditions that also present with increased levels of inflammation, have found a significant improvement in the vasodilator response to aerobic exercise training. In patients with chronic heart failure, 4 weeks of cycle training performed 6 times/day for 10mins at 80% HRmax, improved ACh in the brachial artery and FMD response in the radial artery. [176] The same training program performed for 6 months improved ACh and tonic NO release as assessed by L-NMMA infusion in the femoral artery. [177] Furthermore, 8 weeks of circuit training consisting of alternating between resistance exercises (45sec) and cycling (45sec) performed for 1hour 3times/week increased forearm blood flow to ACh. [178] While 8 weeks of a combined aerobic and resistance circuit training program performed 3 days/week for 30-45 min, increased brachial FMD by 2.7%. [153] Similar improvements have also been reported in patients with coronary artery disease. [177] Although the exercise interventions and methodology in assessing vascular function in these studies do not exactly mimic ours, these findings support the notion that vascular function can be improved in other chronic conditions characterized by increased levels of systemic inflammation. This suggests that COPD may be unique in that the level of systemic inflammation may be too great or cannot be sufficiently reduced with 8 weeks of aerobic exercise training. One big difference in COPD compared to other heart diseases is that the chronic lung inflammation that is primarily responsible for COPD may continually contribute to the systemic inflammation that has been linked to the

endothelial dysfunction in these patients. As such if 8-weeks of exercise cannot reduce lung inflammation it may not be able to affect "downstream" vascular function.

This is not to say that the vascular endothelium in COPD may not be amenable to the benefits of exercise training in longer duration exercise programs. Although, not clinically relevant we observed that 7 out of the 10 (70%) patients had an improvement in FMD % peak following exercise training compared to 3 patients who showed no change or a reduction in FMD peak (Figure 2.3, panel C). These observations support that in the majority of COPD patients, the endothelium is able to improve following training but these changes were relatively small. We choose 8 weeks of aerobic training, as 6-12 weeks is the typical length of current pulmonary rehabilitation programs and as such, may not be long enough to reduce cardiovascular disease risk through modification of vascular function in the COPD population.

Unlike previous studies,[148-150] the current study found no relationship between FMD % peak and FEV_1 % predicted (r=0.31, p=0.37), suggesting that endothelial dysfunction does not necessarily get worse as disease severity advances. [142-144] Additionally, these previous studies also reported that COPD patients had impaired endothelial dependent and independent function. [142-144] However, all of these previous studies have inherent methodological limitations. In a study performed by Eickhoff et al., [143] cuff placement was situated above the ultrasound probe in which the FMD response has been shown to be predominantly mediated by pathways other than NO. [80] Another study performed Barr and colleagues [142] did not measure endothelial independent dilation and therefore reductions in FMD could be localized to the endothelium alone with little or no change in smooth muscle function. Finally, Moro et al., [144] included COPD patients

with co-morbidities such as diabetes, cerebrovascular disease and coronary artery disease, which have also been associated with vascular dysfunction so the vascular dysfunction specific to COPD alone cannot be determined.

The endothelium is highly influenced by other regulating mechanisms that may play a role in the vasodilatory capacity of blood vessels in COPD patients. The vasodilatory response measured by FMD is thought to be primarily NO mediated however with increasing age and potentially exercise, other vasoactive pathways of the endothelium become activated [179,180] (ie. PGI2 and EDHF) and may play a larger role in mediating vasodilation and therefore may account for our unchanged FMD response. Also increased circulating levels of ROS are known to affect vascular function. [35] The maintained or improved vasodilator response in older adults who regularly partake in aerobic exercise is thought to be mediated by enhanced NO bioavailability and secondary to reduced oxidative stress. Infusions of vitamin C (an antioxidant) as well as acute administrations of BH_4 improved endothelial dependent dilation in sedentary but not endurance trained older adults. [102,124] Also, older adults who habitually exercise have a higher total oxyradical scavenging capacity and a related increase in FMD when compared to sedentary controls. [103] These findings suggest that we may not have been able to change the oxidant/anti-oxidant capacity in our COPD patients following 8 weeks of training, therefore leading to no observed changes in FMD.

2.4.2 The Effect of 8-weeks of Aerobic Exercise Training on Changes in Vascular Function in Patients with COPD and Healthy Controls

No significant differences were observed in endothelial dependent dilation measured at baseline, and there was no difference in the change in FMD with exercise training in the COPD patients compared to controls. These findings suggest that vascular function is not impaired in COPD patients greater than that normally associated with healthy aging. Our findings are support by one study performed by Maclay et al., in which no difference in endothelial dependent and independent dilation was found between COPD patients and controls assessed by the gold standard technique of venous plethysmography. [138] Furthermore, it is suggested that COPD patients may respond in a similar way to exercise training as healthy controls as 4 out of 6 controls (67%) had an improvement in FMD % peak, which was similar to 7 out of 10 (70%) in patients with COPD (Figure 2.3, panels B and C). One postulation for this similarity between groups may be due to the fact that our healthy controls were classified as sedentary (did not participate in a regimented exercise program in the last 6 months and were physically active <2days/week), prior to entering the study, and therefore did not have any enhanced vascular function associated with chronic physical activity/ exercise. Also, our COPD cohort consisted of those ranging from mild-to-severe disease. It may be possible that those patients with mild COPD respond similarly to exercise training as sedentary healthy age-matched controls. However, there was not a significant correlation observed between FMD% change and disease severity in the COPD group ($r=0.31$, $p=0.37$) suggesting that responses to exercise training may be similar across different disease severities but this needs to be accurately assessed in a larger cohort of patients.

2.4.3 The Effect of 8-weeks of Aerobic Exercise Training on Changes in Arterial Stiffness in Patients with COPD

Peripheral PWV significantly decreased in COPD patients following 8 weeks of aerobic exercise training; however there were no significant changes in any other measure of arterial stiffness. Our findings are in contrast to the previous findings of Gale et al., who reported that central PWV decreased from 9.8 ± 3.0 m/s to 9.3 ± 2.7m/s but peripheral PWV was unchanged after 7 weeks of pulmonary rehabilitation, which consisted of combined resistance and aerobic training. [147] The reduction in central PWV reported by Gale and associates was significantly correlated to a reduction in peripheral systolic and diastolic blood pressure. [147] However, carotid pulse pressure which is a better predictor of cardiovascular outcomes [67,68] did not change. In the present study, we observed an improvement in peripheral but not central PWV, however carotid pulse pressure in our COPD patients also remained unchanged following exercise. Carotid distensbility, compliance and β stiffness index also did not significantly change indicating that we were unable to modify central arterial stiffness following 8 weeks of aerobic exercise training.

Although the COPD patients in the Gale et al., study were more severe (FEV_1% predicted = 45%) compared to our more moderate group (FEV_1% predicted = 65%), our COPD cohort had a higher baseline central PWV of ~10%. This may suggest that the stiffer the central arteries are, the harder it may be to modify through shorter-term exercise programs. Furthermore, our exercise prescription was vastly different than that of Gale and colleagues. Pulmonary rehabilitation consisted of an unstructured combination of aerobic exercise (performed on a treadmill at 80% of maximum walking speed for 10mins and cycling at a self selected pace for 10mins) and resistance training (upper body exercises, performing as many reps as possible in 2 min) for a total of

30mins, 3times/week. Therefore differences in arterial stiffness could be due to differences in training programs such as combined (resistance and aerobic) versus high volume aerobic exercise. Literature comparing the effects of different training modes on PWV is limited. However, one study examined the effects of a 13 week strength training program (10 upper and lower body exercise, performed 3 days/week, at 70% 1 rep max., 1 set for 8-12 reps) compared to a combined strength and aerobic training program (walking or cycling at 60-75% of heart rate reserve, for 30-45mins, 2 days/week) in middle-older age adults. [181] No change in central PWV was found following either intervention, however the effects of resistance training versus aerobic training on arterial stiffness remains to be studied in COPD. It should also be noted that Gale and colleagues used the radial pulse wave to generate a central arterial waveform and determine the augmentation index as measures of central arterial stiffness. This indirect measure of central arterial stiffness has been shown to be inaccurate when assessing the effects of a vasodilator therapy, such as exercise, since it is predicted from peripheral vascular function. [65] Therefore exercise related changes such as endothelial function, sympathetic nerve activity and blood pressure are reflective of changes in peripheral vascular function rather than central vascular function and are not true reflections of changes in central arterial stiffness.

Our results, however were similar to those of Vivodtzev et al., in which a more structured 4 week lower body cycling training program (5days/week for 18-30mins at 38-65% peak workload) reduced peripheral PWV by ~10% in COPD patients. [148] Multiple regression analysis revealed an independent relationship between reductions in peripheral systolic blood pressure and peripheral PWV. However, they did not measure central

arterial stiffness which is the more clinically relevant measure associated with cardiovascular and cerebrovascular risk. With our exercise program we observed an 11% reduction in peripheral PWV, which was of a similar magnitude to Vivodtzev and collegues (Figure 2.5, panels A and C). [148] We found no significant reduction in systolic blood pressure across the COPD group, but also similar to Vivodtzev et al., there was a significant relationship between the change in systolic blood pressure and the reduction in peripheral PWV (r=0.74, p=0.02). Interestingly, resting diastolic blood pressure was significantly reduced in our COPD patients following exercise training, but was not significantly related to reductions in peripheral PWV (r=0.56, p=0.12).

Potential mechanisms for this reduction in peripheral PWV include alterations in the vasomotor tone of vascular smooth muscle cells. Peripheral arteries contain a larger ratio of vascular smooth muscle cells compared to central arteries, and are highly governed by the sympathetic nervous system. COPD is associated with increase sympathetic nerve activity, and a reduction in baroreflex sensitivity, which can both increase blood pressure. [182] Exercise training by reducing basal sympathetic nerve activity (SNA), enhancing vagal activity and restoring baroreflex sensitivity may decrease blood pressure, [182,183] thus reducing PWV.

Differences in the intrinsic properties and regulatory elements of central verses peripheral arteries may explain the regional difference observed in PWV. Central arteries stiffen progressively with age due to the fraying and fragmentation of elastin fibers with an associated increase in collagen and calcium deposits within the vascular wall. [60] Although older adults who habitually partake in aerobic exercise show reduced central PWV, [111,113] it seems unlikely that a short-term exercise program (8-weeks) would modify

the structural properties such as cross-linking and fragmentation which has occurred over time, therefore having little impact on central arterial stiffness. It may also be that larger changes in SNA are needed to alter central arterial stiffness, as peripheral arteries are more responsive to spontaneous changes in SNA at rest. [184]

2.4.4 The Effect of 8-weeks of Aerobic Exercise Training on Changes in Arterial Stiffness in Patients with COPD and Healthy Controls

There was a significant difference in baseline central PWV in COPD patients compared to controls, but not in peripheral PWV, carotid disensibility, compliance or β stiffness index between groups. Following exercise training the changes in measures of central and peripheral stiffness were not different in the patients with COPD compared to controls. Baseline central PWV was 10.9 ± 1.5 m/s in the COPD patients compared to 8.9 ± 2.1 m/s in the controls and this is consistent with previous studies that have reported that central PWV is elevated in COPD compared to healthy age-matched controls. [137,138] To our knowledge no study has previously assessed peripheral PWV in COPD patients compared to controls and therefore our findings showing no difference in peripheral PWV demonstrates that peripheral arterial stiffness in COPD is similar to healthy aging.

A central PWV of ≥11.8 m/s is associate with a 48% increased risk of first major cardiovascular disease event (ie. myocardial infarction, unstable angina, heart failure and/ or stroke) [70] and it was observed that 33% of our COPD cohort had central PWV's ≥11.8m/s compared to 0% in the control group (Figure 2.4, panel B and C). This finding indicates that the known increased risk of cardiovascular disease and stroke in COPD may be related to changes in central arterial stiffness and blood pressure rather than from abnormal changes in the vasoreactivity of the blood vessels.

2.4.5 The Effect of 8-weeks of Aerobic Exercise Training on Changes in Carotid IMT in Patients with COPD

No study has examined the effects of exercise training on carotid IMT in COPD. Carotid IMT increases with age and is an independent predictor of cardiovascular and cerebrovascular disease. [85-87] A change as small as a 0.1mm increase in carotid IMT has been associated with an age- and sex-adjusted relative risk of 18% for stroke and 15% for myocardial infarction. [88] Our findings suggest that 8 weeks of aerobic exercise, such as the typical length of pulmonary rehabilitation, is not long enough to reduce carotid IMT in COPD. Remodeling of the arterial wall is believed to occur over a time frame of months and years, and therefore the benefits of brief exercise training (i.e. 8-12 weeks) may initially include functional arterial adaptation, followed by structural adaptations with continued training.

A significant relationship was found between baseline carotid IMT and central PWV (r=0.72, p=0.03), indicating that those patients with the largest carotid IMT had the fastest central PWV. Since PWV is dictated by the structural components of the arterial wall, it would seem reasonable that those with increased carotid IMT, which is suggestive of subclinical vascular disease, had the fastest central PWV. Two other trends were also observed between changes in carotid IMT following exercise training and FEV_1 % predicted (r=0.47, p=0.08) and baseline relative VO_{2peak} (r=0.59, p=0.07). These associations suggest that those with the least obstruction and/or have the highest aerobic fittness are more likely to benefit from shorter term exercise programs in reducing the associated carotid IMT related cardio and cerebrovascular risk. In contrast there is little change in carotid IMT in those with more severe disease or those with significantly

reduced fitness and these patients may required longer training durations to achieve improvements.

2.4.6 The Effect of 8-weeks of Aerobic Exercise Training on Changes in Carotid IMT in Patients with COPD and Healthy Controls

No significant differences were observed between baseline carotid IMT in COPD and healthy controls, which is in agreement with a previous study by Moro et al. [144] This suggests that the age related increase in carotid IMT in COPD patients is comparable to sedentary aging. Furthermore, we observed no significant difference in change scores following 8 weeks of exercise, indicating that our COPD patients and sedentary controls had a similar response to training. When combining both groups together there was a trend between changes in carotid IMT and changes in relative VO_{2peak} (r=0.36,p=0.17) suggesting that those with the biggest improvements in fitness had the greatest reduction in carotid IMT, however a larger sample size is needed to confirm this relationship.

Exercise training is associated with outward remodeling of the arterial lumen and decreases in wall thickness and it is thought to be largely mediated by shear stress. [185] One proposed mechanisms to explain this trend observed between relative VO_{2peak} and carotid IMT may include increased anti-atherogenic changes in the arterial wall, such as up-regulation of eNOS and NO production following short-term cyclic elevations in blood pressure and increased shear stress. [117] Also, animal studies have shown that sustained sympathetic nerve activity (SNA) stimulates smooth muscle cell hypertrophy and therefore exercise related reductions in SNA may lead to reductions in carotid IMT. [186] Increased levels of inflammation and ROS, which are highly involved in the development of atherosclerosis may also be reduced following exercise where by lowering oxidative stress in the arterial wall and consequently contributing to decreased

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arterial wall thickness. However, more studies need to directly examine the potential link between these proposed mechanisms and arterial remodeling as a result of exercise training.

2.4.7 The Effect of 8-weeks of Aerobic Exercise Training on Blood Lipids, Chemistry and Biomarkers of Systemic Inflammation in Patients with COPD

Following 8 weeks of aerobic exercise no significant changes were observed in blood lipids, chemistry or biomarkers of systemic inflammation in COPD. This finding supports three previous studies. Bolton et al., examined the effects of 8 weeks of pulmonary rehabilitation consisting of combined resistance and aerobic exercise on systemic inflammation and found no changes in IL-6 or TNF-α. [187] Similarly, Rabinovich et al., utilized an 8 week cycling program consisting of intermittent cycling between 60-90% peak work rate for 60 min, 5 days/week, and found it did not induce changes in plasma TNF- α or IL-6 levels in COPD. [188] However, in the later study no information regarding the effectiveness of exercise training on physiological parameters (ie. VO_{2peak} or maximum watts) was provided and therefore it remains unclear whether the training program was appropriate to elicit a training response. Following a 10 week training program in COPD consisting of constant cycle ergometer exercise (60% peak workload for 30mins) and interval training (100% peak workload at 30s work 30s rest for 45mins) 3times/week, fitness levels improved however there were no changes in IL-6, TNF- α and CRP. [189]

Although these studies indicate that exercise may have little effect on biomarkers of systemic inflammation in COPD, it is interesting to note that all inflammatory biomarker tended to be reduced pre to post training in the present study with a ~20% reduction occurring in IL-8. IL-8 is produced early in the atherosclerosis process by

lesion macrophages, endothelial cells and vascular smooth muscle cells. [190] It is primarily responsible for inducing chemotaxis, causing the migration of additional monocytes and neutrophils into areas of damage in the vascular wall there by promoting the inflammatory response. [190] It has also been shown to enhance leukocyte activity and promote adhesion of monocytes to the endothelium potentiating plaque angiogenesis. [190] Studies have shown IL-8 to be an independent predictor of cardiovascular and overall mortality in patients with coronary artery disease, [191] end-stage renal disease [192] and following invasive cardiac surgery. [193] Therefore reductions in IL-8 may be of clinical importance in reducing the overall cardiovascular risk and mortality, especially in the COPD population in which cardiovascular disease is prevalent. However, due to large individual variations associated with measuring cytokines in peripheral blood of COPD patients, we are likely to be underpowered to detect a significant change in IL-8. In fact, a post-hoc power analysis demonstrated that only two more patients would be required to detect a significant reduction in IL-8 following training in our COPD group. As such, we are confident that well structured, high volume aerobic exercise can have a positive benefit on inflammatory mediators that are associated with the development and progression of atherosclerotic plaques.

A trend was also observed between baseline relative VO_{2peak} to baseline IL-8 (r=0.61, p=0.08) as well as the change in IL-8 following training (r=0.58,p=0.1) suggesting that those patients with the lowest fitness level had the highest circulating level of IL-8, but experienced the largest reduction with exercise. A similar relationship was found between FEV_1 % predicted and IL-8 (=0.54, p=0.10), in that those with the most severe obstruction had the greatest reduction following exercise. Additionally, a

significant relationship was found between baseline relative VO_{2peak} and IL-10 (r=0.77, p=0.02) indicating that the least fit patients also had the lowest level of anti-inflammatory biomarkers, however IL-10 did not significantly improve following exercise training. These finding suggest that reductions in systemic inflammation are related to fitness level and disease severity in COPD.

Although Eickhoff et al., reported CRP as an independent predictor of FMD we did not find this relationship to exist between these variables within our COPD cohort. [143] We also did not find any relationships between any other biomarkers of inflammation and FMD. Our findings potentially support that inflammation does not play a critical role in endothelial function in COPD, however this statement remains speculative due to the limited amount of research in this area and the small sample size in the present study. Our findings support the literature in which no relationship has been reported between peripheral or central PWV and CRP at baseline or following exercise training in COPD. [138,139,148] However, it is in contrast to Sabit et al., who reported a significant relationship between baseline IL-6, and TNF-α soluble receptor with central PWV. Interestingly, following stepwise multiple regression analysis only IL-6 was predictive of arterial stiffness. [137] We did not find this relationship to exist in our COPD group, nor was there a significant relationship between any of our inflammatory biomarkers and other measures of arterial stiffness.

2.4.8 The Effect of 8-weeks of Aerobic Exercise Training on Blood Lipids, Chemistry and Biomarkers of Systemic Inflammation in Patients with COPD and Healthy Controls.

No significant differences were observed between baseline measures or change scores of blood lipids, chemistry and biomarkers of inflammation between COPD and healthy

controls. Cross sectional studies have shown that exercise is associate with reduced pro-inflammatory cytokines such as IL-6, TNF-alpha and CRP and improved anti-inflammatory cytokines such as IL-10 in habitual exercising older adults, [194-196] however available data reflects less robust findings in randomized control trials. Nicklas et al., examined the effects of 12 month of moderate walking (150mins/week, at a 12-13 rating of perceived exertion) and reported a significant reduction in IL-6 (16%) and although CRP was reduced (32%) it was not statistically significant. [197] Contrarily, Campbell et al., demonstrated that a 12 month aerobic training program (45mins, 60-75% heart rate max, 5times/week) reduced CRP but not IL-6 in postmenopausal women. [198] Interestingly, the reduction in CRP was not significant at 3 month, but only at 12month and was related to total fat loss. [198] Another study performed by Kohut et al., randomized sedentary older age adults into an aerobic training group (65-80% heart rate reserve, 3times/week, for 30-45mins) or flexibility control for 10 months. [199] The exercise group had significant reductions in CRP, IL-6, and TNF-alpha, without a change in BMI. [199] Additionally, an 8-month progressive aerobic program (40-85% heart rate reserve, for 45mins, 3times/week) decreased CRP by 51% in older adults, [200] however 6 months of cardiovascular exercise (80% VO_{2max}, for 45 min, 4day/week) did not improve CRP levels. [201]

Although subjects used in these above mentioned studies were sedentary older adults, the majority did not exclude for co-morbidities (i.e. diabetes, osteoporosis, atherosclerosis), which are associated with increased levels of systemic inflammation and therefore are not comparative to our control group, which was free of known inflammatory diseases. However, it appears that when the duration of the intervention is 6 months or shorter, exercise training may have little effect on inflammation in healthy

individuals and therefore 8-weeks of aerobic training may not be long enough to reduce inflammation in our control group.

2.4.9 The Effect of 8-weeks of Aerobic Exercise Training on Cardiovascular Responses to Exercise in Patients with COPD and Healthy Controls

A significant improvement in relative $VO_{2\,peak}$ occurred in both groups (10% and 30% in COPD and controls, respectively) following 8 weeks of aerobic exercise training, indicating that our exercise prescription and duration of 8 weeks was appropriate to elicit a physiological adaptation to exercise. It is important to note that there were no significant differences in BMI following training in either group indicating that improvements in exercise capacity are not due to reductions in body mass.

Numerous studies have demonstrated that exercise training increases exercise tolerance in constant load submaximal exercise in COPD, [202-204] however relatively few have been able to significantly improve VO_{2peak} with 8 weeks of training, thus supporting the quality of our training program. Consistent with previous studies, [202-204] peak exercise dyspnea and leg fatigue were significantly reduced following exercise training indicating that the COPD patients experienced less shortness of breath at a higher workload. This is a relevant finding as it suggests that the COPD patients can now exercise for a longer duration at a higher intensity, without feeling as short of breath and therefore obtain more cardiovascular benefits of exercise.

As would be expected, in our COPD patients, the total training volume was significantly related to the change in relative VO_{2peak} (r=0.8, p<0.01) indicating that those who performed the largest volume of training had the greatest improvements in fitness. It was also found that baseline relative VO_{2peak} was significantly related to the change in relative VO_{2peak} (r=0.86, p<0.01), such that those who were the least fit achieved the

greatest improvement in VO_{2peak} following training. Despite these improvements, 8 weeks of training did not elicit changes in peripheral vascular structure and function or markers of systemic inflammation as previously stated suggesting that improvements in aerobic capacity are not necessarily associated with changes in vascular structure and function.

No differences in baseline relative $VO_{2\ peak}$ was observed between COPD and healthy controls. Interestingly, under the same relative stimulus, the controls had a greater overall improvement in VO_{2peak} than in COPD. The improvements observed in VO_{2peak} are likely attributable to improvements in musculoskeletal or cardiac adaptation as lung function does not change with training. Reasons for this discrepancy between increases in VO_{2peak} between the COPD and the control group may be due to inherent musculoskeletal abnormalities, increased afterload, reduced compliance, reduced contractility or heart-lung interactions. Another postulation may be the absolute difference in training intensity between COPD and healthy controls, which may influence the stimulus elicited for adaptation, however these speculations are not known and require further investigation.

2.4.10 Limitations and Considerations

It should be noted that there are limitations within our present study. Perhaps the largest limitation is the small sample size utilized in the study. However, this study was designed as a pilot study to power a future randomized controlled trial to supply data to investigate the benefits of exercise training for improving vascular structure and function. To this end, we have achieved this goal as from the present data a sample of 98 patients per group would be needed to investigate changes in FMD %peak with 8-weeks of aerobic exercise training in COPD. As such, we are confident that although our finding of no

significant difference in FMD with exercise-training may be a type II error, the effect is small and on average likely not clinical relevant.

Flow mediated dilation is accepted as a robust measure of endothelial function; however endothelial function may be influenced by a myriad of external and internal stimuli. Although all vascular measures were performed following a fast ($\geq$4 hours), we did not record any dietary changes, which may have occurred over the 8 weeks. Dietary changes, such as eating a higher or lower amount of fatty foods may change hemodynamics and blood properties altering the FMD response. Also, intrinsic changes in sympathetic mediation such as emotions and stress level, may influence resting vascular tone and the FMD response. There are also inherit methodological limitation with FMD such as anatomical probe placement, cursor angle, sample volume used, and time of day effects. However, tests were standardized pre and post training by recording the ultrasound settings from the baseline test, as well as using anatomical features for consistent probe placement. Additionally, tests were performed at the same time of day to account for any diurnal variation.

Finally, although none of the participants presented with a history of major cardiovascular disease, participants were still included even if they were currently taking cardiovascular medications (ie. anti-hypertensive medications and statins). We felt that inclusion of these participants was acceptable as excluding them would result in the misrepresentation of the overall COPD and aging population and therefore decrease the translatability of our findings.

2.5 Conclusion

This is the first study to comprehensively examine the effects of an 8-week aerobic exercise training program on vascular structure and function in patients with COPD. Peripheral PWV was the only measure of vascular structure or function, which improved following exercise training and all other markers of arterial stiffness (central PWV, β stiffness index, carotid IMT) and biomarkers of systemic inflammation remained unchanged despite an increased in relative VO_{2peak}. Our findings suggest that shorter term exercise programs common in pulmonary rehabilitation across Canada and around the World may not be long enough or a strong enough stimulus to reduce the cardiovascular risk associated with vascular structure, function or systemic inflammation in COPD. Future studies should aim to determine the time course for vascular adaptation to exercise and as well as varying modes, intensities, and frequencies of exercise in order to modify cardiovascular risk outcomes and maximize exercise prescriptions for patients with COPD.

Chapter 3: Extended Discussion

3.1 Vascular Function in Response to 8 Weeks of Aerobic Exercise Training in COPD

Contrary to our primary hypothesis, endothelial dependent vasodilation was not significantly altered following 8 weeks of aerobic exercise training in COPD. Patients with COPD have higher circulating levels of pro-inflammatory markers, such as IL-6, IL-8, TNF-α, and CRP. [35,36] These inflammatory biomarkers are known to exert their effects on the vascular endothelium by interfering with basal NO production. [9,54,55] NO not only regulates blood flow but confers significant vasoprotective effects such as inhibition of platelet aggregation and inflammatory cell adhesion to the endothelium, disruption of pro-inflammatory cytokine-induced signaling pathways and the inhibition of apoptosis and vascular smooth muscle cell proliferation. [205] A reduction in NO renders the endothelium more susceptible to insult and injury by making it more permeable to factors such as circulating lipoproteins, and the development of atherosclerosis. It is possible that the unchanged FMD response we observed was mediated by unchanged inflammatory biomarkers leading to reduce NO availability through altered endothelial function.

Cytokines, such as IL-8, and IL-6 are released in response to vascular injury and promote chemotaxis and the infiltration of other inflammatory cells to the site of injury. [54,190] They also promote the expression of adhesion molecules on the endothelium causing disruption of endothelial functioning. [190] In addition, the production of CRP is stimulated by IL-6 released following vascular damage, and has been shown to up-regulate other inflammatory cytokines, promote the uptake of LDLs by macrophages, stimulate the production of endothelin-1 (a potent vasoconstrictor) from endothelial cells and inhibit eNOS activity. [54] TNF alpha, the master cytokine, is produced by

macrophages and monocytes during acute inflammation and is related to vascular dysfunction as it promotes endothelial apoptosis, and the up regulation of NADHP oxidase, [95,96] ultimately contributing to ROS production and further reducing NO bioavailability.

We observed no reductions in pro-inflammatory, or an increase in anti-inflammatory biomarkers suggesting that we were unable to change the inflammatory/anti-inflammatory balance through 8 weeks of aerobic exercise in patients with COPD. One postulation for this may be due to the chronic "spill-over" affect of inflammatory mediators from the lungs. The inflammatory response within the lungs of COPD patients has been shown to persist for many years even after smoking cession. [23,24] It is possible that even if acute reductions in inflammation occurred following exercise training, the spill-over from the lungs into the systemic circulation would provide a continuous supply of circulating inflammatory biomarkers thus negating the effects of exercise. The role of chronic exercise for reducing airway inflammation in COPD remains to be explored.

In addition, differences in training stimulus have been associated with varying oxidant/antioxidant effects and associated vasodilatory responses. Three months of sustained (1 hour) moderate-high intensity running (70-80% VO_{2max}) 4times/week, resulted in a ~35% reduction in vasodilator response to acetylcholine with increased circulating levels of measured oxidants in healthy younger males. [131] Additionally, moderate-high intensity cycling (75%VO_{2max}) for 30minutes, 5-7 times/week for a total of 4months showed no improvement in forearm blood flow with increased concentrations of oxidative stress markers. [132] These studies suggest that moderate-to-high intensity

training is associated with elevated levels of oxidative stress and impaired endothelial dependent dilation if the individual's bodies do not have the oxidant defenses to protect against the greater oxidative insult.

Our high intensity interval prescription differed from the protocols used above whereby alternating between high intensity and low intensity (performed at 80-90% of maximum watts, for 3mins, and 35-40% of maximum watts for 3mins off for a total of 30-35mins) rather than just sustained high intensity training. Additionally, our subjects were only progressed to intervals after completing one month of steady state aerobic exercise at moderate intensities which was achieved through appropriate progressive overload. Thus if changes in anti-oxidants had occurred during this phase, that would have protected against the above-mentioned oxidant response. However, it is also possible that there was not an increase in anti-oxidants levels with exercise training. It may also be possible that certain individuals with COPD have considerably elevated levels of ROS to start with, so even at low levels of exercise the oxidant/anti-oxidant balance may already be shifted to the oxidant side, and therefore high intensity exercise may worsen this imbalance, which could have affected any positive adaptation in vascular function.

Age and oxidant induced changes to endothelial vasodilator pathways may also contribute to the lack of improvement in endothelial dependent dilation following 8 weeks of exercise training in COPD. FMD measures vasodilation of the brachial artery in response to reactive hyperemia which is largely NO mediated, however other vasodilator factors such as prostacyclin (PGI_2) and endothelial-derived hyperpolarizing factor (EDHF) are also released in response to increased shear stress.[206,207] An age associated

shift in cyclooxygenase signaling results in reduced PGI_2 mediated vasodilation to enhanced PGI_2 mediated vasoconstriction in older adults. [180] This was demonstrated by the infusion of a COX inhibitor, which had little effect on the acetylcholine-mediated response in younger adults, but showed enhanced vasodilation in older adults. [180] Increased oxidative stress, in particular the chronic production of O_2^-, has also been shown to decrease the activity of calcium-activated potassium channels involved in EDHF-mediated vasodilator response. [208] Likewise, ONOO- has been shown to decrease the EDHF component of flow-mediated dilation in the coronary arteries or mice. [209] ET-1 produced by the vascular endothelium. ET-1 mediated vasoconstriction and plasma concentrations are augmented in older adults [210,211] Expression of ET-1 is increased in vascular endothelial cells obtained from the brachial arteries and antecubital veins of older compared to younger adults, and is inversely related to endothelial dependent dilation and positively related to oxidative stress [212] Oxidative stress plays an inhibitory role in vasodilation, as in the case of EDHF, and has enhanced vasoconstrictor effects such as in ET-1. If circulating levels of ROS remained unchanged in our COPD cohort, coupled with an age related shift in cyclooxygenase signaling, the balance of vasodilator and vasoconstrictor substances may be unequal. The shear stress induced increase in NO may not be enough to counteract these enhanced vasoconstrictor pathways of the endothelium. These changes may be reflected in our FMD measures however the degree to which each contributes to the total vasodilatory response cannot be elucidate from FMD alone.

3.2 Arterial Stiffness in Response to 8 Weeks of Aerobic Exercise Training in COPD

Arterial stiffness is governed by intrinsic elastic properties of the arterial wall (structural determinants) and/or vasomotor tone of the smooth muscle cells (functional determinates) as related to changes in vasoconstrictor or dilator pathways. The vascular wall of larger conduit arteries is comprised largely of connective tissue (elastin and collagen) compared to smaller resistive peripheral arteries that are more muscular. With age, central arteries stiffen progressively due to the fraying and fragmentation of elastin fibers with an associated increase in collagen and calcium deposits within the vascular wall. [60] More muscular, peripheral arteries are more resistant to age related stiffening, however they remain highly modulated by endothelial function, and the sympathetic nervous system. [213] These structural and functional properties (in particular the sympathetic nervous system) may provide a possible explanation for the regional differences observed in PWV and measures of arterial stiffness in our study.

Since biochemical changes in elastin-collagen composition of the arterial wall are believed to occur over years, it is unlikely that short-term aerobic exercise such as 8 weeks would a reduction in central PWV. Although, it may be possible that increased pulse pressure and mechanical distension during exercise may stretch collagen fibers and modify their cross-linking leading to increased arterial compliance, such that older adults who habitually partake in aerobic exercise have reduced central PWV and increased arterial compliance compared to sedentary peers. [89,111,113] Additionally, sedentary older aged adults who participated in a short-term (14 weeks) aerobic program (3-6 days/week, for 30-45mins at 60-75% heart rate max) had a 25% increase in arterial compliance, and a ~20% reduction in β stiffness index. [89] These findings indicate that aerobic exercise may

slow down or potentially even reverse the age-related increased in central arterial stiffness. No studies have examined the effects of an exercise program shorter than 14 weeks on arterial stiffness, however our findings suggest that 8 weeks of aerobic exercise is not a long enough stimulus to elicit changes in the intrinsic properties which contribute to central arterial stiffness, particularly in the COPD population.

Peripheral arteries, which are governed by the functional rather than structural determinants, may respond better to the effects of exercise training in reducing arterial stiffness. Vascular smooth muscle tone is highly regulated by the sympathetic nervous system (SNA) and COPD is associated with autonomic dysfunction and increased sympathetic activation.[214] The autonomic nervous system dysregulation is thought to be caused by a combination of numerous factors characteristic of COPD such as hypoxemia, poor exercise tolerance, increased work of breathing, dyspnea, reduced muscle strength and systemic inflammation.[214] Exercise training has been well documented to improve exercise tolerance at constant load submaximal and muscular strength with associated reductions in dyspnea in COPD.[202-204,215] It has also been shown to reduced the work of breathing by reducing the ventilatory demand and rate of dynamic hyperinflation.[202,216] Therefore since exercise has shown to improve factors which attribute to increased SNA in COPD it may serve as a potential mechanism in reducing peripheral arterial stiffness.

In support of the role of some of these physiological mechanisms in the regulation of peripheral arterial stiffness, a 6 week aerobic exercise program consisting of treadmill exercise set to 70% of maximum speed, performed 3 times/ week, showed marked improvements in parasympathetic activity and a reduction in sympathetic activity at rest and during submaximal exercise as indicated by heart rate variability in COPD.[183]

Similarly, following an 8 week out patient pulmonary rehabilitation program, which consisted of cycling at 60-75% of maximum exercise capacity for 30-40mins, baroreflex sensitivity as measured by heart rate variability, improved suggesting improved sympathetic modulation following exercise. [182] Although the effects of exercise training on reducing SNA and arterial stiffness has not been studied in COPD, these association may provide insight to our observed reduction in peripheral PWV whereby reducing peripheral vascular tone and improving autonomic dysfunction through modifications with exercise.

An increase in shear stress stimulus elicited through exercise may also affect arterial stiffness through a NO mediated inhibitory effect on SNA. [217] NO released by the endothelium in response to increased shear stress has been shown to reduce both the release and vasoconstrictor effect of noradrenaline induced by increased SNA. [218,219] Interestingly, we observed a significant relationship between the change in FMD % peak and change in peripheral PWV (r=0.76,p=0.01) in COPD following 8 weeks of exercise training. This strong relationship suggests that in COPD patients, those with the greatest improvement in FMD% peak had the largest reduction in peripheral PWV. Furthermore, this relationship ceased to exist when the control group was included to the analysis. This suggests a prominent relationship may exist between the sympathoinhibitory effect of NO due to increased shear stress stimulus and the reduction in SNA resulting in improved peripheral PWV and that the magnitude of the relationship is greatest in COPD in which SNA is commonly elevated. [214]

3.3 Modifications in Vascular Function with Exercise

The vascular endothelium is highly modulated by a myriad of physiological factors, which could potentially explain the lack of a positive change in FMD with a relatively high volume aerobic exercise program. Studies have established that increased production of ROS leads to endothelial dysfunction in aging.[99,103,212] In humans, the age-associated decline in endothelial dependent dilation is mediated in part by an oxidative stress reduction in NO activity and availability through increased concentrations of the free radical superoxide anion (O_2^-) arising from increased NADPH oxidase production. [98,99,102,124] Tetrahydrobiopterin (BH_4) is an essential cofactor for eNOS and has been shown to reduce with age. [107] A BH_4 deficiency results in eNOS uncoupling and the production of ROS including peroxynitrite (ONOO-), superoxide anion (O_2^-) and hydrogen peroxide (H2O2). [220] These reactive species are not only associated with further down regulation of eNOS, and subsequent reduced NO production but also increased inflammation, cell proliferation, hypertrophy and apoptosis, all of which directly influence the vascular endothelium. [220]

Endothelial dependent dilation is greater in middle and older aged adults who regularly perform aerobic exercise compared to their sedentary peers. [102,121,124] Aerobic exercise training interventions have reported improved endothelial dependent dilation in older sedentary adults [121] as well as in patients with metabolic disease, congestive heart failure and hypertension. [120,145,153,177,221] Reduced oxidative stress following exercise may provide possible insight into these findings as older trained adults have a higher oxyradical scavenging capacity and improved B_{H4} activity, accompanied by greater endothelial dependent dilation compared to sedentary controls [103] [102,106,124] Patients with

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coronary artery disease who participated in an aerobic exercise program (30min on a row ergometer, 30min on a treadmill, daily for 4 weeks) also exhibit decreased production of ROS and suppression of NADPH oxidase in the mammary artery. [222] These studies demonstrate that aerobic exercise can be used as a means to reduced oxidative stress and therefore enhance NO activity and improve endothelial dependent dilation.

Mechanisms related to the improvements in reduced oxidative stress remain up for debate, however a link has been established between eNOS expression and superoxide dismutase (SOD), a powerful antioxidant. In the aortas of mice lacking eNOS, SOD was reduced more than 2-fold compared to controls. [223] However treadmill exercise training increased eNOS and SOD expression in controls but not in eNOS lacking mice, suggesting that SOD is mediated by eNOS expression. [223] Exercise induced NO bioavailability is associated with increased eNOS gene expression in animals and in the coronary arties of humans following exercise training. [224,225 222] It may be plausible that the mechanical alterations/stress on the endothelium during exercise as a result of increased systemic arterial pressure and pulsatile flow contribute to eNOS upregulation and enhances SOD production leading improved antioxidant defenses, and NO availability. However, the increased levels of ROS found in COPD may be too great to be overcome by the eNOS regulated increase in SOD resulting in no change in oxidant/antioxidant capacity. Furthermore, the ability of aerobic exercise to increase eNOS expression, SOD production and subsequent NO availability in the peripheral vasculature in COPD remains to be determined.

Another mechanism leading to reduced vascular improvement following training may include a reduction in endothelial progenitor cells (EPCs). Circulating levels of

EPCs are decreased in the systemic vasculature of patients with COPD. [226] In patients with cardiovascular risk factors and coronary artery disease, aerobic exercise (moderate intensity running performed 3time/week for 12 weeks) was shown to increase EPC numbers and was positively correlated to improvements in FMD. [227] A single bout of moderate aerobic exercise (70% VO_{2peak} for 20mins) in COPD patents resulted in no increase in circulating EPCs [226] and the effects of aerobic exercise training on EPCs in COPD remains to be studied. However it may be possible that exercise may not increase EPCs in COPD leading to an inability to repair vascular damage and enhance vascular endothelial function.

3.4 Clinical Relevance and Future Considerations

Despite a structured 8-week, high volume aerobic exercise training program in the present study, clinically relevant markers of vascular function and structure and inflammation remained unchanged in patients with COPD. Given that the typical length of pulmonary rehabilitation in Canada is ~8 weeks, and that the volume of exercise is considerably lower and more generic than the present study, it may not be long enough to reduce cardiovascular disease risk through modification in vascular function and/or structure and through reducing systemic inflammation in patients with COPD. Future studies should aim to examine the time course for vascular adaptation in function and structure to exercise training in different severities of COPD as well as different training modes such as aerobic versus resistance training. Additionally, the acute and chronic effects of varying intensities, frequencies and durations of training interventions should be investigated to try and understand the mechanisms responsible for vascular adaptation and to allow more optimal exercise prescription. Finally, variations in phenotypic

responses to exercise training and modifications of vascular function and structure should also be explored in order to better individualize exercise prescription and achieve the maximum benefit of exercise training for COPD patients. Through the evidence gained in this study, it may be possible to change pulmonary rehabilitation practice and focus this highly effective therapy not only on changing dyspnea and quality of life but on altering the cardiovascular disease risk that is the primary cause of morbidity and mortality in this population.

3.5 Conclusion

This is the first study to comprehensively examine the effects of an 8-week aerobic exercise training program on vascular structure and function in patients with COPD. We were unable to change clinically relevant markers of vascular function and structure and inflammation in patients with COPD, suggesting that shorter term exercise programs, such as pulmonary rehabilitation, may not be long enough to reduce the cardiovascular disease risk associated with COPD. Future studies are needed to determine the effects of varying type and durations of exercise prescriptions on modifying these outcomes in order to achieve the maximum benefits of exercise and reduced the burden of cardiovascular disease in the COPD population.

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Appendices

Appendix A- Informed Consent

a place of mind

THE UNIVERSITY OF BRITISH COLUMBIA

Subject Information and Informed Consent

Title of Project: The Effects of Aerobic Exercise Training on Peripheral Vascular, Cardiac and Cerebral Vascular Function in Patients with Chronic Obstructive Pulmonary Disease

Principal Investigator: *Dr. Neil Eves, PhD*
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Director of Respiratory Medicine Kelowna General Hospital and Associate Director of Critical Care Medicine
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Institution: Faculty of Health and Social Development
School of Health and Exercise Sciences

University of British Columbia- Okanagan

Contact Person: Ms. Jinelle Gelinas
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                           Phone: (250) 807-8860

INTRODUCTION

You are being invited to partake in this research study because you have chronic obstructive pulmonary disease (COPD). COPD is a lung disease, which makes breathing difficult especially during exercise. COPD is also associated with inflammation in the blood, and can affect the health of your blood vessels and heart. Aerobic exercise has been shown to prevent the adverse effects of inflammation and can reduce the risk of heart and brain disease by improving blood vessel and heart function. This study will help us to understand the benefits of aerobic training on inflammation in people with your condition, and its affect on blood vessel function. Please read the following information carefully before deciding to participate in the study. If you have any questions, please do not hesitate to ask. The information collected during this study will be used for the Masters thesis of Jinelle Gelinas at the University of British Columbia.

YOUR PARTICIPATION IS VOLUNTARY

Your participation is entirely voluntary. Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts. If you wish to participate, you will be asked to sign this form. Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide. If you choose not to participate in this study, you will not be penalized in any way, nor do you need to disclose why you have chosen not to participate.

WHO IS CONDUCTING THE STUDY?

You are being invited to consider taking part in a collaborative study run under the School of Health and Exercise Sciences in the Faculty of Health and Social Development at the University of British Columbia Okanagan. This study will be conducted and overseen by Dr. Neil Eves (Principal Investigator), Jinelle Gelinas (Co-Investigators and MSc candidate), Dr. Philip Ainslie (Co-Investigator) and Dr. Douglass Rolf (Co-Investigator) who is a physician with Interior Health.

BACKGROUND

Chronic obstructive pulmonary disease or COPD, is one of the most prevalent forms of lung disease affecting 8.2% of the Canadian adult population over 40 yrs. Smoking is the primary cause of COPD and leads to inflammation in the lungs and blood that

can lead to secondary problems such as blood vessel disease, high blood pressure and heart disease. These conditions can reduce blood flow to the heart and brain, increasing the risk of heart attack or stroke. Aerobic exercise training, such as walking or biking, has been shown to reduce the risk of heart and brain disease in healthy individuals and in some other conditions. However, it is currently unknown whether exercise training can have the same affect in patients with a chronic inflammatory condition such as COPD. It is likely that the benefits of exercise for reducing the risk of heart and brain disease are due to changes in inflammation and how the blood vessels function but this has never been studied. The aim of this study is to investigate if eight weeks of aerobic exercise training improves blood vessel function and brain blood flow in patients with COPD. By exploring the effects of an 8 week aerobic exercise program, we hope to find new evidence to support the benefits of exercise in the management of COPD which could prevent the development of other health problems and greatly improve your quality of life. This study is a pilot study, which means that it is a small-scale preliminary study, in our case with 18 participants, designed to study the effects of aerobic exercise training on blood vessel health in patients with COPD.

WHAT IS THE PURPOSE OF THE STUDY?

The purpose of this study is to determine the effects of exercise training on blood vessel health in patients such as yourself with COPD, and how the benefits of exercise relate to the function of the heart, blood vessels and brain. This will help us to better understand the benefits of aerobic exercise in patients with lung conditions like yours and allow for more optimal exercise prescriptions and therapies to be designed to help improve health status and quality of life for individuals with COPD.

WHO SHOULD NOT PARTICIPATE IN THE STUDY?

If you have had an increase in your sputum production and a worsening of shortness of breath, or have been admitted to hospital in the last six weeks you should not participate in the study until you have returned to your previous condition for at least six weeks. If you have a heart or brain condition that limits your ability to exercise safely or you suffer from pains in your muscles and joints that regularly stop you from exercising, you will also not be able to participate in this study. If you are diabetic, have known problems with you breathing during sleep (obstructive sleep apnea) or are currently smoking or have quit for less than 3months, you will not be able to participate in the study. Additionally, if you are taking supplemental oxygen or have a large reduction in oxygen levels in your blood during exercise, you will not be able to participate. Finally, if your body weight is a lot greater than normal you may not be able to participate because body fat can also affect some of the measurements being studied independent of your lung disease.

WHAT DOES THE STUDY INVOLVE?

Visit 1 (2 hours): A breathing test (pulmonary function test), to confirm the severity of your condition and an incremental cycling exercise test, to determine your current fitness level and your safety to perform exercise in a community setting. During this visit we will also assess your current health-related quality of life using a questionnaire. This visit will be carried out at Kelowna General Hospital.

Visit 2 (90 min maximum): After fasting overnight, on the following day a trained health professional will take a blood sample from your arm which will be used to measure inflammation in your blood. While you are resting, we will also use a non-invasive technique called ultrasound (similar to that used when looking at an unborn fetus in a mothers stomach) and tonometry (a small pressure gauge that looks like a pen) to examine the blood vessels in your neck, arms and legs. This visit will be carried out at the integrative Clinical Cardiopulmonary Physiology Laboratory (iCCP) at the University of British Columbia Okanagan Campus.

Visit 3 (2 hours): On the final visit we will measure your heart function and brain blood flow with a similar non-invasive ultrasound device to that used in visit two and then you will exercise on a stationary exercise bike at a light, moderate and heavy intensity while we continue to make measurements of your heart function and brain blood flow. This visit will also be carried out at the integrative Clinical Cardiopulmonary Physiology Laboratory (iCCP) at the University of British Columbia Okanagan Campus.

Exercise training Sessions (3 hours/week, total 24 hours): Following all baseline measurements you will come to our exercise laboratory three times a week for 8 weeks to exercise on a stationary bike. All aerobic exercise training sessions will be supervised by a trained exercise specialist. Following the completion of the training we will ask you to repeat visits 1-3 again to see how all of the measurements have changed. All training sessions will be carried out at the integrative Clinical Cardiopulmonary Physiology Laboratory (iCCP) at the University of British Columbia Okanagan Campus.

* All tests and associated time commitments are described in full below. Testing will likely occur in the afternoons, evenings, or weekends. Following exercise training, tests 2-6 will be repeated.

(1) Pulmonary Function Test
What is this? This test is similar to those you have performed on a regular basis to monitor your condition. A registered respiratory therapist will run and supervise this test. You will sit in a comfortable chair in a large clear chamber and breathe through a mouthpiece while wearing a nose clip. You will be asked to breathe normally and sometimes you will inhale all the way and exhale all the way as fast as you can. After the initial test is performed you will be given a bronchodilator medication (**bronchodilators are given by inhalation [with a puffer] to open or relax the breathing tubes or airways**) to be sure that your airways are fully open

and the breathing test will be repeated. The bronchodilator we will be giving you is called Ventolin, and you will take 2 puffs of it (200mcg). In the unlikely event of an emergency, a physician nearby in the hospital will be contacted immediately.
Time commitment: 45 minutes
Location: Kelowna General Hospital
Why is this important? The breathing test (spirometry) will be done to measure your lung function (how strong the lungs are).

(2) Symptom limited exercise test
What is this test? This test is an exercise test, which slowly gets harder until shortness of breath and/or tired legs stops you exercising. This test will be done on a stationary bicycle and you will breathe through a mouthpiece while wearing a nose clip to collect your expired air. Small stickers (electrodes) will also be stuck to your chest so that we can monitor your heart.
Time commitment: 45 minutes (only exercising for 10-12 minutes)
Location: Kelowna General Hospital
Why is this test important? This test is an indication of your current fitness level. We will use your values obtained from this test to determine your aerobic training intensity level.

(3) Blood collection
What is this? A blood sample will be collected from the forearm by the physician or trained medical staff. Twenty milliliters (1 table spoon full) will be collected. This is the same as a standard blood test.
Time commitment: 10 minutes
Location: integrative Clinical Cardiopulmonary Physiology Laboratory (iCCP)
Why is it important? This test allows us to measure the inflammation in your blood.

(4) Blood Vessel Function Assessment (Flow mediated dilation and Tonometry)
What is this? This test is done to assess the function of your blood vessels. In the first part of the test, while lying down a blood pressure cuff will be placed around your left forearm, and pumped up to a level that will reduce blood flow for 5 minutes. At that time the cuff will be released, and using an ultrasound machine, we will monitor the return of blood flow in the arm. During the second part of the test we will place a pen like device with minimal pressure on the right side of your neck, and then on your wrist, back of your ankle, and your upper leg while you are lying down, which will measure stiffness of your blood vessels. We will also give you a small spray of a drug called nitroglycerine beneath your tongue which allows you blood vessels to increase in size and the we will repeat the measurements described.
Time Commitment: 90 min
Location: integrative Clinical Cardiopulmonary Physiology Laboratory (iCCP)
Why is this important? These tests will assess how your blood vessels work.

(5) Transcranial Doppler Test

What is this? During this test we ask you to wear a plastic head band which holds in position two small probes on either side of your temples. The probes non-invasively measure the speed of blood flow in one of the main arteries in your brain. It does this by using very high frequency sound which you cannot hear and by recording the echoes from the moving blood. The headband holds the probes in place and allows you to exercise without moving the probes. We will also assess how the brain responds to changes in blood pressure. This will require you to repeatedly perform a slight squat followed by a stand. Arm support can be provided if needed. Finally, we will evaluate how the brain responds to changes in carbon dioxide. This will involve you to breathe in a gas mixture made up of 5% of carbon dioxide (room air is a usually around 0.03% carbon dioxide) and the same amount of oxygen as you breathe in room air for 3 minutes, while lying down. We will also measure your brain blood flow while you exercise at a light, moderate and heavy load determine specifically for your fitness level from your incremental test described above.

Time Commitment: ~ 1 hour
Location: integrative Clinical Cardiopulmonary Physiology Laboratory (iCCP)
Why is this important? This test will assess your brain blood flow at rest and during exercise

(6) Echocardiography Exercise Test
What is this test? This test is an exercise test that measures the function of your heart at rest before and after you have completed 8 weeks of aerobic exercise training. A small ultrasound probe will be placed on your chest at the level of your heart. The probe uses sound wave to create a moving picture of the heart and involves no radiation exposure. We will repeat these measurements while you exercise at a light, moderate and heavy load determine specifically for your fitness level at the same time as we are measuring your brain blood flow.

Time commitment: 1 hour
Location: integrative Clinical Cardiopulmonary Physiology Laboratory (iCCP)
Why is this important? This test will assess your heart function at rest before and after aerobic exercise training.

(7) Aerobic Exercise Training
What is this? For 3 sessions a week for a total of 8 weeks, you will participate in a supervised aerobic exercise training program. A trained exercise specialist will supervise you throughout the entire session, and will consist of you cycling on a sit-down bike at a moderate intensity. Sessions will last for 20 minutes in the beginning of the 8 weeks and progress to a total time of 45 minutes of exercise. We will non-invasively monitor your heart rate, amount of oxygen in your blood, blood pressure and exertion symptoms throughout exercise.

Time commitment: 30-60 minutes, three times per week for 8 weeks
Location: integrative Clinical Cardiopulmonary Physiology Laboratory (iCCP)
Why is this important? We are trying to understand the effects of exercise on heart and blood vessel function, inflammation and brain blood flow.

WHAT ARE THE RISKS?

The exercise that you will be performing is regarded as safe. All testing will be performed under appropriate supervision and appropriate resuscitation equipment will be available. Stress test data from other investigations suggest that the likelihood of dying from sudden cardiac death is 5 per 100,000 tests. This usually only occurs in people who already have some form of heart disease. Following all of the exercise sessions you may experience muscle soreness, which will disappear within a few days. All ultrasound measurements are non-invasive meaning they are performed on the surface of your skin and are regarded as safe with little risk of injury. The inflation of the cuff around your arm during flow mediated dilation may be painful and give an unpleasant sensation to your arm, similar to pins and needles. However, the sensation disappears when the cuff is deflated. You may terminate the test if you experience a great deal of pain or discomfort. As a consequence of receiving a single dose of nitroglycerine, you may experience a headache, dizziness, hot flush, and in the extreme case fainting, dry mouth and blurred vision. Blood samples will be collected by a trained medical professional; however, taking a sample of blood may result in some discomfort or bruising at the site of sampling. Upon standing out of bed or during the squat stand protocol, you may experience symptoms associated with fainting (light-headedness, dizziness) or may possibly faint. These symptoms should subside, however if they progress and you feel you may faint you will be returned to a lying position. We will be continuously monitoring your blood pressure and brain blood flow, from these measurements we will be able to identify if you are at risk of fainting, and prevent this from occurring. There are no risks associated with the mild changes in carbon dioxide; however you may experience minor headache or dizziness during increases in carbon dioxide. You will be closely monitored throughout the protocol, and in our experience of conducting greater than 2000 of these tests there have been no ill effects reported. All of these techniques listed are commonly used techniques, which have been ethically approved and are regarded as safe. Should it be determined during testing that you require emergency medical care, you will be taken to the Emergency Department at Kelowna General Hospital where you may be admitted.

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

If you agree to participate in this study there may not be a direct medical benefit to you. However, exercise training has the potential to improve you heart, blood vessel and skeletal muscle function. It is also well-documented that exercise can improve exercise capacity, reduce fatigue and exertional symptoms and improve quality of life in individuals with COPD. You will also receive an up to date pulmonary function assessment, your current fitness level will be evaluated, and you will receive an individualized aerobic exercise training program and supervised sessions as well as non-invasive assessments of your blood vessel, heart and brain function. The information we get from this study may help us to provide better treatments in the future for patients with COPD as it will allow us to better understand the

mechanisms of aerobic exercise training and its potential benefits for the heart, brain and blood vessels and the implication on exercise tolerance and quality of life.

WHAT HAPPENS IF I DECIDE TO WITHDRAW MY CONSENT TO PARTICIPATE?

Participation in this study is entirely voluntary. You may refuse to participate or you may withdraw from the study at any time without prejudice and without providing any reasons. If you decide to withdraw from the study, there will be no penalty or loss of benefits to which you are otherwise entitled, and your future medical care will not be affected. You do not waive any of your rights by signing this consent form. **If you choose to withdraw from the study, we will ask permission to use your data and/or information, however if you prefer that none of your data and/or information be used, you will not be penalized in any way.**

The study investigators/doctor may decide to discontinue the study at any time, or withdraw you from the study at any time if they feel that it is in your best interest.

WHAT WILL THE STUDY COST ME?

You will be reimbursed for any parking expenses that you incur while participating in the study. You should keep your parking receipts. You will also be given a $50.00 gift card to your business of choice as a gratuity for your participation.

COMPENSATION AND INJURY:

In the event that you suffer injury as a result of participating in this research, the University of British Columbia, or the researchers will not voluntarily provide compensation to you. You still have all your legal rights. Nothing said in this consent form alters your right to seek damages against the sponsor, investigators, or anyone else.

CONFIDENTIALITY:

Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent. All data and information related to you will be assigned a code designation so that personal identification is not possible. However, research records and medical records, such as your past and current health history, which identify you may be inspected in the presence of the Investigator or his or her designate by representatives of Health Canada, the UBC Research Ethics Board, or the Interior Health Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices.

All information collected during the study will be kept confidential and results will be coded and stored in a locked filing cabinet and/or password-protected computer and will be stored for twelve years after completion of this study. Data will be stored

only with patient identifiers and no names, telephone numbers of addresses will be stored with the date. Individual data will be assigned a code designation so that personal identification is not possible. If you decided to withdraw from the investigation, you will be asked if you would like your information included or removed from the study. Any report published as a result of the study will not identify you by name or initials since all findings will be reported as group averages. You will be asked to provide consent, which will allow us to publish our findings.

WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY DURING MY PARTICIPATION?

A researcher will be available on every occasion to explain the procedure and answer any questions. If you have any other questions or desire further information about this study before or during participation, you can contact Ms. Jinelle Gelinas at (250) 807-8860.

WHO DO I CONTACT IF I HAVE ANY QUESTIONS OR CONCERNS ABOUT MY RIGHTS AS A SUBJECT DURING THE STUDY?

Please note that you may ask questions at any time. We will be glad to discuss your results with you when they have become available and we welcome your comments and suggestions. If you have any concerns about your rights as a research subject and/or your experiences while participating in this study, contact the Research Subject Information Line in the University of British Columbia Office of Research Services' at 604-822-8598 or by email at RSIL@ors.ubc.ca or you may contact the Chair of the Interior Health Research Ethics Board through the Research Office at 250-870-4602.

S**UBJECT CONSENT TO PARTICIPATE**

In signing this form you are consenting to participate in this research project. Furthermore, signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

- *I have read and understood the subject information and consent form.*
- *I have had sufficient time to consider the information provided and to ask for advice if necessary.*
- *I have had the opportunity to ask questions and have had satisfactory responses to my questions.*
- *I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.*
- *I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.*
- *I understand that I am not waiving any of my legal rights as a result of signing this consent form.*

- *I understand that there is no guarantee that this study will provide any benefits to me*
- *I have read this form and I freely consent to participate in this study.*
- *I have been told that I will receive a dated and signed copy of this form.*

SIGNATURES

_____

Printed name of subject   Signature   Date

_____

Printed name of principal investigator/
designated representative   Signature   Date

THE UNIVERSITY OF BRITISH COLUMBIA

a place of mind

Subject Information and Informed Consent

Title of Project: The Effects of Aerobic Exercise Training on Cardiac, Peripheral Vascular and Cerebral Vascular Function in Patients with Chronic Obstructive Pulmonary Disease

Principal Investigator: *Dr. Neil Eves, PhD*
Assistant professor, Faculty of Health and Social Development, School of Health and Exercise Sciences
neil.eves@ubc.ca
(250) 807-9676

Co-Investigators: *Ms. Jinelle Gelinas*, *BHK, CPT*
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Dr. Philip Ainslie, PhD
Associate professor, Faculty of Health and Social Development,
School of Health and Exercise Sciences
philip.ainslie@ubc.ca
(250) 807-8089

Dr. Douglass Rolf
Clinical Assistant Professor, UBC Department of Medicine, Associate Member UBC Division of Respiratory Medicine.
Director of Respiratory Medicine Kelowna General Hospital and Associate Director of Critical Care Medicine
(250) 868-2943

Institution: Faculty of Health and Social Development

School of Health and Exercise Sciences
University of British Columbia- Okanagan

Contact Person: Ms. Jinelle Gelinas
Email: jcmgelinas@gmail.com
Phone: (250) 807-8860

INTRODUCTION

You are being invited to take part in this research study because you are a healthy age and activity matched control subject for a Chronic Obstructive Pulmonary Disease (COPD) patient who has been recruited for this study. COPD is a lung disease, which makes breathing difficult especially during exercise. Another aspect of COPD is that it is associated with inflammation in the blood, which can affect the health of the blood vessels and heart. Aerobic exercise has been shown to prevent the adverse effects of inflammation and can reduce the risk of heart and brain disease by improving blood vessel and heart function. This study will help us to understand the benefits of aerobic training on inflammation in people with COPD and its affect on blood vessel function compared to the effects of exercise on healthy subjects. Please read the following information carefully before deciding to participate in the study. If you have any questions, please do not hesitate to ask. The information collected during this study will be used for the Masters thesis of Jinelle Gelinas at the University of British Columbia.

YOUR PARTICIPATION IS VOLUNTARY

Your participation is entirely voluntary. Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts. If you wish to participate, you will be asked to sign this form. Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide. If you choose not to participate in this study, you will not be penalized in any way, nor do you need to disclose why you have chosen not to participate.

WHO IS CONDUCTING THE STUDY?

You are being invited to consider taking part in a collaborative study run under the School of Health and Exercise Sciences in the Faculty of Health and Social Development at the University of British Columbia Okanagan. This study will be conducted and overseen by Dr. Neil Eves (Principal Investigator), Jinelle Gelinas (Co-Investigators and MSc candidate), Dr. Philip Ainslie (Co-Investigator) and Dr. Douglass Rolf (Co-Investigator) who is a physician with Interior Health.

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BACKGROUND

Chronic obstructive pulmonary disease or COPD, is one of the most prevalent forms of lung disease affecting 8.2% of the Canadian adult population over 40 yrs. Smoking is the primary cause of COPD and leads to inflammation in the lungs and blood that can lead to secondary problems such as blood vessel disease, high blood pressure and heart disease. These conditions can reduce blood flow to the heart and brain, increasing the risk of heart attack or stroke. Aerobic exercise training, such as walking or biking, has been shown to reduce the risk of heart and brain disease in healthy individuals and in some other conditions. However, it is currently unknown whether exercise training can have the same affect in patients with a chronic inflammatory condition such as COPD. It is likely that the benefits of exercise for reducing the risk of heart and brain disease are due to changes in inflammation and how the blood vessels function but this has never been studied. The aim of this study is to investigate if eight weeks of aerobic exercise training improves blood vessel function and brain blood flow in patients with COPD. By exploring the effects of an 8 week aerobic exercise program, we hope to find new evidence to support the benefits of exercise in the management of COPD which could prevent the development of other health problems and greatly improve your quality of life. This study is a pilot study, which means that it is a small-scale preliminary study, in our case with 18 participants, designed to study the effects of aerobic exercise training on blood vessel health in patients with COPD. We would also like to recruit 18 control subjects to better understand difference in how COPD patients respond to the proposed exercise training.

WHAT IS THE PURPOSE OF THE STUDY?

The purpose of this study is to determine the effects of exercise training on blood vessel health in patients with COPD, and how the benefits of exercise relate to the function of the heart, blood vessels and brain. This will help us to better understand the benefits of aerobic exercise in patients with lung conditions and allow for more optimal exercise prescriptions and therapies to be designed to help improve health status and quality of life for individuals with COPD.

WHO SHOULD NOT PARTICIPATE IN THE STUDY?

If you have a respiratory, heart or brain condition that limits your ability to exercise safely or you suffer from pains in your muscles and joints that regularly stop you from exercising, you will not be able to participate in this study. If you are diabetic, have known problems with you breathing during sleep (obstructive sleep apnea) or are currently smoking or have quit for less than 3 months, you will not be able to participate in the study. Additionally, if your body weight is a lot greater than what is considered normal you may not be able to participate because body fat can also affect some of the measurements being studied.

WHAT DOES THE STUDY INVOLVE?

Visit 1 (2 hours): A breathing test (pulmonary function test), to measure pulmonary function and an incremental cycling exercise test, to determine your current fitness level and your safety to perform exercise in a community setting. During this visit we will also assess your current health-related quality of life using a questionnaire. This visit will be carried out at Kelowna General Hospital.

Visit 2 (90 min maximum): After fasting overnight, on the following day a trained health professional will take a blood sample from your arm which will be used to measure inflammation in your blood. While you are resting, we will also use a non-invasive technique called ultrasound (similar to that used when looking at an unborn fetus in a mothers stomach) and tonometry (a small pressure gauge that looks like a pen) to examine the blood vessels in your neck, arms and legs. This visit will be carried out at the integrative Clinical Cardiopulmonary Physiology Laboratory (iCCP) at the University of British Columbia Okanagan Campus.

Visit 3 (2 hours): On the final visit we will measure your heart function and brain blood flow with a similar non-invasive ultrasound device to that used in visit two and then you will exercise on a stationary exercise bike at a light, moderate and heavy intensity while we continue to make measurements of your heart function and brain blood flow. This visit will also be carried out at the integrative Clinical Cardiopulmonary Physiology Laboratory (iCCP) at the University of British Columbia Okanagan Campus.

Exercise training Sessions (3 hours/week, total 24 hours): Following all baseline measurements you will come to our exercise laboratory three times a week for 8 weeks to exercise on a stationary bike. All aerobic exercise training sessions will be supervised by a trained exercise specialist. Following the completion of the training we will ask you to repeat visits 1-3 again to see how all of the measurements have changed. All training sessions will be carried out at the integrative Clinical Cardiopulmonary Physiology Laboratory (iCCP) at the University of British Columbia Okanagan Campus.

* All tests and associated time commitments are described in full below. Testing will likely occur in the afternoons, evenings, or weekends. Following exercise training, tests 2-6 will be repeated.

(1) Pulmonary Function Test
What is this? This is a breathing test performed routinely to evaluate lung disease. A registered respiratory therapist will run and supervise this test. You will sit in a comfortable chair in a large clear chamber and breathe through a mouthpiece while wearing a nose clip. You will be asked to breathe normally and sometimes you will inhale all the way and exhale all the way as fast as you can. After the initial test is performed you will be given a bronchodilator medication (**bronchodilators are**

given by inhalation [with a puffer] to open or relax the breathing tubes or airways) to be sure that your airways are fully open and the breathing test will be repeated. The bronchodilator we will be giving you is called Ventolin, and you will take 2 puffs of it (200mcg). In the unlikely event of an emergency, a physician nearby in the hospital will be contacted immediately.

Time commitment: 45 minutes

Location: Kelowna General Hospital

Why is this important? The breathing test (spirometry) will be done to measure your lung function (how strong the lungs are).

(2) Symptom limited exercise test

What is this test? This test is an exercise test, which slowly gets harder until shortness of breath and/or tired legs stops you exercising. This test will be done on a stationary bicycle and you will breathe through a mouthpiece while wearing a nose clip to collect your expired air. Small stickers (electrodes) will also be stuck to your chest so that we can monitor your heart.

Time commitment: 45 minutes (only exercising for 10-12 minutes)

Location: Kelowna General Hospital

Why is this test important? This test is an indication of your current fitness level. We will use your values obtained from this test to determine your aerobic training intensity level.

(3) Blood collection

What is this? A blood sample will be collected from the forearm by the physician or trained medical staff. Twenty milliliters (1 table spoon full) will be collected. This is the same as a standard blood test.

Time commitment: 10 minutes

Location: integrative Clinical Cardiopulmonary Physiology Laboratory (iCCP)

Why is it important? This test allows us to measure the inflammation in your blood.

(4) Blood Vessel Function Assessment (Flow mediated dilation and Tonometry)

What is this? This test is done to assess the function of your blood vessels. In the first part of the test, while lying down, a blood pressure cuff will be placed around your left forearm, and pumped up to a level that will reduce blood flow for 5 minutes. At that time the cuff will be released, and using an ultrasound machine, we will monitor the return of blood flow in the arm. During the second part of the test we will place a pen like device with minimal pressure on the right side of your neck, and then on your wrist, back of your ankle, and your upper leg while you are lying down, which will measure stiffness of your blood vessels. We will also give you a small spray of a drug called nitroglycerine beneath your tongue which allows you blood vessels to increase in size and then we will repeat the measurements described.

Time Commitment: 90 min

Location: integrative Clinical Cardiopulmonary Physiology Laboratory (iCCP)
Why is this important? These tests will assess how your blood vessels work.

(5) Transcranial Doppler Test

What is this? During this test we ask you to wear a plastic head band which holds in position two small probes on either side of your temples. The probes non-invasively measure the speed of blood flow in one of the main arteries in your brain. It does this by using very high frequency sound which you cannot hear and by recording the echoes from the moving blood. The headband holds the probes in place and allows you to exercise without moving the probes. We will also assess how the brain responds to changes in blood pressure. This will require you to repeatedly perform a slight squat followed by a stand. Arm support can be provided if needed. Finally, we will evaluate how the brain responds to changes in carbon dioxide. This will involve you to breathe in a gas mixture made up of 5% of carbon dioxide (room air is a usually around 0.03% carbon dioxide) and the same amount of oxygen as you breathe in room air for 3 minutes, while lying down. We will also measure your brain blood flow while you exercise at a light, moderate and heavy load determine specifically for your fitness level from your incremental test described above.
Time Commitment: ~ 1 hour
Location: integrative Clinical Cardiopulmonary Physiology Laboratory (iCCP)
Why is this important? This test will assess your brain blood flow at rest and during exercise

(6) Echocardiography Exercise Test

What is this test? This test is an exercise test that measures the function of your heart at rest before and after you have completed 8 weeks of aerobic exercise training. A small ultrasound probe will be placed on your chest at the level of your heart. The probe uses sound wave to create a moving picture of the heart and involves no radiation exposure. We will repeat these measurements while you exercise at a light, moderate and heavy load determine specifically for your fitness level at the same time as we are measuring your brain blood flow.
Time commitment: 1 hour
Location: integrative Clinical Cardiopulmonary Physiology Laboratory (iCCP)
Why is this important? This test will assess your heart function at rest before and after aerobic exercise training.

(7) Aerobic Exercise Training

What is this? For 3 sessions a week for a total of 8 weeks, you will participate in a supervised aerobic exercise training program. A trained exercise specialist will supervise you throughout the entire session, and will consist of you cycling on a sit-down bike at a moderate intensity. Sessions will last for 20 minutes in the beginning of the 8 weeks and progress to a total time of 45 minutes of exercise. We will non-invasively monitor your heart rate, amount of oxygen in your blood, blood pressure and exertion symptoms throughout exercise.
Time commitment: 30-60 minutes, three times per week for 8 weeks
Location: integrative Clinical Cardiopulmonary Physiology Laboratory (iCCP)

Why is this important? We are trying to understand the effects of exercise on heart and blood vessel function, inflammation and brain blood flow.

WHAT ARE THE RISKS?

The exercise that you will be performing is regarded as safe. All testing will be performed under appropriate supervision and appropriate resuscitation equipment will be available. Stress test data from other investigations suggest that the likelihood of dying from sudden cardiac death is 5 per 100,000 tests. This usually only occurs in people who already have some form of heart disease. Following all of the exercise sessions you may experience muscle soreness, which will disappear within a few days. All ultrasound measurements are non-invasive meaning they are performed on the surface of your skin and are regarded as safe with little risk of injury. The inflation of the cuff around your arm during flow mediated dilation may be painful and give an unpleasant sensation to your arm, similar to pins and needles. However, the sensation disappears when the cuff is deflated. You may terminate the test if you experience a great deal of pain or discomfort. As a consequence of receiving a single dose of nitroglycerine, you may experience a headache, dizziness, hot flush, and in the extreme case fainting, dry mouth and blurred vision. Blood samples will be collected by a trained medical professional; however, taking a sample of blood may result in some discomfort or bruising at the site of sampling. Upon standing out of bed or during the squat stand protocol, you may experience symptoms associated with fainting (light-headedness, dizziness) or may possibly faint. These symptoms should subside, however if they progress and you feel you may faint you will be returned to a lying position. We will be continuously monitoring your blood pressure and brain blood flow, from these measurements we will be able to identify if you are at risk of fainting, and prevent this from occurring. There are no risks associated with the mild changes in carbon dioxide; however you may experience minor headache or dizziness during increases in carbon dioxide. You will be closely monitored throughout the protocol, and in our experience of conducting greater than 2000 of these tests there have been no ill effects reported. All of these techniques listed are commonly used techniques, which have been ethically approved and are regarded as safe. Should it be determined during testing that you require emergency medical care, you will be taken to the Emergency Department at Kelowna General Hospital where you may be admitted.

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

If you agree to participate in this study there may not be a direct medical benefit to you. However, exercise training is well recognized to improve fitness, reduce fatigue and increase energy levels, improve sleep, and improve quality of life and stress levels. You will also receive an up to date pulmonary function assessment, your current fitness level will be evaluated, and you will receive an individualized aerobic exercise training program and supervised sessions as well as non-invasive assessments of your blood vessel, heart and brain function. The information we get from this study may help us to provide better treatments in the future for patients

with COPD as it will allow us to better understand the mechanisms of aerobic exercise training and its potential benefits for the heart, brain and blood vessels and the implication on exercise tolerance and quality of life.

WHAT HAPPENS IF I DECIDE TO WITHDRAW MY CONSENT TO PARTICIPATE?

Participation in this study is entirely voluntary. You may refuse to participate or you may withdraw from the study at any time without prejudice and without providing any reasons. If you decide to withdraw from the study, there will be no penalty or loss of benefits to which you are otherwise entitled, and your future medical care will not be affected. You do not waive any of your rights by signing this consent form. **If you choose to withdraw from the study, we will ask permission to use your data and/or information, however if you prefer that none of your data and/or information be used, you will not be penalized in any way.**

The study investigators/doctor may decide to discontinue the study at any time, or withdraw you from the study at any time, if they feel that it is in your best interests.

WHAT WILL THE STUDY COST ME?

You will be reimbursed for any parking expenses that you incur while participating in the study. You should keep your parking receipts. You will also be given a $50.00 gift card to your business of choice as a gratuity for your participation.

COMPENSATION AND INJURY:

In the event that you suffer injury as a result of participating in this research, the University of British Columbia, or the researchers will not voluntarily provide compensation to you. You still have all your legal rights. Nothing said in this consent form alters your right to seek damages against the sponsor, investigators, or anyone else.

CONFIDENTIALITY:

Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent. All data and information related to you will be assigned a code designation so that personal identification is not possible. However, research records and medical records, such as your past and current health history, which identify you may be inspected in the presence of the Investigator or his or her designate by representatives of Health Canada, the UBC Research Ethics Board, or the Interior Health Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices.

All information collected during the study will be kept confidential and results will be coded and stored in a locked filing cabinet and/or password-protected computer and will be stored for twelve years after completion of this study. Data will be stored only with patient identifiers and no names, telephone numbers of addresses will be stored with the date. Individual data will be assigned a code designation so that personal identification is not possible. If you decided to withdraw from the investigation, you will be asked if you would like your information included or removed from the study. Any report published as a result of the study will not identify you by name or initials since all findings will be reported as group averages. You will be asked to provide consent, which will allow us to publish our findings.

WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY DURING MY PARTICIPATION?

A researcher will be available on every occasion to explain the procedure and answer any questions. If you have any other questions or desire further information about this study before or during participation, you can contact Ms. Jinelle Gelinas at (250) 863-3268.

WHO DO I CONTACT IF I HAVE ANY QUESTIONS OR CONCERNS ABOUT MY RIGHTS AS A SUBJECT DURING THE STUDY?

Please note that you may ask questions at any time. We will be glad to discuss your results with you when they have become available and we welcome your comments and suggestions. If you have any concerns about your rights as a research subject and/or your experiences while participating in this study, contact the Research Subject Information Line in the University of British Columbia Office of Research Services' at 604-822-8598 or by email at RSIL@ors.ubc.ca or you may contact the Chair of the Interior Health Research Ethics Board through the Research Office at 250 870-4602.

SUBJECT CONSENT TO PARTICIPATE

In signing this form you are consenting to participate in this research project. Furthermore, signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

- *I have read and understood the subject information and consent form.*
- *I have had sufficient time to consider the information provided and to ask for advice if necessary.*
- *I have had the opportunity to ask questions and have had satisfactory responses to my questions.*
- *I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.*

- *I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.*
- *I understand that I am not waiving any of my legal rights as a result of signing this consent form.*
- *I understand that there is no guarantee that this study will provide any benefits to me*
- *I have read this form and I freely consent to participate in this study.*
- *I have been told that I will receive a dated and signed copy of this form.*

SIGNATURES

_____
Printed name of subject   Signature   Date

_____
Printed name of principal investigator/
designated representative   Signature   Date

Appendix B- Ethics Certificates

The University of British Columbia
Office of Research Services
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC
V5Z 1L8

ETHICS CERTIFICATE OF FULL BOARD APPROVAL

PRINCIPAL INVESTIGATOR: Neil Eves	INSTITUTION / DEPARTMENT: UBC/UBCO Health & Social Development/UBCO Health and Exercise Sciences	UBC CREB NUMBER: H11-02770
INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:		

Institution	Site
UBC	Okanagan
Other locations where the research will be conducted: Kelowna General Hospital	

CO-INVESTIGATOR(S):
Jinelle Gelinas
Philip Ainslie

SPONSORING AGENCIES:
- Michael Smith Foundation for Health Research - "The Anti-Inflammatory Effects of Exercise in Patients with Chronic Obstructive Pulmonary Disease"

PROJECT TITLE:
The Effects of Aerobic Exercise Training on Peripheral Vascular, Cardiac and Cerebral Vascular Function in Patients with Chronic Obstructive Pulmonary Disease

THE CURRENT UBC CREB APPROVAL FOR THIS STUDY EXPIRES: December 13, 2012

The full UBC Clinical Research Ethics Board has reviewed the above described research project, including associated documentation noted below, and finds the research project acceptable on ethical grounds for research involving human subjects and hereby grants approval.

This approval applies to research ethics issues only. The approval does not obligate an institution or any of its departments to proceed with activation of the study. The Principal Investigator for the study is responsible for identifying and ensuring that resource impacts from this study on any institution are properly negotiated, and that other institutional policies are followed. The REB assumes that investigators and the coordinating office of all trials continuously review new information for findings that indicate a change should be made to the protocol, consent documents or conduct of the trial and that such changes will be brought to the attention of the REB in a timely manner.

REB FULL BOARD MEETING REVIEW DATE: December 13, 2011			
DOCUMENTS INCLUDED IN THIS APPROVAL:			**DATE DOCUMENTS APPROVED:**

Document Name	Version	Date	
Protocol:			
Proposal	2	December 20,	

		2011	
Consent Forms:			
Consent Form - COPD	2	December 20, 2011	
Consent Form - Controls	2	December 20, 2011	
Investigator Brochures:			
Product Monograph - Nitrolingual GTN	1	January 21, 2011	
Product Monograph - Ventolin	1	October 3, 2007	**January 3, 2012**
Advertisements:			
Poster - COPD	2	December 20, 2011	
Poster - Controls	2	December 20, 2011	
Questionnaire, Questionnaire Cover Letter, Tests:			
HRQOL Questionnaire	1	December 20, 2011	
Letter of Initial Contact:			
Letter of Initial contact	2	December 20, 2011	
Other Documents:			
Cover Letter	1	November 24, 2011	

CERTIFICATION:
In respect of clinical trials:
1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The documentation included for the above-named project has been reviewed by the UBC CREB, and the research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved by the UBC CREB.

Approval of the Clinical Research Ethics Board by one of:

Dr. Peter Loewen, Chair
Dr. Stephen Hoption Cann, Associate Chair

B.2- Interior Healthy Research Ethics Certificate

Interior Health

Certificate of Full Research Ethics Board Approval-Amendment

Principal Investigator: Dr. Neil Eves	Institution of Primary Association UBC Okanagan	IH Research File Identifier 2011-12-037-E
Research Study Title: The Effects of Aerobic Exercise Training on Peripheral Vascular and Cerebral Vascular Function in Patients with Chronic Obstructive Pulmonary Disease		
IH Administrative Contact Sharon Cook John Cabral	**Co-Investigators** Jinelle Gelinas Graduate student UBCO Dr. Douglass Rolf UBC/IH Dr. Philip Ainslie UBCO Dr. Nia Lewis UBCO	
Sponsoring/Funding Agencies MSFBC	**IH Departments Involved in Research Study** Pulmonary Function Laboratory KGH	
Documents Covered by this Approval IH REB Request for Amendment received 24 Oct 24 2012 Notice of Research Study Version date 24 Oct 2012	**Certificate of Approval from Primary REB** UBC CREB Jan 03, 2012	

Certification

The above named documents have been reviewed according to Interior Health Research Ethics Board policy and the procedures were found to be acceptable on ethical grounds for research involving human subjects.

This Certificate of Approval is valid for the term specified below provided there are no changes in the study procedures.

The Interior Health Research Ethics Board is in compliance with the ethical principles presented in the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans

Conditions for Approval

It is the responsibility of the principal investigator to inform the IH Research Office if there are changes to consents or other materials used with human subjects – these must be submitted to the IH Research Office for review and approval prior to implementation.

It is the responsibility of the Principal Investigator to inform the IH Research Office if human subjects experience serious or unexpected events.

Approval Date 01 November 2012	**Approval Term** For current approved term

IH Authorized Signature

B. Ann Ferguson, Chair, Interior Health Research Ethics Board   19 Nov 2012   Date