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The Effect of Exercise Intensity on Airway and Systemic Inflammation in Patients with COPD

by

Wendy S. Verity

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Effect of Exercise Intensity on Airway and Systemic Inflammation in Patients with COPD" submitted by Wendy S. Verity in partial fulfillment of the requirements of the degree of Master of Science.

Supervisor, Dr. Neil Eves, Faculty of Kinesiology, Department of Medical Science

Co-Supervisor, Dr. Warren Davidson, Faculty of Medicine, Department of Community Health Sciences

Dr. Richard Leigh, Departments of Medical Science and Cardiovascular and Respiratory Science

Dr. Gordon Ford, Department of Cardiovascular and Respiratory Science

Dr. Margaret Kelly, Department of Cardiovascular and Respiratory Science

18th MAY 2010

Date

ABSTRACT

Rationale: Airway and systemic inflammation are characteristic features of chronic obstructive pulmonary disease (COPD). A single bout of exercise increases systemic inflammation in patients with COPD, but no study has documented the airway inflammatory response, the anti-inflammatory response, or how different intensities of aerobic exercise influence these inflammatory processes.

Objective: To investigate the effect of exercise intensity on airway and systemic inflammation in patients with COPD.

Methods: Ten patients with moderate-to-severe smoking-related COPD ($FEV_1/FVC<0.7$, FEV_1 30-80% pred) and ten healthy age- and activity-matched controls were recruited for the study. Following pulmonary function and cardiopulmonary exercise tests at an initial visit, sputum and blood samples were collected ~48 hours before, and 0 (blood only) and 2 hours after a high intensity (HIGH) or low intensity (LOW) cycle exercise trial. The order of exercise was randomized and trials were equated for total work. HIGH consisted of eight intervals of 1 min at 100% workload maximum (W_{max}) with 2 min at 30% W_{max}, while LOW consisted of ~32 min of exercise at 40% W_{max}. Sputum and blood samples were assessed for differential cell count, as well as interleukin (IL) 6, 8 and 10.

Results: Sputum differential cell counts were obtained at all time points in eight patients and six controls while blood samples were obtained in ten patients and nine controls. Patients with COPD demonstrated higher absolute sputum neutrophils at all time points. Sputum neutrophils were reduced after LOW in patients with COPD ($p<0.05$), and this reduction was greater than with HIGH (-14.7 vs. -0.8% change in LOW and HIGH respectively, $p<0.05$). This finding was not observed in controls. A decrease in sputum IL-8 occurred after HIGH in the COPD group ($p=0.05$) but not in controls. Systemically, IL-8 was elevated and IL-10 was reduced in the COPD compared to controls at all time points ($p<0.05$). After HIGH, controls had an increase in systemic IL-10 from baseline to immediately after exercise (5.7 vs. 9.2 pg/ml, respectively, $p<0.05$), while the COPD group had no significant increase in IL-10 at any time point after either exercise trial.

Conclusions: This is the first study to investigate the airway inflammatory response to exercise in patients with COPD and demonstrates that high and low intensity exercise

result in differing airway inflammatory responses. Systemically, there is a blunted anti-inflammatory response to high-intensity exercise in patients with COPD, which may have important implications for appropriately prescribing exercise for this population.

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Dedication

To Jesus Christ,

The greatest scientist that ever lived.

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List of Abbreviations

6MWT – Six Minute Walk Test
ATS – American Thoracic Society
BAL – Bronchoalveolar Lavage
COPD – Chronic Obstructive Pulmonary Disease
CPET – Cardiopulmonary Exercise Test
CRP – C-Reactive Protein
CTS – Canadian Thoracic Society
DL_{CO} – Carbon Monoxide Lung Diffusion Capacity
DTT - Dithiothreitol
ECG – Electrocardiogram
GM-CSF – Granulocyte Macrophage-Colony Stimulating Factor
H_2O_2 – Hydrogen Peroxide
ICS – Inhaled Corticosteroid
IFN-γ - Interferon Gamma
IL-1 – Interleukin 1
IL-1β - Interleukin 1 Beta
IL-1ra – Interleukin 1 Receptor Agonist
IL-4 – Interleukin 4
IL-5 – Interleukin 5
IL-6 – Interleukin 6
IL-8 – Interleukin 8
IL-10 – Interleukin 10
IL-11 – Interleukin 11
IL-13 – Interleukin 13
IL-18 – Interleukin 18
IP-10 – Interferon-Inducible Protein 10
FEV_1 – Forced Expiratory Volume in One Second
FVC – Forced Vital Capacity
LABA – Long Acting Beta-2 Agonist
LIF – Leukemia Inhibitory Factor
LTB4 – Leukotriene B4
MAP – Maximal Aerobic Power
MAPK – Mitogen-activated protein kinase
METs – Metabolic Equivalents
MMP – Matrix Metalloproteinase
MMP-9 – Matrix Metalloproteinase-9
mRNA – Messenger Ribonucleic Acid
NFκB - Nuclear Transcription Factor-kappaB
NK Cells – Natural Killer Cells
PBS – Phosphate Buffered Saline
RPM – Revolutions Per Minute
SP-D – Surfactant Protein-D
SpO_2 – Oxyhemoglobin Saturation
sTNF-r – Soluble Tumor Necrosis Factor Receptor
Tc1 Cells – Type 1 Cytotoxic T Cells
TGF-β - Transforming Growth Factor Beta
Th1 Cells – T Helper 1 Cells
TNF-α - Tumor Necrosis Factor-Alpha
V_E - Minute Ventilation
VO_2 – Oxygen Consumption
W_{max} – Work Rate Maximum

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1 Chronic Obstructive Pulmonary Disease

COPD is the fourth leading cause of death globally and is a continually growing burden to healthcare systems in Canada and across the world [1]. In Canada, COPD is the direct cause of 4% of deaths, and may contribute to increased risk of death from other chronic diseases [2]. Over 750 000 Canadians are currently living with COPD [2]. Over the past thirty years, the death rates of many other diseases such as cardiovascular disease, stroke, and cancer have been decreasing or remaining consistent, while death rates of COPD patients have doubled [3]. COPD is projected to become the third leading cause of death worldwide by 2020 [4]. The economic cost of COPD is substantial, with a direct and indirect costs to the Canadian health care system of approximately 1.7 billion dollars per year [5]. Consequently, the burden of COPD on the health care system is, and will be of significant concern for health care practitioners and individuals affected by this disease.

The Canadian Thoracic Society defines COPD as a progressive yet treatable disease characterized by partially reversible airflow obstruction and lung hyperinflation caused by chronic inhalation of noxious particles or gases [6]. The greatest risk factor identified for COPD is smoking, although workplace or environmental factors may contribute to development of the disease [6]. The airflow obstruction in patients with COPD is caused by two factors, chronic bronchitis, which can be defined as a chronic

cough that produces sputum and mucus and which persists for longer than three months for two consecutive years, and emphysema, which is the destruction of the alveoli and the structures supporting them [7]. Both chronic bronchitis and emphysema are largely a consequence of chronic inflammation in the lung [8]. Along with airflow limitation, which may contribute to dyspnea in patients with COPD, several systemic comorbidities exist, including cardiovascular disease, cachexia, muscle atrophy, osteoporosis, depression, and anemia [9-11]. Several of these systemic effects may be caused by an increased systemic inflammatory state [9]. Thus, because of the many effects of inflammation, COPD has recently been classified as an inflammatory disorder with pulmonary and systemic effects [12].

1.2 Inflammation

Inflammation refers to the response elicited by the body to a stimulus that disrupts the body from homeostasis. This stimulus can exist as a bacteria, fungus, virus (these three are referred to as *pathogens*), or physical disruption. The ultimate purpose of inflammation is to remove the harmful stimuli and set up an environment that is suitable for tissue repair and a return to homeostasis [13].

The process of pathogen clearance and tissue repair is a local response; however, the key players in the inflammatory response are not expressed constitutively in all tissues and must be recruited from other regions of the body. All inflammatory cells are derived from the bone marrow and are formed from hematopoeitic stem cell progenitors [14]. These progenitors differentiate into the diverse array of inflammatory cells, which

include monocytes, macrophages, neutrophils, eosinophils, basophils, mast cells, dendritic cells, T- and B- lymphocytes, natural killer (NK) cells, and plasma cells. All of these cells (excluding macrophages, which are formed when monocytes differentiate in peripheral tissues) circulate through the blood, enter tissues, and can presume an activated or non-activated role in their local environments [14].

Inflammatory cells can be recruited to specific sites upon tissue damage or disruption by the inflammatory messengers known as cytokines and chemokines. Cytokines and chemokines are secreted by numerous sources within the body, including vascular endothelial cells, epithelial cells, and inflammatory cells [15]. Each inflammatory cell displays various receptors for differing cytokines and chemokines, and will therefore respond according to the chemical messengers in an area. Neutrophils, for example, are potently attracted into the lung by the chemokine IL-8 [16], and eosinophils are recruited primarily by IL-5 [17]. Changes to a local tissue environment can cause an immediate release of cytokines from that area, and the release of these cytokines into systemic circulation will draw inflammatory cells from distant regions towards the affected tissue to aid in host defense. In this way, the innate immune system is able to respond rapidly to disruptions in the tissues and act as the first line of the defense against invading pathogens or other harmful stimuli [18].

Cytokine and chemokine release can be stimulated by a wide variety of chemical or mechanical stimuli. Endothelial cells, for example, secrete cytokines in response to shear stress [19], and inflammatory cells such as macrophages will secrete cytokines

upon binding with bacteria [20]. Upon release, cytokines and chemokines will stimulate biochemical and metabolic changes in the local environment [20], as well as in distant areas, including the lymph nodes, kidney, spleen, and bone marrow [21]. Such changes include, but are not limited to: increases in lipolysis [22], glucose uptake [23], catecholamine release [24], T and B lymphocyte development and maturation [25], macrophage, neutrophil and other granulocyte activation [26-28], allergic hypersensitivity [29], vascular permeability [30], and endothelial damage [31]. Thus, an alteration to a local tissue environment may result in a diverse array of physiological changes throughout the body.

The response to a change in a local environment can be divided into two interconnected phases: the pro-inflammatory response, which is aimed at defending and eliminating the pathogen, and the anti-inflammatory response, which is aimed at removing excess inflammatory cells and byproducts that have accumulated during defense of the area. The pro- and anti-inflammatory state in tissue or systemic circulation is a dynamic condition determined by the levels of cytokines and chemokines found in that specific region. Pro-inflammatory cytokines include TNF-α, IL-1, interferon gamma (IFNγ), and IL-8, among many others [32]. These cytokines aid in attracting leukocytes and lymphocytes to the area of tissue damage. They can accomplish this in several ways, such as signaling to endothelial cells to increase expression of adhesion molecules, or simply by acting as chemoattractants to cells as they enter tissues. Anti-inflammatory cytokines include IL-10, IL-4, IL-13, and transforming growth factor beta (TGF-β) [33]. These cytokines often act as a protective

feature of the immune system by preventing abnormally high levels of inflammatory cells from accumulating in peripheral tissues [32]. They do so by signaling for down-regulation of adhesion molecules, preventing synthesis of additional pro-inflammatory cells, or by binding with pro-inflammatory cytokines to prevent downstream signaling [33]. Lastly, there is a third group of cytokines termed "IL-6-type cytokines", which can initiate either pro- or anti-inflammatory pathways, depending on the eliciting stimulus [32]. IL-6 is the most dominant of these cytokines, but others include leukemia inhibitory factor (LIF), and IL-11. An example of pro- and anti-inflammatory action from a cytokine in this group is when, in response to exercise, IL-6 acts in an anti-inflammatory manner and stimulates production of IL-10 (an anti-inflammatory cytokine) [34], and during sepsis, IL-6 acts in a pro-inflammatory fashion by mediating increases in fibrinogin (a pro-inflammatory acute phase protein) [35]. Because of the vast array of agonists, antagonists, and redundancies among cytokine actions, elucidating the control mechanisms by which IL-6 type cytokines exhibit either pro- or anti-inflammatory behaviors has yet to be accomplished, but doing so may provide valuable insight into the pathogenesis of many chronic inflammatory conditions where the pro- versus anti-inflammatory balance has been disrupted.

There is much interplay between pro- and anti-inflammatory cytokines, leukocytes, lymphocytes, and other cells in the immune system. In healthy conditions, the balance between pro- and anti-inflammatory conditions is tightly regulated, but in disease states, the balance becomes disrupted. Understanding how this balance is maintained, and factors that contribute to pro- or anti-inflammatory fluctuations in

disease states, is necessary to slow development and manage progression of many chronic diseases, including COPD.

1.3 Inflammation and COPD

A major feature of COPD is the presence of chronically elevated levels of airway and systemic inflammation. Both airway and systemic inflammation have been correlated with worsening of disease symptoms and comorbidities, including declines in lung function, increased risk of exacerbation, muscle wasting, cardiovascular disease, and endothelial dysfunction [10, 36, 37]. Understanding the specific inflammatory processes that contribute to these pulmonary and systemic effects will assist in developing appropriate treatment strategies for patients at varying severities of disease.

1.3.1 Airway Inflammation in COPD Patients

The inflammatory cascade involved in the progression of COPD begins with the inhalation of cigarette smoke or other noxious particles. Exposure of airway epithelial cells and alveolar macrophages to airway particulate matter has been shown to increase the production of the pro-inflammatory cytokines TNF-α, granulocyte macrophage colony stimulating factor (GM-CSF), IL-1β, IL-6, and IL-8, among many others [38, 39]. Cigarette smoke also enhances leukocyte rolling and adhesion on pulmonary endothelial cells [40, 41]. The release of cytokines paired with increased rolling and adhesion on the endothelium can result in an invasion into the lungs of several types of inflammatory cells, including neutrophils, monocytes, T helper 1 (Th1) cells and type 1

cytotoxic T (Tc1) cells [39, 42]. Inside the lung, neutrophils and macrophages secrete proteases such as matrix metalloproteinase 9 (MMP-9) and neutrophil elastase. These proteases act on goblet cells in the airway epithelium and mucus cells in the submucosal glands, which stimulates mucus hypersecretion [43]. Secretion of proteases by neutrophils and macrophages, paired with the release of perforin and granzymes from CD8+ T-cells can result in degradation of the alveolar wall and breaking of alveolar attachments, leading to the large airspaces indicative of emphysema [44]. Further, release of growth factors, namely TGF-β and fibroblast growth factor, by airway epithelial cells and alveolar macrophages in response to cigarette smoke or other cytokine stimulation, stimulates fibroblasts to proliferate, and upon repeated insult, may result in fibrosis of the airways [8, 44, 45]

Years of repeated exposure to cigarette smoke or other airway irritants results in irreversible damage to the airways. Cessation of smoking or removal of the toxic inhalant often leads to a decrease in airway inflammation [46] and therefore reduction in the risk of developing COPD. In many cases, however, with smoking cessation, airway inflammation persists and individuals still acquire COPD [47]. Several theories exist as to why only certain individuals are susceptible to COPD. One likely cause would be genetic deficiency, as is the case for individuals with alpha-1 antitrypsin deficiency [48]. Another hypothesis for selective development of COPD is because of an alteration to the adaptive immune system in response to chronic inhalation of particles [49]. This hypothesis explains that with repeated exposure to cigarette smoke, airway epithelial cells become damaged and their DNA patterns become altered. Changes to the DNA

patterns of these cells causes dendritic cells to identify them as non-host, thus eliciting an immune response consisting primarily of CD8+ T-cells aimed at repairing the homeostasis of the airway environment. Secretion of perforin and granzymes from CD8+ cells causes further damage to airway epithelial cells and contributes to the pathological changes observed in the airways of COPD patients. As long as epithelial cells are being identified as non-self, this pathway will continue to occur [49].

1.3.2 *Airway Inflammation and Severity of Disease in Chronic Obstructive Pulmonary Disease*

A number of inflammatory markers in the lungs have been related to various markers of disease in patients with COPD [50]. Forced expiratory volume in one second (FEV_1) is one variable used in the diagnosis of COPD and can provide a quantitative measurement of the degree of airflow obstruction in these patients. In relation to disease severity, a decline in FEV_1 has been repeatedly associated with patient mortality [51, 52], and therefore FEV_1 is an important prognostic value for patients with COPD. Lower FEV_1 values in patients with COPD have been correlated with sputum levels of IL-6 [53], bronchoalveolar lavage (BAL) levels of neutrophils, IL-8, and IL-6 [54], and bronchial biopsy levels of CD8+ T lymphocytes [55] and nuclear transcription factor-kappaB (NFκB) [56]. Regarding changes in FEV_1 over time, a greater rate of decline of FEV_1 has been correlated with increased levels of sputum IL-6 and magnitude of sputum neutrophilia [50]. This evidence suggests that the inflammatory cells and cytokines that accumulate in the lungs of patients with COPD play a role in the pathogenesis of airflow

obstruction, yet the precise mechanism by which these changes occur remains to be understood.

Another harmful consequence of airway inflammation may be an increased risk for exacerbations in patients with COPD. While the cause of exacerbations may vary from patient to patient, inflammatory cells may play a role in the development of an exacerbation. The frequency of exacerbations has been found to positively correlate to sputum levels of IL-6 and IL-8 [57], and during exacerbations, an increase in airway eosinophils has been observed [58]. Exacerbation frequency correlates with declines in lung function, measured by FEV_1 over time [59], and exacerbations severely affect COPD patients' quality of life [60], pose great financial burden because of the cost of hospitalization as well as lost days of work [61], and contribute largely to COPD deaths [62]. Thus, preventing exacerbations by managing the inflammatory processes leading to these events is of primary importance in COPD patient care.

1.3.3 Systemic Inflammation in Patients with COPD, and Relationship with Comorbidities

There is growing evidence that COPD is no longer simply a disease of pulmonary manifestations, since a majority of patients with COPD have at least one extrapulmonary comorbidity. The prevalence in patients with COPD living with one or more systemic conditions ranges from approximately fifty-one to eighty-seven percent [60, 63, 64]. Comorbidities and their respective prevalence in COPD patients include,

but are certainly not limited to, hypertension (48%), other cardiovascular diseases, including ischemia, heart failure, and stroke (17%), diabetes (19%), osteoporosis (50-70%), anemia (15-30%) muscle deconditioning (33%) and depression (19-42%) [65-67]. Not only do these conditions escalate the economic cost of COPD, but they have a negative impact on patient health-related quality of life and are major contributors to patient mortality [64]. The presence of the many systemic comorbidities complicates the understanding of the natural progression of COPD, and therefore much of the recent COPD research has focused on elucidating the pathogenesis of both airway and systemic manifestations of this disease. Much of the recent research has investigated how systemic inflammation may play a role in comorbidity development, since in the absence of COPD, many of the abovementioned comorbidities have been attributed to systemic inflammation.

The progression of systemic inflammation in patients with COPD begins with inhalation of cigarette smoke, which leads to an acute inflammatory response in the lungs, and "spillover" of cytokines into systemic circulation [66]. This results in an acute phase response in systemic circulation, marked by elevations in systemic IL-6, TNF-α, and CRP, followed by an increase in blood leukocytes [68]. Continued cigarette smoking results in recurrent elevations in these inflammatory markers. As COPD develops this inflammation becomes abnormally high, and remains chronically elevated [66, 69]. Even with smoking cessation this inflammation is likely to remain high, as evidenced by the persistence of elevated systemic CRP in ex-smokers with COPD [68].

It is this chronically raised systemic inflammatory state that is hypothesized to contribute to the many systemic effects seen in patients with COPD.

It is well accepted that systemic inflammation plays an important role in the progression of cardiovascular disease in the absence of COPD [70-72], but few studies have examined how cardiovascular disease and systemic inflammation are related in when airflow obstruction is present. Sin and Man [73] analyzed data from 6629 study participants and found that individuals who had severe airflow obstruction (FEV_1 < 50% predicted) combined with elevated levels of systemic CRP (defined as >1mg/dL) had nearly 5 times greater risk of ischemic heart disease than those who had normal spirometry values and circulating levels of CRP. Individuals who had moderate airflow obstruction (FEV_1 = 50-80% predicted) combined with elevated levels of systemic CRP had approximately twice the risk of ischemic heart disease compared to those with normal airflow and CRP levels. Systemic levels of fibrinogen and IL-6 were also correlated with cardiovascular disease risk, but these values were not compared to values of airflow obstruction in the study participants. The mechanisms by which CRP is thought to mediate the development of coronary plaques are complex: upregulation of other inflammatory cytokines, initiating complement proteins, stimulation of macrophages to uptake low-density lipoproteins, ultimately forming foam cells, and by stimulating adhesion molecule expression on endothelial cells [74, 75]. There are likely other players in the inflammatory cascades leading to cardiovascular disease in patients with COPD, such as fibrinogen or TNF-α, but the precise mechanisms by which they act are not well understood. Because of the substantial burden of cardiovascular disease on

patients with COPD (or vice versa), much of the upcoming COPD research will likely focus on determining how these diseases concomitantly progress, and how to best mange their progression and improve patient outcomes.

Systemic inflammation may also contribute to a number of the other comorbidities that occur in patients with COPD. Both weight loss and muscle wasting in patients have been correlated with TNF-α [76, 77], decreased maximal and submaximal aerobic exercise capacity are associated with circulating IL-6 and CRP [78], and reduced muscle strength has been correlated with raised levels of IL-8 [79]. IL-6 levels are raised in systemic circulation during COPD exacerbations [57], and in a recent study by Pinto-Plata and colleagues [80], patients who were admitted to hospital for an acute exacerbation of COPD showed decreasing levels of systemic IL-6, IL-8 and leukotriene B4 (LTB4) as they recovered from their exacerbation. Clearly, there is a significant role that systemic inflammation plays in the many systemic effects of COPD. Whether this inflammation occurs primarily as a response to pulmonary inflammation, or as an independent mechanism, is an important topic to address.

1.3.4 Relationship Between Airway and Systemic Inflammation in Patients with COPD

It is well-known that chronic inhalation of cigarette smoke or other toxic particles may induce a local inflammatory response in the lungs [81, 82]. This inflammatory response is elicited in order to protect the surrounding tissues from the harmful effects of the inhaled toxin. It is also understood that patients with COPD who

stop smoking continue to display an elevated airway inflammatory profile, while inflammatory levels decrease in former smokers without COPD. Along with the increased level of inflammation in the lung, COPD patients exhibit an elevated systemic inflammatory profile. Much recent work has aimed at elucidating how airway and systemic inflammation are related and why airway inflammation persists even with smoking cessation in patients with COPD, and two potential mechanisms may help explain these processes.

First, as described briefly in section 1.3.3, increased levels of systemic inflammation could occur as a consequence of elevated levels of inflammation in the lung. The first and most convincing evidence for an airway origin for systemic inflammation is found in recent research investigating the roles of surfactant protein-D (SP-D). SP-D is a surfactant protein which is formed exclusively in the lung but found in measurable quantities in systemic circulation [83]. While BAL levels of SP-D are not found to correlate well with markers of disease in COPD, systemic SP-D levels have been correlated to pack year history of smoking as well as declines in FEV_1 in patients with COPD [84]. It is hypothesized that smoking exposure causes damage to the alveolar-capillary surface, allowing leakage of SP-D into systemic circulation [83]. Once in the blood, SP-D may be involved in the development of atherosclerosis [85]. The high prevalence of cardiovascular disease in patients with COPD, along with the scarcity of current biomarkers able to specifically and non-invasively track the pathogenesis of COPD, including declines in lung function, progression of inflammation, and systemic

effects, make SP-D an important future target for further research regarding the airway-systemic relationship in COPD.

The airway-systemic inflammatory relationship is supported by evidence where inhalation of noxious particles leads to elevation of systemic inflammatory markers. Van Eeden and colleagues [86] observed higher levels of systemic inflammatory cytokines such as IL-6, IL-1β, and GM-CSF in individuals exposed to the environmental pollution of the South East Asia Haze of 1997, and the concentration of these cytokines decreased when the haze resided. A comparison between smokers and non smokers by Yeung and Buncio [87] found increased levels of circulating leukocytes in the smoking group, and the level of circulating leukocytes correlated with the amount of cigarettes smoked per day as well as pack-years of the study participants. Furthermore, leukocyte rolling and adhesion is increased not only on pulmonary endothelial cells but also in systemic microcirculation in response to cigarette smoke [40, 41]. This increased rolling and adhesion may promote increased invasion of inflammatory cells into systemic tissues, and could mediate the systemic tissue damage associated with these inflammatory cells. Therefore, the airway-systemic relationship may develop as follows: (1) cytokines or other biomarkers (such as SP-D) produced by airway cells such as alveolar macrophages and epithelial cells in response to cigarette smoke enter systemic circulation and stimulate the bone marrow to produce inflammatory cells, (2) these cells are released by the bone marrow and contribute to raised levels of inflammation in the blood [38], and (3) increased rolling and adhesion of inflammatory cells on the pulmonary and systemic endothelium may result in increased transmigration, and

consequently increased inflammatory accumulation in these tissues [41]. Chronic inhalation of cigarette smoke will continue to cause this increased inflammatory invasion, and ultimately may result in the inflammation-induced tissue damage observed systemically in COPD.

Alternatively, it is possible that an increase in systemic inflammatory cells are responsible for the elevated airway inflammation [69]. It is possible that several circulating inflammatory factors, such as α1-antitrypsin or fibrinogen [88], may either increase or decrease the affinity of circulating inflammatory cells for migration into the lung. In α1-antitrypsin deficiency, for example, leukocytes have increased chemotactic activity [89], and thus an increased capacity for neutrophil and other leukocyte recruitment into the lungs exists in individuals with this condition. Inflammatory cells in individuals who are predisposed to COPD (through α1-antitrypsin deficiency or other genetic abnormalities) may be more prone to inflammatory cell transmigration into lung tissues even without inhalation of cigarette smoke or environmental toxins. When airway particulate matter is inhaled, the increased affinity for transmigration from systemic circulation into lung tissue may create a larger inflammatory response in the lungs for COPD-predisposed individuals than those who are not predisposed. This increased chemotactic activity may also help to explain why airway inflammation persists in individuals with COPD even with smoking cessation [47].

1.4 Exercise and Inflammation

The benefits of exercise training in both healthy and disease populations are numerous, including improvements in quality of life, exercise capacity, and body composition [90]. Exercise also plays an important role in disease prevention, as higher levels of physical activity have been linked to a decreased risk of type II diabetes, cardiovascular disease, stroke, cancers of the colon and breast, obesity, and depression [91]. From an inflammatory perspective, exercise seems to play an important role, since increased levels of physical activity have been associated with lower levels of systemic inflammation such as CRP, IL-6, and TNF-α in healthy individuals [70, 92-94]. Since these acute phase proteins are major players in eliciting larger inflammatory responses, and have been associated with many of the chronic diseases listed above [95-97], this may help explain why individuals who participate in physical activity on a regular basis are at a decreased risk of chronic disease. In chronic disease populations, new evidence appears to support an anti-inflammatory mechanism in response to aerobic exercise training. Decreases in systemic inflammatory markers have been observed in type II diabetics, individuals with cardiovascular disease (including coronary artery disease and endothelial dysfunction), women with polycystic ovary syndrome and overweight adults [98-104]. The reduction in systemic inflammation with exercise training provides promising preliminary evidence for other inflammatory conditions such as COPD. While it is important to investigate the anti-inflammatory mechanisms to long-term exercise interventions, it is equally important to understand how each acute exercise session may contribute to this effect. Especially in patients with COPD, there has been very little

research done to investigate the acute effects of exercise on inflammation, and thus we must work from this starting point to progress to understanding the full effects of exercise as a therapeutic intervention for these individuals.

1.4.1 The Acute Inflammatory Response to Aerobic Exercise

The systemic inflammatory response to an acute bout aerobic exercise has been well documented in healthy individuals and is often referred to as the "leukocytosis of exercise" [105]. This inflammatory response consists primarily of two interconnected phases known as the early and late inflammatory responses. The early inflammatory response begins during exercise and usually lasts for 1-2 hours after the exercise bout [106]. Immediately after exercise, individuals display elevated levels of total inflammatory cells, mainly as a result of increases NK cells [107, 108] as well as a small increase in circulating neutrophils [109]. The increase in NK cells immediately after exercise likely occurs because of increases in circulating catecholamines, which interact with the dense population of β receptors on the surface of NK cells. Following low to moderate intensity exercise, lymphocytes (and NK cells) decrease from their peak concentrations and 1-2 hours post-exercise they return to baseline values [108]. Following intense (>75% VO_{2max}) exercise sessions, however, lymphocyte levels may drop below baseline values. This post-exercise immunosuppression usually returns to baseline by approximately 24 hours after exercise [110].

Along with increases in NK cells, cytokines such as TNF-α, IL-1β, interleukin-1 receptor agonist (IL-1ra), IL-6, soluble tumor necrosis factor receptor (sTNF-r) and IL-10 are increased systemically immediately following exercise [106]. Most of these cytokines reach a peak immediately after exercise and then decrease from that concentration in first 1-2 hours after an exercise bout. However, sTNF-r remains elevated and IL-1ra peaks at 1.5 hours after exercise, indicating a shift towards an anti-inflammatory state at this time which may last for several hours [106]. Neutrophils continue to rise post-exercise, and are the main inflammatory cell present in what is termed the "late inflammatory response", which usually lasts from 2 to approximately 24-48 hours after exercise [111].

1.4.2 Variables in Exercise that May Affect Inflammation

The systemic inflammatory response to exercise in healthy individuals varies with many factors relating to the specific exercise prescription, including exercise intensity [112], duration [113, 114] mode [115], and muscle damage [116]. Several mechanisms may exist that cause an increase in inflammation following these varying exercise stresses. First, the early inflammatory response may occur partially because of cells released from the "marginal pool" in the lungs [117]. In pulmonary circulation, leukocytes often become trapped in the pulmonary capillaries because their diameter is greater than the diameter of the capillaries, and must deform in order to make their way through the pulmonary vasculature, a process that is much slower than their transit time in larger vessels [118]. During exercise, these cells may be freed from these small

vessels because of increased mechanical stress from increased cardiac output [119, 120] which may contribute to an immediate increase in systemic leukocytes. Following exercise, a return to normal levels of cardiac output results in less mechanical force propelling the leukocytes out of the pulmonary capillaries, and the level of circulating leukocytes decline as they are re-sequestered in the lung.

Second, increased levels of catecholamines such as epinephrine contribute to the increase in systemic inflammation during the early response. Catecholamines act on the beta-receptors found on neutrophils, lymphocytes and endothelial cells, and as catecholamine levels rise during an exercise bout, they free leukocytes from their attachments to endothelial cells. An increased level of plasma catecholamines with exercise has been correlated with increased levels of inflammatory cells in systemic circulation in healthy individuals, [121] and van Helvoort and colleagues [122] support this mechanism with the finding that the increase in norepinephrine following an acute bout of maximal exercise in COPD patients was correlated with the increase in blood lymphocytes. Furthermore, catecholamines increase in systemic circulation in relation to exercise intensity [123], and this may help explain why inflammatory cells increase systemically with increases in exercise intensity [124]. While catecholamines play a large role in stimulating inflammation, especially in regards to the early response, it is important to note that other mechanisms leading to increases in systemic inflammation may exist. This is supported by evidence that exercise will stimulate a greater inflammatory effect than epinephrine administration alone [125], and that blocking beta receptors will reduce but not abolish the inflammatory response to exercise [121].

Third, muscles may produce cytokines that initiate the sequence of inflammatory events observed during and after exercise. The first experimental evidence for skeletal muscle release of cytokines appeared in 2000 from Pedersen's Muscle Research Group in Copenhagen [126]. Experimenters reported net increases in leg muscle arterial-venous differences in IL-6 in response to continuous leg extension exercise, a finding that isolated skeletal muscle as the origin for IL-6 production during exercise. This finding was supported by Hiscock et al [127] using both immunohistochemistry and in situ hybridization to examine IL-6 mRNA and protein expression of skeletal muscle before and after exercise. Using these techniques, the authors were able to visualize IL-6 mRNA within myocytes, and the detection of this IL-6 mRNA served as important evidence that muscles are indeed potent producers of cytokines. These cytokines, termed "myokines", have in later research been found to include IL-6, IL-8 and IL-15 [128, 129]. While the effects of skeletal muscle-induced IL-6 are widespread and with many pleiotropic effects on both pro- and anti-inflammatory cells and cytokines, the functions of IL-8 and IL-15 have been limited to local angiogenic and muscle-tissue repair mechanisms, respectively [129]. Likely several more "myokines" will be added to this interesting list in the future, and will allow us to further understand the immune responses to acute and chronic exercise in both healthy and disease populations

1.4.3 Acute Aerobic Exercise, Inflammation, and COPD Patients

To date, no studies have addressed the airway inflammatory response to acute aerobic exercise in patients with COPD. However, recent work has investigated the

airway oxidative stress responses to acute exercise in these individuals. Two studies by Mercken and colleagues have investigated the airway oxidative stress responses, measured by hydrogen peroxide (H_2O_2) levels in exhaled breath condensate, to various exercise stresses performed by patients with COPD [130, 131]. The earlier of these two studies compared the oxidative stress responses in patients with COPD and controls following a CPET and a constant load endurance trial at 60% maximal aerobic power (MAP) which lasted approximately 12 minutes. Results showed that patients with COPD had higher levels of H_2O_2 compared to control subjects at rest and 4 hours after both high and low intensity exercise. Significant increases in H_2O_2 were observed after the CPET, but not after the low intensity trial. Increased levels of oxidative stress after exercise were also seen in the latter study by Mercken et al [130]. Patients with COPD and healthy subjects performed single leg ergometry at 40% MAP for approximately 11 minutes, and in response to this exercise, increases in exhaled H_2O_2 were observed at 0 and 2 hours after exercise in the COPD group, but not the control group. Notably, no differences in resting pulmonary oxidative stress were observed between the healthy and COPD groups in this study. These two studies, taken together, show that patients with COPD experience greater pulmonary oxidative stress responses to exercise compared to controls, and even when ventilatory demands are diminished (during single leg exercise), an oxidative stress response still occurs. The source of this oxidative stress is unknown, but may be at least partially attributed to increased inflammatory cell activity in the lungs. Determining the mechanisms of airway oxidative stress, and of equal interest to our research team, airway inflammation, in response to acute exercise, will likely be the focus of much future research in the COPD literature.

While the systemic inflammatory response to an acute bout of exercise has been quite well established in healthy individuals, the response in patients with COPD is less well understood. Rabinovich and colleagues [132] were the first to investigate the systemic inflammatory response to acute exercise in COPD patients. They found that although COPD patients and healthy controls had similar resting levels of TNF-α, levels of this cytokine increased significantly in COPD patients in response to 11 minutes of cycle ergometry at 40% MAP while TNF-α levels did not change in healthy controls. No changes in IL-6, the primary cytokine present in response to exercise, were observed in healthy subjects or COPD patients [132]. As this exercise session was performed at 40% MAP, which corresponds to the energetic demands of activities of daily living, and was of short duration, the stimulus was likely not great enough to elicit a significant IL-6 response.

Similar to the initial study by Rabinovich et al [132], van Helvoort and colleagues have conducted several studies that examine the acute systemic inflammatory response to exercise in COPD patients. First, this group examined the systemic inflammatory response to a CPET in patients with COPD and healthy controls [122]. Both groups had very similar changes in circulating inflammatory cells including total leukocytes, neutrophils, and monocytes, but because patients with COPD had raised inflammatory levels at baseline, the inflammatory cells in the COPD group reached higher absolute values. Of note in this study is the greater increase in NK cells and lymphocytes in healthy individuals at their peak exercise. This likely occurred because

of the higher workloads achieved in the healthy individuals and the contribution of catecholamines to NK cell release during these higher work rates.

A second study by van Helvoort and colleagues [133] examined the systemic inflammatory and oxidative stress responses to two sessions of cycling at 40 watts breathing a randomly assigned gas until exhaustion in patients with COPD. One exercise trial consisted of cycling breathing compressed air, while the other consisted of cycling while breathing supplemental oxygen. The mean exercise time to exhaustion in the compressed air trial was 15 minutes, and the mean exercise time to exhaustion for breathing supplemental oxygen during exercise was 14 minutes. This difference was not statistically significant, but the low duration of exercise completed by these patients reveals the extreme state of deconditioning that occurs during the disease. In both arms of the study, an increase in blood leukocytes and IL-6 were observed. The magnitude of increase in leukocytes did not differ between treatments, while circulating IL-6 increase was lower in the supplemental oxygen trial. Breathing supplemental oxygen also attenuated the systemic oxidative stress response (measured by lipid peroxidation and production of reactive oxygen species) that was observed during the exercise trial using compressed air. This study confirms that COPD patients experience an increase in systemic inflammatory cells, cytokines, and oxidative stress during exercise breathing air, and these responses may be attenuated by breathing supplemental oxygen. Further, these findings suggest that the increased inflammatory response to exercise in COPD patients compared to healthy individuals observed in van Helvoort's first study [122] may be a result of hypoxemia during exercise. However, because no healthy control

group was used in this study, it is difficult to determine whether the inflammatory and oxidative responses were higher than values observed in healthy populations. Regardless, this study is helpful in elucidating a potential mechanism for exercise-induced oxidative stress and inflammation in patients with COPD.

Two of van Helvoort and colleagues' studies have attempted to investigate the systemic inflammatory response to varying intensities of exercise in COPD patients [134, 135]. The first study [134] compared the systemic inflammatory response in a submaximal 6MWT to a maximal CPET in muscle-wasted patients with COPD. Both exercise sessions elicited increased levels of inflammation, measured by increases in IL-6, total leukocytes, neutrophils, and lymphocytes, as well as oxidative stress, but total leukocytes and lymphocytes reached higher circulating levels in response to the CPET than in the 6MWT. The authors therefore claimed that, with an increase in exercise intensity, an increase in systemic inflammation may occur in patients with COPD. However, several limitations to this conclusion must be mentioned. CPETs and 6MWT vary in both duration (the CPETS were likely longer) and the muscle groups used, and work cannot be equated for the 6MWT. Furthermore, for some individuals with very reduced exercise capacities, a 6MWT is a near-maximal test, which may be similar or greater in intensity to a CPET. It is therefore impossible to conclude that the increased inflammatory response to the CPET occurred strictly because of a higher exercise intensity, and may instead have been a results of modality or duration.

The second study investigating the systemic inflammatory response to differing exercise intensities compared the systemic inflammatory response from a CPET to 30 minutes of cycle ergometry at 50% MAP [135]. While the total leukocytosis of exercise was greater in response to maximal exercise than submaximal exercise in healthy individuals, the increase in blood leukocytes was the same for both exercise intensities in COPD patients. While this may indicate that the systemic inflammatory response does not depend on exercise intensity in COPD patients, one must note that this test was not standardized for the amount of work performed in each exercise trial. Therefore, the increased duration and amount of work performed in the low intensity exercise session may have contributed to the equivalent level of inflammation observed in the high intensity trial. Even though lactate levels, minute ventilation (V_E), and VO_2 were all higher in the high intensity trial, these values would only have been this high for a duration of approximately 1-2 minutes, and therefore may not have been long enough to cause an increased inflammatory response compared to the low intensity trial. Equating the volume of work performed in the low and high intensity trials will determine whether intensity does in fact play a role in the inflammatory response to exercise in COPD patients.

From the studies that have been conducted to date examining the systemic inflammatory response to acute exercise in patients with COPD, several hypotheses may be made. First, these patients display elevated levels of inflammatory cells, such as neutrophils, at rest. Therefore, in response to exercise, even if the change in inflammatory cells from baseline to post-exercise is the same as in healthy individuals, a

greater absolute concentration of an inflammatory marker may be present systemically in patients with COPD. Next, COPD patients may have a decreased lymphocyte response to exercise compared to control subjects. This is likely because of a decreased capacity to exercise at the intensities that healthy individuals usually participate, and is likely tied to a lower level of circulating catecholamines at these lower intensities. Exercise intensity may have an effect on the inflammatory response in patients with COPD, but since no previous studies have equated work, the intensity-related responses to exercise in these patients remains to be determined. Lastly, patients with COPD display elevated levels of airway oxidative stress compared to healthy individuals and the level of baseline oxidative stress may increase in response to exercise. The source of this oxidative stress is unknown, but it may occur because of increased invasion and activity of inflammatory cells in the airways. In the pathophysiology of COPD, airway inflammation and oxidative stress appear to upregulate each other through a large variety of mechanisms, resulting in a vicious cycle of airway damage and remodeling [136]. Likely, if increased oxidative stress appears in the lungs in response to exercise, an increase in airway inflammation may have caused that oxidative stress, or will appear through signaling mechanisms stimulated by the oxidative stress. Therefore, it is a likely hypothesis that an increase in inflammatory cells and cytokines will be observed following exercise.

1.5 Significance of the Project

COPD is a disease characterized by chronic airway inflammation combined with an elevated systemic inflammatory state. Examining how airway and systemic

inflammation change over the course of this disease, and determining factors that will help us understand the progression and development of inflammation in these patients is essential for optimizing patient health and functional capacity. While it has been widely accepted that exercise training for patients with COPD is beneficial for a variety of health- and quality of life-related reasons [137-141], determining the optimal acute aerobic stimulus for these individuals is not well understood. Low intensity, long duration continuous aerobic exercise has often been used as the exercise stimulus for training disease populations [91]. During continuous aerobic exercise, ventilatory demands increase to match metabolic demands. However, because patients with COPD are expiratory flow limited, gas trapping and dynamic lung hyperinflation frequently occur during relatively low to moderate intensity continuous exercise, and patients often stop exercising due to intolerable levels of dyspnea [142]. High intensity interval exercise has been recommended as a solution to avoid dynamic hyperinflation in patients with COPD. Using this method of training, individuals will perform a similar amount of work as in a continuous aerobic training session, but dynamic hyperinflation is avoided because the rest intervals interspersed between high intensity repetitions will allow ventilatory rates to return to lower levels [143, 144]. High intensity interval training has been repeatedly found to be safe and tolerable for COPD patients [144, 145], and is an effective stimulus for improving aerobic capacity and symptoms of dyspnea [143], but the immune response to this type of exercise is not well understood in COPD patients.

Understanding how an acute bout of exercise will affect a patient's disease status or immune function is important, as inducing too much exercise stress on a patient may result in excess fatigue or soreness, and may even increase their risk of infection or exacerbation. In fact, several training studies as well as anecdotal evidence have reported that some patients are unable to adhere to exercise training prescription because of exacerbations during their training program [143, 146]. It is also important to note that the cause of exacerbations during these training studies has never been investigated and could very likely be from a stimulus other than exercise. Regardless, understanding the acute immune response to exercise in patients with COPD is essential for maximizing functional and health-related outcomes.

While the acute systemic inflammatory response to exercise in these patients has been examined by a few studies, to date no study exists that has observed the airway inflammatory response to exercise in this disease. Inflammation in the lungs relates to disease progression as well as risk of exacerbations, and therefore examining the acute effects of exercise on this inflammation is an essential first step to understanding the complete immunological effect of exercise on this patient population.

van Helvoort's two studies [134, 135] that have previously attempted to examine the effect of high and low intensity exercise in patients with COPD have utilized a CPET protocol to represent the high intensity exercise stimulus. Traditionally in a CPET protocol, individuals are only exercising at a high intensity for a short duration, and in patients with COPD, the duration of high intensity exercise during this test is especially

short. Our project utilized high intensity interval training to represent the high intensity stimulus; the inflammatory response to this type of exercise has never been investigated in patients with COPD. Despite its physiological benefit, evidence exists that excessive high intensity training may alter immune function in some individuals [149]. The disease status of patients with COPD relies heavily on the absence or presence of inflammatory cells; therefore, better understanding of the inflammatory responses to different exercise intensities is of critical importance.

This project was also novel because it examined both the pro- and anti-inflammatory events following varying intensities of exercise in patients with COPD. Although anti-oxidant capacity has been examined in response to exercise in patients with COPD by van Helvoort et al [134], no study has ever examined the anti-inflammatory response to exercise in this population. Resting sputum levels of IL-10 are reduced in patients with COPD [150], and IL-10 release from lung tissue is impaired in these patients compared to individuals without lung obstruction [151]. This has lead to the hypothesis that an impaired anti-inflammatory capacity that leads to an imbalance between pro- and anti-inflammatory cytokines may contribute to the progression of airway inflammation and lung damage in patients with COPD. Since exercise is known to invoke an inflammatory response, and patient health relies largely on inflammatory balance in COPD, it is important to understand both the pro- and anti-inflammatory responses in order to safely prescribe exercise for these patients.

Lastly, we compared the airway and systemic inflammatory responses in patients with COPD to healthy age and activity-level matched controls. Understanding the healthy response to exercise stimuli allows us to determine whether or not patients with COPD elicit an altered inflammatory response to exercise, and if so, exercise prescriptions may need to be designed to avoid an excessive pro-inflammatory state or promote an elevated anti-inflammatory state for these patients.

This thesis project was designed to address several important questions regarding the airway and systemic pro- and anti-inflammatory responses to acute aerobic exercise in COPD patients. The purpose of this project was to examine the airway and systemic inflammatory responses to high intensity interval exercise and continuous low intensity exercise in patients with COPD compared to healthy age- and activity-level-matched controls. In this project we recorded baseline inflammatory levels of airway and systemic inflammation, and patients performed high and low intensity exercise in a random order. Airway and systemic inflammatory biomarkers were measured after exercise to quantify the acute changes in inflammatory biomarkers to both stimuli. The information obtained from this study will guide future research investigating the immunological response to exercise in COPD patients, and will help guide and optimize exercise prescriptions in pulmonary rehabilitation programs for this population.

1.6 Objectives and Hypothesis

1.6.1 Primary Objective

Our primary objective was to determine the airway neutrophil response to high and low intensity exercise in patients with COPD compared to healthy age- and activity level-matched controls.

1.6.2 Secondary Objective

Our secondary objective was to determine the response of other airway and systemic inflammatory biomarkers in response to high and low intensity exercise in patients with COPD compared to healthy age- and activity level-matched controls.

1.6.3 Hypothesis

We hypothesized that there would be an increased pro-inflammatory and a reduced anti-inflammatory response in both airway and systemic biomarkers with high intensity exercise compared to low intensity exercise in patients with moderate-severe COPD compared to healthy age- and activity-matched controls.

CHAPTER 2

THE EFFECT OF EXERCISE INTENSITY ON AIRWAY AND SYSTEMIC INFLAMMATION IN PATIENTS WITH COPD

2.1 INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is an inflammatory condition associated with chronically elevated levels of airway and systemic inflammation, which are linked to the pathophysiology of the disease and the development of a number of secondary comorbidities [9, 152]. It is well documented that exercise training has an anti-inflammatory effect in healthy individuals and a number of clinical populations [153] but whether this is the case in COPD is not well understood. An important preliminary step in determining whether exercise training could be a beneficial anti-inflammatory treatment for patients with COPD is to understand the acute response to an exercise bout.

Pulmonary rehabilitation programs now utilize a combination of aerobic exercise training modalities, including continuous low intensity training and high intensity interval training. Both training modalities are effective in increasing exercise capacity in patients with COPD, but how they affect the inflammatory responses to exercise is not well understood. Recent work has shown that patients with COPD have similar changes in circulating inflammatory markers in response to aerobic exercise compared to healthy individuals, but because of elevations in baseline inflammatory levels, patients reach

higher absolute levels of systemic inflammation compared to their healthy counterparts [122]. An increased systemic inflammatory response to high intensity exercise, measured following an incremental cardiopulmonary exercise test (CPET), compared to a lower intensity six-minute walk test, has also been observed [134].

Despite the advances in our understanding of the systemic inflammatory responses to exercise in patients with COPD, several key areas remain to be examined. Most importantly, the airway inflammatory response to exercise has not been examined in these patients, and since airway inflammation is largely responsible for the primary pathophysiology of this disease, it is necessary to understand how exercise may affect the airway inflammatory processes. Secondly, the anti-inflammatory response to exercise has not been examined. Understanding the anti-inflammatory response to exercise is important since a reduced anti-inflammatory state at rest has been observed in patients with COPD and may contribute to an increased pro-inflammatory state. Thirdly, the inflammatory responses to exercise sesssions of differing intensities but equivalent work rates remains to be studied. Varying intensities of exercise are regularily employed in pulmonary rehabilitation programs, and previous work has shown that the inflammatory response may depend on the intensity of exercise performed [122, 134]. In an inflammatory condition such as COPD, where perturbations in an individual's inflammatory state may result in negative health outcomes, it is necessary to understand the airway and systemic pro- and anti-inflammatory responses to varying exercise stimuli. Therefore, we sought to determine the airway and systemic inflammatory responses to high and low intensity exercise training in COPD patients versus healthy

individuals. It was hypothesized that there would be an increased pro-inflammatory and a reduced anti-inflammatory response in both airway and systemic biomarkers with high intensity exercise compared to low intensity exercise in patients with moderate-severe COPD compared to healthy age- and activity-matched controls.

2.2 METHODS

2.2.1 Subjects

Thirteen patients with moderate to severe COPD based on Canadian Thoracic Society Guidelines (post-bronchodilator $FEV_1/FVC < .70$, FEV_1 30-80% predicted, ≥10 pack-year of smoking) who were exacerbation free for at least 8 weeks were recruited for this study. Thirteen healthy age- and activity- matched individuals, were recruited to serve as control subjects. Patients with COPD were recruited through a respiratory outpatient database as well as from physician referral and by poster advertisement, and healthy subjects were recruited through word of mouth from the local Calgary community. All subjects were required to be non-smokers before participation in the study. Exclusion criteria for both groups included a history of asthma or allergic rhinitis; use of oral or parenteral corticosteroids and/or theophylline within 8 weeks of study entry; diagnosed upper respiratory tract infection within 4 weeks of study participation; required use of supplemental oxygen; history of any other respiratory or inflammatory disease considered by the investigators to be clinically significant, including autoimmune disorders, infectious diseases, immunodeficiency conditions, malignancies or clinically significant cardiovascular, neurological, endocrine or hematological disorders; contraindications to exercise; or unable to provide consent. Additional exclusion for the COPD group included an acute exacerbation of COPD (defined as increased cough, sputum production, or dyspnea requiring increased doses of baseline medications, addition of new inhaled medications, addition of oral/parenteral corticosteroid, addition of oxygen, or admission to hospital emergency) within 8 weeks

of study entry. Patients with COPD who were currently using inhaled corticosteroid (ICS) therapy were included in the study but were required to withdraw from their ICS treatment for 4 weeks prior to study entry and remain off the ICS treatment for the duration of the study protocol (approximately 2.5 weeks). Subjects were informed of the entire research protocol, and written consent approved by the local ethics board was obtained from all subjects before participation.

2.2.2 Study Design

This study utilized a prospective randomized crossover design which required all participants to come to the pulmonary exercise research lab at the University of Calgary on five separate occasions. Additionally, COPD patients who were currently taking inhaled corticosteroid (ICS) medication were required to attend one visit prior to the protocol visits in which they were instructed to stop their ICS therapy and replace that medication with a long-acting bronchodilator. Patients who were being withdrawn from their ICS therapy were questioned on their baseline respiratory symptoms (see Appendix A) at this visit, and were questioned weekly until they had completed the entire study protocol, they had been returned to their regular medications, and the study physician had determined that their respiratory symptoms were comparable to baseline.

During the first visit, informed consent was obtained followed by pulmonary function testing and a CPET. These tests were conducted according to American Thoracic Society (ATS) guidelines [154-157] as measures to identify any cardiovascular contraindications to exercise and to determine appropriate exercise intensities for the

high and low intensity exercise trials. On the second visit, venous blood samples were taken followed by induced sputum collection, both of which were used as baseline values. This visit occurred approximately one week after the first to ensure that any airway inflammation caused by pulmonary function testing or the CPET was negligible when measuring baseline data. Some subjects were unable to produce adequate sputum samples on their first baseline visit. In this circumstance, visit 2 was repeated to obtain baseline samples. If they could not produce an adequate sample on their second attempt, they completed the remainder of the study without sputum sampling. The third visit was scheduled 48 hours after visit two at which time subjects performed either a) a high intensity interval training session or b) a continuous low intensity exercise trial for a duration that equated to the same amount of work performed in the high intensity trial. Rationale for selection of these two training sessions is provided in Appendix B. Blood was collected immediately after and 2 hours following each exercise bout, while sputum was collected once, 2 hours following each exercise test. The fourth visit occurred approximately 5 days following visit 3 and consisted of a second baseline blood and sputum measurement. Visit 5 occurred 48 hours after visit 4, and required each subject to complete the remaining exercise session. The order of the exercise sessions was randomized. Random assignment was achieved using computer-generated sequences and individuals were randomized to begin with a high or low session after completing their CPET. In order to minimize the influence of external factors on the inflammatory responses of each subject, each participant was instructed to perform the same routine of activity, nutrient intake, medication use, and sleep throughout the testing period, and testing occurred at the same time of day for each experimental trial.

2.2.3 Outcomes:

The primary outcome was the change in sputum neutrophils from baseline to 2 hours following either high or low intensity exercise in patients with COPD compared to controls. Secondary outcomes were the changes in other airway and systemic inflammatory biomarkers, including sputum macrophages and eosinophils, serum neutrophils, monocytes, lymphocytes and eosinophils, and sputum and serum interleukin (IL)-6, IL-8, IL-10, and interferon-inducible protein-10 (IP-10), in response to either low or high intensity exercise in the COPD group compared to control subjects. We also aimed to determine the impact of exercise intensity on airway and systemic inflammation, and therefore compared low and high intensity trials in both COPD and control groups.

2.2.4 Experimental Procedures/Protocols:

Pulmonary Function Testing

Pulmonary function testing included spirometry, static lung volumes and diffusion capacity for carbon monoxide (DL_{CO}) were performed according to ATS criteria [154, 155, 157].

Incremental Cardiopulmonary Exercise Test

To ensure patients were free from clinically significant ischemic heart disease and other cardiovascular contraindications to exercise, and to calculate workloads for the subsequent exercise sessions, each patient performed an incremental exercise test to

symptom-limitation with 12-lead electrocardiogram (ECG) monitoring. Participants performed the test on an electrically braked cycle ergometer (Ergoline 800S, SensorMedics, Yorba Linda, CA) and expired gases were utilized to document peak oxygen consumption (SensorMedics Vmax 29C, SensorMedics, Yorba Linda, CA). The test protocol was performed using ATS recommendations [156]. After stable resting metabolic values were achieved, subjects cycled unloaded for two minutes before the load was increased by 10 $W \cdot min^{-1}$ until symptom-limitation. During each test, oxyhemoglobin saturation and heart rate were monitored continuously by pulse oximetry and ECG, respectively, while blood pressure was measured every two minutes using a manual sphymomanometer. At the end of each workload, exertional dyspnea and leg discomfort was evaluated using the 10-point modified Borg scale.

Measurements of Airway and Systemic Inflammation

Sputum induction was performed using escalating doses of hypertonic saline (3%, 4%, 5%) as previously described [158]. After induction, samples were placed on ice and processed for differential cell counts within 2 hours of collection using modifications on standardized methods [159]. In brief, the sputum sample was placed into a petri dish and placed on a black background, mucus plugs were selected from saliva and swirled around on the lid of the petri dish to remove contaminating salvia. The plugs were then weighed and phosphate buffered saline (PBS) was then added to the plugs at eight times the sample weight. Samples were vortexed for 15 seconds, rocked for 15 minutes at room temperature and then centrifuged at 700 x g for 10 minutes. After centrifugation the supernatant was removed, aliquoted and stored at -80°C for

subsequent quantification of IL-6, IL-8, IL-10 and IP-10. Specific biomarkers were chosen given their association with disease severity (IL-6, IL-8) [54], their role as neutrophil and T-cell chemoattractants (IL-8, IP-10, respectively) [16, 160], and their anti-inflammatory action (IL-10) [33]. Following removal of the supernatant, the cell pellet was resuspended with four times the sample weight of PBS containing 0.1% DTT, vortexed for 15 sec, then rocked for 15 minutes at room temperature. Finally an equal volume of PBS was added to the sample bringing the final DTT concentration to 0.05. Total cell counts were obtained manually using a Bright-line neubuer hemacytometer (Hausser Scientific, Horsham, PA), and the viability of each sample was assessed using the trypan blue exclusion method [161]. The cells were diluted to a concentration of 1x 10^6/mL in PBS. Cytospin slides were prepared by placing slides, filter cards and sample chambers into the cytospin slide clip, securing it and loading 70μL of cell suspension into the sample chamber. The slides were then centrifuged at 450rpm for 6 minutes in a Thermo Scientific Shandon cytospin 4 and then stained with congo red for sputum cell counts.

All cytospin slides were coded with patient initials and dates prior to determination of differential cell counts, allowing the cell counts to be performed 'blind' with regards to patient group and exercise treatment. 400 non-squamous cells were counted. Differential cell counts are expressed as number (10^9/g) and percent of total cells. Samples were considered adequate for analysis if there was less than 30% squamous cell contamination.

For systemic inflammatory measures, at each sample collection a 5mL sample of venous blood was collected and direct assessment of systemic inflammation involved a differential cell count using accepted techniques. Additional samples were centrifuged and the serum was stored at -70°C for later analysis of inflammatory mediators. These markers again included IL-6, IL-8, IL-10, and IP-10.

Inflammation-relevant analytes were measured in sputum and serum samples with a Luminex 200 apparatus (Applied Cytometry Systems, UK) using a multiplex human kit from Millipore (Massachusets, USA; for IL-6, IL-10 and IP-10) and an R&D Systems (for IL-8) kit according to manufacturer's instructions. Briefly, the beads were distributed into each well and washed once. For the serum samples, the serum matrix provided with the kit was used to generate the standard curve. For the standard curve of the sputum samples, the same batch of PBS used to dilute the sputum was used as the matrix solution for the standard curve. The samples and the standards were then added to the beads and incubated either overnight at 4°C with agitation (Millipore kits) or at room temperature for three hours, with agitation (R&D Systems kit). After the incubation, the beads were washed three times with wash buffer and incubated with the detection antibodies for one hour, at room temperature with agitation. Then, the beads were washed again three times and incubated for thirty minutes at room temperature with phycoerythrin. Finally, the beads were washed three times and resuspended in 75 ul of sheat fluid. 50ul of beads (a minimum of 50 events per beads set) were captured by the Luminex 200 apparatus. The data was analyzed with the StarStation V.2.3 software from Applied Cytometry Systems (UK).

High Intensity Test

Subjects performed the same warm-up consisting of 5 minutes of unloaded pedaling for each trial. All cycling was performed with a pedal rate between 50-70 rpm. After warm-up, subjects performed 8 intervals of one minute cycling at 100% of their maximum work rate (W_{max}) determined from the CPET test, followed by 2 minutes recovery at 30% W_{max}. Blood pressure was taken at the end of each one-minute high intensity workload, while ECG and oxyhemoglobin saturation were monitored continuously throughout the work intervals.

Low Intensity Test

After the standardized warm-up, subjects cycled at 40% of their W_{max} for 30-32 minutes. Oxyhemoglobin saturation, heart rate, and blood pressure were again monitored continuously throughout the test.

Godin Physical Activity Score

The Godin Leisure Time Exercise Questionnaire [162] was used to match physical activity levels between patients with COPD and controls. This questionnaire is a simple and valid assessment tool which asks participants to record the frequency of strenuous, moderate, and light activities performed for more than 15 minutes throughout a regular week. These values are multiplied by nine, five, and three, respectively, which correspond to approximate metabolic equivalents (METs) performed during these

different intensities of exercise. The sum of these three products is recorded as arbitrary units, with higher values corresponding to greater levels of leisure time physical activity.

2.2.5 Statistics

Statistical analysis was performed using SPSS Statistics 17.0 for Mac (SPSS Inc, Chicago, IL). As data for sputum inflammatory cells and both airway and systemic cytokines were not normally distributed, the Mann-Whitney U test was employed for analysis between groups, and the Wilcoxon sign-ranked test was used for within-group comparisons for these samples. Pulmonary function, cardiopulmonary exercise test and systemic inflammatory cells data was normally distributed; therefore, independent t-tests were used for comparisons between groups, and dependent samples t-tests were performed for within-group comparisons. Non-parametric data is displayed as median (interquartile range), and parametric data is shown as mean ± SD.

2.3 RESULTS

Participant flow through the study is presented in Figure 1. Patients were enrolled between November 2008 and October 2009. Of the subjects assessed for eligibility 40/192 patients with COPD and 41/307 control subjects were potentially eligible for the study. From these, 13/40 patients and 13/41 controls were recruited and 20/26 participants (10 COPD, 10 controls) completed all baseline tests. Three patients with COPD and three controls withdrew from the study after randomization. The primary reasons for withdrawal in the COPD group included worsening of respiratory symptoms while being washed out from ICS therapy (n=3). Sputum samples from 14/20 subjects were included in the final analysis, while blood samples were analyzed in 19/20 subjects. One control subject was excluded from all analyses based on the presence of elevated sputum eosinophilia in the baseline sample, and five individuals could not produce acceptable sputum samples for cell and cytokine measurement.

Baseline Subject Characteristics

Subject characteristics at baseline are listed in Table 1, and individual data for patients with COPD and controls is presented in Tables A2 and A3, respectively. There were no differences in demographic characteristics between groups. As expected, COPD patients had a reduced FEV_1 and FEV_1/FVC, increased residual volume, and impaired diffusion capacity compared to controls. Similar levels of physical activity were reported in patients with COPD and controls (36 ± 15 COPD vs. 32 ± 13 controls, $p>.05$). Five of

the 10 patients with COPD were removed from their ICS therapy prior to participating in the study.

Results of Cardiopulmonary Exercise Tests

All subjects successfully completed the graded exercise test and there were no adverse events. Individual CPET scores are shown in tables A4 and A5. Results from the CPET showed that the samples of patients with COPD and healthy controls had similar absolute and relative levels of peak oxygen consumption; however, controls were able to achieve higher absolute work rates in their CPETs. Due to the higher maximal work rates achieved, controls were prescribed more work in both the high and the low intensity trials (90.12 ± 22.98 kJ in controls vs. 59.52 ± 27.18 kJ in COPD $p< 0.05$).

Results of High and Low Intensity Exercise Tests

No adverse events were observed in the high and low intensity exercise sessions. Two subjects (1 COPD, 1 Control) ended their low intensity exercise sessions early because of muscle and joint discomfort. This did not significantly alter the amount of work completed in the low intensity trial by either the COPD group (60.0 ± 27.9 kJ high intensity vs. 58.9 ± 28.1 kJ low intensity, $p>0.05$) or controls (90.1 ± 23.0 kJ high intensity vs. 88.3 ± 23.5 kJ low intensity, $p>0.05$).

Changes in Airway Inflammation After High and Low Intensity Exercise.

The change in sputum inflammatory cells is depicted in Figure 2, while sputum cell counts are shown in table 2, and cytokine data is presented in table 3. Total cell

count data and individual airway inflammatory responses are presented in tables A6-A13. There was no difference in the change in percent sputum neutrophils after high or low intensity exercise between patients with COPD and controls ($p>0.05$). Patients with COPD had higher total cell counts compared to controls at all time points, except at the baseline sample before the high intensity exercise trial. As expected, significantly higher levels of percent and total sputum neutrophils were observed in the COPD group at all time points compared to controls ($p<0.05$). Control subjects displayed raised levels of percent sputum macrophages at both baselines, but sputum macrophage counts did not differ between patients with COPD and control subjects ($p>0.05$) except at two hours after high intensity exercise, where patients with COPD displayed elevated macrophage counts ($p<0.05$). No significant differences were observed in the percent or total sputum eosinophils or in any of the sputum cytokines between groups.

The most marked result regarding changes in airway inflammation after exercise was the decrease in percent sputum neutrophils in patients with COPD after low intensity exercise ($p<0.05$; Table 2). This change was significantly greater than the change after high intensity exercise ($p<0.05$; Figure 2). Controls also showed a decline in sputum neutrophils after both low and high intensity exercise trials, although not statistically significant. The only other change in sputum markers was a decrease in IL-8 after high intensity exercise in the COPD group ($p<0.05$). No significant changes in total inflammatory cell count, or in sputum neutrophil, macrophage or eosinophil number from baseline to 2 hours post-exercise were observed after either exercise session in either group ($p>0.05$).

Changes in Systemic Inflammation after High and Low Intensity Exercise

Changes in systemic inflammatory variables are depicted in figure 3, absolute levels of systemic cells and cytokines are outlined in tables 4 and 5, respectively, and individual systemic inflammatory responses are listed in tables A14-A21. A significant increase in serum neutrophils was observed in both groups after both high and low intensity exercise. This effect was seen at 0 and 2 hours after low intensity exercise, but only at 2 hours after exercise in the high intensity trial for both COPD and healthy groups. Patients with COPD showed an elevation in serum eosinophils immediately after high intensity exercise ($p<0.05$), which returned to levels similar to baseline by 2 hours after exercise. In the healthy group, serum eosinophils were lower than baseline at 2 hours after low intensity exercise ($p<0.05$). This effect was not seen in the high intensity trial. Blood lymphocytes were significantly elevated immediately after high intensity exercise in both groups ($p<0.05$), but this did not occur after low intensity exercise. A rise in blood monocytes was observed immediately after both high and low intensity exercise in both groups, which returned to baseline at 2 hours post-exercise.

There were no differences in changes in circulating cytokines between patients with COPD and controls. IL-8 was elevated at the majority of time points, while IL-10 was reduced at all time points in the COPD group compared to controls ($p<0.05$) (Table 5). Immediately after high intensity exercise, controls had an increase in IL-10 from baseline ($p<0.05$), while the COPD group had no significant increase in IL-10 at any time point after either exercise trial. Two hours after high intensity exercise, controls had

an increase in IL-6 ($p<0.05$), which was not observed in patients with COPD. This change in IL-6 was greater than the change observed with low intensity exercise ($p=0.05$), and no such change was observed in the COPD group. An increase in IP-10 was observed in patients with COPD immediately after high intensity exercise ($p<0.05$), and this was not observed in controls.

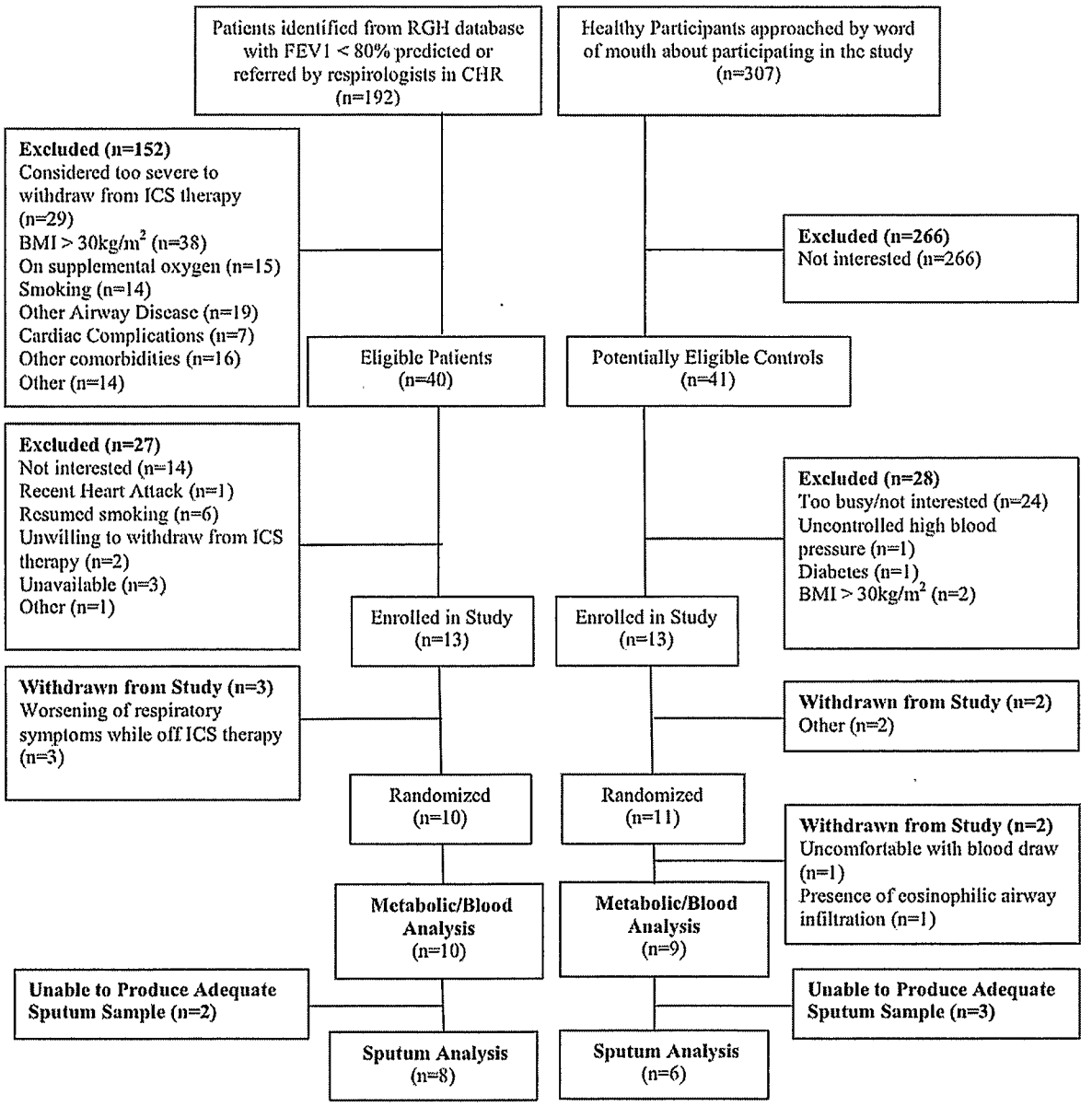

FIGURE 1 – Study Flow

Table 1 – *Subject Characteristics at Baseline*

Characteristics	COPD Group		Control Group		
	Value	% Predicted	Value	% Predicted	p Value
Demographic					
Male/Female sex, #	4/6		5/4		
Withdrawn from ICS, #	5		N/A		
Age, yr	67 ± 8		65 ± 9		n/s
Height, cm	166 ± 9		170 ± 9		n/s
BMI, kg/m^2	24.4 ± 3.9		26.5 ± 3.0		n/s
Smoking History, pack-yrs	41 ± 20		3 ± 5		p<0.05
Time since quit smoking, yrs	12 ± 9		19 ± 18*		n/s
Godin Score, units	36 ± 15		32 ± 13		n/s
Pulmonary Function Testing					
FEV_1, L	1.73 ± 0.53	68 ± 17	2.76 ± 0.36	99 ± 15	p<0.05
FVC, L	3.10 ± 0.69	96 ± 11	3.59 ± 0.56	102 ± 15	n/s
FEV_1/FVC, %	56.0 ± 11.8	69 ± 14	77.6 ± 7.3	96 ± 8	p<0.05
TLC, L	6.59 ± 1.23	113 ± 14	5.93 ± 0.83	99 ± 15	n/s
FRC, L	4.09 ± 0.89	129 ± 27	3.23 ± 0.67	99 ± 19	p<0.05
RV, L	3.35 ± 0.70	149 ± 28	2.21 ± 0.56	101 ± 20	p<0.05
RV/TLC, %	51 ± 6	131 ± 15	37 ± 5	100 ± 12	p<0.05
DLCO, mL/min/mm Hg	17.7 ± 5.3	93 ± 23	26.8 ± 3.7	131 ± 25	p<0.05
Cardiopulmonary Exercise Testing					
$\dot{V}o_2$peak, mL/kg/min	18.9 ± 5.0	91 ± 25	22.8 ± 5.3	111 ± 29	n/s
$\dot{V}o_2$peak, L/min	1.30 ± 0.51	86 ± 16	1.75 ± 0.48	104 ± 29	n/s
V_E max, L/min	54 ± 19	88 ± 17	71 ± 20	75 ± 24	n/s
W_{max}, Watts	77 ± 36	58 ± 17	118 ± 29	88 ± 20	p<0.05
HR_{max}, Beats/min	126 ± 17	82 ± 11	151 ± 24	97 ± 13	p<0.05
ΔSpO_2	3 ± 4		1 ± 2		n/s
ΔIC, L	-0.26 ± 0.34**		0.08 ± 0.25		n/s
Peak Dyspnea, Borg Units	5 ± 2		6 ± 3		n/s
Peak Leg Discomfort, Borg Units	6 ± 2		8 ± 2		n/s
Reasons for ending exercise, #					
Dyspnea	0		1		
Leg Discomfort	7		7		
Other	3		1		

Data are presented as mean ± SD or number. All spirometry values are post-bronchodilator. *Abbreviations:* DLCO = diffusion capacity of the lung for carbon monoxide; FEV_1 = forced expiratory volume in one second; FVC = forced vital capacity; FRC = functional residual capacity; IC = inspiratory capacity; ICS = inhaled corticosteroids; TLC = total lung capacity; RV = residual volume; $\dot{V}O_2$ = volume of oxygen consumption; ΔSpO_2 = change in oxyhemoglobin saturation from rest to end-exercise; ΔIC = change in inspiratory capacity from rest to end-exercise.

* Time since quitting in the three control subjects who were previous smokers.

** Negative values denote a decrease in IC from baseline to peak exercise

Table 2 – *Airway Inflammatory Cell Response to High and Low Intensity Exercise*

	LOW		HIGH	
	Baseline	2H	Baseline	2H
TOTAL LEUKOCYTES (10^6/g)				
COPD	12.30 (7.70-15.58)*	7.65 (3.68-17.43)*	10.25 (3.73-20.68)	14.55 (8.00-25.55)*
Control	5.37 (1.30-7.09)	1.76 (0.58-3.74)	3.80 (1.75-9.81)	1.87 (0.80-3.25)
NEUTROPHILS (%)				
COPD	85.4 (79.9-93.6)*	73.5 (68.5-91.0)*#	76.3 (68.4-80.9)*	73.5 (67.5-84.6)*
Control	58.3 (17.7-71.0)	9.5 (4.6-51.1)	52.9 (36.6-63.7)	31.5 (3.6-58.5)
NEUTROPHILS (10^6/g)				
COPD	10.02 (7.00-13.84)*	4.75 (2.06-11.53)*	7.46 (2.84-16.12)*	10.69 (5.06-21.58)*
Control	3.06 (0.24-5.04)	0.09 (0.06-1.99)	2.65 (0.73-5.36)	0.70 (0.05-1.81)
MACROPHAGES (%)				
COPD	11.9 (5.6-18.5)*	13.0 (6.4-27.8)*	19.0 (11.4-29.3)*	19.6 (8.8-38.6)
Control	23.6 (17.7-59.8)	90.5 (42.8-95.3)	43.8 (32.0-62.3)	44.5 (20.1-90.4)
MACROPHAGES (10^6/g)				
COPD	1.24 (0.66-2.20)	0.60 (0.34-2.63)	1.86 (0.60-3.71)	2.04 (1.33-3.93)
Control	1.32 (0.30-2.39)	1.13 (0.51-1.67)	1.15 (0.84-4.22)	0.89 (0.62-1.40)
EOSINOPHILS (%)				
COPD	0.5 (0.8-1.3)	1.5 (0.0-6.5)	1.5 (0.5-11.25)	1.8 (0.3-3.6)
Control	0.3 (0.0-0.8)	0.0 (0.0-0.6)	0.8 (0.2-2.8)	0.5 (0.2-0.8)
EOSINOPHILS (10^6/g)				
COPD	0.05 (0.01-0.17)	0.07 (0.00-0.28)	0.10 (0.05-0.27)	0.14 (0.04-0.56)
Control	0.01 (0.00-0.05)	0.00 (0.00-0.02)	0.03 (0.02-0.10)	0.01 (0.00-0.02)

Data are presented as median (interquartile range). * $p<0.05$ vs. Control; # $p<0.05$ vs. baseline.

Table 3 – *Airway Inflammatory Cytokine Response to High and Low Intensity Exercise*

	LOW		HIGH	
	Baseline	2H	Baseline	2H
IL-6 (pg/mL)				
COPD	381 (119-518)	128 (16-463)	298 (199-692)	175 (99-537)
Control	111 (56-277)	75 (41-353)	181 (90-560)	137 (54-261)
IL-8 (ng/mL)				
COPD	8.3 (3.5-15.4)	4.7 (0.4-23.3)	19.7 (13.4-25.3)	8.2 (3.1-12.0)#
Control	12.1 (1.7-25.5)	4.2 (1.4-10.9)	18.6 (7.5-22.6)	3.6 (1.5-7.0)
IL-10 (pg/mL)				
COPD	15.0 (10.5-26.3)	7.5 (1.0-35.5)	19.0 (12.3-26.8)	16.5 (5.5-40.5)
Control	10.8 (5.4-39.3)	9.9 (3.6-21.8)	23.4 (16.7-29.1)	15.8 (5.2-27.0)
IP-10 (ng/mL)				
COPD	18.4 (8.1-43.6)	7.4 (0.9-39.2)	19.7 (13.2-48.0)	13.6 (7.8-56.7)
Control	9.7 (5.5-39.4)	7.7 (3.4-17.1)	29.9 (11.2-35.6)	9.0 (5.5-27.4)

Data are presented as median (interquartile range). # $p<0.05$ vs. baseline. IL-6 = interleukin-6, IL-8 = interleukin-8, IL-10 = interleukin-10, IP-10 = interferon-inducible protein-10.

Table 4 – *Systemic Inflammatory Cell Response to High and Low Intensity Exercise*

	LOW			HIGH		
	Baseline	0H	2H	Baseline	0H	2H
NEUTROPHILS (10^9/L)						
COPD	4.2 ± 1.0	4.8 ± 1.1#	4.8 ± 1.4#	4.2 ± 1.3	4.7 ± 1.2	4.9 ± 1.1#
Control	3.2 ± 0.5	4.2 ± 0.6#	4.9 ±1.6#	4.2 ± 0.9	4.2 ± 0.5	5.2 ± 1.6#
EOSINOPHILS (10^9/L)						
COPD	0.18 ± 0.18	0.23 ± 0.16	0.14 ± 0.10	0.13 ± 0.11	0.21 ± 0.14#	0.12 ± 0.09
Control	0.18 ± 0.07	0.16 ± 0.09	0.13 ± 0.07#	0.16 ± 0.10	0.18 ± 0.08	0.12 ± 0.04
LYMPHOCYTES (10^9/L)						
COPD	1.8 ± 1.1	2.0 ± 1.0	2.0 ± 1.4	1.8 ± 1.0	2.2 ± 1.0#	1.7 ± 1.0
Control	2.0 ± 0.9	2.2 ± 1.0	2.0 ± 0.8	1.9 ± 0.8	2.9 ± 1.1#	1.9 ± 0.5
MONOCYTES (10^9/L)						
COPD	0.55 ± 0.16	0.63 ± 0.16#	0.57 ± 0.16	0.54 ± 0.16	0.75 ± 0.18#	0.55 ± 0.18
Control	0.50 ± 0.16	0.63 ± 0.17#	0.57 ± 0.11	0.58 ± 0.19	0.71 ± 0.20#	0.56 ± 0.17

Data are presented as mean ± SD. # $p<0.05$ vs. baseline.

Table 5– ***Systemic Inflammatory Cytokine Response to High and Low Intensity Exercise***

	LOW			HIGH		
	Baseline	0H	2H	Baseline	0H	2H
IL-6 (pg/ml)						
COPD	3.0 (1.8-11.9)	4.9 (2.7-14.8)	4.9 (2.3-8.8)	2.7 (2.3-8.4)	4.6 (3.2-9.4)	5.0 (2.0-12.6)
Control	7.4 (3.5-56.1)	4.6 (2.0-48.4)	7.2 (3.1-24.1)	3.0 (1.5-14.1)	5.4 (2.6-62.3)	5.6 (2.5-37.6)$^{\#}$
IL-8 (pg/ml)						
COPD	3.0 (1.8-11.9)	9.4 (4.5-22.2)*	8.8 (5.8-12.1)*	9.7 (6.7-17.5)*	9.7 (5.4-12.0)*	9.3 (7.3-11.5)
Control	3.2 (2.7-5.9)	2.6 (0.8-7.6)	4.3 (2.0-6.3)	4.3 (1.8-8.4)	3.0 (0.1-7.4)	4.0 (1.8-9.0)
IL-10 (pg/ml)						
COPD	2.2 (1.7-3.4)*	2.3 (1.7-3.4)*	2.1 (1.8-2.9)*	2.0 (1.5-3.5)*	2.4 (2.0-3.8)*	1.9 (1.6-3.5)*
Control	5.0 (4.1-14.3)	5.2 (3.3-9.4)	5.2 (3.4-10.0)	4.4 (3.4-7.0)	6.3 (4.7-12.5)$^{\#}$	6.5 (4.1-25.2)
IP-10 (pg/ml)						
COPD	341 (200-404)	390 (226-435)	338 (226-418)	327 (186-434)	348 (236-523)$^{\#}$	285 (236-433)
Control	220 (183-297)	199 (177-310)	226 (188-274)	222 (163-272)	196 (175-396)	208 (183-330)

Data are presented as median (interquartile range) ± SD. * $p<0.05$ vs. Control; # $p<0.05$ vs. baseline. IL-6 = interleukin-6, IL-8 = interleukin-8, IL-10 = interleukin-10, IP-10 = interferon-inducible protein-10.

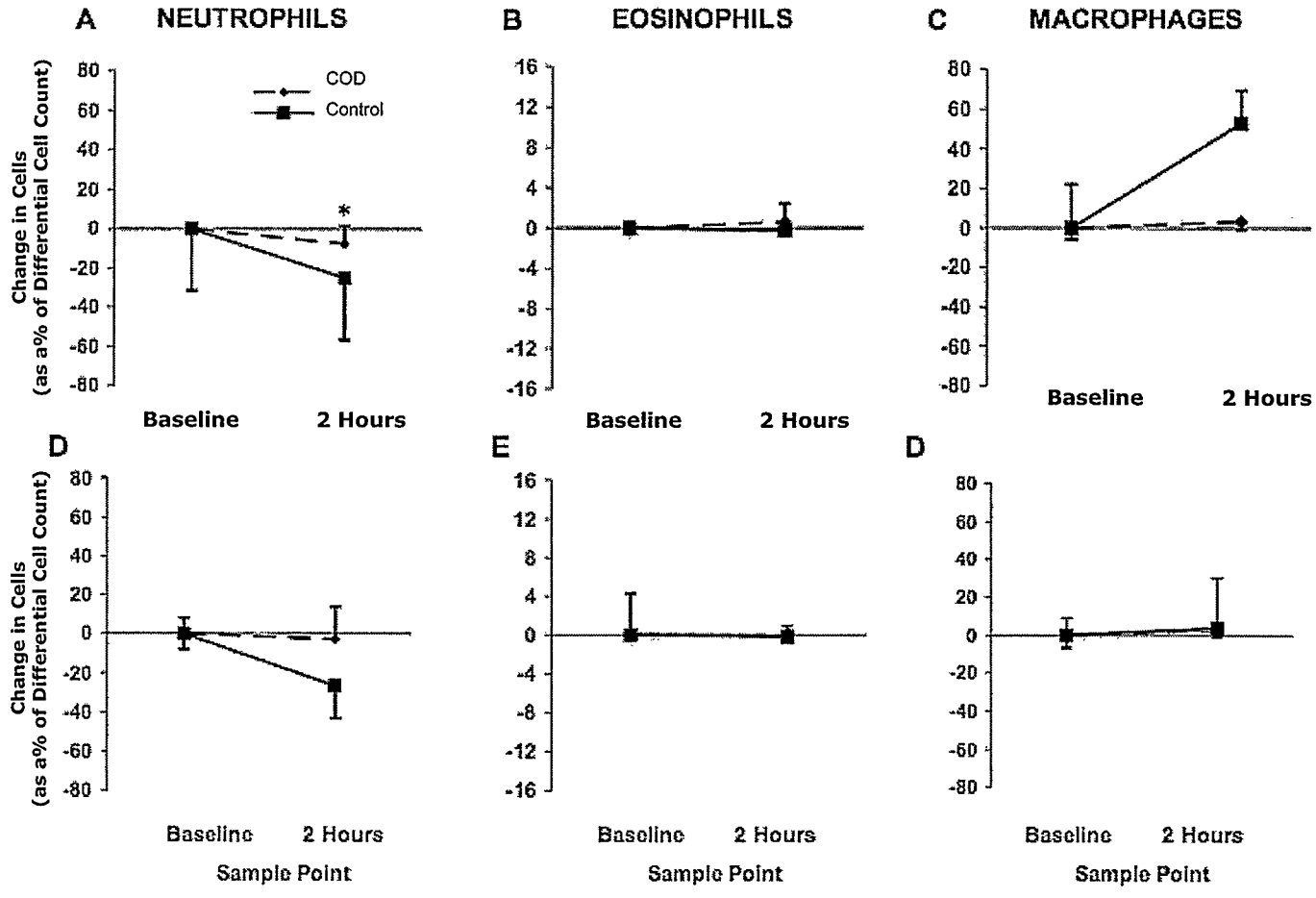

FIGURE 2. Baseline sputum inflammatory differential cell count and change in sputum differential cell counts at 2 hours after low *(top panels)* and high *(bottom panels)* intensity exercise. All graphs: COPD – dashed line, Healthy – solid line. All values are presented as mean ± SD. *$p<0.05$ change score of COPD low trial vs. high trial.

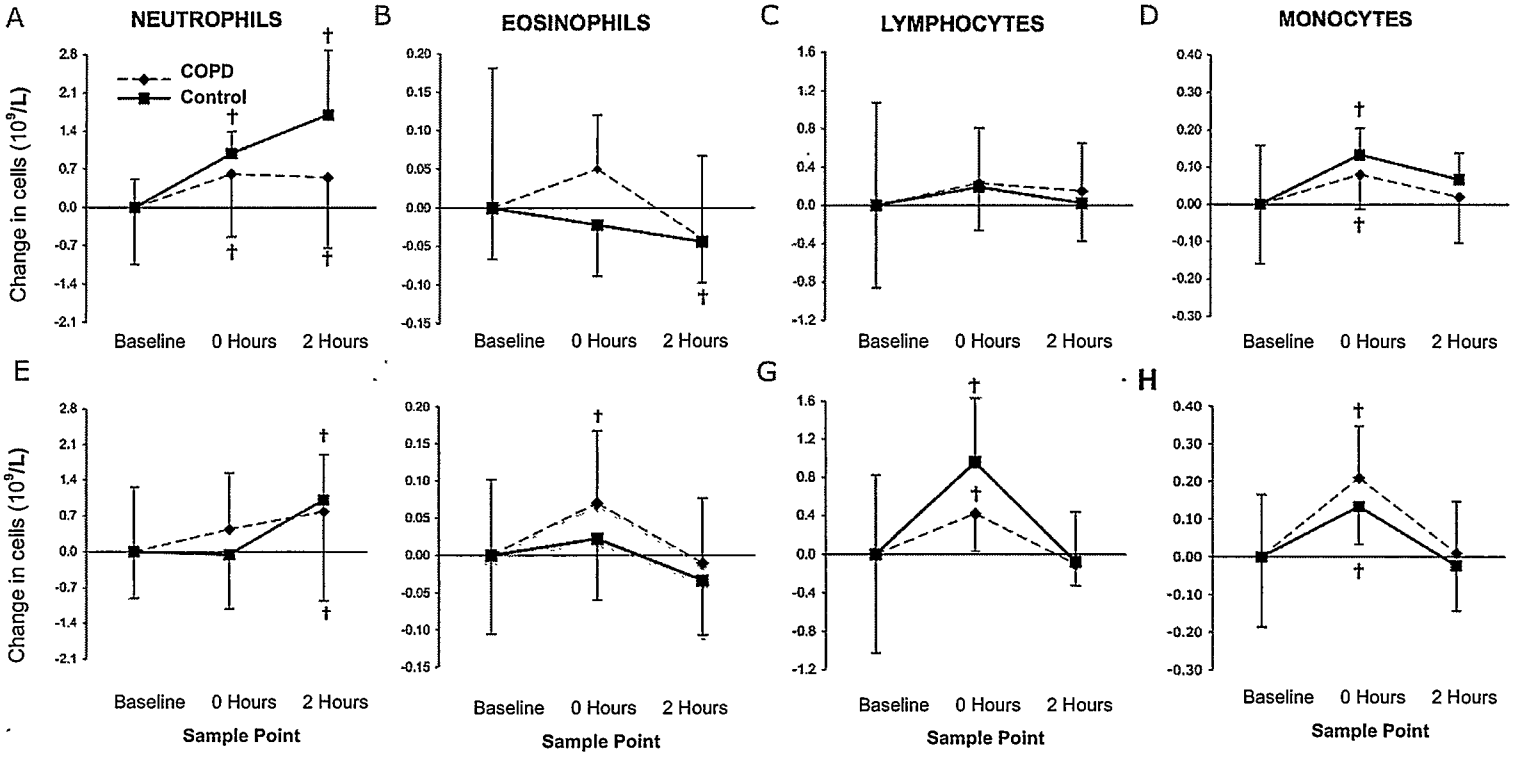

FIGURE 3. Change from baseline in serum inflammatory cell concentrations at 0 and 2 hours after low *(top panels)* and high *(bottom panels)* intensity exercise. All graphs: COPD – dashed line, Control – solid line. All values are presented as mean ± SD. † $p<0.05$ cell count post-exercise vs. baseline.

2.4 DISCUSSION

This is the first study to document the airway inflammatory and anti-inflammatory responses to acute exercise of different intensities but equal amounts of work in patients with COPD. The three unique findings of this study were: (1) sputum neutrophils were reduced after low intensity exercise in patients with COPD, (2) there was a decrease in sputum IL-8 in patients with COPD after high intensity exercise, and (3) serum IL-10, an anti-inflammatory cytokine, was unaffected by either low or high intensity exercise in patients with COPD but increased after high intensity exercise in controls.

Airway Inflammation

Contrary to our primary hypothesis, we did not observe a difference in the changes in sputum neutrophils between patients with COPD and healthy controls after high or low intensity exercise. Instead, we observed a reduction in percent sputum neutrophils in response to low intensity exercise in patients with COPD, and this response was greater than that observed after high intensity exercise. We did not observe a significant change in the total cell counts of any sputum inflammatory cells after high or low intensity exercise in either group. This finding demonstrates that the decrease in percent neutrophils observed after low intensity exercise in the COPD group did not occur simply as a result of an increase in the number of other sputum inflammatory cells

(i.e.macrophages), but is primarily due to a reduction in the absolute sputum neutrophil numbers.

While no other study has examined airway inflammation in response to exercise, Mercken and colleagues reported an increase in pulmonary oxidative stress after high intensity aerobic exercise in patients with COPD, and no change in oxidative stress after low intensity exercise [131]. It is generally accepted that increased levels of oxidative stress in the airways increases activation of NFκB and the production of a variety of proinflammatory cytokines, including the neutrophil chemoattractant IL-8 [163]. It is therefore feasible that reduced levels of pulmonary oxidative stress might result in decreased production of IL-8 by NFκB, and an attenuation of neutrophil migration into the airways. Such a mechanism would explain the decrease in sputum neutrophilia observed after low-intensity exercise in patients with COPD in our study.

While it is possible that a decrease in oxidative stress in the low intensity trial may have contributed to the decrease in sputum neutrophils observed in the COPD group, it is unlikely that oxidative stress is the only factor contributing to this decline. Because oxidative stress acts by upregulating NFκB, resulting in increased IL-8 transcription and increased neutrophil recruitment, any increase in pulmonary oxidative stress, even if the change were small, would contribute to an increase, as opposed to the decrease that we observed, in sputum neutrophils. Thus, a decline in sputum neutrophils must have been mediated by some other mechanism. Toll-like receptors (TLRs) are transmembrane proteins known to upregulate NFκB and ultimately induce transcription

of proinflammatory signalers [164]. Notably, reductions in TLR1, TLR2, TLR3 and TLR4 expression has been observed systemically following acute aerobic exercise in healthy individuals [165]. If a reduction in TLR expression occurs in lung inflammatory cells following acute aerobic exercise in patients with COPD, reductions in proinflammatory cytokine production through NFκB or other gene transcriptors would slow the recruitment of neutrophils into the lungs and might account for the reduction in sputum neutrophils observed after low intensity exercise in the COPD group in our study. Conversely, a reduction in sputum neutrophils may have occurred because of an increase in neutrophil death and not a reduction in neutrophil recruitment. Stepovaya and colleagues [166] reported increases in neutrophil apoptosis with exposure to H_2O_2, and since Mercken observed increases in airway H_2O_2 with acute aerobic exercise, this may also be a likely pathway in the reduction in sputum neutrophils observed in our study.

This is the first study to demonstrate that an acute stimulus, in our case, aerobic exercise, can decrease percent sputum neutrophils in patients with COPD. We did not observe significant increases in total cell counts of other airway inflammatory cells, which may have accounted for this decrease. Thus we believe that acute aerobic exercise in patients with COPD can directly reduce sputum neutrophils. These results are exciting because to date, only a handful of pharmaceutical studies have investigated therapies that may cause decreases in sputum neutrophils, which are key inflammatory cells involved in the progression of COPD. There is conflicting evidence for combination ICS/long acting beta-2 agonist (LABA) therapy to reduce sputum neutrophils. Barnes [167] reported a decrease from ~80 to 76 percent in sputum neutrophils after 12 weeks

of treatment with salmeterol/fluticasone proprionate, while other studies have not shown decreases [168, 169]. Theophylline has been shown to decrease sputum neutrophils from 34 to 14% of the differential cell count after 12 months of treatment [170]. Another trial found a decrease in sputum neutrophil number (in millions/ml) by 22% [171], but these authors did not find a decrease in neutrophils as a percent of the differential cell count. The observation that a single bout of exercise can reduce sputum neutrophilia shows that patients with COPD may experience beneficial short-term immunological effects even before the well-known changes to exercise capacity, quality of life, and skeletal muscle function occur. Whether the short term reductions in sputum neutrophilia persist with continued exercise training for patients with COPD, how long these effects last, and whether they may have beneficial effects on lung function or defense against pathogens, remains to be determined in future randomized control trials.

Aside from the decrease in sputum neutrophils in the COPD group with low intensity exercise, no other changes in airway cells were observed. Noteworthy is the significant decrease in sputum IL-8 in the high intensity trial in patients with COPD. A reduction in sputum IL-8 in patients with COPD was also seen in the low intensity trial; however, this reduction did not reach statistical significance. Given the role of IL-8 as a potent chemoattractant for neutrophils, the declines in sputum IL-8 coupled with the reduction in sputum neutrophils after exercise provides us with an interesting potential pathway for the control of airway inflammation in response to exercise in patients with COPD. This study was not powered to detect changes in sputum IL-8 after exercise, nor was it designed to examine relationships between inflammatory cells and cytokines such

as neutrophils and IL-8, but future studies with larger sample sizes are now needed to investigate the mechanisms responsible for the reductions in neutrophils and IL-8 with different intensities of exercise in this patient population.

Systemic Inflammation

The systemic inflammatory responses observed in this study add to the knowledge obtained from the several previous studies that have to date provided the foundation for the understanding of systemic inflammation in response to acute aerobic exercise in patients with COPD. We observed an increase in blood lymphocytes after high intensity exercise, an increase in neutrophils after low intensity exercise, and an increase in blood monocytes after both high and low intensity exercise. These results are similar to the findings of van Helvoort and colleagues [122], who observed increases in blood neutrophils, monocytes, and lymphocytes after a CPET in patients with COPD and control subjects. They have also demonstrated that the blood lymphocyte response is associated with increases in catecholamine concentrations, which are increased with higher intensities of aerobic exercise [122, 134]. The relationship of exercise intensity to catecholamine release and subsequent increases in circulating blood lymphocytes likely explains why we did not observe an increase in blood lymphocytes in response to low intensity exercise in either group.

In previous work by van Helvoort and colleagues [134], an increase in systemic IL-6 was observed after CPET and 6MWT in patients with COPD. This finding is in contrast with our study, since no change in IL-6 after either of our exercise sessions was

observed. Several possibilities exist for the difference in our results. We utilized a Luminex 200 system to quantify cytokines, while the van Helvoort group used the ELISA technique. Compared to van Helvoort's work, our study showed lower levels of baseline circulating IL-6 and larger variance in this measure (3.0 (1.8-11.9) and 2.7 (2.3-8.4) pg/ml at our two baselines compared to approximately 5.5 to 6.0 pg/ml at baseline in van Helvoort's study). Both of our studies had only modest increases in IL-6 (approximate increases of 0.71-0.82 pg/mL observed in the van Helvoort study vs median increases of 0.3 and 1.8 pg/mL in low and high intensity exercise, respectively), but the greater levels of variance detected in our study prevented these changes from being significant. Furthermore, the patient population involved in the van Helvoort study displayed more severe airflow obstruction, were less fit, and were muscle-wasted. Thus, differences in measurement techniques and patient samples may account for the disparity in the findings of our two studies.

Blood neutrophils were increased significantly in both groups after the low intensity trial, and this did not occur after the high intensity trial. Many studies have shown that neutrophils can continue to rise past two hours of exercise [172, 173], and many others have shown that neutrophils display a "biphasic" response, where a small increase in neutrophils is observed immediately after exercise, followed by greater increases from two hours onward [174, 175]. The immediate increase in blood neutrophils observed with exercise has been attributed to neutrophil release from the marginal pool, which is usually stimulated by increases in blood flow or from catecholamine interactions, while the delayed increase in blood neutrophils may be

stimulated by cortisol release, cytokine stimulation, or markers of muscle damage such as creatine kinase [176]. A biphasic neutrophil response has been previously reported in healthy individuals and patients with COPD after a CPET, with immediate increases in blood neutrophils observed immediately after exercise, followed by a slight decline at 30 minutes into recovery, which then led to a second elevation in blood neutrophils by two hours after exercise [122]. We did not measure past two hours after our exercise sessions, but sampling at time points after two hours post-exercise may have allowed us to determine whether the neutrophil response to high intensity exercise was delayed or simply blunted compared to the low intensity trial.

We did not observe a change in systemic IL-8 after either intensity of exercise in neither patients with COPD nor healthy subjects. While IL-8 is produced by skeletal muscle after exercise, the magnitude of its release from skeletal muscle is much less than IL-6 and thus only local increases in IL-8 are usually observed in response to exercise [128]. We also observed an increase in IP-10 in patients with COPD immediately after high intensity exercise. IP-10 is an "angiostatic" chemokine, and although there is considerable variability in systemic IP-10 at rest, this chemokine is highly responsive to treatment with corticosteroids and resultantly has been implicated as a potential systemic biomarker for corticosteroid therapy in patients with COPD [181, 182]. In our study, despite quite large variance in baseline IP-10, we observed a significant increase in IP-10 following high intensity exercise in patients with COPD, and a trend towards this same increase in the control group. Thus we can see that IP-10 is a highly responsive element sytemically and although our study was not designed to examine relationships

between IP-10 and inflammatory markers in response to exercise, this may be a subject of interest in future studies.

The Anti-Inflammatory Response to Exercise

To our knowledge, this study is the first to examine the anti-inflammatory response to aerobic exercise in both the airways and systemically in patients with COPD. We observed no difference in resting levels of sputum IL-10 between patients with COPD and control subjects, and no changes were observed with high or low intensity exercise in either of these groups. Contrary to our study, Takanashi and colleagues revealed reduced levels of sputum IL-10 in patients with COPD compared to healthy non-smokers [150]. In this previous study, patients presented with a more pronounced degree of airflow obstruction (FEV_1 52.4 ± 4.1 % predicted), were compared to a much younger sample of healthy individuals, and it is unclear whether these subjects were current smokers or not. Given that our subjects were similar in terms of age, and all subjects were required to be non-smokers, we may have eliminated some confounding variables which may have contributed to the differences observed by Takanashi and colleagues. The lack of an observed sputum IL-10 response to exercise suggests that the airway anti-inflammatory response may be less sensitive than the systemic response. It may also indicate that the initiation of an IL-10 response in the lungs depends on an elevation of pro-inflammatory mediators. Petersen and Pedersen [183] have explained that systemically, the initiation of the anti-inflammatory response may be mediated by pro-inflammatory cytokines, and this same mechanism may occur

in the lungs. The lack of an increase in any of the pro-inflammatory cytokines measured in sputum may therefore explain why IL-10 was not elevated in this study.

Although IL-10 levels were similar in sputum samples of controls and COPD subjects, circulating IL-10 levels were lower in patients with COPD throughout the study. Furthermore, an increase in circulating IL-10 was observed in healthy subjects two hours after high intensity exercise, but this response was not observed in patients with COPD. As discussed in Chapter 1, after aerobic exercise in healthy individuals, an acute pro-inflammatory cytokine response is generally countered by a compensatory anti-inflammatory response [106, 184]. For example, Petersen and Pedersen explained that post-exercise elevations in IL-6 may be responsible for stimulating IL-10 and IL-1ra release, and decreasing TNF-α [34]. In our study, we did not observe an increase in IL-6 post-exercise, and this may partially explain why an anti-inflammatory response, marked by IL-10, was not observed in patients with COPD. It has also been hypothesized that patients with COPD display a reduced anti-inflammatory capacity, indicated by a reduced pro- versus anti-inflammatory cytokine balance [185]. The lack of an anti-inflammatory response in patients with COPD to either forms of exercise in our study, coupled with the depressed baseline levels of circulating IL-10 and elevated levels of IL-8 compared to controls, supports the theory that patients with COPD have an imbalance in pro and anti-inflammatory cytokines that may perturb their response to exercise.

2.5 Study Limitations

There are a number of limitations to the pilot study performed. The first limitation that we must address is that sputum could only be taken once every 48 hours. Repeated induced sputum tests have been found to cause an increase in airway inflammation, specifically, in sputum neutrophils [186, 187]. Because neutrophils are a prominent inflammatory cell associated with the progression of COPD, and an important aim of the research in our laboratory is to obtain a clear understanding of neutrophil dynamics in response to exercise, we did not want to confound the inflammatory response by repeated sputum induction on the same day. Therefore, we could only perform one sputum induction on days when subjects performed exercise trials and baseline measurements had to be completed at least 48 hours in advance. However, as patients were stable, non-smoking individuals and had no changes in FEV_1 or symptoms between baseline measurements and testing days we are confident that our baseline measurements accurately reflect pre-exercise levels of airway inflammation. Determining the best time to sample for sputum after exercise is important in understanding the airway inflammatory response in patients with COPD. Systemically and in sputum, an increase in neutrophils has been observed two hours post exercise in individuals with exercise-induced bronchoconstriction [188]. Assuming that this same response would occur in patients with COPD, we chose to sample for sputum two hours after exercise. It is important to note that the timeline of release of other leukocytes and lymphocytes into systemic circulation and into the airways may occur at a different rate than neutrophils, but because neutrophils are largely involved in the pathology of

COPD, and measuring the neutrophil response to exercise was our primary outcome, our timing of sputum induction revolved around when we believed we would observe the greatest rise in these inflammatory cells.

Next, we chose the induced sputum technique to obtain inflammatory cell samples from the lungs, and this technique is limited to obtaining cells from the bronchial compartments of the airways. In order to fully understand COPD, it is important to investigate the inflammatory processes occurring in other areas, primarily the lower airways, interstitial space and parenchyma, since these areas are most involved in the disease [53, 57]. However, induced sputum has been correlated with many of the clinical symptoms in COPD, including degree of airflow obstruction and exacerbation frequency [50, 189], and is a safe, repeatable, and valid measure [190]. For the repeated measuring involved in this study, we required a safe, tolerable, and reliable measure, and induced sputum was chosen above other sampling techniques such as bronchoalveolar lavage or lung biopsy for these reasons. Thus, we believe that sputum induction was a sufficient measure of airway inflammation in this first study investigating the acute airway inflammatory response to exercise.

Lastly, to allow us to study the effects of exercise independent of other anti-inflammatory interventions patients who were receiving inhaled corticosteroids were removed from this component of their therapy for a period of 4 weeks before study participation and remained off them for the two weeks of study protocol. Recently, there has been much discussion regarding the methodological implications of removing

individuals from ICS treatment before an intervention. Suissa and Barnes [191] have explained that individuals who have been removed from ICS therapy are phenotypically different from steroid-naïve patients, and trials that show benefits after removing individuals from ICS and applying an intervention are only showing the effect of ICS cessation and not actually a benefit from the intervention applied. To investigate whether withdrawal of inhaled steroids may have resulted in different inflammatory phenotypes of patients with COPD, we compared sputum neutrophil, eosinophil, and IL-10 baseline levels and responses to exercise in steroid-withdrawn and steroid-naïve patients. No differences were found in any of these inflammatory parameters. Thus, we believe that the steroid-withdrawn and steroid-naïve subjects were very similar in terms of airway inflammatory profiles, and the acute changes to sputum markers in response to aerobic exercise were a result of the exercise stimulus and not simply a result of the removal of ICS therapy.

Future Directions

The results of this study have a number of practical and theoretical applications. A primary goal of our laboratory is to effectively optimize the prescription of training programs for individuals entering and continuing pulmonary rehabilitation programs. The data from the acute inflammatory responses to two different exercise sessions in patients with COPD show that, because of a reduced airway inflammatory response, low intensity training may be a preferable form of exercise for individuals who are not currently performing a regular amount of structured physical activity. High intensity

training sessions may need to be prescribed only after the patient has become accustomed to the exercise.

A second direction, and also a very important aim of our research team, is to investigate how long-term exercise training in patients with COPD can affect the progression of airway and systemic inflammation over time. As discussed previously, the journey towards finding effective therapies aimed at slowing airway and systemic inflammatory processes associated with the primary and secondary pathologies of this disease has been a laborious one with few advances made towards slowing disease progression. The results of this study, especially in regards to airway inflammation, provide preliminary evidence to support the potential role of exercise training as an anti-inflammatory treatment for COPD. Future investigations into how airway inflammation changes with long-term exercise training in patients with COPD, and how exercise training may act synergistically with the current pharmacological therapies for patients with COPD are necessary in order to continue the progression towards management of this very complex disease.

Conclusions

The results from this study indicate that the airway inflammatory response to low but not high intensity exercise may have an anti-inflammatory effect in patients with COPD. Systemically, patients with COPD and control subjects displayed similar inflammatory responses to exercise of different intensities but it appears that COPD patients have a blunted anti-inflammatory response compared to healthy age and activity

matched controls. The reduction in sputum neutrophils observed after low intensity exercise in patients with COPD may have considerable benefit in the management of this disease if this effect can be maintained with exercise training. Further research is now needed to investigate the chronic effect of exercise on airway inflammation in this patient population.

APPENDIX A – COPD Washout questionnaire

PATIENT ID:
Date: -_____ Phone Interview # _____
Medications: _____

1. How are you feeling since the time we first met? (When you were on your usual medications?)

_____

2. Do you have shortness of breath? _____
   a) compared with first visit when you were on your original medications? (is it the same, better, or worse?) _____

3. Do you have a regular cough?
   a) compared with the first visit when you were on your original medications? (is it same, better, or worse?)
   b) Are you bringing up any phlegm/mucus? What are you coughing up?
   c) What color?
   d) Is that the same as what you were coughing up before, or has the color changed?
   e) Are you coughing up blood? _____

4. Do you have chest tightness?
   a) Is this the same, better, or worse than when we first saw you?

5. Do you wheeze (noisy breathing)?
   a) Is this the same, better, or worse than when we first saw you?

6. Had you had any fevers or chills?
   a) If yes, ask if they took their temperature?
   b) Were the fevers/chills like an infection/pneumonia?
   c) *If they're having a tough time answering these questions: How many episodes have you had this last week? How many? How often?*

7. How many times over the past week did you use your rescue medication?

8. Do you have any other concerns that you are worried about?

Signed: _____ Date: _____

APPENDIX B – Justification and Calculation of Work Rates and Total Work Achieved

The exercise intensities and durations described above were chosen because they mimic exercise sessions that frequently occur in pulmonary rehabilitation. The work rate and duration of the low intensity exercise session was chosen because it is a sustainable exercise intensity for these patients [132], and corresponds with with the energic demands of activities of daily living [192]. The high intensity exercise session was designed based on previous work with COPD patients [145] and is aimed at providing a strenuous but attainable workload. The high and low intensity exercise sessions were equal in the amount of work performed, thus allowing a clear observation of the effect of exercise intensity on the inflammatory response. Both the low and the high intensity exercise sessions included a warm-up which consisted of 5 minutes of cycling between 0 and 30 watts, depending on subject fitness level, which was kept consistent between the two trials. All cycle ergometry was performed between 50 and 70 revolutions per minute (rpm).

The duration of the high intensity interval session was held constant at 8 sets of 1 minute at 100% Wmax followed by 2 minutes at 30% Wmax. Total work (in watt*minutes) for the high intensity exercise session was calculated as

Total Work (w*min) = 8 x [(100% watts * 1 minute) + {30% watts * 2 minutes)]

*Note that the watts for these calculations were rounded to the nearest multiple of 5, as this was the lowest increment of power change on our cycle ergometer.

The duration of the low intensity trial was then calculated based on the total work achieve in the high intensity trial. Assuming an equal amount of work achieved, duration for the low intensity trial was calculated as

Duration = Total work (Watt*minutes)/ 40% watts

Ending exercise 5 minutes early resulted in a 300 w*min and a 100 w*min decrease in work performed in the healthy and COP both groups during the low intensity trial, but this difference is not statistically different, nor was it likely to produce a much different inflammatory response between high and low intensity trials.

APPENDIX C – Tables and Figures

Table A1. Average amounts of work performed in High vs Low intensity trials: Healthy and COPD patients

	Watts*minutes (Average)	Change noted
Healthy High	1502	No changes
Healthy Low	1472	1 subject ended 5 minutes early
COPD High	1000	2 subjects had 1 extra minute of rest
COPD Low	982	1 subject ended 5 minutes early

Table A2. Pulmonary Function Test - Individual Values - COPD

SUBJECT ID:	1	2	3	4	5	6	7	8	9	10
FEV_1 (L)	1.59	2.53	1.18	1.55	1.23	2.49	1.61	2.21	1.07	1.89
FEV_1 (% Pred)	45	72	50	70	68	83	69	99	44	79
FVC (L)	4.07	3.96	2.41	2.21	2.58	4.03	2.93	3.32	2.69	3.13
FVC (% Pred)	93	90	81	80	105	104	100	124	89	104
FEV_1/FVC (%)	39	64	49	70	48	62	55	67	40	60
FEV_1/FVC (% Pred)	49	81	60	85	59	80	67	79	49	75
TLC (L)	8.00	7.68	u/a	4.96	6.84	8.07	5.30	u/a	5.94	5.91
TLC (% Pred)	115	107	u/a	101	143	116	103	u/a	116	101
RV (L)	4.25	3.22	u/a	2.55	4.27	3.85	2.61	u/a	3.14	2.87
RV (% Pred)	193	132	u/a	122	189	149	120	u/a	150	140
FRC (L)	5.46	3.94	u/a	2.88	5.09	4.67	3.43	u/a	3.72	3.56
FRC (% Pred)	152	103	u/a	103	185	124	117	u/a	127	119
RV/TLC (%)	53	42	u/a	51	62	48	49	u/a	53	48
RV/TLC (% Pred)	164	121	u/a	120	132	126	116	u/a	129	141
DLCO (mL/mmHg/min)	18.6	28.5	13.0	15.4	15.9	16.6	13.7	17.0	12.6	25.7
DLCO (% Pred)	88	121	65	76	97	89	82	113	66	133

All values are post-bronchodilator.
u/a = Unable to perform body plethysmography according to ATS criteria

Table A3. Pulmonary Function Test – Individual Values – Control

SUBJECT ID:	1	2	3	4	5	6	7	8	9
FEV_1 (L)	3.01	2.28	3.06	2.24	2.81	2.43	3.19	2.77	3.06
FEV_1 (% Pred)	114	98	87	94	124	78	89	110	93
FVC (L)	3.69	3.39	3.81	2.65	3.31	3.34	4.06	3.37	4.66
FVC (% Pred)	114	118	86	89	122	84	90	104	111
FEV_1/FVC (%)	82	67	80	85	85	73	78	82	66
FEV_1/FVC (% Pred)	98	81	102	102	100	92	100	105	85
TLC (L)	5.46	6.00	6.05	4.49	5.21	5.80	6.19	6.96	7.19
TLC (% Pred)	105	122	84	88	116	85	84	108	100
RV (L)	1.61	2.38	1.98	1.64	1.87	2.15	2.14	3.33	2.83
RV (% Pred)	85	122	82	80	114	92	85	136	110
FRC (L)	2.33	3.48	3.24	2.56	2.89	2.86	3.33	4.05	4.37
FRC (% Pred)	80	126	85	89	116	80	85	118	113
RV/TLC (%)	29	40	33	36	36	37	35	48	39
RV/TLC (% Pred)	81	100	95	90	100	106	98	125	106
DLCO (mL/mmHg/min)	23.2	27.8	27.8	23.9	19.8	28.6	29.3	30.6	30.0
DLCO (% Pred)	96	142	121	109	103	134	149	173	149

All values are post-bronchodilator.

Table A4. Cardiopulmonary Exercise Test – Individual Values - COPD

SUBJECT ID:	1	2	3	4	5	6	7	8	9	10
VO_2 (mL/kg/mL)	23.8	25.4	10.7	15.6	13	21.7	18.5	21.8	15.9	23
VO_2 (% Pred)	96	122	53	76	76	136	90	84	72	107
VO_2 (L/min)	1.609	2.316	0.765	1.175	0.789	1.683	0.972	0.903	1.015	1.725
VO_2 (% Pred)	66	96	64	107	91	85	80	80	80	114
V_E (L/min)	65.2	89.4	30.3	47.1	26.1	71.4	50.6	62.1	41.2	52
V_E (% Pred)	117	101	73	87	61	82	90	81	110	79
RER	1.17	1.07	0.88	1.06	0.89	1.03	1.04	1.13	1.10	1.04
SPO_{2max}	93	96	91	88	96	100	93	96	88	99
HR_{max} (Beats/min)	136	145	120	118	110	114	123	106	127	162
W_{max} (Watts)	100	150	30	70	50	90	60	50	60	110
Peak Borg Dyspnea	7	7	5	6	5	3	5	1	3	5
Peak Borg Muscular Discomfort	7	7	7	7	6	4	7	2	9	7

Table A5. Cardiopulmonary Exercise Test – Individual Values - Control

SUBJECT ID:	1	2	3	4	5	6	7	8	9
VO_2 (mL/kg/mL)	21.40	22.30	20.9	15.9	25.20	34.40	17.7	25.3	22.00
VO_2 (% Pred)	88	97	98	72	97	169	89	164	126
VO_2 (L/min)	1.811	1.489	1.790	1.264	1.568	2.841	1.225	1.996	1.764
VO_2 (% Pred)	123	122	75	99	122	135	51	126	79
V_E (L/min)	68.40	51.7	83.8	63.8	61.80	107	43.2	82.1	75.5
V_E (% Pred)	65	65	78	81	63	126	39	85	70
RER	1.08	1.12	1.15	1.18	1.06	1.21	1.06	1.05	1.13
SPO_{2max}	99	98	98	95	98	96	97	99	98
HR_{max} (Beats/min)	171	165	132	148	177	179	113	124	148
W_{max} (Watts)	130	90	130	90	110	180	90	120	120
Peak Borg Dyspnea	10	5	9	8	6	2	0	7	3
Peak Borg Muscular Discomfort	10	7	9	10	9	6	3	8	8

Table A6 – Sputum Total Cell Counts

	LOW		HIGH	
	Baseline	2 Hours	Baseline	2 Hours
COPD				
Total Cell Count (10^6/g)	12.04 ± 4.75	10.20 ± 8.45	12.43 ± 9.48	17.45 ± 12.98
Sputum Weight (g)	0.51 ± 0.32	0.37 ± 0.37	0.53 ± 0.37	0.49 ± 0.49
Total Cells in Suspension (10^6)	5.43 ± 3.16	2.09 ± 1.85	3.14 ± 3.03	7.34 ± 8.20
Control				
Total Cells (10^6/g)	4.75 ± 3.07	2.21 ± 1.97	5.12 ± 4.20	1.98 ± 1.24
Sputum Weight (g)	0.34 ± 0.25	0.25 ± 0.15	0.29 ± 0.20	0.44 ± 0.48
Total Cells in Suspension (10^6)	1.52 ± 1.03	0.59 ± 0.78	1.31 ± 0.95	1.22 ± 1.85

Table A7. Sputum Neutrophils (%) – Individual Values

Subject	NEUTROPHIL LOW BASE	2H Post	HIGH BASE	2H Post
Healthy				
1	74.4	13.5	16.0	53.0
2	69.5	5.0	43.5	10.0
3	47.0	3.5	56.8	4.0
4	10.8	5.5	49.0	2.5
5	69.8	63.5	59.3	63.0
6	20.0	47.0	76.7	57.0
COPD				
1	84.0	76.0	74.0	82.0
2	94.0	94.0	66.5	75.0
3	92.5	70.0	80.5	66.0
4	75.0	68.0	63.8	50.0
5	85.3	86.7	77.0	96.7
6	95.0	92.3	87.5	85.5
7	85.5	71.0	81.0	72.0
8	78.5	14.0	75.5	72.0

Table A8. Sputum Eosinophils (%) – Individual Values

Subject	EOSINOPHIL LOW BASE	2H Post	HIGH BASE	2H Post
Healthy				
1	1.1	0.0	0.0	0.5
2	0.5	0.0	1.5	1.5
3	0.0	0.5	0.3	0.0
4	0.7	0.0	0.3	0.3
5	0.0	1.0	6.5	0.5
6	0.0	0.0	1.3	0.5
COPD				
1	4.3	8.0	15.0	4.0
2	0.5	0.0	0.5	0.3
3	1.5	1.0	1.5	2.0
4	0.3	0.0	1.5	1.5
5	0.7	1.9	3.0	0.3
6	0.0	0.0	0.0	0.0
7	0.5	26.5	14.0	21.0
8	0.0	2.0	0.5	2.5

Table A9. Sputum Macrophages (%) – Individual Values

Subject	MACROPHAGE LOW BASE	2H Post	HIGH BASE	2H Post
Healthy				
1	23.9	86.5	84.0	46.5
2	23.3	95.0	55.0	88.5
3	53.0	96.0	42.0	96.0
4	21.2	94.5	45.5	13.5
5	7.0	12.3	35.3	22.3
6	80.0	53.0	22.0	42.5
COPD				
1	11.7	16.0	11.0	14.0
2	5.5	6.0	33.0	24.7
3	6.0	29.0	18.0	32.0
4	24.7	32.0	31.0	48.5
5	14.1	10.0	20.0	3.0
6	5.0	7.7	12.5	14.5
7	12.0	2.5	5.0	7.0
8	20.0	24.0	24.0	25.5

Table A10. Sputum IL-6 ($pg*mL^{-1}$) – Individual Values

Subject	IL-6 LOW BASE	2H Post	HIGH BASE	2H Post
Healthy				
1	67.6	70.5	110.2	161.7
2	388.4	80.0	27.4	111.4
3	239.3	174.1	178.3	179.3
4	23.7	44.4	184.3	17.0
5	154.3	30.0	362.3	66.1
6	67.4	889.5	1151.8	508.0
COPD				
1	890.8	110.2	358.2	401.3
2	56.3	145.2	196.6	112.2
3	79.9	63.1	202.6	94.6
4	537.8		727.0	582.1
5	388.1	519.4	86.3	153.0
6	459.3	294.4	586.6	197.1
7	235.6	2926.9	237.1	2590.7
8	374.4		1831.7	68.1

Table A11. Sputum IL-8 ($ng*mL^{-1}$) – Individual Values

Subject	IL-8 LOW BASE	2H Post	HIGH BASE	2H Post
Healthy				
1	20.9	1.5	0.5	2.9
2	28.1	1.0	32.0	1.6
3	24.6	21.5	17.8	5.2
4	1.9	2.3	19.3	1.0
5	3.2	7.3	9.8	12.3
6	1.1	6.1	19.4	4.2
COPD				
1	8.8	3.4	19.7	8.6
2	4.2	5.9	30.9	37.0
3	7.8	1.4	12.6	2.2
4	16.6		19.6	7.8
5	11.8	26.9	3.4	5.8
6	349.1	12.4	24.5	9.5
7	3.3	80.7	15.7	12.8
8	3.2		25.5	2.1

Table A12. Sputum IL-10 ($pg*mL^{-1}$) – Individual Values

Subject	IL-10 LOW BASE	2H Post	HIGH BASE	2H Post
Healthy				
1	37.9	6.3	1.8	12.6
2	9.9	3.6	24.3	5.4
3	43.3	13.5	23.4	18.9
4	1.8	3.6	23.4	4.5
5	11.7	23.4	21.6	35.2
6	6.6	21.2	43.3	24.3
COPD				
1	17.1	9.0	19.8	7.2
2	9.0	4.5	26.1	23.4
3	9.9	6.3	11.7	5.4
4	23.5		33.0	36.1
5	27.4	33.7	12.7	10.4
6	694.7	1089.1	11.9	430.0
7	12.7	36.1	18.1	41.7
8	11.9		26.7	2.9

Table A13. Sputum IP-10 ($ng*mL^{-1}$) – Individual Values

Subject	IP-10 LOW BASE	2H Post	HIGH BASE	2H Post
Healthy				
1	67.9	4.2	0.6	8.6
2	11.2	2.3	38.6	6.5
3	29.9	11.1	14.7	9.4
4	5.8	3.8	33.9	2.5
5	8.1	18.5	25.8	45.8
6	4.4	16.6	34.6	21.3
COPD				
1	7.5	3.6	12.8	6.1
2	6.1	6.9	14.5	13.7
3	18.8	7.9	27.0	12.9
4	50.3		55.0	52.0
5	23.5	39.6	8.6	13.4
6	110.2	66.8	67.7	133.3
7	18.0	37.9	17.4	58.2
8	10.0		22.0	3.6

Table A14. Serum Neutrophils ($10^9 * L^{-1}$) – *Individual Values*

Subject	NEUTROPHIL LOW BASE	0H Post	2H Post	HIGH BASE	0H Post	2H Post
HEALTHY						
1	4.1	5.2	7.9	6.0	4.0	4.2
2	2.8	3.3	4.7	3.7	3.9	4.7
3	3.1	3.9	3.4	3.5	4.2	4.5
4	2.6	4.3	3.8	3.9	3.7	3.6
5	3.2	4.1	4.4	4.1	4.1	7.9
6	3.2	4.1	5.0	4.0	5.0	6.4
7	5.7	7.9	7.1	6.1	7.5	9.7
8	4.7	5.2	5.8	4.7	3.9	4.8
9	4.1	4.2	5.4	3.5	5.5	4.5
COPD						
1	5.2	3.9	3.9	6.0	4.4	5.3
2	4.5	3.2	3.2	2.7	3.3	3.5
3	3.7	5.8	3.7	4.9	3.8	4.4
4	5.0	6.2	6.9	5.2	5.9	6.0
5	5.7	6.5	6.7	4.9	5.8	5.2
6	4.8	5.6	5.6	5.3	6.5	6.3
7	2.7	4.0	5.4	3.1	3.4	6.5
8	2.9	4.6	4.3	2.9	4.8	3.8
9	3.1	3.8	2.9	2.5	2.5	3.7
10	4.5	4.6	5.0	4.1	5.6	4.7

Table A15. Serum Monocytes ($10^9 * L^{-1}$) – *Individual Values*

Subject	MONOCYTE LOW BASE	0H Post	2H Post	HIGH BASE	0H Post	2H Post
HEALTHY						
1	0.4	0.6	0.5	0.5	0.7	0.7
2	0.3	0.5	0.4	0.5	0.7	0.4
3	0.7	0.8	0.7	1.0	1.1	0.8
4	0.5	0.5	0.5	0.5	0.6	0.5
5	0.4	0.5	0.5	0.4	0.5	0.4
6	0.3	0.4	0.5	0.4	0.6	0.3
7	0.6	0.8	0.6	0.6	0.5	0.5
8	0.6	0.7	0.7	0.6	0.8	0.7
9	0.7	0.9	0.7	0.7	0.9	0.7
COPD						
1	0.6	0.7	0.5	0.6	0.7	0.5
2	0.4	0.5	0.4	0.4	0.6	0.3
3	0.4	0.4	0.5	0.5	0.6	0.4
4	0.9	0.9	0.9	0.9	1.1	0.9
5	0.7	0.8	0.6	0.7	0.9	0.5
6	0.6	0.7	0.6	0.5	0.8	0.7
7	0.4	0.6	0.5	0.6	0.8	0.7
8	0.5	0.6	0.5	0.4	0.7	0.5
9	0.5	0.4	0.4	0.4	0.4	0.4
10	0.5	0.7	0.8	0.4	0.9	0.6

Table A16. Serum Lymphocytes ($10^9 * L^{-1}$) – *Individual Values*

Subject	LYMPHOCYTE LOW BASE	0H Post	2H Post	HIGH BASE	0H Post	2H Post
HEALTHY						
1	1.3	1.6	1.5	1.6	1.9	1.5
2	1.1	1.5	1.3	1.3	2.4	1.4
3	1.9	1.8	1.6	1.9	2.7	1.6
4	3.0	2.2	2.1	1.9	3.2	2.2
5	1.7	1.8	1.8	1.4	2.0	1.8
6	1.5	1.6	1.6	1.7	3.4	1.4
7	2.1	2.8	2.4	2.5	2.2	2.0
8	1.7	2.0	2.1	1.3	2.7	2.0
9	3.8	4.5	3.9	3.9	5.6	2.9
COPD						
1	1.0	1.6	1.2	1.0	1.4	1.2
2	0.9	0.9	0.8	1.0	1.7	0.8
3	1.9	3.3	1.9	1.8	1.6	1.3
4	4.3	3.5	4.7	4.2	4.3	4.0
5	1.9	2.2	2.2	2.0	2.5	2.0
6	0.7	0.6	0.7	0.6	0.9	0.8
7	1.4	1.8	1.1	1.4	2.5	1.1
8	2.5	2.3	2.4	2.0	2.5	2.1
9	1.1	1.5	0.8	1.4	1.4	1.2
10	2.4	2.7	3.8	2.6	3.4	2.5

Table A17. Serum Eosinophils ($10^9 * L^{-1}$) – *Individual Values*

Subject	EOSINOPHIL LOW BASE	0H Post	2H Post	HIGH BASE	0H Post	2H Post
HEALTHY						
1	0.1	0.1	0.1	0.2	0.3	0.2
2	0.2	0.2	0.1	0.1	0.2	0.1
3	0.3	0.2	0.2	0.3	0.3	0.2
4	0.2	0.1	0.2	0.1	0.1	0.1
5	0.1	0.0	0.0	0.0	0.1	0.1
6	0.2	0.2	0.2	0.2	0.2	0.1
7	0.2	0.3	0.2	0.3	0.1	0.1
8	0.2	0.2	0.1	0.1	0.2	0.1
9	0.1	0.1	0.1	0.1	0.1	0.1
COPD						
1	0.1	0.2	0.1	0.0	0.1	0.1
2	0.0	0.0	0.0	0.0	0.1	0.0
3	0.3	0.4	0.2	0.1	0.3	0.1
4	0.2	0.2	0.2	0.2	0.2	0.2
5	0.6	0.6	0.3	0.3	0.5	0.2
6	0.0	0.1	0.0	0.0	0.0	0.0
7	0.1	0.2	0.1	0.2	0.2	0.1
8	0.1	0.2	0.2	0.2	0.2	0.1
9	0.3	0.2	0.2	0.2	0.2	0.3
10	0.1	0.2	0.1	0.1	0.2	0.1

Table A18. Serum IL-6 ($pg*mL^{-1}$) – Individual Values

Subject	LOW BASE	0H Post	2H Post	HIGH BASE	0H Post	2H Post
HEALTHY						
1	3.6	4.4	2.9	2.3	1.8	2.1
2	10.9	1.3	28.3	0.8	86.9	29.6
3	3.5	4.8	3.6	3.3	5.1	3.8
4	0.7	1.6	2.1	1.2	1.4	1.7
5	3.8	3.1	4.5	2.6	5.3	4.2
6	69.0	61.3	9.9	4.5	5.4	7.0
7	254.2	303.1	72.3	314.6	79.2	339.9
8						
9	17.5	9.7	11.3	17.3	11.4	40.2
COPD						
1	9.8	3.5	9.7	9.7	2.1	12.2
2	52.1	108.5	8.5	70.5	116.0	14.4
3	7.1	6.3	7.6	7.9	6.7	7.4
4	2.7	3.1	3.9	2.6	4.3	2.2
5	3.2	3.4	2.3	2.8	3.6	2.5
6	18.2	25.8	14.7	6.7	17.3	13.2
7	0.6	0.6	0.3	0.5	0.3	0.8
8	1.4	11.1	5.8	2.2	6.7	12.4
9	2.4	7.2	2.3	2.3	4.2	1.3
10	1.9	1.6	2.3	2.5	4.9	2.6

Table A19. Serum IL-8 ($pg*mL^{-1}$) – Individual Values

Subject	LOW BASE	0H Post	2H Post	HIGH BASE	0H Post	2H Post
HEALTHY						
1	5.9	7.6	6.7	7.6	4.6	4.7
2	10.7	10.4	6.3	10.6	7.1	10.6
3						
4	2.7	0.3	2.0	1.3	0.1	0.3
5	4.6	4.1	4.3	4.8	8.2	3.3
6	2.1	2.6	1.7			
7	3.1	0.8	4.5	2.0	0.1	8.4
8	3.2	1.6	2.9	3.8	1.3	2.3
9						
COPD						
1	9.4	8.8	9.2	9.7	8.5	8.3
2	5.5	5.3	2.7	4.9	1.2	5.8
3	2.3	2.8	5.6	5.9	9.7	5.9
4	11.1	10.9	16.3	7.7	6.0	7.4
5	8.7	6.2	7.7	11.8	8.9	9.2
6	24.3	16.0	18.6	11.0	10.7	20.8
7						
8	20.1	13.2	11.9	12.2	9.3	9.1
9						
10	6.9	8.1	7.2	6.5	5.8	13.8

Table A20. Serum IL-10 ($pg*mL^{-1}$) – Individual Values

Subject	LOW BASE	0H Post	2H Post	HIGH BASE	0H Post	2H Post
HEALTHY						
1	10.3	10.6	7.3	6.3	5.3	5.5
2	20.3	5.2	37.3	3.7	27.8	43.7
3	6.3	8.2	5.6	7.6	8.4	6.6
4	3.1	3.2	3.1	2.8	3.0	2.6
5	5.0	4.1	4.6	4.8	6.3	6.5
6	5.0	5.4	5.2	4.4	5.2	6.5
7	1.5	2.1	3.6	3.0	4.2	1.5
8	5.0	3.4	2.6	3.9	6.4	6.6
9	18.3	10.9	12.6	14.8	16.6	50.4
COPD						
1	39.5	50.6	36.9	33.9	40.2	27.1
2						
3	2.0	2.5	2.1	1.5	2.2	1.6
4	3.6	3.7	2.7	3.8	4.1	3.7
5	2.7	2.1	2.1	2.6	2.5	2.2
6	1.7	1.7	1.7	1.4	1.6	1.6
7	1.5	1.4	1.5	1.6	1.9	1.5
8	1.7	2.6	3.0	2.2	2.1	3.0
9						
10	2.4	1.7	1.9	1.7	2.7	1.6

Table A21. Serum IP-10 ($pg*mL^{-1}$) – Individual Values

Subject	LOW BASE	0H Post	2H Post	HIGH BASE	0H Post	2H Post
HEALTHY						
1	220.1	199.4	190.5	161.7	196.2	184.4
2	207.5	189.6	205.4	170.9	159.8	199.8
3	245.1	333.3	294.5	221.5	347.5	292.1
4	348.3	286.9	253.7	290.5	385.3	317.1
5	170.9	165.0	185.8	164.2	194.8	181.5
6	184.2	191.9	225.8	239.5	191.0	208.3
7	181.2	162.2	157.6	157.8	141.5	128.8
8	234.0	242.5	247.6	254.0	407.1	342.0
9	729.0	559.2	661.7	568.9	479.0	749.5
COPD						
1	507.7	439.6	445.5	428.8	515.9	459.2
2	280.2	364.0	335.2	347.8	328.5	290.7
3	397.2	433.0	488.3	306.5	373.5	375.0
4	423.3	424.4	311.4	451.0	608.5	452.6
5	371.5	415.4	387.3	431.9	544.6	426.2
6	192.7	240.6	238.0	193.0	240.4	187.3
7	202.8	176.7	153.7	157.6	199.3	174.7
8	177.7	181.1	190.6	166.8	223.9	279.0
9	341.3	480.9	408.5	442.2	366.9	251.9
10	340.4	257.6	341.1	231.0	290.6	266.5

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