University of Alberta

Cardiovascular Risk in Asthma

by

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Abstract

Introduction: Asthma is a chronic inflammatory disease characterized by intermittent episodes of acute airway inflammation and bronchoconstriction. Although asthma is generally considered a disease of the airways, asthma is also associated with an increased risk of developing cardiovascular (CV) diseases such as coronary artery disease, cerebrovascular disease, and heart failure. The reasons for the increased CV risk in asthma are not well understood; therefore, the purpose of this dissertation was to evaluate different aspects of asthma which may contribute to CV risk: physical inactivity; exertional dyspnea; airway inflammation; and asthma medications.

Methods: This dissertation consists of four projects. In Chapter III, to assess the impact of physical activity and fitness on CV health, the endothelial function (flow-mediated dilation (FMD)), microvascular function (velocity time integral (VTI) during reactive hyperemia), and arterial stiffness (pulse wave velocity (PWV)) were evaluated in 16 asthmatics and 16 physical activity and fitness-matched controls. In Chapter IV, the physiological reasons for exertional dyspnea were evaluated in 16 asthmatics and 16 age and sex-matched controls. The participants completed two incremental bicycle exercise tests to exhaustion, and the dyspnea responses (modified Borg (0-10) scale) were evaluated in relation to operating lung volumes. In random order, salbutamol was given prior to exercise to evaluate the impact on the sensory and physiological responses to exercise. In Chapter V, 12 asthmatics completed three bronchial challenge tests (placebo, mannitol, and methacholine) in random order on separate days, and the impact of increased pulmonary inflammation and bronchoconstriction was assessed. Prior to, and within one hour after each bronchial challenge, FMD, VTI, PWV, and systemic inflammation (serum C-reactive protein (CRP)) levels were evaluated. In Chapter VI, FMD, VTI, and PWV were evaluated at baseline and

following 400 mcg of inhaled salbutamol or placebo (delivered on separate days, order randomized) in 14 asthmatics and 14 controls. Subsequently, to further examine the systemic vascular differences between asthmatics and controls observed following salbutamol inhalation, the systemic vascular responses to a large sympathetic stimulus (delivered via the cold pressor test (CPT) were evaluated in a subset of 10 asthmatics and 10 controls, and compared to placebo (body temperature water hand submersion).

Results: When matched for physical activity and fitness, asthmatics and controls displayed similar endothelial function; however, arterial stiffness remained elevated in asthmatics (PWV: 7.3 m/s vs. 8.7 m/s, $p<0.05$). During incremental exercise, asthmatics experienced intensified exertional dyspnea compared to controls, which was explained by reduced inspiratory reserve volume, throughout exercise in asthmatics. Neither the sensation of dyspnea nor the operating lung volumes were affected by salbutamol usage. Both mannitol and methacholine challenges resulted in significant bronchoconstriction (reduction in forced expiratory volume in one second from baseline of 11.5% and 19.3%, respectively), but only the mannitol challenge caused elevated systemic inflammation (CRP levels increased by 60.4% following mannitol, versus -20.6% following methacholine, $p<0.05$). Neither challenge lead to systemic vascular changes. In the last study, salbutamol inhalation resulted in reduced endothelial function and increased arterial stiffness in asthmatics but not controls (FMD: -3.0% vs. 0.5%, $p<0.05$, PWV: 0.66 m/s vs. -0.16 m/s, $p<0.05$ asthmatics vs. controls). Both asthmatics and controls showed similar responses to a non-specific increase in sympathetic nervous activity, indicating that the differences in vascular responses with salbutamol observed in asthmatics were not explained by altered neurovascular transduction.

Conclusion: Physical inactivity and reduced fitness levels likely contribute to the increased CV risk seen in stable asthmatics. Asthmatics do show greater exertional dyspnea, and thus to reduce physical activity avoidance, further research should focus on normalizing operating lung volumes during physical activity in asthma. Acutely increasing pulmonary inflammation, but not bronchoconstriction, leads to elevated systemic inflammation and therefore increased CV risk. Furthermore, salbutamol acutely impairs vascular function, and the increased usage of asthma medications during times of reduced asthma control likely contributes to elevated CV risk.

Preface

This doctoral dissertation is the original work by myself, Linn E. Moore. The research projects in Chapter III-VI received research ethics approval from the University of Alberta Research Ethics Board (Chapter III: Pro00029773; Chapter IV: Pro00047054; Chapter V: Pro00047054: Chapter VI: Pro00029773 and Pro00063459). The research project in Chapter V additionally received approval from Health Canada (#9427-G0890-88C) and is registered on ClinicalTrails.gov (NCT02630511).

A version of the research project in Chapter III has been published in the Journal of Allergy and Clinical Immunology, as Moore LE, Bhutani M, Petersen SR, McMurtry MS, Byers BW, Tedjasaputra V & Stickland MK, *Physical activity, fitness, and vascular health in patients with asthma*. 136, 809-811 e803, 2015. I was responsible for all parts of the study, including design, subject recruitment, data collection, data analysis, and manuscript preperation. Bhutani M, Petersen SR, McMurtry MS assisted with study design and manuscript preparation. Byers BW and Tedjasaputra V assisted in data collection and analysis. Stickland MK was the principal investigator for the study.

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Abbreviations

ACQ	Asthma control questionnaire
BD	Bronchodilator
BMI	Body mass index
BP	Blood pressure
BT	Body temperature
COPD	Chronic obstructive pulmonary disease
CPT	Cold pressor test
CRP	C-reactive protein
CV	Cardiovascular
DBP	Diastolic blood pressure
ECB	Exhaled breath condensate
ECG	Electrocardiography
EELV	End expiratory lung volume
EFL	Expiratory flow limitation
EILV	end inspiratory lung volume
eNOS	Endothelial nitric oxide synthase
FeNO	Fraction expired nitric oxide
FEV_1	Forced expiratory volume in one second
FMD	Flow-mediated dilation
f_R	Breathing frequency
FVC	Forced vital capacity

GINA	Global initiative for asthma
HDL	High density lipoprotein
HR	Heart rate
HRV	heart rate variability
IC	Inspiratory capacity
ICS	inhaled corticosteroids
IgE	Immunoglobulin E
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IRV	Inspiratory reserve volume
LABA	Long-acting β_2-agonists
MAP	Mean arterial pressure
MSNA	muscle sympathetic nerve activity
nNOS	Neural nitric oxide synthase
NO	Nitric oxide
PC_{20}	Provocative concentration causing a 20% reduction in FEV1
PD_{10}	Provocative dose causing a 10% reduction in FEV1
PD_{15}	Provocative dose causing a 15% reduction in FEV1
PEF	Peak expiratory flow
PP	Pulse pressure
PWV	Pulse wave velocity
RH	Reactive hyperemia

ROS	Reactive oxygen species
RV	Residual volume
SABA	short-acting β_2-agonists
SBP	Systolic blood pressure
SSRH	Shear stress due to reactive hyperemia
TLC	Total lung capacity
TNFα	Tumor necrosis factor alpha
$\dot{V}CO_2$	rate of carbon dioxide production
$\dot{V}E$	Ventilation
$\dot{V}O_2$	rate of oxygen consumption
VRH	velocity during reactive hyperemia
V_T	tidal volume
VTI	Velocity time integral

1 CHAPTER I

Introduction

1.1 General introduction

Asthma is a chronic inflammatory disease characterized by intermittent episodes of wheezing, shortness of breath, and it affects approximately 300 million people worldwide[1]. Although asthma is generally considered a disease of the airways, there are important systemic consequences associated with asthma. Epidemiology studies have reported an increased mortality rate among asthmatics, predominately due to cardiovascular (CV) disease[2-10]. Systemic inflammation has been shown to predict future risk of CV events in both asymptomatic[11-13] and in diseased populations[14-16]. Among the most established markers of systemic inflammation are interleukin-6 (IL-6), C-reactive protein (CRP) and tumor necrosis factor-alpha (TNFα), which have all been shown to be elevated in asthmatics[17-20]. Brachial endothelial function mimics coronary endothelial function[21], can assist in predicting CV risk[22-26], and has also been shown to be compromised in asthma[27]. Arterial stiffness is an indicator of arterial structure, vascular tone, and atherosclerotic buildup, is a predictor of future CV risk[28] and is elevated in asthmatics[29]. The reason(s) for the increased CV risk in asthma is currently unknown, and warrants further investigation.

Several studies have looked at the effects of physical activity on systemic inflammation and CV health, and there is consensus regarding the long-term benefits of physical activity; people who live an active lifestyle have lower levels of systemic inflammation[30] and better vascular health[31-33] than sedentary people, hence lower overall risk of morbidity and mortality[34]. Aerobic exercise training performed to increase physical activity levels has been shown to both reduce signs of pulmonary inflammation in asthma[35, 36] and improve asthma control[35, 37]. Despite this, people with asthma are generally less active than non-asthmatics[38, 39], which may be due to the perceived risk of asthma exacerbations associated with physical activity[40]. Thus, part of the increased CV risk associated with asthma may be due to reduced physical activity levels among

these patients, and finding out why asthmatics experience respiratory discomfort while exercising may help reduce the risk of CV disease in asthma in the future.

Furthermore, systemic inflammation, arterial stiffness, and endothelial impairment seem to be related to the level of asthma control[17, 19, 20, 27, 29, 41]. Animal studies have identified potential mechanisms by which pulmonary inflammation increases systemic inflammation and impairs vascular function[42, 43]; however, it is unknown if asthma exacerbations directly affect CV risk in humans. Additionally, while respiratory infections themselves have been associated with an increased acute CV risk[44], data suggest that the usage of commonly prescribed asthma medications used to relieve acute bronchoconstriction may increase CV risk[3, 45], and it is thus plausible that the main aid in treating airflow obstruction may be contributing to impaired CV health in asthma.

1.2 Purpose of dissertation

This dissertation consisted of four separate studies investigating factors potentially involved in CV risk in asthma. The first study (Chapter III) was a cross-sectional study in which the influence of cardiopulmonary fitness and everyday physical activity levels on systemic inflammation and vascular function in asthmatics was evaluated. The second study (Chapter IV) was a case-control study, investigating the underlying physiological mechanisms to why asthmatics experience intensified exertional dyspnea. A secondary purpose of this study was to further evaluate the effects of the commonly prescribed β_2-agonist salbutamol on the ventilatory responses to higher physical activity intensities. The third study (Chapter V) was an experimental study which investigated the potential link between pulmonary inflammation, bronchoconstriction, systemic inflammation, and systemic vascular health. The fourth study (Chapter VI) was also an

experimental study, separated into Phase I and Phase II. In Phase I, the systemic vascular effect of the β_2-agonist asthma medication salbutamol was evaluated in asthmatics and controls, and in the Phase II of the study, the vascular response to increased sympathetic vasoconstrictor outflow was examined in asthmatics and controls to determine if asthmatics had an altered vascular response to a sympathetic stimulus.

1.3 Summary of dissertation

Asthma is a heterogenous disease, affecting people of all ages, worldwide[46]. Although asthma is generally considered a disease of the airways, people with asthma also suffer from increased risk of developing CV disease[2-10], but the reasons for the increased CV risk in asthma are unknown. The results presented in Chapter III of this dissertation suggest physical activity and fitness to be of major importance for maintaining CV health in asthma. Further, physical activity avoidance, seen as a result of elevated exertional dyspnea[40, 47], was in Chapter IV found to likely stem from an upward shift in operating lung volumes. To reduce exertional dyspnea and thus enable asthmatics to live a physically active life with good CV health, further research is needed to evaluate how to normalize operating lung volumes in asthma during higher intensity physical activities. Chapter V of this dissertation reported increased systemic inflammation as a direct effect of increased pulmonary inflammation, but not bronchoconstriction alone. It is thus likely that asthma exacerbations may play a key role in CV health in asthma. Lastly, the systemic effect of the commonly used asthma symptom reliever medication salbutamol was evaluated in Chapter VI. Asthmatics, but not healthy controls, showed an impairment in endothelial function as well as clinically important elevations in arterial stiffness acutely following salbutamol administration.

Salbutamol, taken in times of reduced asthma control, appears to add further burden to an already stressed CV system. While no difference in the vascular responses to an increase in sympathetic outflow was observed between asthmatics and controls, further investigation into the mechanisms behind CV impairment during naturally occurring asthma exacerbations, and when using salbutamol, is warranted.

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2 CHAPTER II

Review of the literature

2.1 Introduction

Asthma is a chronic inflammatory airway disease characterized by intermittent episodes of bronchoconstriction with symptoms such as chest tightness, shortness of breath, difficulty breathing, wheezing, and cough[1]. The cause of asthma is heterogeneous, and symptoms can be triggered by airborne allergens as well as viral respiratory tract infections, emotional stimuli, exercise, and pollution. The symptoms often worsen during nighttime or during shifts in seasons or temperatures, and the disease has been shown to result in reduced quality of life among those affected[2]. Asthma is diagnosed based on a medical history of asthma like symptoms in combination with spirometry tests showing at least a 12% and 200 mL increase in forced expiratory volume in 1 second (FEV_1) post-bronchodilator[1]. Airway hyperresponsiveness, another common feature of asthma, may not appear on a lung function test. Instead, airway hyperresponsiveness is commonly diagnosed by a positive methacholine challenge test whereby the person tested exhibits significant airway constriction (FEV_1 reduced $\geq$ 20% as compared to baseline spirometry values) in response to a provocative concentration (PC_{20}) of methacholine of $\leq$ 4 mg/mL[3]. In addition, a decrease in $FEV_1 \geq 10$ % following an exercise challenge is currently used in Canada to assist in the diagnosis of exercise-induced asthma and bronchoconstriction[4].

While the test for reversibility of airway constriction may be the most commonly used test to evaluate asthma clinically, other tests can be used for diagnosis as well as in evaluation of asthma disease severity and responses to asthma medications. There are two main classes of airway challenge; direct and indirect. The methacholine challenge and the histamine challenge are considered direct challenges as methacholine and histamine act directly on muscarinic and histamine receptors on the smooth muscles in the airways and thereby cause bronchoconstriction. The classification for indirect challenges is based on a mediated response and includes exercise

challenges, hypertonic saline challenges, dry air hyperpnea challenges, and mannitol challenges. The mechanism by which the indirect challenges cause bronchoconstriction is based on an increase in airway osmolarity, which in turn triggers cells within the airway mucosa to release inflammatory mediators[5]. The indirect challenge using hypertonic saline to evaluate bronchial hyperreactivity was first evaluated in 1981[6] and has been used extensively since. The bronchial responses to the indirect mannitol challenge have been shown to be attenuated with the use of both mast cell stabilizers[7] and inhaled corticosteroids[8] which strengthen the belief that the mechanisms by which mannitol acts are dependent on inflammatory pathways within the airways. Although similar mechanistically, the mannitol challenge has been shown to cause dose-dependent bronchoconstrictive responses[9, 10] and thus exhibits important advantages over other indirect challenges from a safety perspective[11].

The methacholine challenge is currently the recommended method for evaluating airway hyperreactivity in North America[3, 4], meanwhile, the usefulness of mannitol challenges is being verified in Europe and Australia[11]. Recent studies have evaluated the relationship between the airway responses seen during methacholine and mannitol challenges, and there seems to be consistency in airway hyperreactivity between the two tests when using PC_{20}-value for the methacholine challenge and either a provocative dose yielding a 10% (PD_{10}; used among subjects on inhaled corticosteroid medication[12]) or a 15% (PD_{15}) reduction in FEV_1 as the cut-off values for a positive mannitol challenge[9, 13]. While bronchoconstriction following positive methacholine challenges seems to occur via pathways not directly associated with an acute increase in inflammation[14, 15], both urine and plasma markers of mast cell activation have been found to be elevated following inhalation of mannitol[16, 17]. Thus, the mannitol challenge may be the more appropriate method for evaluation of conditions associated with pulmonary inflammation.

2.1.1 Asthma control

The term asthma control is based on the clinical status of the person with asthma, and the risk of future exacerbations[18]. As the severity of the underlying disease varies, asthma control is not constant, and changes often occur in combination with a change in seasons, and/or a change in climate, or simultaneously with respiratory infections. Due to the variable nature of the disease, asthma control is recommended to be assessed over time (1 to 4 weeks) rather than at a single-point of assessment[18]. The Global Initiative for Asthma (GINA) classifies asthma based on level of control as: a) controlled, b) partly controlled, and c) uncontrolled asthma. Patients with controlled asthma are not limited in their everyday lives, and their lung function (defined as peak expiratory flow (PEF) or FEV_1) is considered normal when not experiencing an exacerbation. The probability of severe exacerbations in controlled asthma is low and daytime symptoms are infrequent. Patients with partly controlled asthma, as per GINA definition, experience any of the following: reduced lung function (PEF or FEV_1 < 80% predicted), asthmatic symptoms or need of reliever medication more than twice a week, or activity limitations. Uncontrolled asthma is defined as three or more features of partly controlled asthma per week, preferably over 4 weeks, and the risk of exacerbation is considerably higher than in controlled or partly controlled asthma[1]. The Asthma Control Questionnaire (ACQ) was developed to enable easy assessment and classification of asthma control and consists of 7 questions (ranked 0 – 6) which cover asthma symptoms, activity limitations, lung function, and the use of reliever medication, and are based on the 7 days leading up to the day of assessment[19]. Validation studies have shown that by using the ACQ, small differences in asthma control both between and within subjects can be detected, and the questionnaire can be used for identification of subjects with controlled (ACQ score <0.75), partly controlled (ACQ score 0.75 – 1.50), and uncontrolled asthma (ACQ score >1.50)[19, 20].

2.1.2 Asthma exacerbations

Asthma is a heterogeneous disease with a wide array of mediators; however, the clinical symptoms such as airway constriction and pulmonary inflammation are present independently of initial triggers during asthma exacerbations. The results from bronchial biopsies have shown that people with asthma have higher levels of inflammatory cells, such as eosinophils, granulocytes[21], mast cells[21], and macrophages, than non-asthmatics, and that the degree of inflammation correlates positively to disease severity[22]. The inflammatory profiles in asthma are not fully understood and it seems to vary between different phenotypes, which are generally divided as atopic and non-atopic (intrinsic) asthma. People with atopic asthma have high levels of immunoglobulin E (IgE) and cells expressing IgE-binding receptors[21]. The elevated inflammation seen in atopic asthma is thought to be driven by T2-helper (Th2) cells (referred to as Th2 asthma[23]) which through the release of interleukin (IL)-4, IL-5 and IL-13 regulate the production of antigen-binding IgE associated with eosinophilia[24]. In atopic asthma, an exacerbation starts when inhaled allergens (such as dust particles, animal fur, grass, certain food articles, and perfumes, among others) bind to inflammatory cells within the airways, leading to a release of pro-inflammatory mediators. The release of inflammatory mediators, such as histamine, leukotrienes, and interleukins, from stimulated inflammatory cells during an asthma exacerbation causes leukocyte tissue migration and constriction of bronchial smooth muscle cells, mucous secretion, and excessive vasodilation of the intra-pulmonary vasculature. The airflow obstruction is enhanced by leakage of plasma protein into the airways and compromised endothelial integrity with further build-up of airway mucous. Levels of oxidative stress, measured as plasma levels of the lipid peroxidation marker 8-isoprostane, have been shown to be elevated in plasma in asthmatics during exacerbations and may play a role in pulmonary tissue remodeling[25]. The mechanisms for non-atopic asthma is

predominately associated with the migration of neutrophil leukocytes to the lungs and elevated levels of tumor necrosis factor alpha (TNF-α), IL-8 and activation of toll-like receptors[26] and can be referred to as non-Th2 asthma[23]. Some data suggest that severe refractory asthma is associated with the non-atopic neutrophilic asthma phenotype[27-29], although other research underlines the fact that there seems to be a higher degree of complexity in the disease pathology and that there is substantial overlap in inflammatory features between phenotypes[30].

2.1.2.1 Assessment of airway inflammation

Several techniques have been developed to evaluate airway inflammation in people with asthma. While invasive techniques (*i.e.* bronchial biopsies, collection of bronchial lavage fluid, and sputum induction) enable direct measurement of the quantity of inflammatory cells and cell types, they are often perceived as uncomfortable for the patient and may therefore not be suitable for consecutive measurements. Non-invasive techniques such as the evaluation of exhaled nitric oxide (NO), and measurement of inflammatory markers in exhaled breath condensate and urine have been developed with the purpose of enabling lung disease patients and health care providers to more easily assess current respiratory status and disease progression[31, 32], and responsiveness to medication[33].

2.1.2.1.1 Non-invasive evaluation of airway inflammation

One of the more common ways to evaluate airway inflammation non-invasively is to measure NO in exhaled breath[34]. NO is a volatile gas which is produced by inflammatory cells within the airways and has been shown to be present in exhaled breath in higher amounts in asthmatics than

in non-asthmatics[35]. Exhaled NO correlates well with asthma control[31] and has been found to predict changes in asthma control better than common measures of lung function alone[32, 36]. The amount of NO in exhaled breath can be evaluated via single breath sampling or in series during which the patient breathes into the analyzer at a constant and pre-determined flow rate, often set to 0.05 L/second[34]. There are currently no established guidelines regarding normal ranges for NO measurements, however; cut-off values between 20 and 30 parts per billion (ppb) have previously been used in research studies to separate high and low exhaled NO-values[13, 36]. Although exhaled NO has been associated with asthma control[31] and prediction of future exacerbations[32, 36], several studies have failed to show differences in exhaled NO between stable and exacerbated asthma[37-39]. Connections have been made between levels of exhaled NO and the responsiveness to mannitol challenges[40] but only a limited number of studies have been done evaluating changes in exhaled NO directly following bronchial challenges; levels of NO declined following both mannitol[41, 42] and methacholine challenges[43], thus, although the level of exhaled NO seems to be a good indicator of asthma control, it may not be an appropriate evaluation method for measuring acute changes in pulmonary inflammatory levels.

The analysis of inflammatory markers in exhaled breath condensate (EBC) is another non-invasive tool used to measure airway inflammation in asthma; by letting the patient breathe into a cooled air chamber through a one-way valve, EBC can be produced in sufficient quantity in approximately 10 minutes for detection of several markers of airway inflammation[44], including C-reactive protein[39], pH-levels[45], and markers of oxidative stress[46, 47]. Several inflammatory markers from the airways can be detected in urine[16], although caution has to be taken due to potential systemic contamination. A recent study by Pelclova et al[48] evaluated inflammatory markers in plasma, urine, and EBC in subjects with asthma and in non-asthmatics before and after

methacholine challenges. At baseline, the EBC samples revealed elevated levels of markers of oxidative stress (8-iso-prostaglandin, leukotriene cysteinyl leukotriene and leukotriene C_4) in asthmatics compared to non-asthmatics. Elevated levels of oxidative stress could also be seen in plasma samples among the asthmatics, although there were no measurable differences in plasma markers of leukotriene activity. Following the methacholine challenge, the levels of 8-iso-prostaglandin declined in EBC, and plasma Cysteinyl leukotriene and pH decreased slightly but statistically significantly. Markers of leukotriene activity were also measured in urine, although no differences were observed between patient groups or after bronchial challenges with methacholine. To date, no data are available regarding the suitability of measuring changes in airway inflammation following mannitol challenges using EBC, however; it has been demonstrated that levels of urinary leukotriene C_4 and markers of mast cell activation increase following bronchial challenges with mannitol[16, 17] which is consistent with the theory that the responses to mannitol, as opposed to methacholine, are dependent on pulmonary inflammatory responses.

2.2 Cardiovascular disease in asthma

Asthma is primarily a pulmonary disease in which the aim of treatment is to reduce pulmonary inflammation, improve lung function, and reduce the risk of any asthma exacerbations. However, concerns about systemic complications in asthma have been raised in the last decade as asthma has been linked to increased rates of cardiovascular (CV) disease such as coronary heart disease, cerebrovascular disease, and heart failure[49-57]. The *odds ratios* for developing these kind of CV diseases if diagnosed with asthma have been reported to be between 1.43 and 2.66[49-52, 54], with the highest odds of developing CV disease being among women[50] and those with "active" asthma[52].

The largest study looking at CV risk in asthma to date was a longitudinal study of a cohort of over 400,000 asthmatics and age, gender, and ethnicity-matched non-asthmatics, published in 2012[54]. This study revealed that the odds ratio for adults with asthma were 1.45 for coronary heart disease, 2.14 for heart failure, and 3.28 for all-cause mortality, after adjustment for comorbid allergies, and the associations were stronger in women than in men. Similarly, the *hazard ratio* for developing CV disease among people with asthma is between 1.47 and 2.00[55, 57], and the *relative risk* of CV disease in asthma also reveals a gender difference (RR women vs. men; 2.5 vs. 1.4)[56]. Interestingly, studies in which asthma status has been based on self-reported asthma have failed to show increased rates of CV disease in asthma[55, 58], suggesting there is some discrepancy in CV disease prediction in asthma due to the inaccuracy of the asthma diagnosis. To date, the reason(s) for the increased prevalence of CV disease in asthma is unclear. While factors such as gender and allergies have been studied previously[54, 56], other factors, such as disease severity, medications[50, 59], and physical inactivity[56], have been suggested to potentially influence the risk of CV disease in asthma.

2.2.1 Systemic inflammation and cardiovascular risk

When examining CV comorbidities in asthma, connections have been made between the pulmonary and the systemic inflammatory response, suggesting that inflammation in asthma is not limited to the respiratory system[14, 27, 39, 60]. Systemic inflammation is characterized by elevated levels of pro-inflammatory mediators such as C-reactive protein (CRP), tumor necrosis factor-alpha (TNFα), and a shift in the balance between pro- and anti-inflammatory interleukins. Interleukin-6 (IL-6) is a pro-inflammatory cytokine which is in disease produced by activated immune cells, including alveolar macrophages[61], mast cells[62], and bronchial epithelial cells[62].

When present in the circulation, IL-6 stimulates hepatocytes to produce pro-inflammatory mediators, among them the acute phase protein CRP[63]. Clearance of CRP is unaffected by disease and plasma levels of CRP are therefore in direct proportion to CRP synthesis[64], which links IL-6 to the acute phase of the inflammatory response and high levels of CRP can be used as a clinical marker of acute phase systemic inflammation[65-67]. TNFα is a pro-inflammatory cytokine which plays an important role in the innate immune defense, but it has also been shown to be involved in systemic disease as an established predictor of CV disease[68, 69]. Markers of systemic inflammation have been shown to correspond well to risk of future CV events and mortality in diseased and elderly populations[70-72]; for example, high levels of both IL-6 (≥3.19 pg/mL) and CRP (≥2.78 mg/L) were related to increased risk of mortality in elderly people in a population-based study. This study also revealed an additional risk among those with high combined levels of both IL-6 and CRP[73]. In a study evaluating the risk of recurring myocardial infarction in stable heart patients, the people with the highest levels of circulating TNFα following the onset of myocardial infarction were reported to be at a 2.7-fold increased risk of having an additional event during a 5-year follow-up period[68]. Interestingly, the risk of CV disease among apparently healthy people at younger ages also correlate well with markers of systemic inflammation; asymptomatic men have been shown to be 2.3 times as likely to develop future myocardial infarction if they have high (>2.28 pg/mL) circulating levels of IL-6[74]. The same study demonstrated a 38% increased risk with increasing IL-6 quartiles among healthy people[74] and circulating levels of IL-6, TNF-α, and CRP have all been shown to be indicative of future cardiovascular events over a follow-up period of 3.6 years in healthy people[69]. Because of the strong connection between chronic systemic inflammation and CV risk, it has been suggested that inflammatory markers such as IL-6, TNF-α,

and CRP can be used to evaluate risk of CV disease in conditions associated with elevated inflammation[75].

2.2.2 Systemic inflammation in asthma

Circulating levels of IL-6, CRP, and TNFα are higher in asthmatics than in non-asthmatics[14, 39, 60] and serum levels of both IL-6[14] and CRP[25, 39] are higher in exacerbated or severe asthma than in stable or mild asthma. CRP is also elevated in condensed exhaled air of asthmatics[39] and remains elevated up to 24 hours following intense exercise in subjects with allergic asthma[76]. Furthermore, serum levels of CRP positively correlate to asthma disease severity[39, 77] which indicates that there is a connection between the degree of pulmonary inflammation and acute systemic inflammation. The peak in CRP seen during asthma exacerbations declines as the patient regains control of the disease[25], and some studies examining CRP levels in stable asthma have reported that there were no differences between asthmatics and healthy controls[60, 77, 78].

Levels of circulating IL-6 are elevated in stable asthma[14, 60] and increase during naturally occurring asthma exacerbations[14] and following allergen challenges[14]. Interestingly, methacholine-induced bronchoconstriction does not result in increased serum levels of IL-6[14]. This suggests that IL-6 is chronically elevated in stable asthma and that there is excessive release of IL-6 in exacerbated asthma, but the mechanism for this appears to be independent of airway hyperresponsiveness. Adults with stable neutrophilic asthma, defined as no exacerbation within 4 weeks and sputum neutrophils ≥64 % of total cell count, have elevated circulating levels of both IL-6 and IL-8-associated cytokines compared to both non-asthmatic controls and subjects with non-neutrophilic asthma[27], suggesting that asthma phenotypes affect the systemic inflammatory

profile in asthmatic adults. The same study also showed that the prevalence of CV comorbidities was higher in neutrophilic asthma; again, highlighting that systemic inflammation in asthma may be linked to unfavorable CV manifestations. CRP has been shown to be elevated in neutrophilic asymptomatic asthma, but not in non-neutrophilic asymptomatic asthma compared to healthy controls[27].

While clinical studies have shown that TNF-α is present in sputum of asthmatics patients[79], several studies have investigated levels of circulating TNF-α in stable asthma, but the results are variable. Serum levels of TNF-α were reported to be elevated in patients with stable asthma compared to both stable COPD and controls without lung disease[60]. However, no differences were observed between either neutrophilic stable asthma, non-neutrophilic stable asthma, and controls[27], or between adult or pediatric asthma and controls[80]. Berry and colleagues[81] showed that levels of membrane-bound TNF-α, TNF-α receptor-1, and its converting enzyme were all found to be up-regulated in blood from subjects with severe refractory asthma. After 10 weeks of anti-TNF-α treatment, Berry et al. could also report a reduced airway hyperresponsiveness (increased minimum dose of methacholine required to reach PC_{20}) and improved FEV_1 post-bronchodilator in people with severe asthma[81]. The results from this study are supported by previous studies assessing levels of TNF-α in people with uncontrolled asthma[82], and the up-regulation of this pro-inflammatory cytokine in severe asthma could play a potential role in the pathogenesis of asthma comorbidities.

Although elevated levels of both pulmonary and systemic inflammation can be observed in asthma[14, 83], the connection between pulmonary and systemic inflammation is still not completely understood. In summary, people with asthma are at increased risk of developing CV

disease[49-57]. Systemic inflammation is an established marker of future risk of CV disease[67, 68, 74] and levels of circulatory pro-inflammatory cytokines have been shown to be increased in asthma, among them IL-6[14, 27], TNF-α[60, 81, 82], and the acute phase protein CRP[27, 39, 77]. While both TNF-α and CRP are increased in severe asthma[39, 81], TNF-α has also been shown to be directly implicated in bronchial hyperresponsiveness[81]. IL-6 is elevated in neutrophilic asthma[27], during naturally occurring symptomatic asthma[14], and in response to allergen challenges[14], suggesting there may be several mechanisms leading to an increased release of IL-6 in asthma, but that it is separate from bronchoconstriction induced by methacholine. Proposed mechanisms which could assist in explaining the increase in systemic inflammation in asthma and following an asthma exacerbation are translocation of pulmonary inflammation into the systemic circulation[84], activation of immune cells potentially due to viral infections, pollutions and smoking, or immune cells in asthma may be chronically sensitized whereby they would produce inflammatory mediators in a disproportionate manner. Several studies have shown that the degree of systemic inflammation is in direct relation to CV morbidity[68, 69, 74], but little is known regarding systemic inflammation and CV health in stable and/or exacerbated asthma.

2.2.3 Vascular function

In health, the vascular system allows for distribution of oxygen and nutrients to tissues, and removal of waste products from our bodies. Changes in vascular tone are firmly regulated to allow for optimal flow distribution throughout the body and local regulation of blood flow is highly dependent on endothelial secretion of vasoactive substances. Nitric oxide (NO) is the main local vasodilator and is produced through the reaction between L-arginine and endothelial nitric oxide

synthase (eNOS) in endothelial cells. The production of NO can be stimulated by several triggers, common ones being sheer stress, acetylcholine, and bradykinin. Once released by the endothelium, NO diffuses into the smooth muscle-rich tunica media in conducting vessels, where it reduces intracellular Ca^{2+} through the interaction with soluble guanylate cyclase, and thereby causes vasorelaxation. NO can also be produced from nNOS (neural) and iNOS (inducible). eNOS and nNOS are both important for a well-functioning endothelium; however, increased iNOS activity has been linked to the production of reactive oxygen species (ROS), oxidative stress, and vascular damage[85]. Other important factors for vascular tone and blood distribution are the sympathetic and parasympathetic nervous systems, the renin-angiotensin system, and numerous vasoactive substances which are orchestrated by these systems.

2.2.3.1 Endothelial function

NO-dependent endothelial dysfunction of the larger conducting arteries can be used as an indicator of more wide-spread vascular disease[86] and has been shown to occur in concert with the prevalence of other established risk factors for CV disease[87]. For research purposes, several pharmacological interventions have been conducted to assess endothelial dysfunction, such as intra-arterial infusion of acetylcholine, bradykinin, or epinephrine. Flow mediated dilation (FMD) of the brachial artery is a method which has been shown to accurately predict NO-mediated endothelial function[88] and has become the standard for assessment of vascular function[89-91]. FMD has the advantage over other mentioned methods of estimating endothelial function since it is a non-invasive technique with few contraindications. Blood flow is the main physiological trigger for vasodilation, and normal endothelial response to increased blood flow is crucial for proper vascular function, which

further enhances the importance of FMD testing. FMD is generally measured as the peak dilatory response of the artery in response to an increase in sheer stress (flow perpendicular to the vessel wall) due to reactive hyperemia (RH), and is commonly expressed as the percent change in diameter from baseline[91, 92]. Reduced FMD in asymptomatic subjects have in larger studies been shown be present together with established risk factors of CV disease[87], and is associated with future risk of CV disease[93], and the time to peak dilation has been shown to correlate well with signs of atherosclerosis[94]. Impairment of the endothelium in the larger arteries has also been demonstrated in people at high risk of CV disease[95] and in people with previously diagnosed CV disease[86], and meta-analyses looking at the predictive value of FMD found that the risk of CV events decrease by 7-13 % with every 1% increase in FMD[89, 96]. However, a few studies have reported that other vascular parameters may predict CV risk better than the traditional FMD measurement; Philpott et al.[97] found that the both shear stress due to reactive hyperemia (SSRH) and peak blood velocity during reactive hyperemia (VRH) show stronger associations with CV risk than FMD expressed as percent change from baseline diameter, and argue that by reporting these parameters rather than reporting FMD alone, the development of future CV disease can be detected earlier[97]. In addition, since shear stress is an important determinant of FMD[98] magnitude, it has been recommended that FMD should be normalized to the shear stress stimulus for a more accurate estimation of endothelial function, and FMD/SSRH is therefore considered a more appropriate FMD outcome[99, 100].

In summary, impaired vascular function has been seen in association with other established risk factors of CV disease and has therefore been suggested to be an early indicator of future CV risk[87, 93, 94, 101, 102]. However, it is recommended that the % baseline FMD responses be normalized to the hyperemic stimulus for a more accurate estimate of endothelial function[99, 100].

2.2.3.2 Systemic inflammation & endothelial injury

As previously mentioned, markers of systemic inflammation can be used as predictors of future risk of CV disease in both presumably healthy[69, 74] and diseased populations[65, 67, 68]. The presence of chronically elevated levels of systemic inflammation is thought to be closely linked to the development and progression of atherosclerosis[103] which directly links inflammation to endothelial dysfunction and CV disease. In addition, inflammation is an important mediator that when present in the systemic circulation has been shown to negatively affect endothelial integrity and thus its function; high levels of CRP have been shown to reduce bioavailable NO by suppressing *enos* expression and reduce endothelial nitric oxide synthase activity[104], and thereby impair endothelium-dependent vasodilation. CRP can also promote endothelial cell adhesion by up-regulating the expression of cell adhesion markers[105], allowing for increased leukocyte migration and consequently elevated oxidative activity. TNF-α has been shown to be important in vascular disease pathogenesis; TNF-α can reduce bioavailable NO[106] and limit endothelium-dependent vasodilation in both murine models[107] and in humans[108], which has been linked to its ability to increase oxidative stress by activation of iNOS increased levels of superoxide[109]. TNF-α can also up-regulate the secretion of other pro-inflammatory cytokines[110] and cell adhesion markers[111], and induce cellular apoptosis[112]. Aged rats have been shown to express naturally elevated levels of TNF-α, accompanied by adverse endothelial dysfunction (shown as significantly reduced vasodilatory capacity in the presence of acetylcholine, as compared to younger animals without TNF-α elevation). Anti-TNF-α treatment eliminates the vascular dysfunction seen in older rats, decreases levels of oxidative stress in the vessel wall, and reduces the up-regulated expression of iNOS and cell adhesion molecules[109].

The connection between systemic inflammation and endothelial dysfunction has previously been investigated experimentally in healthy humans. In 2000, Hingorani et al.[113] compared the vascular dilatory response to pharmacologically and RH-induced vasodilation and before and after *Salmonella typhi* vaccine was administered to healthy volunteers. Although the vaccination only resulted in a weak increase in systemic inflammation (elevated serum levels of IL-6 and IL-1Ra), there was a detectable reduction in FMD following RH, and impaired pharmacologically induced endothelium-dependent vasodilation compared to baseline values before vaccination. The dilatory reaction to endothelium-independent vasodilators did not change following vaccination, indicating that impaired vascular function in systemic inflammation-associated conditions is indeed due to endothelial impairment.

Arterial stiffness and how arteries respond to pressure differences have also used for the assessment of vascular function and future cardiovascular risk[114]. The gold standard method for evaluating arterial stiffness is to measure the velocity of the pulse wave (PWV) between the common carotid artery and the femoral artery[115] and this has been shown to be an independent predictor of future risk of both coronary artery disease and stroke in people without known disease[114]. In a meta-analysis, an increase in aortic PWV of 1m/s was shown to correspond to a 15% increased risk of CV disease, with the risk of CV events linear between tertiles[116]. Similarly to the study performed by Hingorani et al., in 2000, Vlachopoulos et al.[117] assessed how inflammatory responses to *Salmonella typhi* vaccinations affect different markers of arterial stiffness in healthy people and found a causal relationship between acute increases in systemic inflammation (CRP increased by 0.16 mg/L at eight hours post vaccination) and increased aortic PWV (PWV increased by 0.43 m/s at eight hours post-vaccination), which according to the meta-analysis corresponds to an increase in CV risk of approximately 7%[116]. Together these results

would suggest that acute inflammatory episodes, such as those that would be seen with an asthma exacerbation, may cause impaired vascular function secondary to increased systemic inflammation.

2.2.4 Vascular dysfunction in asthma

The relaxation of the aorta in response to acetylcholine has previously been evaluated and shown to be reduced in animal models of allergic asthma[118] and following pulmonary exposure to particulate matter[84]. Importantly, the studies demonstrated increased airway reactivity and inflammation, with the increase in systemic inflammation associated with the degree of aortic impairment. To date, little is known regarding vascular function in people with asthma. Only one study has examined endothelial function in asthma[119], in which forty-nine subjects with mild and moderate asthma (mean age 33 ± 12 years) and age-matched healthy underwent brachial ultrasonography and lung function testing. FMD was measured 60 seconds following five minutes of forearm ischemia and was expressed as percent change in diameter compared to baseline. At baseline, there was no difference in brachial artery diameter between groups. Following five minutes of forearm ischemia, the endothelium-dependent vasodilatation in asthmatics was significantly lower than in controls, and subjects with moderate asthma had further impairment in endothelial function compared to subjects with mild asthma. The degree of change in brachial diameter following transient ischemia was also correlated to FEV_1, forced vital capacity (FVC), and PEF in asthmatics subjects, suggesting that there is a relationship between endothelial dysfunction and reduced lung function. No data on systemic or pulmonary inflammatory profiles where reported in this study[119].

Arterial stiffness has been evaluated previously in asthma, but with inconsistent results. In a study with 85 severe asthmatics, 85 stable asthmatics, and 85 non-asthmatic controls matched for age (40 ± 4 years), gender, BMI, and smoking status, the brachial-ankle (ba) PWV was significantly higher in the asthmatics than in the controls, and the highest arterial stiffness could be seen in people with severe asthma. The same study also reported a strong relationship between elevated baPWV and reduced FEV_1 and FEV_1/FVC which remained significant after adjusting for age, gender, BMI and smoking status[120]. Measures of arterial stiffness have also been associated with lung function in younger asthmatics (age 19-39), although this group did not demonstrate higher overall arterial stiffness compared to controls[121]. The differences in findings could potentially be explained by differences in age or be due to dissimilar methodology for evaluating arterial stiffness.

In summary, both systemic inflammation and vascular dysfunction are predictors of future risk of CV disease[68, 69, 74, 86, 87, 122]. There is an established relationship between systemic inflammation and reduced vascular health[84, 106-108]; however the connection between systemic inflammation and vascular dysfunction in asthma is unknown. Both systemic inflammatory levels and the degree of vascular impairment have been shown to be associated with disease severity in asthma[14, 39, 119-121]. Acute increases in systemic inflammation reduces vascular function in otherwise healthy people[113, 117], and although there are animal studies indicating that acute pulmonary inflammation does indeed impact the cardiovascular system[84, 118], little is known regarding the CV consequences of an acute asthma exacerbation.

2.3 Physical activity and asthma

Physical activity is well-known for being beneficial for CV health in the general population[123, 124] and many disease conditions[124, 125]. The relationship between physical activity and CV risk in the general population is inversely linear, and is suggesting an approximate relative risk of 0.8 for CV events for those in the 50th percentile of physical activity, compared to the most inactive part of the population[123]. Increasing physical activity levels from low to moderate (defined as activities at an intensity of 4.5 METs) has been seen to independently improve the CV risk outcome by 23% among healthy men[126]. Cardiopulmonary fitness is closely related to physical activity and can be measured through cardiopulmonary exercise testing[127]. Improved cardiopulmonary fitness has been shown to be one of the most efficient ways to improve CV health in previously sedentary populations[128] and has been suggested to be a stronger indicator of CV risk than physical activity alone[123]. Canadian physical activity guidelines for adults recommends a minimum of 150 minutes of moderate to vigorous physical activity and two sessions of muscle and bone strengthening exercise per week[129]. However, adherence to the recommendations is low[130, 131]; Statistics Canada report that as little as 15.4% of adult Canadians meet the weekly recommendations for physical activity, and only about a third has a daily step count over 10.000 steps[131]. Furthermore, even after adjusting for age and body mass index, people with asthma are significantly less active than non-asthmatics[132, 133] and when they do perform physical activity, it is less vigorous than the physical activity of their age-matched counterparts[132, 133]. Low physical activity levels may thus contribute to added CV risk in asthma, and the reason(s) for physical activity avoidance in asthma requires investigation.

2.3.1 Physical activity and vascular health

A potential mechanism by which reduced physical activity and fitness levels contribute to CV disease risk in asthma is the negative impact inactivity has on vascular function[134] and systemic inflammation[135]. Multiple studies have been conducted in order to assess potential immune-modulating effects of physical activity, cardiopulmonary fitness, and exercise. While the *acute effect* of physical activity, depending on intensity and duration, may allow for both beneficial temporary changes in immune-responses[136] and an increased short-term susceptibility to infections, the *long-term effect* of physical activity is associated with overall fewer signs of systemic stress[137] and a reduced number of reported sick-days[138]. Physical activity is often evaluated using self-reporting questionnaires or activity monitors and has in several studies been shown to correlate well to markers of systemic inflammation, among them CRP, TNF-α, and IL-6[135]. To evaluate how physical activity influences chronic circulating levels of IL-6, and subsequently CRP, Hamer et al[135]. examined levels of physical activity and cytokine profiles from a large cohort including both men and women from the general population. The results showed that circulating levels of IL-6 and CRP were inversely correlated to physical activity levels, and follow-up measurements 10 years later revealed that subjects who had remained active had lower levels of systemic inflammation than those who had adapted a more sedentary lifestyle[135]. Similar results were reported from a cross-sectional study on CRP levels in middle-aged men stratified based on self-reported leisure-time physical activity. Circulating levels of CRP were significantly lower among both subjects who performed moderate and vigorous physical activity than in sedentary subjects[139], suggesting there is an association between CRP-mediated chronic inflammation and inactivity. Moderate intensity physical activity has been shown to improve the inflammatory profile in apparently healthy people, especially among those with high baseline

levels of system inflammation (CRP > 3mg/l at baseline)[140,141]. In high-risk populations, including people with coronary heart disease[142] and atherosclerosis[143], CRP has also been shown to decrease with increased physical activity.

Endothelial dysfunction is, as previously mentioned, a common feature of the vascular dysfunction frequently seen in association with increased risk of CV disease[87, 97, 101]. Both regular physical activity[143, 144] and higher levels of cardio-pulmonary fitness[145, 146] have been shown to be important factors for a healthy endothelium, which could potentially be attributed to lower levels of systemic inflammation and improved NO-bioavailability. In young healthy men, cardio-pulmonary fitness is strongly correlated to FMD[145], and healthy FMD responses are maintained in aging in physically fit people[146]. Low exercise tolerance, defined as low six minutes walking distance, has also been shown to be a strong predictor of endothelial dysfunction in chronic lung disease[147]. Importantly, endothelial function can be improved with increased physical activity, both in healthy[144], and diseased populations[143].

While both human[148, 149] and animal[150, 151] work show that exercise training at a moderate level can reduce airway inflammation[150, 151], there is currently no consensus on whether physical activity and fitness have substantial systemic anti-inflammatory and beneficial vascular effect in asthma, and whether physical inactivity and low fitness levels contribute to CV risk in asthma.

2.3.2 Exertional dyspnea in asthma

The reduced physical activity levels in asthma has been suggested to be due to the fear of physical activity-associated exacerbations[152]. However, with proper disease management, the risk of severe asthma exacerbations due to physical activity is minimal[153]. Aerobic training in asthma has been

associated with both reduced pulmonary inflammation[149], improved asthma control[148, 149], and enhanced quality of life[148]. Asthmatics do however report greater perceived exertional dyspnea[152, 154], which cannot be explained by traditional ventilatory parameters such as increased ventilation (V_E), breathing frequency (f_R), ventilatory equivalent for carbon dioxide, or breathing reserve (V_E/maximal voluntary ventilation)[155, 156]. During methacholine-induced bronchoconstriction at rest, changes in inspiratory capacity (IC) account for 74% of the variation in dyspnea (measured on the modified Borg scale), and most asthmatics report inspiratory difficulty, inability to take deep breaths, "unrewarded" inspiration, and enhanced work of breathing to be the main sensations, rather than expiratory difficulties[157]. One explanation is that the acute increase in airway resistance seen with a reduced FEV_1 during bronchoconstriction makes tidal expiratory breathing time to be insufficient, which contributes to dynamic hyperinflation, seen as a reduction in IC. This compromises the inspiratory muscle pressure generation, which likely contributes to the sensation of insufficient ability to inhale rather than exhale during bronchoconstriction at rest[157]. While there is no relationship between dyspnea, PC_{20} and reductions in FEV_1 present at rest[157], significant dynamic hyperinflation (>0.30L reduction in IC compared to baseline) can be seen even during mild bronchoconstriction (FEV_1 reduced by 20%) at rest[158]. Additionally, IC has been shown to be reduced by as much as 0.70L during peak exercise in asthma[159]. In other lung diseases such as chronic obstructive pulmonary disease, dynamic hyperinflation manifesting as an inspiratory reserve volume (IRV) <0.5L or below 10% of total lung capacity (TLC) has been shown to be of clinical importance for dyspnea perception[160]. Furthermore, asthmatics performing constant workload exercise who reach a V_T/V_E inflection point at an IRV of approximately 0.5L or below 10% of TLC have significantly higher Borg-dyspnea scores than those who have an IRV within normal ranges [159]. It is thus likely that dynamic hyperinflation seen as an upward shift in operating

lung volumes substantially contributes to exertional dyspnea in asthma. To further understand the physiology behind the increased exertional dyspnea in asthma, and to reduce physical activity avoidance, this area of research needs investigation.

2.4 Asthma medications and CV risk

To manage asthma symptoms, depending on severity, control, and asthma phenotype, asthmatics are prescribed a variety of different medications to suppress airway inflammation and dilation of the airways[1]. Short-acting asthma medications binding to the beta-2(β_2)-adrenoceptors were first introduced in the early 1900s. Today, short-acting β_2-agonists (SABAs) are one of the mainstream treatment steps for asthma management in the western world[1]. Long-acting β_2-agonists (LABA) taken on their own has been associated with increased serious adverse asthma events[161], and thus today are only prescribed as an add-on medication together with another concurrent anti-inflammatory controller medication[1]. The definite structure of be β_2-agonists are not completely known, but LABAs and SABAs are believed to have identical active domains binding to the adrenoceptors, however, the LABA-molecules have a longer tail which attach to binding sites near the receptor and allows them to work over longer periods of time. Once attached to the β_2-adrenoceptor on the bronchial smooth muscle, there is an influx of intracellular potassium as well as activation of cyclic monophosphate with a subsequent reduction of intracellular calcium, together causing smooth muscle relaxation, and thus bronchodilation. There are three types of β-receptors; β_1, β_2, and β_3. The β_1-adrenoceptor can be found predominantly in tissues such as the heart, whereas β_2-adrenoceptors are more wide-spread and can be found in smooth and striated muscle tissues throughout the body; importantly, the β_1-β_2-adrenoceptor ratio is 4:1 in the heart[162].

The β_3-adrenoceptors can be found predominantly in adipose tissue. To minimize side-effects, the β_2-agonists used for asthma management have purposely been designed to have high selectivity for the β_2-adrenoceptor only[163].

The vast majority of studies evaluating CV disease risk in asthma have found an increased risk of heart disease of approximately 40-80% after adjusting for traditional risk factors such as age, body mass index, and smoking status[49, 51, 52, 54-57]. To further investigate the reason for CV disease risk in obstructive diseases such as asthma, in 2002 Au et al.,[59] conducted a study evaluating β_2-agonist usage prior to hospitalizations for acute coronary syndrome (myocardial infarction or unstable angina). The authors found that filled prescriptions for medications containing β_2-agonists over the three months leading up to the hospitalization was in a dose-dependent manner (1-2 canisters OR:1.38; 3-5 canisters OR: 1.58; and >6 canisters OR: 1.93) associated with acute coronary syndrome. This relationship remained significant even after adjusting for CV disease, age, hypertension, diabetes, smoking status, and pack-years. Following this study, an additional three epidemiological studies have used β_2-agonists usage as a parameter to adjust CV disease risk in asthma. Two of the studies did not find β_2-agonists to affect the increased CV risk[51, 54]; however, in 2009 a study by Appleton et al.,[50] found β_2-agonist usage to be a determinant for CV disease, especially in women where the odds ratio for CV disease among asthmatics regularly using β_2-agonists was as high as 2.66.

2.4.1 β_2-agonists and CV function

Due to the abundance of extrapulmonary β_2-adrenoceptors and the small, but possible, capacity of β_2-agonists to also act on β_1-adrenoceptors, the systemic CV effects of short-acting β_2-agonists

have been extensively evaluated. The first studies evaluating the systemic effects of salbutamol specifically found it to increase both heart rate and blood pressure in healthy people, but only at high doses (800 mcg). This high dose of salbutamol also increases cardiac QT intervals and plasma potassium levels[164]. Later studies have found that salbutamol affects measures of sympathetic and parasympathetic autonomic control in healthy individuals, without concurrent changes in blood pressure, suggesting unchanged baroreflex activity[165, 166]. The elevating effects of short-acting β_2-agonists on sympathetic outflow in healthy people have been confirmed by others who found nebulized albuterol to increase plasma norepinephrine levels in the absence of blood pressure changes[167].

The first study to directly evaluate muscle sympathetic nerve activity (MSNA) following salbutamol administration was done by Beloka et al., in 2011[168]. Conversely to what has previously been seen, they found that intravenous injections of low (10 mcg) and high (20 mcg) doses of salbutamol increased heart rate, blood pressure, and peripheral chemosensitivity, but it did not change MSNA burst frequency. Around then same time, the radial argumentation index (AI) was evaluated as a measure of arterial stiffness following 400mcg salbutamol inhalation in healthy people. Indeed, as systemic vascular resistance declined, radial AI was also reduced, assuming relaxation of the artery. Similar to previous studies, these two studies found salbutamol to increase heart rate and cardiac output[169, 170].

As the results from the previous studies are contradictory, we recently conducted a study in healthy people where we evaluated the impact of 400 mcg inhaled salbutamol on MSNA and central and peripheral pulse wave velocity[171] as measures of arterial stiffness and future risk of CV disease[114]. Similar to previous studies, we found salbutamol to cause a moderate but statistically

significant elevation in heart rate, but without simultaneously affecting blood pressure. In our sample, salbutamol also increased total MSNA by approximately 23%, but this elevation in MSNA did not translate to acute increases in arterial stiffness. It was thus concluded that salbutamol does indeed affect sympathetic outflow acutely. Even though we did not see this leading to increased arterial stiffness in healthy individuals, it is plausible that regular salbutamol usage causes long-lasting elevations in MSNA, and/or changes in vascular responses to increased MSNA due to modulations of the β_2-adrenoceptor, which would both affect the structure and function of the systemic blood vessels[172] and hence CV risk.

While the CV effects of SABA has been extensively studied in healthy people, very little work has been done among people with asthma. Repeated β_2-agonist usage lead to reduced bronchodilator responses with the same drug. This was first proposed during the 1980s and 1990s when an upswing in asthma mortality was observed[173, 174]. Today, it is suggested that the resistance to β_2-agonists stemming from repeated regular usage is caused by a desensitization as well as a reduction in the density of β_2-adrenoceptors[175, 176]. It is now known that daily salbutamol usage (200 mcg/day) leads to significantly reduced bronchoprotection to methacholine-induced bronchoconstriction after only four days of usage[177], but it is unknown how the systemic vascular β_2-adrenoceptors change their sensitivity to inhaled doses of salbutamol in asthmatics who use salbutamol regularly. To date, only two studies evaluating CV effects of inhaled salbutamol have been conducted, both utilizing doses larger than what is commonly recommended for regular asthma management[1], and neither having a healthy control group for comparison. Burggraaf et al., found that salbutamol inhaled during hypoxic conditions (SpO_2=82%) caused a substantial decrease in peripheral vascular resistance[178], which downstream could result in reduced venous return to the heart, and thus explain CV risk during asthma exacerbations. The other study

evaluating CV effects of salbutamol in asthma found those with the Gly16-Glu27 versus the Arg16-Gln27 polymorphism to have higher cardiac plasma potassium levels following salbutamol inhalation, and would thus be at higher CV risk[179]. The same mutation has been shown to be implicated in reduced bronchodilator responses to β_2-agonists[180].

In summary, the usage of inhaled short-acting β_2-agonists has been suggested to play a major role in CV disease risk in asthma. Experimental studies evaluating the CV impact of salbutamol, the most commonly prescribed SABA today, have found it to increase sympathetic outflow, seen both as increased heart rate, norepinephrine levels[167], and plasma potassium levels[164], and total MSNA[171]. The absence of simultaneous changes in blood pressure indicates that salbutamol does not activate the baroreflex. While the increased sympathetic outflow appears to be transient, it does not cause an acute increase in arterial stiffness among healthy people. While there is evidence of changes to the sensitivity and density of β_2-adrenoceptor in the lungs caused by repeated β_2-agonist usage[176], it is currently unknown if similar changes can be seen on the β_2-adrenoceptors in the systemic vasculature, and if this has a downstream effect on CV risk among those asthmatics who use β_2-agonists regularly.

2.5 Summary

People with asthma are at increased risk of developing CV diseases such as coronary heart disease, cerebrovascular disease, and heart failure[54, 56]; however the cause(s) remain unknown. Endothelial dysfunction, FMD[86, 92, 97], is an early marker of CV disease[94, 97, 102, 181] and endothelial function has been shown to be reduced in asthmatics[119]. Systemic inflammation is a pathological condition present in many disorders associated with CV risk[69, 71, 74, 75, 182], including asthma[14, 27, 39, 82], and is

involved in vascular impairment [106, 108, 109, 113]. However, whether systemic inflammation is related to vascular dysfunction in asthmatics is unknown. People with asthma are generally less physically active than non-asthmatics[152] and physical inactivity is associated with increased systemic inflammation[135] and impaired vascular function[134]. Increased cardiopulmonary fitness and physical activity have been shown to reduce signs of systemic inflammation[136, 137, 142] and vascular dysfunction[143, 144] but the influence of physical activity and cardiopulmonary fitness on systemic inflammation and vascular function have not been investigated in asthma. Intensified dyspnea has been identified as one of the main barriers to physical activity in asthma[152, 154], but the physiological reason(s) for this is unknown. Thus, the purpose of Chapter III was to examine if there is a relationship between systemic inflammation and endothelial dysfunction in asthma and to evaluate if the reducted endothelial funtion[119] and arterial stiffness[120, 121] previously demonstated in asthmatics are dependent on cardiopulmonary fitness and/or physical activity levels. In Chapter IV, the physiological mechanisms as to why people with asthma experience increased exertional dyspnea were evaluated.

During asthma exacerbations there is an increase in pulmonary inflammation[14, 25, 39], which in animal studies have been shown to translate to systemic inflammation[84] and negatively affect the dilatory responses in larger arteries[84, 118]. It has previously been reported that acute systemic inflammation in otherwise healthy humans attenuates vascular function[113] and elevates arterial stiffness[117]. Furthermore, although vascular function correlates well with disease severity in asthma[119-121], and respiratory exacerbations increases CV risk[183], little is known regarding the direct influence of asthma exacerbations on vascular health. Research also suggests some involvement between medications taken to relieve asthma symptoms and increased CV risk[50, 59]. Thus, the purpose of Chapter V and Chapter VI was to evaluate how increased pulmonary

inflammation and bronchoconstriction affects the CV system in asthma, and if there are additional impact on CV risk following the use of the β_2-agonist salbutamol.

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3 CHAPTER III

The role of fitness and physical activity for vascular health in asthma

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3.1 Introduction

Asthma is associated with important systemic consequences such as an increased risk of cardiovascular (CV) diseases such as coronary heart disease, cerebrovascular disease, and heart failure[1-3]. While the reasons for the increased CV risk in asthma are unknown, increased systemic inflammation associated with asthma may play a role. In particular, high levels of the pro-inflammatory cytokines interleukin-6 (IL-6)[4-6], C-reactive protein (CRP)[5, 7, 8], and tumor necrosis factor-alpha (TNFα)[4, 9, 10] are elevated in asthma, and all of these markers are associated with increased risk of CV disease[11-17].

Reduced endothelial vasodilation has been seen in early stages of CV development and is incrementally predictive of CV risk beyond traditional markers of CV risk alone[18-21]. Endothelial function is commonly defined as the percent increase in brachial artery diameter in response to reactive hyperemia[22]. In addition, the changes in blood velocity following reactive hyperemia and the stiffness of arteries have both been shown to be indicators of CV risk in asymptomatic populations[23-25]. Importantly, in addition to increased systemic inflammation, asthmatics have reduced endothelial function and increased arterial stiffness compared to non-asthmatics[26, 27]. High levels of systemic inflammation impair endothelial function and increase arterial stiffness in otherwise healthy populations[28-30], but it is unknown if there is a connection between systemic inflammation and vascular function and stiffness in subjects with asthma.

Both low aerobic fitness and physical inactivity are established risk factors for overall CV morbidity and mortality[31-33], and are directly implicated in both elevated systemic inflammation[34] and impaired endothelial function[35]. Furthermore, increased physical activity is associated with improved aerobic fitness and exercise training has been shown to reduce systemic inflammation[36, 37] as well as improve endothelial function[38, 39] in both healthy people and in populations at high

risk of CV disease. Exercise training has also been shown to reduce pulmonary inflammation in asthma[40, 41], suggesting that there are additional beneficial anti-inflammatory effects associated with fitness and physical activity for asthmatics.

To date, little is known in regard to what degree the predisposition to CV risk in asthma is due to low aerobic fitness and physical activity levels versus the disease itself. People with asthma are generally more inactive than their age-matched counterparts[42], and based on previous work this would independently impair vascular function[35] and increase CV risk[33]. Thus, the main purpose of this study was to evaluate parameters of vascular function and systemic inflammation in asthmatics and non-asthmatics who were matched for physical activity and aerobic fitness. We hypothesized that asthma would be associated with both higher markers of systemic inflammation, impaired endothelial function, and increased arterial stiffness than in controls matched for physical activity and aerobic fitness.

3.2 Methods

3.2.1 Subjects

Asthmatic men and women (n= 16) between the ages of 18 and 45 years old and with a body mass index (BMI) of ≤ 30 kg/m^2 were recruited from the University of Alberta Asthma Clinic and The Lung Health Clinic, Edmonton, Alberta, Canada. Asthma diagnosis was based on previous history of asthma symptoms, and either: 1) $\geq$ 12% and 200 mL improvement in FEV_1 post bronchodilator[43, 44], 2) a positive methacholine challenge ($PC_{20} \leq 4$ mg/mL[44]), or 3) $\geq$ 10% decrease in FEV_1 following an exercise challenge[44, 45]. Asthma control was evaluated according to

the Asthma Control Questionnaire[46, 47] at the time of the first visit and only controlled and partly controlled asthmatics were included. Age, BMI, and physical activity/fitness-matched control subjects (n=16) without asthma (as demonstrated by negative responses to all three tests described above) were recruited from the general population. Subjects were excluded if demonstrating the following cardiovascular risk factors: hypertension defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90[48], a family history of sudden cardiac death or known coronary heart disease, elevated cholesterol levels (measured with CardioCheck capillary blood analyzer)[49, 50], diagnosed diabetes or undiagnosed pre-diabetes, smoking history (current smokers or persons who quit smoking within less than 6 months were excluded)[48], or one or more reason(s) to withhold from physical activity according to the Par-Q and You Questionnaire[49]. As coexisting allergies do not increase the risk of CV disease in asthma [1] but may influence the systemic inflammatory profile[5, 7], both atopic and non-atopic subjects were recruited and previously known allergies noted. Exhaled nitric oxide (FeNO; NIOX MINO, Aerocrine Inc., Solna, Sweden) was measured to evaluate pulmonary inflammation, and risk of obstructive sleep apnea was defined as having ≥ 3 occurring risk factors on the STOP-bang questionnaire[51, 52]. Nine of the asthmatics were using short-acting beta-agonists, three were using inhaled corticosteroids (ICS), and seven were using combined ICS and long-acting beta-agonists. Short-acting and long-acting medications were withheld for a minimum of eight and forty-eight hours, respectively, prior to the tests.

This study (protocol number Pro00029773) was approved by the University of Alberta Health Research Ethics Board. Written consent was obtained from all subjects prior to participation in the study.

3.2.2 Measurements

3.2.2.1 Aerobic fitness

A graded cardiopulmonary exercise test was performed to determine the rate of oxygen consumption at maximal exercise (VO_{2max})[53]. The test was performed on a cycle ergometer (Ergoselect 200, Ergoline, Bitz, Germany) at incremental stages of 25 watts increase/2 minute until the ventilatory threshold was reached, and then in steps of 25 watts/minute until exhaustion. Breath to breath analysis of pulmonary gases was performed throughout the test using the Vmax Metabolic Cart (CareFusion, Yorba Linda, CA, USA). The subjects were asked to rate the level of perceived exertion at two-minute intervals during the test, expressed on the modified Borg scale from 1 (nothing at all) to 10 (maximal). VO_{2max} was confirmed if the following criteria were met: 1) plateau in VO_2, 2) respiratory exchange ratio ≥ 1.1, 3) HR ≥ 90 % of predicted maximum, 4) patient exhaustion/Borg scale > 9/10, or 5) evidence of respiratory limitations. In the absence of a plateau in VO_2 where remaining criteria for a maximal test were met, the highest recorded VO_2 value was referred to as VO_{2peak} and used as a substitute for VO_{2max} as a measurement of cardio-pulmonary fitness[54].

3.2.2.2 Physical activity

Physical activity was quantified as number of steps taken and energy expenditure through the use of physical activity monitors (biaxial accelerometers, SenseWear Pro3 Armband, Bodymedia). SenseWear Pro accelerometers have previously been shown to accurately measure energy expenditure[55, 56] and have been used in previous conducted research with respiratory patients[57, 58]. The subjects were instructed to wear the activity monitors on the back of their dominant upper arm

for the entire day for a total of 3 days, preferably two week-days and one day of the weekend. Data from the armbands was analyzed using SenseWear® software (version 7.0.0.2378, BodyMedia, Inc.).

3.2.2.3 Endothelial function

The endothelial function of the brachial artery was evaluated after 10 minutes of rest in the supine position using ultrasound imaging (8L-RS 4.0-13.0MHz probe, Vivid q, GE Healthcare, Mississauga, ON). Baseline diameter of the brachial artery was established, whereby the blood flow of the forearm was occluded distally of the measuring site for the duration of 5 minutes. The blood velocity and the brachial diameter were monitored for 20 seconds prior to and three minutes after cuff-release, and later analyzed offline (Medical Imaging Applications, LLC, Coralville, IA, USA; EchoPAC PC software, version 110.x.x, GE Healthcare, Horten, Norway). Microvascular function was evaluated as the velocity time integral-envelope of the first heartbeat of reactive hyperemia (VTI; m/s)[24]. The total shear stress until peak dilation ($SSRH_{total}$) was calculated as 8 x mean velocity until peak FMD/(baseline diameter/10)[59]. All tests were conducted at the same time in the morning after a 12 hour fasting period. When applicable, the subjects were asked to withhold any long-acting asthma medication for a minimum on 48 hours prior to the test, and short-acting asthma medications for 12 hours prior to the test. All FMD tests were done by the same person, and changes within the vessel diameters were evaluated by two independent investigators. The inter-interpreter correlation coefficients for baseline, peak and peak % baseline diameter were r = 0.96, 0.97, and 0.74, respectively.

3.2.2.4 Arterial stiffness

Arterial stiffness was evaluated as the pulse wave velocity (PWV) between the radial and the carotid arteries using applanation tonometry. The carotid and radial pulses were obtained following 10 minutes of supine rest, whereby PWV was recorded simultaneously over the two measuring sites. Approximately 30 waveforms were recorded from each subject, and 10 consecutive waveforms were selected for analysis (LabChart version 7.3.5 ADInstruments). The distance between measuring points were divided by the average difference in time between corresponding carotid-radial waveforms, as per guidelines[60].

3.2.3 Blood collection

Blood was collected via an antecubital venipuncture immediately following the vascular function test. Once collected, the blood was left allowed to clot in room temperature for a minimum of 30 minutes before being centrifuged at 1000g for 10 minutes at 4 degrees Celsius. Aliquots were outsourced to Eve Technologies, Calgary, AB, for analysis of inflammatory markers (*i.e.* IL-6, CRP, and TNF-α).

3.2.4 Statistical analysis

Subject characteristics and vascular parameters were compared between asthmatics and controls using 2-tailed independent samples t-tests. Binominal variables were compared between asthmatics and controls using the Chi-square test. The relationships between serum levels of TNF-α, IL-6, and CRP, and FMD/$SSRH_{total}$ and PWV were evaluated in asthmatics using the Pearson

product-moment correlation coefficient (r). Correlations with an r^2-value of 0.09-0.25 were considered to be of medium strength, while correlations of $r^2 \geq 0.25$ were considered strong. Based on previous studies[27], a large effect size in FMD was expected between groups, thus a sample of 16 subjects per group was considered sufficient to detect a difference in FMD between asthmatics and non-asthmatics with a power of 80%. An α-level of 0.05 (2-tailed) was used as significance level for all statistical analysis, and results are reported as mean ± standard deviation unless indicated otherwise. All statistical analysis was performed using Statistical Package for The Social Sciences (SPSS Statistics, IBM, version 20.0).

3.3 Results

A total of 16 confirmed asthmatics and 16 confirmed non-asthmatics, matched for age, gender, BMI, physical activity and aerobic fitness (Table 1) were enrolled and completed all parts of the study. The prevalence of allergies was higher among asthmatics than non-asthmatics (57.1 versus 18.8%, $p<0.05$), but no between-group differences were seen in FeNO (37.3 ± 40.9 versus 17.9 ± 8.5 ppb) or FEV_1 post bronchodilation (91.6 ± 12.6 versus 100.9 ± 13.2 % predicted), suggesting that the asthma sample obtained consisted of subjects with mild or well-controlled asthma. Asthma control, evaluated according to the Asthma Control Questionnaire, indicated that the asthmatics were within the ranges of controlled to partly controlled asthma (ACQ-score for asthmatics: 1.0 ± 0.4). No differences in indices of blood pressure, fasting glucose, lipid profiles, or systemic inflammatory markers were seen between groups (Table 2).

While there were no differences in endothelial function ($FMD/SSRH_{total}$: 0.05 ± 0.02 versus 0.06 ± 0.02, p=NS, figure 1a) or microvascular function (VTI: 82.5 ± 22.0 versus 81.5 ± 18.5 m/s,

p=NS, figure 1b) between asthmatics and non-asthmatics when matched for physical activity and fitness, people with asthma had significantly higher arterial stiffness than non-asthmatics (PWV: 7.3 ± 1.5 vs. 8.7 ± 1.5 m/s, $p<0.05$) (Figure 1c).

There was a strong positive correlation between PWV and IL-6 among asthmatics ($r^2=0.33$, $p<0.05$, Figure 2a), but no association between PWV and TNFα or CRP (Figure 2b-c). There was no relationship between endothelial function and measured markers of systemic inflammation in asthma (Figure 2d-f).

3.4 Discussion

When matched on age, BMI, physical activity, and aerobic fitness, there were no differences in endothelial function, microvascular function, and systemic inflammation, between asthmatics and non-asthmatics. The asthmatics had significantly higher arterial stiffness, which suggests that structural changes within the vessel walls of asthmatics occur to a higher degree than in age-matched non-asthmatics, and that this is largely independent on physical activity and aerobic fitness.

Previous work has found that FMD was reduced in asthmatics as compared to controls[27]. While the participants in the previous study were matched on age and gender, they did not report fitness or physical activity levels, and based on previous work[42] it is likely that the asthmatics were less active and as a result would have lower fitness and impaired vascular health[34, 35]. In addition, while both baseline vessel diameter and blood velocity have been shown to affect the magnitude of the shear stress generated and subsequently the FMD[61, 62], the previous study did not report shear stress, and hence the FMD was not normalized to its stimulus. Thus, it is possible that the

differences seen in FMD in the previous study were due to either a difference in shear stress and/or physical activity and fitness levels between the asthmatics and the non-asthmatics, rather than asthma alone. Differences could also potentially be due to differences in study power or asthma duration and severity. Our findings indicate that when matched for fitness and physical activity, asthmatics do not appear to have reduced vascular function as compared to healthy controls.

Two previous studies examined arterial stiffness in asthmatics[26, 63]. Weiler et al.[63] evaluated the relationship between indices of arterial stiffness (radial wave form analysis and systemic vascular resistance) and found associations between stiffness and FEV_1, but no differences were seen based on asthma status. Similar to our study, Sun et al.[26] measured brachial-ankle arterial PWV and found a difference in arterial stiffness between asthmatics and non-asthmatics. Sun et al. did not evaluate physical activity or fitness, which based on previous work[42] were likely reduced in the asthmatics and could have contributed to an increase in arterial stiffness. Previous studies have reported that structural vascular changes are related to reduced lung function[64]. Our findings would indicate that the increase in arterial stiffness in asthmatics is related to the lung disease, and not secondary to reduced physical activity or fitness.

Increased levels of systemic inflammation are known to adversely affect vascular function and arterial stiffness[28, 30]. For example, both CRP and TNFα are known to enable the initiation and progression of atherosclerosis by up-regulating the expression of cell endothelial adhesion markers[65, 66], as well as reduce the bioavailability of nitric oxide[67, 68]. IL-6 assists in the inflammatory response by stimulating the production of other inflammatory mediators[69], and elevated levels of IL-6 have been linked to the increased macrophage lipid-uptake seen in atherosclerosis[70]. In the current study, we evaluated the relationship between endothelial function

and markers of systemic inflammation but did not find any associations. Other studies have found that higher levels of systemic inflammation can be seen in relation to asthma disease severity[6, 8, 26]. Thus, the absence of associations between endothelial function and systemic inflammation in the current study could be due to the composition of the sample of asthmatics, consisting predominantly of people with milder controlled or partly controlled asthma. There was also large variability in systemic inflammation, which limits the ability to detect a difference. While the asthma group had greater arterial stiffness compared to controls, there was no increase in any systemic inflammatory marker in asthmatics as compared to controls, indicating that the increase in PWV occurred independent of systemic inflammation. We did however see an association between IL-6 and arterial stiffness within the asthmatics, suggesting that systemic inflammation may be related to arterial stiffness in asthmatics, but it does not explain the elevation relative to controls. As examining the relationship between arterial stiffness and inflammatory markers was not the primary goal of this study, this study was likely underpowered, and a larger study would be warranted in order to further explore this.

Another possible mechanism that has been suggested to affect cardiovascular health in asthma is the use of asthma medications[1, 71]. For example, the use of beta-agonists has been associated with changes in both heart rate, vascular resistance[72, 73] and studies have suggested an influence of beta-agonists on sympathetic nerve activity[73, 74]. Elevated sympathetic nerve activity is associated with enhanced vascular remodeling and could therefore potentially contribute to the development of structural changes within the vasculature among asthmatics. Thus, the differences seen in arterial stiffness between asthmatics and non-asthmatics may be due to asthma medication usage, however; the chronic effects of asthma medication on vascular health are mostly still unknown and more research is needed in this area.

Potential limitations in this study may come from variations in asthma phenotypes between subjects, since different types of asthma are associated with different inflammatory profiles[5]. Vascular function is a diverse construct; we only evaluated endothelial function and microvascular function, while other features, such as endothelium-independent function, or the renin-angiotensin-aldosterone system, may influence vascular function in asthma. We also measured peripheral rather than central arterial stiffness, although central arterial stiffness has been shown to accurately reflect CV risk in other populations[25]. While one previous study has reported on differences in endothelial function between asthmatics and non-asthmatics[27], no such variance was seen in the current study.

All subjects included in the current study were carefully screened for the presence or absence of asthma. However, while all individuals with asthma exhibited a clinical history of asthma and met at least one of the three criteria for an asthma diagnosis listed in the methods, the asthmatics were not required to show baseline obstruction as defined as a lower than normal FEV_1/FVC-ratio. In addition, the sample recruited may not have been sufficient to detect differences in endothelial dysfunction between groups. Nevertheless, based on the variances seen in $FMD/SSRH_{total}$ in the current study, it is unlikely that the similarities in endothelial function between asthmatics and non-asthmatics would be due to low study power. Our original study was powered to detect a 10% difference in FMD between asthmatics and controls[27] which would correspond to more than a 50% increase in relative risk of CV between the groups based on previous work[75]. Importantly however, the mean value for vascular function between asthmatics and controls was very similar (0.061 versus 0.054, p=0.408), and a power calculation would indicate 340 subjects would have been required to detect a difference which, while statistically significant, would have been well below any clinical significance.

While asthma is more common among adult women than men[43], more men than women with asthma volunteered to participate in the current study, which compromises the generalizability of the results to the actual asthma population. Additionally, CV risk in asthma tends to be greater in women than men[1, 2] and as there were more male than female asthmatics in the current study, the results may underestimate the effect of asthma on CV risk.

All asthmatics were asked to withhold all asthma medications for a minimum of eight and 48 hours for short-acting and long-acting medications, respectively. As there are currently no available guidelines for abstaining from asthma medications prior vascular function or arterial stiffness testing, this timeframe was based on clinical guidelines for pulmonary function testing[43]. The exact washout times for all available asthma medications are not known, and residual components from asthma medications may have affected the vascular outcomes in the current study.

3.5 Conclusion

When matched for physical activity and aerobic fitness, no differences were seen in endothelial function, microvascular function, or systemic inflammation between people with controlled or partly controlled asthma and non-asthmatic controls. This would suggest that in mild and controlled asthma there is no increased systemic inflammation and/or impaired vascular function. However, structural changes within the systemic arterial vessel walls appear to occur in young controlled and partly controlled asthmatics compared to controls, which result in an increased arterial stiffness that seem to be independent of physical activity, aerobic fitness and inflammation.

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3.8 Tables and figures

Table 3.1. Subject characteristics. Values are expressed as mean ± standard deviation.

	Control	Asthma
Sample size - n (male/female)	16 (8/8)	16 (10/6)
Age (years)	26.6 ± 5.2	27.8 ± 6.1
BMI (kg/m^2)	24.7 ± 3.5	24.6 ± 4.6
FEV_1 pre-bronchodilator (% predicted)	96.3 ± 12.1	81.3 ± 15.1*
FEV_1 post bronchodilator (% predicted)	100.9 ± 13.2	91.6 ± 12.6
FeNO (ppb)	17.9 ± 8.5	37.3 ± 40.9
Asthma control questionnaire score	0.1 ± 0.2	1.0 ± 0.4*
Allergies (% yes)	18.8	57.1*
Asthma confirmed with positive methacholine challenge (%)	0.0	25.0*
Asthma confirmed with positive reversibility test (%)	0.0	75.0*
Physical activity (steps/day)	10711.2 ± 2675.3	11125.1 ± 5487.8
Fitness (mL/kg/min % predicted)	106.1 ± 15.9	97.1 ±23.3

BMI; body mass index, FEV_1; Forced expiratory volume in 1 second, FeNO; Fraction exhaled nitric oxide. *$p<0.05$ between asthma and control.

Table 3.2. Vascular parameters. Values are expressed as mean ± standard deviation.

	Control	Asthma
Systolic blood pressure (mmHg)	104.2 ± 7.0	109.3 ± 12.0
Diastolic blood pressure (mmHg)	66.4 ± 5.3	68.3 ± 10.0
Mean arterial pressure (mmHg)	79.2 ± 5.2	82.0 ± 10.3
Total cholesterol (mmol/L)	3.7 ± 0.9	3.9 ± 1.4
HDL (mmol/L)	1.4 ± 0.2	1.4 ± 0.4
Fasting glucose (mmol/L)	3.9 ± 0.4	3.9 ± 0.4
CRP (mg/L)	2.3 ± 3.8	3.6 ± 3.5
IL-6 (pg/mL)	8.6 ± 5.9	6.9 ± 3.5
TNFα (pg/mL)	16.5 ± 5.1	16.3 ± 2.8

HDL; High density lipoprotein, CRP; C-reactive protein, IL-6; Interleukin-6, TNFα; Tumor necrosis factor-alpha.

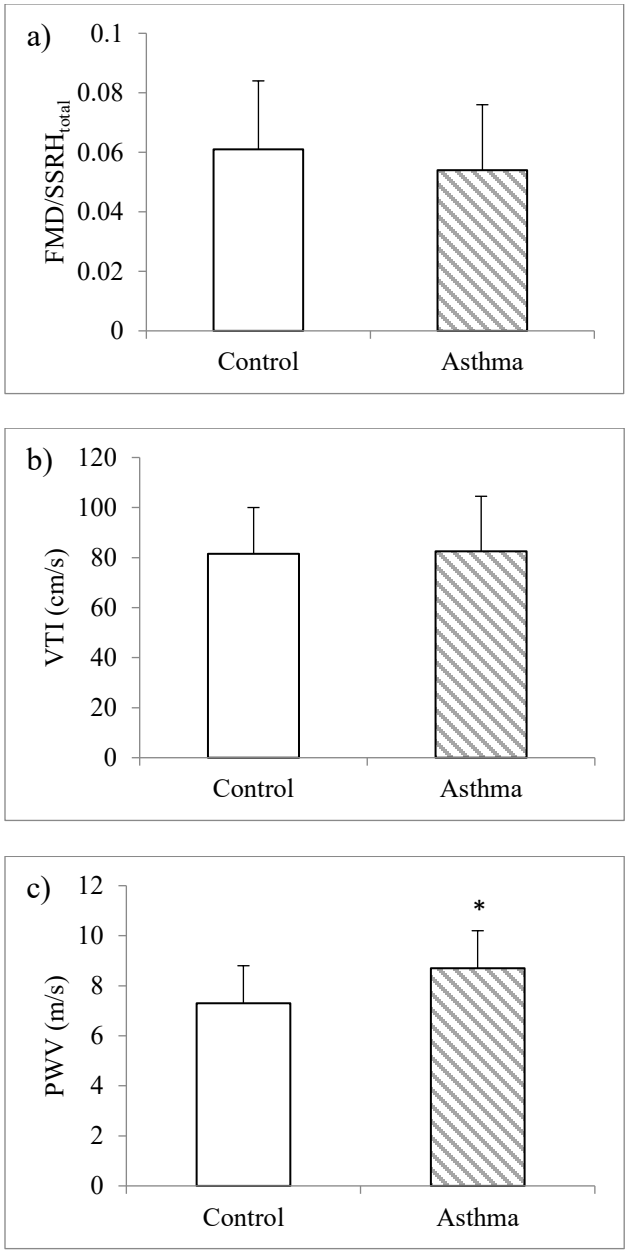

Figure 3.1. Endothelial function (a), microvascular function (b), and arterial stiffness (c) among controls and asthmatics, *indicates $p<0.05$ between groups.

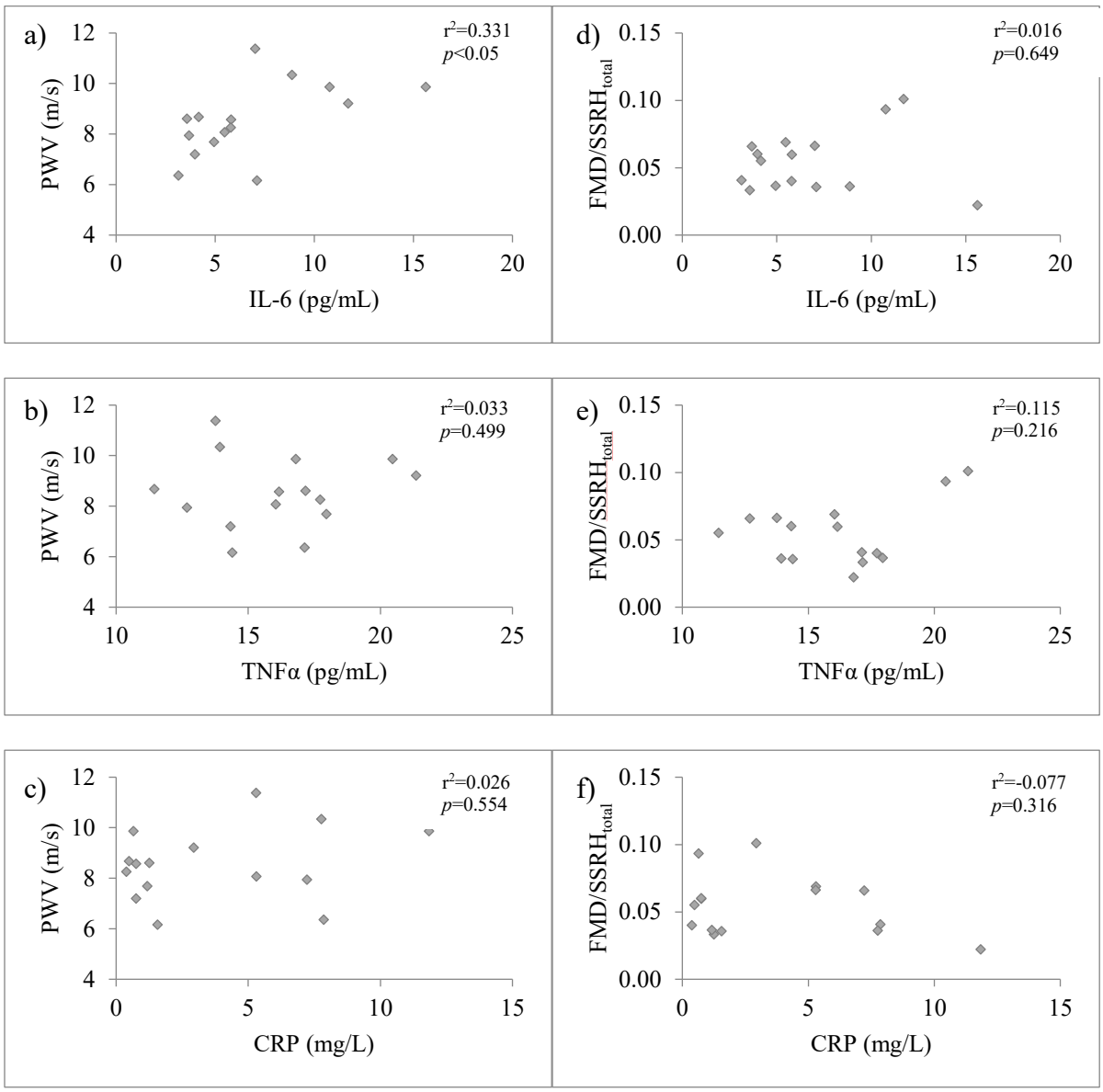

Figure 3.2. Relationship between arterial stiffness and systemic inflammation (PWV vs. (a) IL-6, r^2=0.33, p<0.05, (b) TNFα, p=NS, and (c) CRP, p=NS) and endothelial function and systemic inflammation ($FMD/SSRH_{total}$ vs. (d) IL-6, (e) TNFα, and (f) CRP, p=NS for all) in asthmatics.

4 CHAPTER IV

Understanding the increased exertional dyspnea in asthma

4.1 Introduction

Asthma, a chronic inflammatory airway disease characterized by recurrent airway narrowing, is associated with reduced physical activity levels[1, 2]. Physical activity is important for disease management in asthma in that it improves cardiovascular health[3], reduces airway inflammation, and improves asthma symptoms[4]. Physical activity avoidance in asthma is suggested to be due to intensified perceived breathlessness, (*i.e.* dyspnea) during exertion[5, 6]. However, traditional markers of ventilation during high intensity physical activity, such as peak minute ventilation ($\dot{V}_E$), breathing reserve ($\dot{V}_E$ /maximal voluntary ventilation), and ventilatory equivalents for oxygen ($\dot{V}_E/\dot{V}O_2$) and carbon dioxide ($\dot{V}_E/\dot{V}CO_2$), do not differ between controlled asthmatics and healthy individuals[7, 8], and do not explain the increased perceived dyspnea in asthma.

Expiratory flow limitation (EFL) occurs when the tidal breath increases to the extent where it becomes limited by the maximal expiratory flow of the lungs, and can be seen as the tidal breath overlapping with the outer border of the maximal flow volume loop[9]. EFL is believed to result in an upward shift towards higher operating lung volumes *(i.e.,* reduced inspiratory capacity (IC)) and the development of dynamic lung hyperinflation[9]. The occurrence of high operating lung volumes has been linked to intensified dyspnea during bronchoconstriction at rest in asthma[10, 11], and been shown to be a powerful determinant of dyspnea in chronic obstructive lung disease (COPD)[12-14]. Importantly, during constant workload exercise, asthmatics with a critically low inspiratory reserve volume (IRV) report greater dyspnea than asthmatics with larger IRV[15], but it is unknown if the reduction in exertional IRV during different levels of physical activity explains the increased sensation of dyspnea in asthma as compared to healthy controls.

Short-acting β_2-agonists are currently used as the mainstream treatment for acute asthma symptoms and their usage has been implemented in asthma guidelines worldwide as part of standard asthma management[16]. Many asthmatics have mild airflow obstruction at baseline which is reversed with β_2-agonists, and while most asthmatics do not experience symptoms of dyspnea at rest, dyspnea typically develops during more intense physical activity. Although prescribed liberally, it is not well known how short-acting be β_2-agonists affect operating lung volumes and thus sensory responses to physical activity in asthma.

The purpose of this study was to better our understanding of the mechanisms of exertional dyspnea in patients with asthma. It was hypothesized that asthmatics would breathe at higher operating lung volumes compared to healthy controls, as demonstrated by a reduced IC and IRV during incremental exercise, and that this would be associated with the increased sensation of dyspnea in asthmatics. Further, it was proposed that the administration of the β_2-agonists salbutamol would prevent reductions in IC or IRV and, as a result, reduce exertional dyspnea.

4.2 Methods

4.2.1 Study subjects

This study was approved by the University of Alberta Ethics Board, and all subjects were required to provide written informed consent prior to participating. Asthma (n=16) was confirmed if the participant had a clinical history of asthma and met at least one of the following criteria: a) ≥200 mL and 12% improvement in forced expiratory volume in one second (FEV_1) following β_2-agonist administration, b) ≥10% reduction in FEV_1 following exercise, or c) a positive bronchial challenge

test resulting in a reduction in FEV_1 of ≥20% from baseline at a provocative concentration of ≤4 mg/mL methacholine (PC_{20}≤4 mg/mL)[16, 17]. Control subjects (n=16) all had no clinical history of asthma, normal lung function, no reversibility with β_2-agonists, and no significant reduction in FEV_1 following exercise. All participants were free from known cardiovascular disease or lung disease other than asthma. Twelve of the asthmatics were using short-acting beta-agonists, 3 were using inhaled corticosteroids (ICS), and 5 were using combined ICS and long-acting beta-agonists. Asthmatics were requested to withhold long-acting controller medication for a minimum of 48 hours and short-acting reliever medication for eight hours prior to each study visit. All participants were asked to withhold caffeine, heavy exercise, and alcohol the days of the study.

4.2.2 Study design

Each participant reported to the laboratory on two different days, at least 48 hours apart, for this randomized case-control crossover study. Asthma control was evaluated according to the asthma control questionnaire[18, 19], and a physical activity readiness questionnaire was used to screen for known contraindications to exercise. In addition, medical history and demographics were obtained from all participants prior to any physiological testing. On one of the testing days, a complete pulmonary function test[20-22] followed by an incremental exercise test to exhaustion was completed. On the second day, the participants completed only spirometry and airway resistance testing at baseline, followed by a second incremental exercise test to exhaustion. In random order on one of the days, airway reversibility was assessed during the spirometry testing following 400 mcg of salbutamol (Ventolin®, Glaxo-SmithKline, Mississauga, Canada). Data from this day was also

used to evaluate the potential impact of salbutamol on operating lung volumes and dyspnea during exercise. The subjects were not blinded to salbutamol administration.

4.2.3 Pulmonary function testing

Baseline spirometry[23], lung volume[24], and diffusion capacity[25] were measured as per current guidelines[20-22] on the Vmax Spectra V29System (SensorMedics, Yorba Linda, CA, USA). Additionally, airway resistance was measured using the forced oscillation technique (IOS, Impulse Oscillometry, CareFusion, Yorba Linda, CA, USA)[26].

4.2.4 Incremental exercise test

A cardiopulmonary exercise test was performed on a stationary cycle ergometer (Ergoselect 200, Ergoline, Bitz, Germany) starting at steady state rest, followed by 25 W step-wise increases in workload every two minutes until volitional exhaustion. Metabolic breath-by-breath data was recorded (Vmax Spectra V29System; SensorMedics, Yorba Linda, CA, USA) continuously throughout the test[27].

IC measurements were completed during the last 30 s of each stage to calculate operating lung volumes. End expiratory lung volume (EELV) was calculated as the total lung capacity (TLC)-IC, end inspiratory lung volume (EILV) as EELV+the tidal volume (V_T), and the inspiratory reserve volume (IRV) as IC-V_T expressed as a percentage of TLC. EFL was evaluated at each workload using the IC data and spirometry data by determining the percentage the V_T curve overlapped that with the maximal flow-volume curve obtained at baseline of the same testing day[9].

Dyspnea was assessed according to the modified Borg scale (0-10) for perceived intensity of breathlessness[28] during the last 20 second of each exercise workload and at peak exercise. An average of the ventilatory and metabolic data obtained over 30 seconds at the start of the second minute of each stage was used for analysis[29].

4.2.5 Statistical analysis

Baseline demographics and lung function were compared between asthmatics and controls using unpaired student's t-tests for continuous variables and the chi-square test for discontinuous variables. Spirometry values were compared between disease conditions (factor 1) and before and after the use of bronchodilator (factor 2: repeated factor) with 2-way repeated measure ANOVA. Remaining pulmonary function parameters were evaluated between groups using student's unpaired t-test. Three-way complex mixed design ANOVAs were used to assess the interactions between disease condition (between-group factor), bronchodilator usage (repeated factor 1) and sensory (dyspnea, leg discomfort), ventilatory, and metabolic parameters at baseline, 50 watts, 75 watts, 100 watts, 125 watts, and individual peak workload (repeated factor 2). Significant interactions were further evaluated with multiple comparisons (group averages compared at each workload and peak exercise) corrected with Bonferroni's post-hoc. Further comparisons were made between disease conditions (bronchodilator usage not taken into consideration due to lack of effect) and dyspnea in relation to operating lung volumes at the same workloads as above with a 2-way repeated measure ANOVA, followed by Bonferroni-corrected multiple comparisons (group averages compared at each workload and peak exercise). Graphs were plotted using Sigma plot (version 13.0, Systat Software Inc., San Jose, CA, USA) and statistical tests were performed

using SPSS Statistical software v. 24.0.0.0 (IBM Corp™, Armonk, NY, USA). Statistical significant was set to $p<0.05$ *a priori*.

4.3 Results

Subjects

Sixteen asthmatics and 16 non-asthmatic controls participated in the study, and were group-matched for age, sex, and fitness (%$\dot{V}O_{2peak}$ predicted), see Table 1. All participants were non-smokers, and the asthmatics were classified as controlled (68.8%) or partly controlled (31.3%) as per the asthma control questionnaire[18, 19].

Ventilatory responses to salbutamol at baseline and during exercise

The differences in FEV_1 seen at baseline between asthmatics and controls were attenuated with 400 mcg salbutamol (Table 2). Salbutamol administration did not affect the $\dot{V}O_{2peak}$, dyspnea, or the ventilatory responses to incremental exercise in either asthmatics or controls (Figure 1 and 2), nor did it affect peak EFL (Table 3). As there was no effect of salbutamol, all further comparisons between asthma and controls are reported from the non-salbutamol condition.

Ventilatory and metabolic responses to exercise

Asthmatics and controls showed similar responses in $\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$ at both submaximal and peak workloads (Figure 1a and b, and Table 3). Ventilation was similar between groups throughout exercise, however, asthmatics adopted a more rapid and shallow breathing pattern at peak exercise as demonstrated by a lower V_T and greater f_R (Figure 1).

Dyspnea and ventilatory mechanics during exercise

Asthmatics reported greater dyspnea at submaximal workloads as compared to controls, however; peak dyspnea was not different between asthmatics and controls. While there were no differences in IC or IRV at rest, as seen in Figure 3a, both IC and IRV were lower in asthmatics than controls at all exercise workloads. Dyspnea remained greater in asthmatics when expressed relative to $\dot{V}_E$, V_T, or f_R (Fig 4a-c). However, the between-group differences in dyspnea disappeared when dyspnea was expressed relative to IRV, seen as the two graphs superimposed on each other (Fig 4).

4.4 Discussion

This study evaluated the ventilatory and sensory responses to incremental exercise in asthmatics and controls. As a secondary purpose, the impact of salbutamol on operating lung volumes and dyspnea during exercise was evaluated. The main findings of this study are two-fold: 1) exertional dyspnea is higher in asthmatics as compared to controls. These data suggest that higher operating lung volumes, seen as reduced IC and IRV, are key contributors to exertional dyspnea in asthma. 2) While improving airflow limitation in asthmatics, an acute beta-agonist does not change the breathing pattern, operating lung volume, EFL or dyspnea response to incremental exercise in either asthmatics or controls.

Similar to previous research[7, 8], we did not find group differences in traditional markers of ventilatory efficiency (elevated $\dot{V}_E/\dot{V}CO_2$) or peak ventilation ($\dot{V}_{Epeak}$) during exercise. Despite this, and in contrast to previous studies[7], the asthmatics in the current study reported significantly

higher degrees of dyspnea at submaximal work rates, compared to controls (Fig 1C). IRV has previously been reported to be a main determinant of dyspnea in COPD[12-14], but the relationship between operating lung volumes and exertional dyspnea in asthma has thus far been unknown. Previous studies suggest that inspiratory effort is a main contributor to breathlessness during the onset of asthma-associated bronchoconstriction at rest[10, 11] and the sensation of breathlessness and increased inspiratory effort link well with low resting IC measures[11]. However, these conclusions are limited to resting conditions, and it is unknown how applicable the results are when increasing physical activity intensities. This study expanded on results from the resting studies by evaluating breathing patterns, operating lung volumes and dyspnea during exercise in asthma. While asthmatics did not show evidence of dynamic hyperinflation at any point during exercise, the increased operating lung volumes likely resulted in a mechanical constraint on V_T expansion with increasing exercise intensity, which resulted in asthmatics adopting a more rapid and shallow breathing pattern (Fig 1). The reduced IRV positioned V_T closer to TLC, which increases the elastic loading of the functionally weakened inspiratory muscles[30, 31]. Combined with the accompanying tachypnea (increased velocity of muscle shortening), it is logical to suggest that the mechanical abnormalities present in asthmatics may have led to a disparity between the increased neural drive to breath simultaneous with compromised lung volume expansion, termed neuromechanical uncoupling[32], and may help to explain the mechanism behind the increased perceived dyspnea in asthmatics[30]. When dyspnea was plotted against IRV there was a complete superimposition of the graphs in asthmatics and controls, suggesting that the increased perceived dyspnea is secondary to reduced IRV during incremental exercise. Critically low IRV values during constant workload exercise have also previously been linked to increased dyspnea in

asthma[15], and our findings provide additional support demonstrating a link between IRV and dyspnea in asthmatics.

Lower physical activity levels in asthma are in part due to a fear of increased symptoms sensations such as dyspnea following exercise[5, 6]. Previous work has shown that, when matched for physical activity levels, mild sedentary asthmatics and sedentary controls do not differ in their sensation of dyspnea during incremental exercise[7]. On the contrary to the previous study, this study reports significantly higher dyspnea scores at submaximal workloads than controls, with the maximal difference of 1.6 units on the modified Borg (0-10) scale[28] at 125 W. Our subjects achieved on average a 50% higher relative $\dot{V}O_{2peak}$ (+17 mL/kg/min) than what was reported in the previous study[7], and the $\dot{V}_{Epeak}$ among the asthmatics in the current study was 45 L/min (59%) higher than previously reported. However, the $\dot{V}_E$ at the corresponding workloads where the discrepancy in dyspnea between asthmatics and controls becomes apparent (75, 100, 125 watts) are well within the range of the obtained $\dot{V}_E$ of the asthmatics in the previous study (~76 L/min). It is thus reasonable to argue that differences in dyspnea between the two studies are not due to aerobic fitness, and further research on other factors, such as asthma severity and control, is warranted to further assess why some groups of asthmatics, but not others, experience increased exertional dyspnea.

Salbutamol is a commonly prescribed short-acting bronchodilator which acts on the beta-2-receptors on the bronchial smooth muscles and thereby causes relaxation and opens constricted airways. While salbutamol is thought to prevent exercise-induced bronchoconstriction among susceptible individuals, or relieve asthma symptoms brought on by physical activity[16, 17], this is the first study to evaluate the direct impact of using salbutamol on operating lung volumes and

dyspnea in asthma. As seen in Table 3, there were no differences in operating lung volumes following salbutamol (Fig 2B). The lack of effect of salbutamol on operating lung volumes during higher intensity physical activity in asthma likely explains why dyspnea in asthmatics does not improve with salbutamol.

Limitations

While other studies have further characterized breathlessness associated with obstructive lung disease to be attributed to inspiratory effort[10, 11] increased diaphragmatic muscle effort[33], and increased ventilatory neural drive[33], this study did not evaluate subjective or physiological measures of work of breathing, and further work is needed in this area.

Asthma is a heterogenous disease with various triggers, inflammatory phenotypes, and severity levels, and a larger scale study would be needed to address further differences in lung volumes and dyspnea responses to exercise between distinct phenotypes of asthma. The sensation of dyspnea is likely associated with asthma severity, and an even larger difference in dyspnea may be present in severe or uncontrolled asthma. Additionally, the asthmatics were not required to exhibit baseline obstruction; however, all demonstrated either reversibility, a positive methacholine challenge, or a positive exercise challenge. Thus, the reduced IRV at peak exercise in asthmatics may be more pronounced in those with baseline airflow obstruction. The current study only included participants with controlled and partly controlled asthma, and to our knowledge, no data on exertional dyspnea and lung volume in uncontrolled asthma is currently available addressing this knowledge gap.

Consistent with previous work[34], the operating lung volume data in the current study were expressed in relation to TLC. While TLC has been shown to be stable during bronchoconstriction at rest[35], to our knowledge, no data are available evaluating TLC before and after exercise in asthma. The results from the operating lung volume graphs in the current study are therefore subject to the potential inaccuracy of the assumption that TLC does not change with exercise in asthma.

Recent work has documented sex differences in the ventilatory and dyspnea responses to exercise[36, 37]. Our study purposely included an equal number of men and women in both groups to account for any potential differences between men and women. Future work examining sex differences in breathing mechanics is needed to better understand the pathophysiology of exertional dyspnea in asthma.

Neither the research participants nor the research staff were blinded to the administration of salbutamol in this study. As salbutamol is used to reverse bronchoconstriction, and thus reduce the sensations of breathlessness and chest tightness in asthma[16], the lack of blinding to the drug condition introduces a potential bias towards lower dyspnea and better exercise tolerance following beta-agonist. However, as the administration of salbutamol did not affect operating lung volumes nor dyspnea during incremental exercise, it is unlikely that the lack of change with beta-agonist is explained by a lack of blinding.

4.5 Conclusion

Asthmatics exhibit higher operating lung volumes (seen as low IC and IRV) than controls during incremental exercise. The high operating lung volumes explain why asthmatics experience increased exertional dyspnea, and appear to be unaffected by salbutamol usage prior to exercise. To reduce physical activity avoidance in asthma, the focus of future research should be on ways normalize dynamic lung volumes, and thus improve exertional dyspnea in asthma.

4.6 References

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4.7 Tables and figures

Table 4.1. Subject characteristics.

	Control	Asthma
Number % women	16 (50)	16 (50)
Age years	24 ± 3	25 ± 4
Height m	1.7 ± 0.1	1.7 ± 0.1
Weight kg	75.7 ± 17.3	67.7 ± 12.6
Body mass index kg/m^2	25.4 ± 24.1	23.6 ± 3.0
Smoking history pack years	0.0 ± 0.0	0.5 ± 2.0
Allergies %	44	94
ACQ score	0.0 ± 0.0	0.7 ± 0.5*
Asthma confirmation		
Reversibility (%)	0.0 ± 0.0	43.8*
Positive bronchial challenge (%)	0.0 ± 0.0	37.5*
Positive exercise challenge (%)	0.0 ± 0.0	18.8*
Medications		
SABA %		75
ICS %		19
Combination medication (LABA+ICS) %		31

Values are expressed as mean ± standard deviation. ACQ: asthma control questionnaire; SABA: short-acting beta-agonist; ICS: inhaled corticosteroid; LABA: long-acting bronchodilator. *$p<0.05$ between disease conditions.

Table 4.2. Pulmonary function without and with bronchodilator in control and asthma.

	Control		Asthma	
	Pre	Post	Pre	Post
FEV_1 L/min	4 ± 1	5 ± 1	4 ± 1	4 ± 1**
FEV_1 % predicted	107 ± 9	112 ± 9	94 ± 8	101 ± 10**
FVC L	6 ± 2	6 ± 1	5 ± 1	5 ± 1
FVC % predicted	111 ± 11	111 ± 11	105 ± 11	105 ± 10
FEV_1/FVC	81 ± 7	85 ± 6	76 ± 8	82 ± 7
FEV_1/FVC % predicted	96 ± 8	101 ± 7	90 ± 10	96 ± 8
R5 % predicted	96.4 ± 33.8		125.8 ± 45.4*	
R20 % predicted	122.4 ± 26.3		143.6 ± 42.6	
TLC % predicted	103 ± 13		102 ± 17	
RV % predicted	82 ± 43		98 ± 62	
IC % predicted	107 ± 15		97 ± 17	
DLCO % predicted	95 ± 11		91 ± 9	

Values are expressed as mean ± standard deviation. FEV_1: forced expiratory volume in the first second; FVC: forced vital capacity; R5: total respiratory resistance; R20: proximal respiratory resistance: TLC: total lung capacity; RV: residual volume; IC: inspiratory capacity; DLCO: diffusion capacity of the lungs for carbon monoxide.

*$p<0.05$ between disease conditions,

**$p<0.05$ interaction between disease conditions and with/without bronchodilator.

Table 4.3. Peak exercise data, with and without bronchodilator.

	Control		Asthma	
	Without BD	With BD	Without BD	With BD
$\dot{V}O_2$ L/min	3.55 ± 0.96	3.64 ± 0.98	3.27 ± 0.69	3.26 ± 0.70
$\dot{V}O_2$ mL/kg/min	47.36 ± 8.42	48.43 ± 8.80	48.37 ± 6.91	48.16 ± 7.08
Workload w	247 ± 57	242 ± 54	220 ± 43*	219 ± 41*
Workload % predicted	121 ± 19	119 ± 18	116 ± 17	118 ± 20
HR % predicted	102 ± 5	101 ± 4	98 ± 6*	97 ± 11*
RQ	1.17 ± 0.08	1.16 ± 1.16	1.14 ± 0.06	1.14 ± 0.07
$P_{ET}CO_2$ mmHg	41.6 ± 3.5	42.6 ± 3.0	41.2 ± 2.9	40.6 ± 2.8
$\dot{V}_E$ L/min	128.8 ± 27.7	132.1 ± 30.6	120.9 ± 28.4	120.2 ± 27.7
V_T L	2.74 ± 0.58	2.81 ± 0.66	2.28 ± 0.43*	2.37 ± 0.48*
f_R breaths/min	48.8 ± 8.1	47.9 ± 7.1	51.5 ± 6.9*	53.9 ± 12.4*
EFL % V_T	30.3 ± 26.4	22.8 ± 28.9	38.3 ± 32.7	23.7 ± 23.3

Values are expressed as mean ± standard deviation. BD: bronchodilator; $\dot{V}O_2$: rate of volume of oxygen uptake; HR: heart rate: RQ: respiratory quote; $P_{ET}CO_2$: partial pressure of end-tidal carbon dioxide; $\dot{V}_E$: minute ventilation; V_T: tidal volume; f_R: respiratory frequency; EFL: expiratory flow limitation.

*$p<0.05$ between disease conditions,

**$p<0.05$ between disease conditions and with/without bronchodilator.

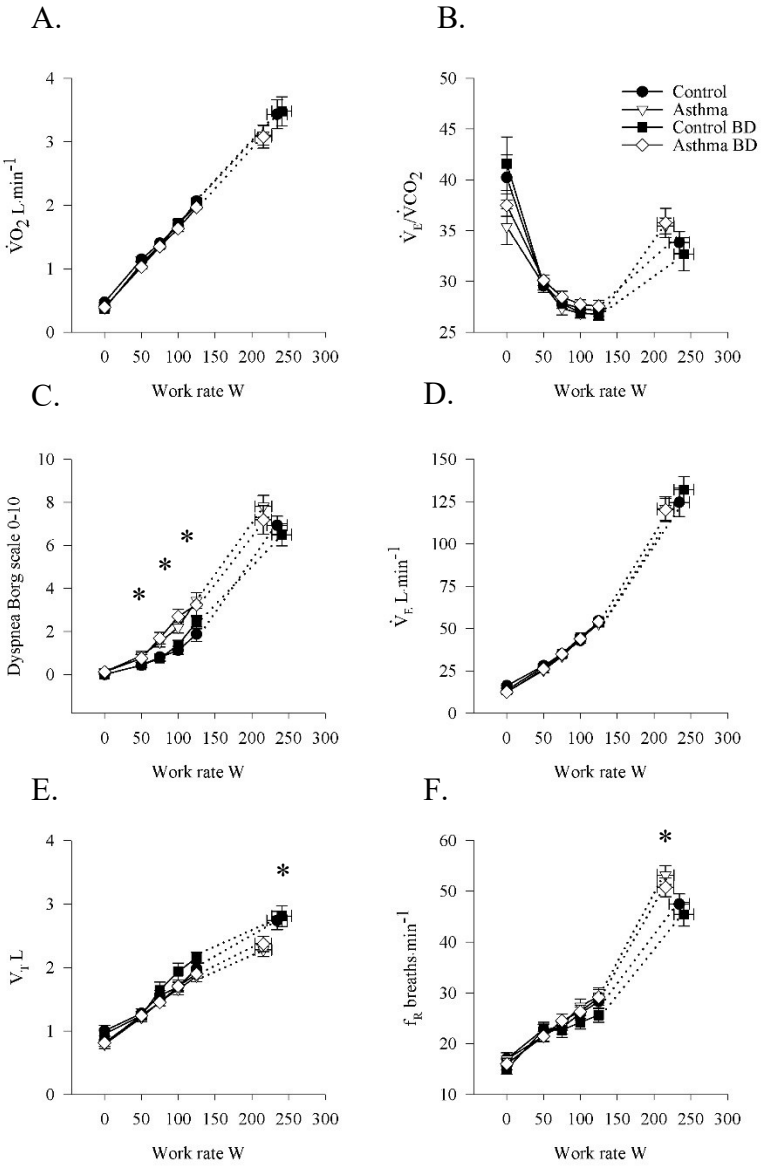

Figure 4.1. Ventilatory responses to exercise in asthmatics and controls with and without salbutamol. BD: bronchodilator; $\dot{V}O_2$: rate of the volume oxygen uptake; $\dot{V}_E$ / $\dot{V}CO_2$: ventilation over the rate of volume carbon dioxide production; $\dot{V}_E$: minute ventilation: V_T: tidal volume; f_R: respiratory frequency. Graphs display means ± standard error of mean.

*p<0.05 between asthma and controls at given workloads

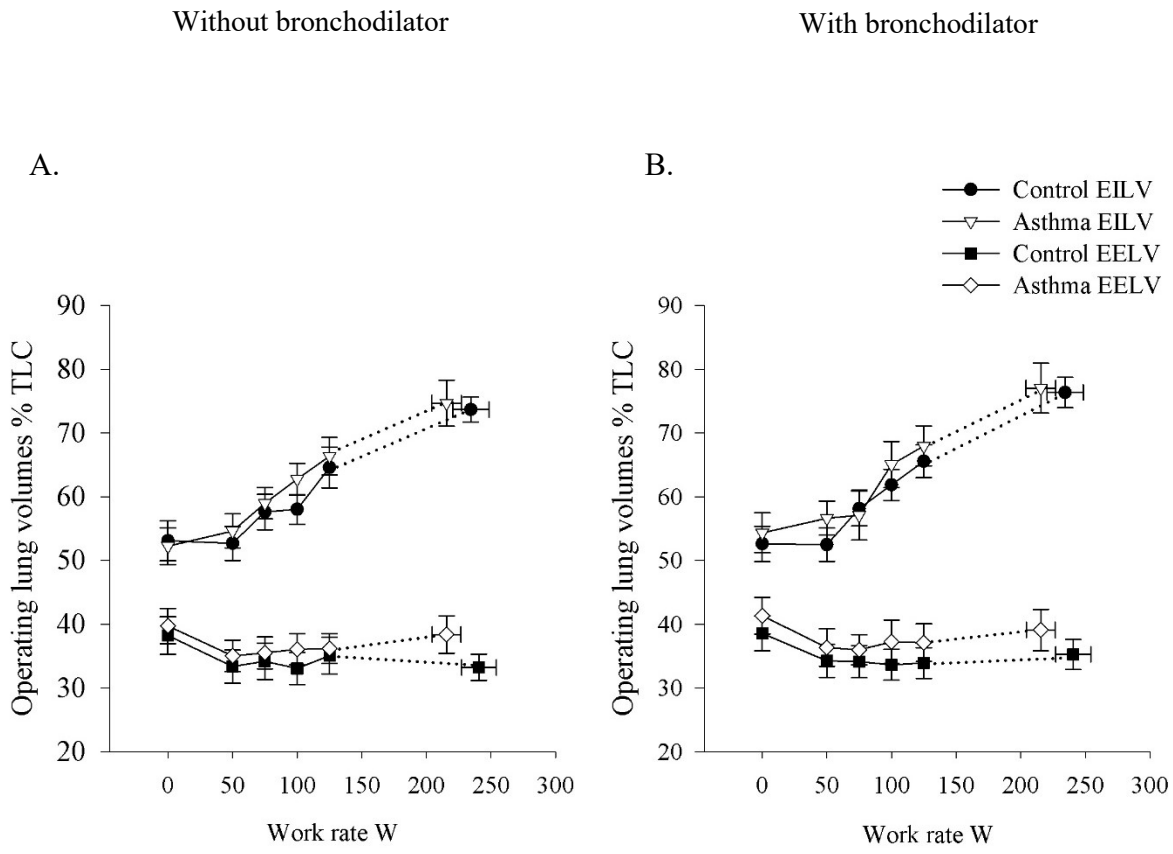

Figure 4.2. Operating lung volumes in asthma and controls during incremental exercise (A) without and (B) with bronchodilator. EILV: end-inspiratory lung volume; EELV: end-expiratory lung volume; TLC: total lung capacity. Graphs display means ± standard error of mean.

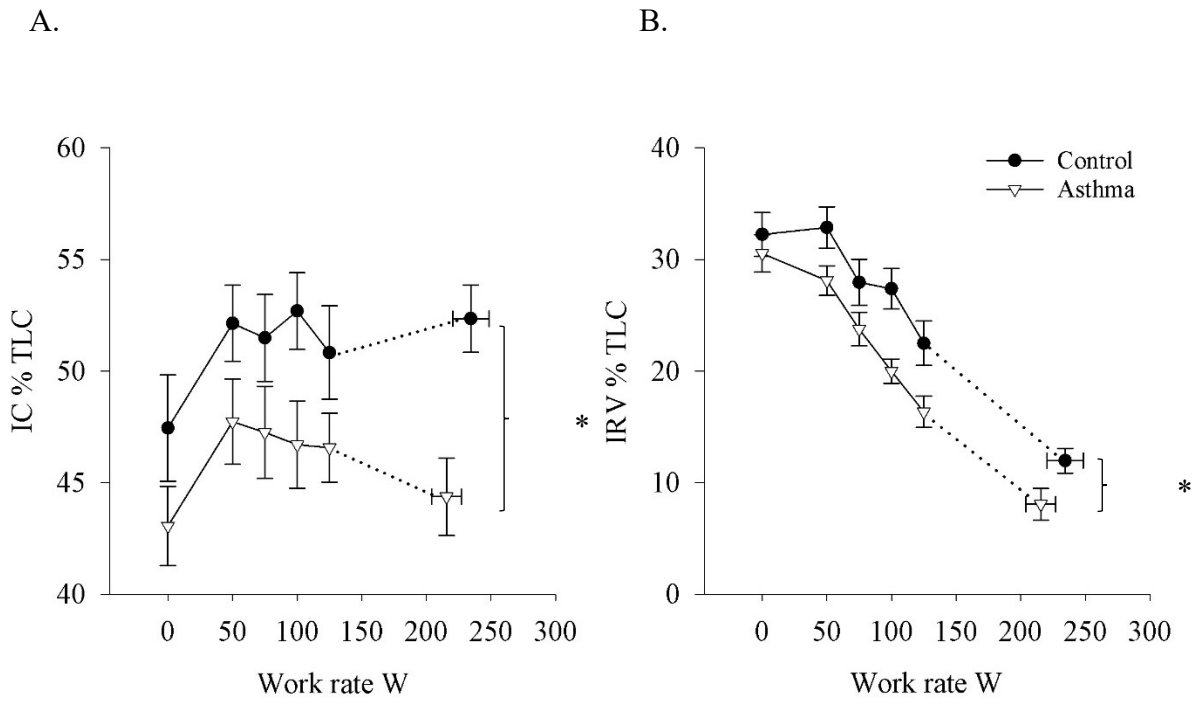

Figure 4.3. Inspiratory capacity and inspiratory reserve volume during incremental exercise in asthma and controls. IC: inspiratory capacity; IRV: inspiratory reserve volume; Graphs display means ± standard error of mean.

*p<0.05 between asthma and controls.

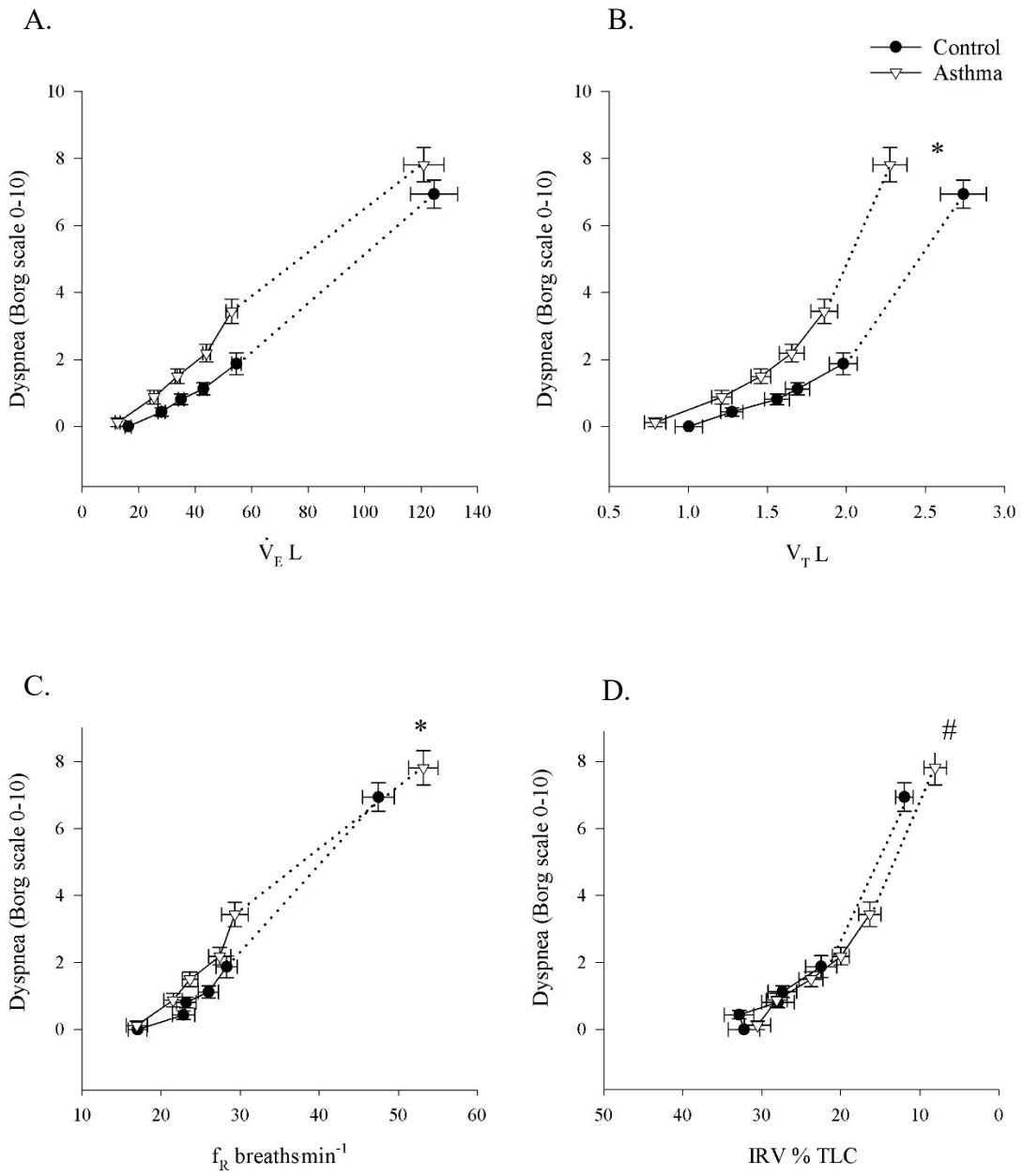

Figure 4.4. Dyspnea response to exercise in asthma and controls in relation to selected respiratory parameters. $\dot{V}_E$: minute ventilation; V_T: tidal volume; f_R: respiratory frequency; IRV: inspiratory reserve volume; TLC: total lung capacity. Graphs display means ± standard error of mean. *p<0.05 for given variable on x-axis between asthma and control, $^{\#}p$<0.05 for main group effect, asthma versus control.

5 CHAPTER V

Acute airway inflammation increases systemic inflammation in young adults with asthma

5.1 Introduction

Asthma is a chronic disease of the airways characterized by recurrent episodes of pulmonary inflammation leading to bronchoconstriction and symptoms such as breathlessness, wheezing, and chest tightness[1]. Those diagnosed with asthma are at an elevated risk of developing cardiovascular (CV) disease such as coronary heart disease, cerebrovascular disease, and heart failure[2, 3], and the CV risk tends to be exaggerated with increasing asthma severity and during exacerbations[4-8]. The reasons for the increased CV risk with asthma are not known.

Elevated systemic inflammation has been shown to acutely compromise CV health in otherwise healthy individuals[9, 10]. Results from animal studies suggest that the increased CV risk seen in asthma may be due to an inflammatory "over-spill" from the lungs during an exacerbation, which in turn negatively impacts the systemic vascular function[11, 12]. Acute inflammatory markers, such as C-reactive protein (CRP), are elevated in exhaled breath condensate during naturally occurring asthma exacerbations in humans, and this correlates well with CRP levels measured in serum at the same time-points[8, 13]. However, it is unknown if the increased systemic inflammation seen in an acute asthma exacerbation is in response to the acute exacerbation itself and whether this results in increased CV risk, as the physiological link between an acute asthma exacerbation/airway inflammation and CV risk in humans has not been established.

The mannitol airway challenge technique activates mast cells within the airways[14, 15], similarly to what is seen during a naturally occurring asthma exacerbation[16], leading to secretion of inflammatory mediators and cytokines[17], and bronchoconstriction among people with asthma[14, 15, 18, 19]. The purpose of this study was to evaluate the impact of airway inflammation induced from an airway challenge (i.e. mannitol) on CV risk parameters in a controlled laboratory environment.

To control for the direct impact of bronchoconstriction alone, the systemic vascular responses to bronchoconstriction induced by methacholine and placebo (saline) were also evaluated. We hypothesized that pulmonary inflammation induced by mannitol would lead to increased systemic inflammation and an impairment in vascular function, and thus help to explain why people with asthma are at increased CV risk.

5.2 Methods

This study was approved by the University of Alberta Ethics Board (Pro0054047), Health Canada (#9427-G0890-88C) and registered on ClinicalTrails.gov (NCT02630511). All participants were required to sign informed consent prior to research participation.

5.2.1 Study subjects

Patients with physician-diagnosed asthma between the ages of 18 and 45 years were identified by chart review from the University of Alberta Asthma Clinic and The Lung Health Clinic, Edmonton, Alberta. Subjects with known lung disease other than asthma or known CV disease, and a body mass index of more than 35 kg/m^2 were excluded from the study. In total, over 400 potential participants were initially identified as having physician-diagnosed asthma through our clinical research database. Twenty-six patients fit the inclusion criteria and consented to participate in the initial screening. Of the original 26, 12 subjects were confirmed to have asthma as demonstrated by a) more than 12% and 200 mL reversibility in the forced expiratory volume in 1 second (FEV_1) with salbutamol[20], b) a reduction in FEV_1 of more than 20% at a provocative

concentration (PC_{20}) of methacholine of less than 4 mg/mL[20], or c) 10% reduction in FEV_1 following exercise[20], and were included in the study. Nine of the asthmatics were using short-acting beta-agonists, two were using inhaled corticosteroids (ICS), five were using combined ICS and long-acting beta-agonist, and 1 was using ipratropium bromide medication.

5.2.2 Study design

Initially, each subject performed a full pulmonary function test[21-26] and a cardiopulmonary exercise test[27] to characterize study participants, identify unknown underlying CV disease, and to evaluate cardiopulmonary health.

The study consisted of three experimental days where the participant received either: 1) mannitol airway challenge, 2) methacholine airway challenge, or 3) standard saline airway challenge (i.e. placebo). The order of challenges was randomized, and the subject was blinded to the type of airway challenge.

Each visit occurred a minimum of one week apart to allow for recovery and to minimize potential carry-over effects. All participants were asked to withhold caffeinated drinks, food, alcohol, and exercise for a minimum of 12 hours prior to each study visit. While no changes were made to individual medication plans, all participants withheld short-acting beta-agonists for eight hours and long-acting controller medication for 48 hours prior to each test day[28]. All tests were done at the same time of day in the morning to minimize circadian influences on testing outcomes.

Each test visit started with the participant resting in the supine position for 10 minutes in a dimly lit room. Baseline brachial blood pressure was measured in duplicate, and a stable baseline

was established when the variance between the systolic blood pressure measurements was less than five percent. Arterial stiffness and vascular function were then evaluated. Serum was collected for systemic inflammatory measurements. Following baseline measurements, the participants completed one of three bronchial challenges. For consistency, independent of the airway response to the given challenge, each participant received 400 mcg salbutamol as an inhalation powder at the end of each challenge. Follow-up testing occurred immediately (within one hour) after each bronchial challenge, and all measurements were repeated in the same order as at baseline.

5.2.3 Bronchial challenges

- *Mannitol*

The mannitol challenge was performed according to guidelines[29]. The subject received incremental doses of mannitol (0, 5, 10, 20, 40, 80, 160, 160, and 160 mg) every two minutes, up to a maximal cumulative dose of 365 mg. Briefly, following baseline spirometry measurements, the subject was instructed to fully inhale the content of the inhaler provided from the manufacturer containing the mannitol capsule (Aridol®, Pharmaxis, Frenchs Forest, NSW, Australia). The subject then held their breath for five seconds, exhaled fully, then performed two forced vital capacity maneuvers one minute later. A challenge was considered positive if the forced expiratory volume in the first second (FEV_1) was reduced more than 10 % from baseline at a provocative dose of ≤365 mg mannitol (PD_{10}).

- *Methacholine*

The methacholine challenge was performed using the typical incremental two-minute tidal breath-protocol[28]. Following baseline spirometry measurements, the participant was instructed to breathe through a facemask connected to a nebulizer with saline (baseline) for two minutes. Following saline, a small amount of methacholine was added to the nebulizer (0.03 mg/mL), and the patients breathed through the facemask for another two minutes. For subsequent stages, the concentration of methacholine was doubled for each round of inhalations (0.031, 0.062, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 mg/mL). Spirometry was performed at 30 seconds and 90 seconds following each concentration. The test ended when either a) FEV_1 was reduced more than 20 percent of baseline values, or b) completion of the 16 mg/mL methacholine concentration.

- *Placebo*

The placebo challenge was performed identical to the methacholine challenge; however, no methacholine was added to the inhaled saline (i.e. participant inhaled saline only). The challenge was terminated following five rounds of saline.

5.2.4 Measurements

5.2.4.1 Vascular function

Vascular function was evaluated with the participant laying in the supine position with the right arm extended and the brachial artery imaged with ultrasound (8L-RS 4.0-13.0MHz probe, Vivid q, GE Healthcare, Mississauga, ON)[30, 31]. Following one minute of baseline diameter imaging and blood flow data collection, a cuff was placed around the forearm distal to the ultrasound measuring site and inflated to supra-systolic pressure for five minutes. Reactive hyperemia was created by a

sudden release of the cuff, and microvascular function was immediately evaluated as the velocity-time-integral (VTI) of the outer envelope of the blood flow profile of the first pulse-wave of reactive hyperemia (EchoPAC PC software, version 110.x.x, GE Healthcare, Horten, Norway). Endothelial function was subsequently assessed as the percent flow-mediated dilation (FMD) of the brachial artery compared to baseline in response caused by the reactive hyperemia, and normalized for sheer stress[32] (Medical Imaging Applications, LLC, Coralville, IA, USA).

5.2.4.2 Arterial stiffness

The stiffness of central (*c*PWV, carotid-femoral) and peripheral (*p*PWV, carotid-radial) arteries was evaluated using automated applanation tonometry (Complior, Alam Medical, Saint Quentin Fallavier, France)[33]. The distance between measuring sites (Δd) was divided by the time difference between the upslope of the pulse-waves at each measuring site (Δt) and arterial stiffness was expressed as the pulse wave velocity (PWV)=Δd/Δt[33]. A higher PWV is indicative of a stiffer arterial section[33] and higher CV risk[34].

5.2.4.3 Systemic inflammation

Ten mL of blood was collected from a vein in the antecubital fossa using standard venipuncture technique. The samples were allowed to coagulate for a minimum of 30 minutes at room temperature, then centrifuged at 12,000 rpm for 10 minutes. Serum was then collected and aliquoted into samples of 100 μL and stored in a -80 degrees Celsius freezer. Analysis of CRP

levels was done in duplicates using immunofluorescent technique (CRP DuoSet ELISA, R&D Systems, Bio-Techne Corporation, Minneapolis, MN, USA).

5.2.5 Statistical analysis

All statistical analyses were performed in SPSS Statistical software (version 24.0.0.0 IBM Corp™, Armonk, NY, USA) and graphs made using Sigma plot (version 13.0, Systat Software Inc., San Jose, CA, USA). Change in FEV_1 with each challenge, and percent change in inflammatory markers (CRP), were each assessed with a one-way ANOVA followed by LSD-corrected multiple comparisons. The vascular responses (FMD, VTI, *c*PWV, and *p*PWV) to each bronchial challenge (mannitol, methacholine, and placebo) were evaluated with a two-way repeated measure ANOVA. Pearson's correlation was used to evaluate the relationship between percent change in FEV_1 during any of the challenges and the individual vascular response, as well as the relationship between changes in CRP and vascular function during the mannitol challenge. All values are expressed as mean ± standard deviation unless otherwise specified. For all inferential analyses, the probability of type I error was set to 0.05.

5.3 Results

Subject characteristics

Baseline subject characteristics are outlined in Table 1. Complete testing was done on 10 individuals, while the remaining two participants completed parts of the testing (the mannitol day and the placebo day).

Pulmonary responses to bronchial challenges

Seven out of 12 (58.3%) and 9 out of 12 (75%) of participants had a positive airway response to mannitol and methacholine challenges, respectively. The mean maximal reduction in FEV_1 was -11.5% for mannitol and -19.3% for methacholine. The placebo challenge resulted in a mean change of -0.1% compared to baseline (Fig. 1).

Systemic responses to bronchial challenges

The mannitol challenge, but not the methacholine or placebo challenges, resulted in an increase in serum CRP levels compared to pre-challenge baseline (placebo: -7.5% (0.50±0.98 to 0.55±1.00 mg/L), mannitol 60.4% (0.39±0.50 to 0.46±0.46 mg/L), methacholine: -20.6%, $p<0.05$ (0.86±0.91 to 0.75±0.86 mg/L), Fig. 1). For each of the challenges, there was an overall time-effect in VTI and *c*PWV (86.6±18.7 to 74.2±14.1 m and 6.5±1.0 to 7.0±1.2 m/s, respectively), which is likely attributed to the inhalation of salbutamol following each challenge. As seen in figure 2, there was no difference in FMD, VTI, *c*PWV or *p*PWV response between mannitol, methacholine or placebo.

Relationship between bronchoconstriction and systemic vascular health

There was no relationship observed between the percent change in FMD, VTI, *c*PWV, or *p*PWV and bronchoconstriction, expressed as peak percent decline in FEV_1 compared to baseline, following either the mannitol or the methacholine challenge (Table 2).

Relationship between systemic inflammation and systemic vascular changes

No correlations were observed between changes in CRP with mannitol and changes in vascular parameters following mannitol (Table 3)

5.4 Discussion

This study evaluated whether the mannitol and methacholine challenges resulted in increased systemic CRP levels and vascular impairment in young people with confirmed asthma. The mannitol inhalation resulted in an increase in systemic CRP; however, this increase in systemic inflammation did not translate into vascular impairment, as no differences in endothelial function, microvascular function, or arterial stiffness were observed between any of the airway challenges. This was the first study to demonstrate that the mannitol inhalation challenge can adversely impact systemic CRP levels and thus potentially increase CV risk in asthmatics.

To date, only a handful of clinical studies have examined the potential physiological mechanism(s) for the increased CV risk seen in asthma. Vascular markers of CV risk tend to be impaired in asthma compared to controls[5-7, 35], and the severity of CV risk correlates well with asthma disease severity[4-7, 13]. However, all previous studies investigating CV risk in asthma have been observational in nature, and thus it has not been possible to make any direct links between asthma exacerbations and CV risk from their results. This was the first study of its kind to show that pulmonary inflammation from the mannitol inhalation challenge[14, 15], but not the methacholine-induced bronchoconstriction, directly leads to increased systemic CRP in humans, a known marker of future CV risk[36-38]. These findings provide the physiological link between asthma and elevated CV risk seen in the earlier observational studies[2-4], and translates work

conducted in animal models demonstrating that pulmonary inflammation leads to systemic inflammation, and thus increased CV risk[11, 12].

Both endothelial[32, 39] and microvascular function[40], as well as arterial stiffness[34], are markers of future risk of CV disease. Cross-sectional studies have shown that each of these CV risk markers are impaired in asthma[5-7]. Previous research looking at the causative effect of increased systemic inflammation on vascular function and arterial stiffness in healthy people did observe small, but statistically significant, changes in systemic levels of CRP and the pro-inflammatory mediator interleukin-6, which corresponded to elevated arterial stiffness[10] and impairments in endothelial function[9]. Although the increase in CRP in the current study was larger than what was seen before[10], the induced airway inflammation that lead to systemic inflammation did not translate to changes in systemic vascular function or arterial stiffness (Fig 2). A potential reason for the discrepancy in results between our study and the previous studies in healthy people is that asthmatics have impaired vascular function[6] and elevated arterial stiffness[5, 7, 35] at baseline, and the relatively small change in CRP may have been inadequate to inflict further changes in vascular function. However, previous work has observed a 300% increase in serum CRP levels during a naturally occurring asthma exacerbation (compared to remission)[8]. While the increase in CRP of 60.4% seen acutely with mannitol observed in the current study was insufficient to cause detectable changes in vascular function or arterial stiffness in asthma, it is likely that the large inflammatory insult following a naturally occurring asthma exacerbation would be detrimental to CV function. Research on the influence of naturally occurring asthma exacerbations on vascular health is needed to fully understand the impact of pulmonary inflammation on vascular function and CV risk.

In the current study, young asthmatics with no CV disease were purposely recruited to allow for a careful examination of the link between airway inflammation, systemic inflammation and vascular function. The risk of developing CV disease increases with age[41]. Thus, it is likely that older asthmatics would have lower baseline vascular function, and potentially a larger inflammatory and vascular response to airway inflammation. Similarly, greater systemic responses may have been observed following airway inflammation among those asthmatics with pre-existing CV disease. Further research is needed to evaluate the impact of asthma exacerbations on systemic vascular health among elderly asthmatics with and without pre-existing CV disease.

In the current study, saline was used as the placebo inhalant, which could have caused inflammation and bronchoconstriction because of the change in the humidity of the inhaled air[42]. However, the overall change in FEV_1 with placebo was less than 1% (Fig. 1A) and no increase in CRP was seen (Fig. 1B). It is therefore unlikely that the lack of change in systemic vascular responses with mannitol and methacholine relative to placebo was due to the choice of placebo inhalant in this study.

To normalize lung function after each challenge and to minimize any discomfort from bronchoconstriction, each participant received 400 μg salbutamol following each challenge. While salbutamol is not believed to have anti-inflammatory properties, is has been shown to increase sympathetic activity[43, 44]. Elevated sympathetic activity is known to modulate vascular tone[45], it is thus probable that the changes in VTI and *p*PWV observed following all challenges are the result of salbutamol usage, and not the bronchial challenges themselves. Salbutamol inhalation itself may contribute to CV risk in asthma, and additional research is needed in this area.

This study was powered to evaluate systemic vascular changes following mannitol and methacholine challenges. As a sub-analysis, the relationships between bronchoconstriction and vascular health, and systemic inflammation and vascular health were evaluated. As the latter were not the primary analyses of the study, the post-hoc sample size calculation to obtain adequate statistical power (β=0.80) based on current correlation coefficients of r^2=0.14 revealed a required sample size of over 500 participants, which was not feasible in the current study.

The mannitol inhalation challenge has previously been shown to increase markers of mast cell activation[14, 15], and anti-inflammatory asthma treatment significantly reduces the responsiveness to mannitol among asthmatics[18, 46]. A key assumption of the current work is that mannitol challenge results in airway inflammation, and that the observed systemic increase in CRP is the result of this increase in airway inflammation. As the increase pulmonary inflammation following mannitol has been established previously [14, 15], pulmonary inflammation was not directly measured in this study.

In the current study, all follow-up measurements were done within the first hour following each challenge. Previous studies evaluating the effect of increased systemic inflammation on vascular function and arterial stiffness both saw detectable changes in inflammatory levels eight hours after the inflammatory insult[9, 10], with an additional increase in CRP and arterial stiffness reported at 32 hours[10]. It is thus plausible that the serum levels of CRP in the current study may have continued to rise beyond what was seen immediately after each challenge, and have a delayed impact on the vascular function and arterial stiffness.

5.5 Conclusion

This study was the first of its kind to demonstrate that the mannitol challenge, but not the methacholine challenge, results in a small increase in systemic CRP levels in asthmatics. This increased systemic CRP levels did not appear to be sufficient to impair vascular function in young asthmatics. However, based on these findings, a larger inflammatory insult, such as seen during a naturally occurring asthma exacerbation, would likely lead to pronounced systemic inflammation, vascular impairments, and thus increased risk of CV diseases such as coronary heart disease, cerebrovascular disease, and heart failure among asthmatics.

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5.7 Tables and figures

Table 5.1. Baseline subject characteristics, values are expressed as mean ± standard deviations.

	Mean ± SD	Range
Sample size (male/female)	6/6	
Age (years)	26.1 ± 3.8	21.0 - 35.0
Height (m)	1.7 ± 0.1	1.5 - 1.9
Weight (kg)	71.3 ± 13.1	53.5 - 102.0
BMI (kg/m^2)	24.7 ± 3.6	20.0 - 31.5
Allergies (% yes)	91.7	
ACQ score	0.7 ± 0.5	0.0 - 1.7
Positive reversibility (%)	75.0	
Positive methacholine (%)	66.7	
Positive mannitol (%)	50.0	
FEV_1 (L)	3.7 ± 0.6	2.5 - 4.4
FEV_1 (% predicted)	95.0 ± 10.7	82.0 - 117.0
FVC (L)	4.9 ± 1.1	3.3 - 7.3
FVC (% predicted)	104.9 ± 12.6	88.0 - 122.0
FEV_1/FVC	76.3 ± 8.1	58.0 - 88.0
FEV_1/FVC (% predicted)	90.8 ± 9.2	69.0 - 103.0
PEF (L/min)	9.1 ± 2.3	5.0 - 13.5
PEF (% predicted)	108.3 ± 15.0	81.0 - 129.0

BMI: body mass index; ACQ: asthma control questionnaire; FEV_1: forced expiratory flow in one second; FVC: forced vital capacity; PEF: peak expiratory flow.

Table 5.2. Relationship between bronchoconstriction and markers of systemic vascular health

	Change in FEV_1 pre-post challenge	
	r^2	p-value
Inflammatory markers		
CRP (mg/l)	-0.558	0.385
Vascular parameters		
VTI (m)	0.232	0.310
FMD (% baseline)	0.136	0.555
*c*PWV (m/s)	-0.388	0.126
*p*PWV (m/s)	-0.014	0.960

CRP: C-reactive protein; VTI: velocity time integral; FMD: flow mediated dilation; *c*PWV: central pulse wave velocity; *p*PWV: peripheral pulse wave velocity.

Table 5.3. Relationship between percent change in systemic inflammation induced by mannitol and percent change in vascular function

	Percent change in CRP pre-post mannitol challenge	
	r^2	p-value
Vascular parameters		
VTI (m)	-0.061	0.818
FMD (% baseline)	0.136	0.925
*c*PWV (m/s)	0.276	0.488
*p*PWV (m/s)	-0.180	0.052

CRP: C-reactive protein; VTI: velocity time integral; FMD: flow mediated dilation; *c*PWV: central pulse wave velocity; *p*PWV: peripheral pulse wave velocity.

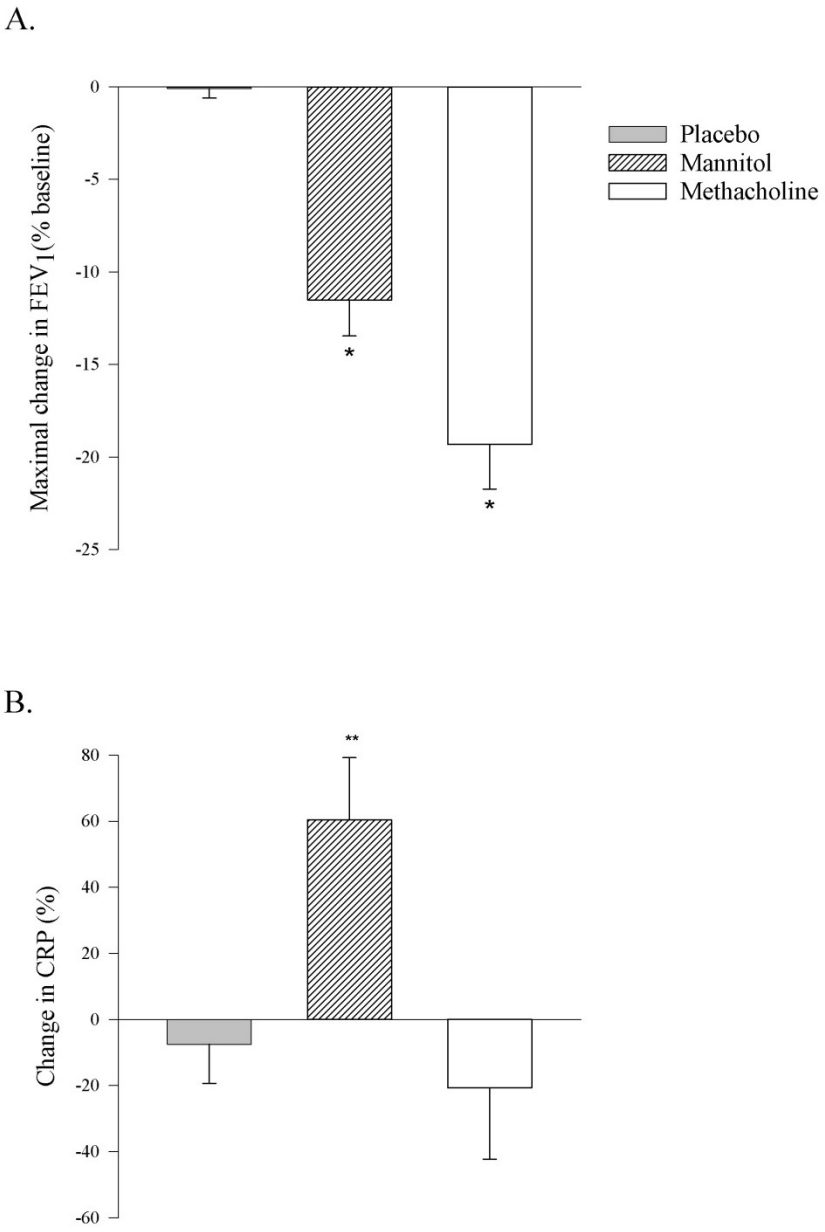

Figure 5.1. Maximal reduction in FEV_1 (A) and changes in CRP (B) and with placebo, mannitol, and methacholine challenges. Graphs display means ± standard errors of mean.

*$p<0.05$ compared to placebo

**$p<0.05$ compared to placebo and methacholine.

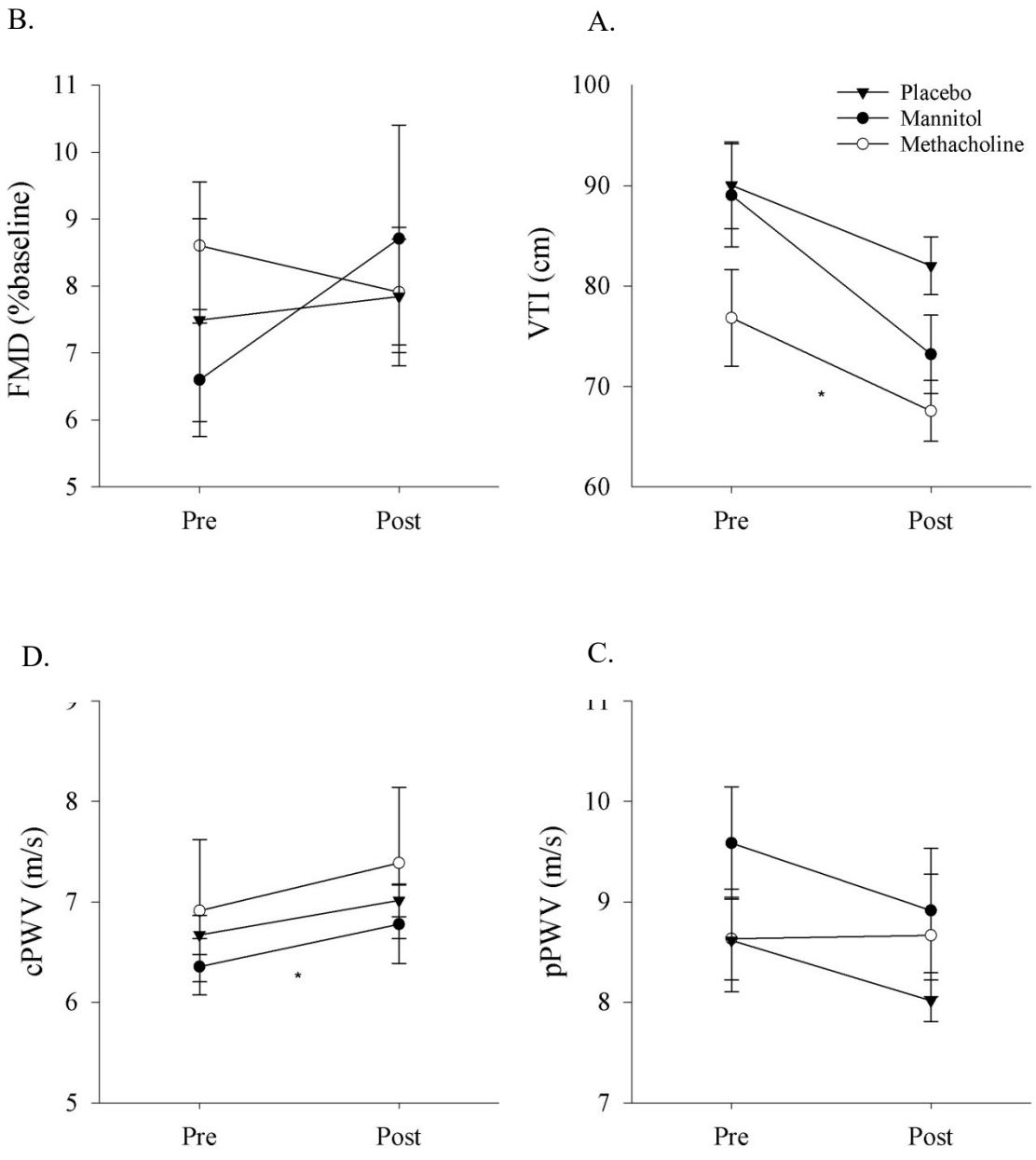

Figure 5.2. Changes in vascular function with placebo, mannitol, and methacholine challenges. A) Endothelial function, B) microvascular function, C) central arterial stiffness, and D) peripheral arterial stiffness. Graphs display means ± standard errors of mean.

*$p<0.05$ for main time effect.

6 CHAPTER VI

Acute effects of salbutamol on systemic vascular function in asthma

6.1 Introduction

Although asthma is primarily considered a disease of the airways, people with asthma are also at an increased risk of developing cardiovascular (CV) diseases such as coronary heart disease, cerebrovascular disease, and heart failure[1-5]. The risk of CV disease increases with decreased asthma control[2, 4, 6, 7], and acute respiratory exacerbations are a significant risk factor for first time CV events[8]. However, the reasons for the increased CV risk in asthma are unknown.

While it is plausible that uncontrolled asthma is contributing to the increased CV risk, reduced asthma control is also linked to greater use of medications[9, 10]. Use of asthma medications containing beta-2(β_2)-agonists has been associated with increased CV risk, independent of other CV disease risk factors, obstructive lung disease, and smoking history[5, 11]. Specifically, acute coronary events (myocardial infarctions and unstable angina) are up to twice as prevalent among those who have filled prescriptions for β_2-agonists within the three months leading up to the CV events[11]. Thus, it has been suggested that asthma medications containing β_2-agonists have deleterious extrapulmonary effects[5, 11].

The short-acting β_2-agonist salbutamol is currently prescribed to asthmatics to be used as part of standard asthma management[10]. It is known that increased use of salbutamol leads to increased airway tolerance[12], likely due to decreased β_2-adrenoceptor sensitivity and density within the lungs[12]. Yet, the vast majority of research examining the CV consequences of salbutamol has been conducted in salbutamol-naïve individuals[13-16]. Both our laboratory and others have found that short-acting β_2-agonists cause acutely increased sympathetic activity in healthy individuals[14-16]. When chronically elevated, sympathetic activity is in itself associated with impaired vascular heath[17] and increased CV risk[18]. It is unknown if regular salbutamol usage and/or having asthma

lead to different systemic vascular responses acutely following salbutamol inhalation as compared to salbutamol-naïve healthy controls. Thus, the purpose of this study was to evaluate the acute effects of a clinically relevant dose of salbutamol on systemic endothelial function and arterial stiffness in asthmatics and non-asthmatic controls (*Phase I*). A secondary aim of the study was to explore potential mechanisms as to why asthmatics react differently to salbutamol than controls (*Phase II*). Here, the cold pressor test (CPT) was used to stimulate a large non-specific sympathetic outflow, to determine if the systemic vascular response to a given sympathetic outflow is altered between asthmatics and non-asthmatics.

6.2 Methods

6.2.1 Research participants

Fourteen patients with physician-diagnosed asthma currently using salbutamol for asthma management, and 14 age, BMI, and sex-matched healthy controls without asthma or prior salbutamol usage, were recruited for Phase I of the study. All participants were required to be free from any known underlying CV disease or respiratory disease other than asthma. Asthmatics were instructed to withhold asthma controller medications for a minimum of 48 hours and short-acting β_2-agonists for eight hours prior to each test day. All participants were asked to withhold food, caffeine, and exercise for eight hours, prior to each test. The asthmatics had a significantly greater history of salbutamol usage, spanning from 2-25 years and 1-10 times/week, than the controls who had no history of salbutamol usage to report. Additionally, six out of the 14 asthmatics were prescribed and currently taking combination medication containing both long-acting β_2-agonists (LABA) and inhaled corticosteroids (ICS). The CPT (Phase II) was completed in a subset of 10

asthmatics and 10 non-asthmatic controls, using the same inclusion and exclusion criteria, and pre-test instructions as for Phase I. The participants in the control and asthma groups of Phase II were matched for sex, age, height, and weight. A higher percentage of the asthmatics had allergies than did the controls. While the controls did not have a history of using asthma medications, the asthmatics were all currently prescribed salbutamol and had a history of using salbutamol spanning from 1-17 years, 1-4 times/week. Three participants were taking daily doses of LABA combined with ICS, two participants were taking ICS only, and one participant was taking leukotrienes inhibitors (data not shown).

6.2.2 Study overview

6.2.2.1 Phase I: The effect of salbutamol on vascular function

Each participant reported to the laboratory in the morning of two separate days, 48 hours apart. After signing informed consent, the participants were instructed to rest for 10 minutes in the supine position in a dimly lit and temperature controlled room. Baseline measures were then recorded, followed by either 400 mcg inhaled salbutamol (Ventolin® inhalation aerosol, Glaxo-SmithKline, Mississagua, Canada) or placebo (placebo inhalation aerosol, Glaxo-SmithKline, Mississagua, Canada) administered orally over four inhalations using a space chamber (ProChamber, Respironics, Parsippany, USA), with each inhalation being held for 10-seconds (Ventolin® HFA Patient Information, Glaxo-SmithKline, Mississauga, Canada). Follow-up measures were recorded 15 minutes after the last inhalation. The order of the two days was randomized, and the participant was blinded to drug administration.

6.2.2.2 Phase II: CPT

The participants reported to the laboratory at the same time of day on two separate days, 48 hours apart. Following informed consent, the participants were instructed to rest in the supine position for 10 minutes in a quiet, dimly lit room. Baseline measures were then collected over a period of six minutes, followed by the participant lowering their left hand into either body temperature (BT) or ice water (CPT) for an additional six minutes while measurements were recorded. As breathing patterns do not influence the sympathetic output during the CPT[19], no breathing data were recorded during either trial. The order of the days of BT or CPT was randomized.

6.2.3 Measurements - *Phase I*

6.2.3.1 Hemodynamic evaluation

Brachial blood pressure, heart rate (BioAmp; ADInstruments, Australia) and finger beat-to-beat blood pressure (Finometer Midi, Amsterdam, Netherlands) were recorded, with heart rate and beat-to-beat blood pressure integrated via LabChart 8.0 (PowerLab 16/30; ADInstruments, Australia).

6.2.3.2 Arterial stiffness

Pulse wave velocity (PWV) between the carotid and radial arteries was assessed as previously described[20]. Briefly, a minimum of 15 consecutive heartbeats were recorded simultaneously at each measuring sites using applanation tonometry (Micro-Tip Catheter Transducer model SPT-301, Millar Instruments, Inc., Houston, USA) and the distance between the sites on the arteries was measured. The time difference (*Δ-time*) between pulse waves was manually analyzed offline

between the foot of the upslope of each pulse wave using LabChart 8.0 (PowerLabs, ADInstruments). PWV was then calculated as *distance/Δ-time* (m/s) and used for evaluation of arterial stiffness[21].

6.2.3.3 Endothelial and microvascular function

Baseline diameter and blood flow of the brachial artery measured at the upper right arm was established over one minute using ultrasonography (8L-RS 4.0-13.0MHz probe, Vivid q, GE Healthcare, Mississauga, ON). Following five minutes of occlusion of the lower limb distal to the measuring site, the same section of the brachial artery was imaged for an additional three minutes for evaluation of changes in diameter and blood flow (Medical Imaging Applications, LLC, Coralville, IA, USA; EchoPAC PC software, version 110.x.x, GE Healthcare, Horten, Norway). Shear stress following reactive hyperemia (SSRH) was calculated as *8 x mean velocity until peak FMD/(baseline diameter/10) (dynes/cm^2) until peak dilation*[22]. Flow-mediated dilation (FMD, % baseline) was assessed as *Δ-diameter/baseline diameter*100* and normalized for SSRH[22]. Microvascular function was evaluated offline (EchoPAC PC software, version 110.x.x, GE Healthcare) as the velocity-time integral (VTI, cm) of the outer envelope of the ultrasound flow profile of the first flow wave following the release of the occlusion cuff[23].

6.2.4 Measurements - *Phase II*

6.2.4.1 Hemodynamic measurements

Beat-to-beat blood pressure (Finometer Midi), heart rate (one-lead ECG, BioAmp), and brachial and carotid arterial blood flow (Vivid q, GE Healthcare) data were obtained throughout the six minutes of baseline and the following six minutes of either BT or CPT, and recorded in LabChart 8.0 (PowerLab, ADInstruments). Data recorded at baseline and 15-second averages for the first two minutes of hand submersion (BT and CPT) were used for analysis.

6.2.4.2 Arterial stiffness

Central (*c*PWV) and peripheral (*p*PWV) arterial stiffness was assessed as the PWV between the carotid – femoral and carotid – radial arterials, respectively, as outlined above. An average of the first minute at baseline, and 15-second averages for the first two minutes of hand submersion during the BT and CPT were used to evaluate peak response.

6.2.4.3 Blood flow and vascular conductance

Carotid and brachial arterial blood flow were evaluated and calculated as the average flow per second * 60 (8L-RS 4.0-13.0MHz probe, Vivid q, GE Healthcare; and EchoPAC PC software, version 110.x.x, GE Healthcare) at baseline and as 15-seconds average segments until two minutes into hand submersion for both BT and CPT. Vascular compliance was calculated as *blood flow/mean arterial pressure* (mL/min/mmHg).

6.2.5 Statistical analysis

All statistical analysis was performed using SPSS Statistical software v. 24.0.0.0 (IBM Corp™, Armonk, USA) and graphs made with SigmaPlot (version 13.0, Systat Softwear, Inc., San Jose, USA). Baseline characteristics and pulmonary function for controls and asthmatics were compared separately for Phase I and Phase II using Student's t-test (continuous variables) and chi-square tests (discrete variable). Vascular responses to salbutamol and placebo (factor 1: drug) for Phase I were compared between groups (factor 2: disease condition) before and after drug administration (repeated factor: time-point) using a three-way repeated measure ANOVA. Hemodynamic, brachial, and carotid responses during Phase II to the CPT or BT between control and asthma (factor 1: disease condition) were evaluated at baseline and in 15 second averages using a two-way repeated measure ANOVA. All statistical significance was set *a priori* to $p<0.05$. Values are expressed as mean ± standard deviation, unless otherwise indicated.

6.3 Results

Subject characteristics

As outlined in Table 1, the controls and asthmatics were well matched on baseline characteristics, including sex, age, height, and weight. Baseline spirometry values did not differ between controls and asthmatics in Phase I. In Phase II, there were no differences in FEV_1 or FVC (% predicted) between groups; however, the asthmatics in Phase II had a significantly lower FEV_1/FVC-ratio than controls.

Phase I

Hemodynamic responses to salbutamol

Following salbutamol inhalation, heart rate increased by 3.1 and 1.9 beats per minute in controls and asthmatics, respectively ($p<0.05$), but no differences were observed between groups and no increase in heart rate was seen with placebo. Blood pressure remained stable in both groups following both placebo and salbutamol (see Table 2).

Vascular responses to salbutamol

Inhalations of salbutamol resulted in a distinctive reduction in FMD in asthmatics but not controls (control: pre 7.0, post 7.5%; asthma: pre 6.1 to post 3.1%, $p<0.05$, Fig 1B), while no changes were seen in either group with placebo (control: pre 5.5, post 6.0%; asthma: pre 6.1, post 8.4%, Fig 1A). Similarly, asthmatics but not controls saw a significant increase in PWV with salbutamol (control: 8.1 to 8.0 m/s, asthma: 8.0 to 8.7 m/s, $p<0.05$, Fig 1D), while no changes were observed during the placebo (control: 7.5 to 7.5 m/s, asthma: 8.3 to 8.5 m/s, $p<0.05$, Fig 1C). When compared to placebo, salbutamol inhalation did not affect microvascular function, as assessed with VTI, in either asthmatics or controls (Fig. 1E & 1F). Individual data can be viewed in Appendix B.

Phase II

Hemodynamic responses to the CPT

There were no differences in HR, SBP, DBP, or MAP at baseline between asthmatics and controls on either the BT day or the CPT day. As seen in figure 3, during BT, SBP and MAP increased by approximately 10% and 5%, respectively, in the asthma group but not the control group ($p<0.05$ for between group effect). During the CPT, the HR and all measured BP parameters increased over

the first two minutes in a similar pattern in both asthmatics and controls (Fig. 2 and 3, right panel), but there were no between-groups effects in HR (p=0.343), SPB (p=0.925), DBP(p=0.608), or MAP (p=0.674).

Brachial, carotid and PWV responses to the CPT

Brachial flow and conductance, as well as *p*PWV, remained stable during BT in both controls and asthmatics (Fig. 4, left panel). During CPT, no significant changes were seen in brachial flow or conductance in either controls or asthmatics; however, *p*PWV increased in both asthmatics and controls over the first two minutes of the test ($p<0.05$ for time effect, Fig. 4f), with no between-group differences observed (p=0.898). As seen in figure 4, there were similarly no overall changes or between-group differences in carotid flow (p=0.905 for between-group effect) or conductance (p=0.904 for between group effect) during the BT or CPT. There was an increase in *c*PWV during the CPT, but not BT, in both controls and asthmatics, with no difference between controls and asthmatics ($p<0.05$ for time effect, p=0.656 for between-group effect).

6.4 Discussion

This study investigated the acute systemic vascular responses to a therapeutic dose of the β_2-agonist salbutamol among people with asthma who are regularly using β_2-agonists, and β_2-agonists naïve controls. The current findings indicate that the systemic vasculature in people with asthma respond differently to salbutamol than in controls, seen as an acute increase in arterial stiffness and an impairment in endothelial function 15 minutes following salbutamol administration. In the second phase of this study, the systemic vascular responses to a large sympathetic stimulus,

delivered via the cold pressor test, were evaluated in a subset of asthmatics and controls. There were no differences in the vasculature response to an elevation in sympathetic nervous activity between people with asthma and controls. These results suggest that differences in the vascular response to salbutamol are not due to increased neurovascular transduction in response to a given sympathetic outflow, but likely due to an increased sympathetic response to salbutamol in asthmatics.

CV disease in asthma has previously been linked to β_2-agonist usage[5, 11]. However, most studies linking asthma, β_2-agonist usage, and CV risk, have been observational, and thus, no cause and effect link has been established. A challenge in understanding the increased CV risk among asthmatics has been to separate medication usage as a risk factor from other features. For example, the use of β_2-agonists has also been associated with increased risk of asthma death[24], but since β_2-agonist-usage usually increases as asthma control decreases[10], the higher asthma mortality rates could be indicating poor asthma control rather than being a side-effect of β_2-agonist-usage. Similarly, the increased CV risk in asthma seen together with β_2-agonist-usage[5, 11] could be due to the reduce asthma control leading to increased β_2-agonist usage, as CV risk typically increases in association with reduced asthma control[2, 6, 7, 25]. However, the results from the current study show adverse changes in both arterial stiffness and endothelial function acutely following salbutamol administration in asthma, independent of changes in lung function. Therefore, the added strain of repeated salbutamol inhalations on the systemic vasculature may be of great clinical significance from a CV risk perspective, and may help explain the link between beta-agonist use and CV events[5, 11].

Numerous studies have examined the vascular responses to both intravenous[13] and inhaled[14-16] β_2-agonists, and the results typically depend on dosage and method of administration. Common responses to β_2-agonists include increased HR[15, 16], reduced vascular resistance[14, 16], but no changes in BP[14-16, 26]. In a previous study from our laboratory, people with no previous salbutamol exposure demonstrated a 23% increase in total muscle sympathetic nerve activity (MSNA) following salbutamol inhalation with concurrent changes in blood pressure[15]. These results indicate that salbutamol administration acutely increases sympathetic drive independently of baroreflex feedback. Increased sympathetic drive can affect vascular remodeling and tone[17], and therefore taking salbutamol regularly may contribute to the chronically elevated arterial stiffness[7, 25, 27] and reduced endothelial function[6] seen in asthma.

The density and sensitivity of the β_2-adrenoreceptor decreases within the airways with repeated agonist exposure[12, 28]. Therefore, it is plausible that modifications in receptor sensitivity and density also occur outside of the airways. Thus, the differences in systemic vascular function and stiffness between controls and asthmatics following salbutamol inhalation were hypothesized to be due to an altered vascular response to a given sympathetic stimulus (i.e. neurovascular transduction). To further investigate the mechanism(s) for the altered vascular responses to salbutamol in asthmatics in *Phase I* of the current study, a non-specific increase in sympathetic nervous activity, delivered by the CPT, was used to examine whether there was a divergent vascular response between asthmatics and controls. The CPT is known to result in a 30-35% increase in MSNA in health[19] and across diseases[29, 30], with the peak response typically seen within the first two minutes of hand submersion[31]. Contrary to our hypothesis, the vascular responses to the CPT during *Phase II* of the current study indicated that there were no differences in the vascular responses to the increase in sympathetic output, suggesting asthmatics maintain a healthy

neurovascular transduction to a given sympathetic stimulus. Thus, the increases in arterial stiffness and reduced endothelial function observed following β_2-agonist inhalation is asthmatics likely stems from differences upstream from the β_2-adrenoceptor-smooth muscle coupling, such as increased sympathoexcitation in response to salbutamol inhalation.

Limitations

While the MSNA responses to the cold pressor test have been shown to be similar in healthy individuals and in other diseased conditions[29, 30], it is to our knowledge unknown if MSNA increases in a comparable manner in asthma. Changes in heart rate variability (HRV) correlate well with MSNA during stable conditions in healthy people, but not during autonomic challenge tests such as the cold pressor test[19] and thus HRV was not used as a surrogate marker of MSNA in the current study. Further research is encouraged to directly measure MSNA in asthma both at rest and in response to salbutamol to further understand the increased CV risk in asthma.

As the first study to-date looking at the CV responses to a therapeutic dose of inhaled β_2-agonists in a population chronically exposed to β_2-agonists, this study would be consistent with epidemiology data that repeated and/or chronic exposure to salbutamol may be detrimental for CV health[32]. However, while the current study evaluated the acute systemic responses to salbutamol among a clinically representable sample of asthmatics, the exact history of previous salbutamol usage was not known. The study was not designed to further evaluate the different impact of length and/or intensity of salbutamol usage, and further research is needed to establish the direct impact of a given salbutamol exposure. Similarly, even though all participants were asked to withhold medications prior to each test day, this study did not control for the long-term use of other asthma medications and it is thus possible that previous medication usage may have influenced the

outcomes in the current study. Additionally, the inclusion criteria for asthmatics in the current study was, as mentioned, self-reported asthma with a history of salbutamol usage. While the emphasis was on previous salbutamol usage when recruiting asthmatics, it is possible that some of the individuals in the asthma groups may not demonstrate confirmed asthma according to current guidelines[10, 33].

The temperature of the water during the CPT or the body temperature day was not monitored with a thermometer, and it is likely that some variability in water temperature existed. While the increase in MSNA with the CPT is rather robust across disease states[29, 30], small changes in water temperatures can influence the sensation of pain[34], and the possible variability in water temperature between individuals could have increased the variability in responses and reduced the ability to detect a difference between asthmatics and controls.

6.5 Conclusions

Patients with asthma and chronic salbutamol use were found to have adverse systemic vascular responses to a therapeutic dose of salbutamol, as indicated by reduced vascular function and increased arterial stiffness. The differences in vascular responses to salbutamol between asthmatics and non-asthmatics were not explained by differences in the vascular responses to increased sympathetic output, suggesting the reason asthmatics respond differently to inhaled salbutamol is likely due to greater sympathoexcitation in response to salbutamol, rather than altered neurovascular transduction. The adverse vascular responses to salbutamol among asthmatics may contribute to the increased risk of CV diseases such as coronary heart disease, cerebrovascular

disease, and heart failure seen in asthma, and clinicians should be cautious of significant β_2-agonist use by their patients.

6.6 References

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6.7 Tables and figures

Table 6.1. Subject characteristics. Values are expressed as mean ± standard deviation.

	Phase I		*Phase II*	
	Control	Asthma	Control	Asthma
Sample size				
(male/female)	14 (7/7)	14 (7/7)	10 (4/6)	10 (5/5)
Age (years)	22.8 ± 3.0	23.1 ± 2.8	23.3 ± 3.0	25.9 ± 4.4
Height (m)	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
Weight (kg)	73.0 ± 13.0	73.8 ± 15.1	73.3 ± 15.7	80.4 ± 19.4
BMI (kg/m2)	24.1 ± 3.0	24.3 ± 3.1	24.3 ± 3.7	27.5 ± 5.3
Allergies (%)	50.0	78.6	20.0	80.0*
FEV_1 (% predicted)	102.1 ± 17.1	96.7 ± 12.8	104.7 ± 0.8	96.9 ± 14.2
FVC (% predicted)	102.6 ± 13.5	105.4 ± 15.0	106.2 ± 16.3	108.0 ± 10.7
FEV_1/FVC	83.0 ± 6.0	77.6 ± 10.1	82.7 ± 4.4	75.4 ± 7.9*
Ventolin usage				
Duration (years)	0.0 ± 0.0	13.1 ± 6.8*	0.0 ± 0.0	10.2 ± 5.3*
Frequency (puffs/week)	0.0 ± 0.0	2.8 ± 2.4*	0.0 ± 0.0	2.1 ± 1.2*

BMI: body mass index; FEV_1: forced expired volume in one second; FVC: forced vital capacity.

*$p<0.05$ between groups

Table 6.2. Hemodynamic responses to placebo and salbutamol among controls and asthmatics during Phase I. Values are expressed as mean ± standard deviation.

	Control				Asthma			
	Baseline 1	Placebo	Baseline 2	Salbutamol	Baseline 1	Placebo	Baseline 2	Salbutamol
HR (bpm)	60.2 ± 6.6	56.3 ± 6.8	61.7 ± 8.5	62.8 ± 9.7*	59.5 ± 8.8	56.4 ± 10.4	59.4 ± 8.8	60.3 ± 9.4*
SBP (mmHg)	106.2 ± 8.4	104.2 ± 9.1	106.8 ± 11.3	109.9 ± 11.6	108.0 ± 12.2	108.9 ± 11.0	106.8 ± 13.1	108.7 ± 12.2
DBP (mmHg)	64.6 ± 8.0	63.3 ± 6.3	64.8 ± 6.7	64.5 ± 6.4	67.0 ± 9.5	69.3 ± 6.3	67.0 ± 10.1	67.7 ± 7.2
MAP (mmHg)	78.4 ± 7.6	76.9 ± 6.6	78.8 ± 6.8	79.6 ± 7.0	80.7 ± 9.8	82.5 ± 7.3	80.2 ± 10.7	81.3 ± 807
PP (mmHg)	41.6 ± 6.5	40.9 ± 6.7	42.0 ± 10.9	45.3 ± 10.4	41.0 ± 8.0	39.7 ± 8.0	39.8 ± 6.9	41.0 ± 6.4

HR: heart rate; bpm: beats per minute; SBP: systolic blood pressure; mmHg: millimeter mercury; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure.

*$p<0.05$ for drug*time interaction

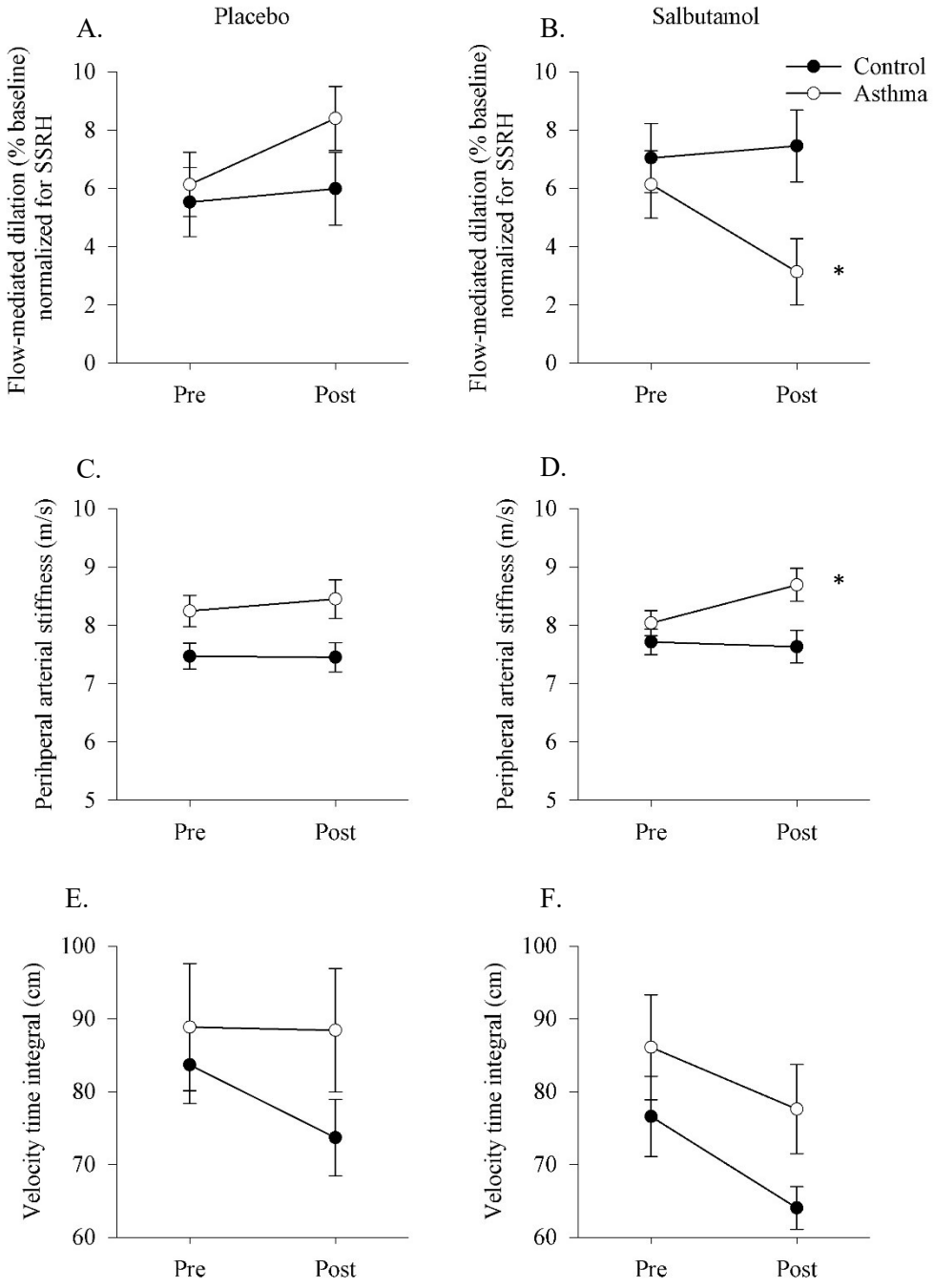

Figure 6.1. Vascular responses to placebo and salbutamol in control and asthma. SSRH: shear stress following reactive hyperemia. Graphs display mean ± standard error of mean.

*$p<0.05$ for interaction between groups and drug conditions.

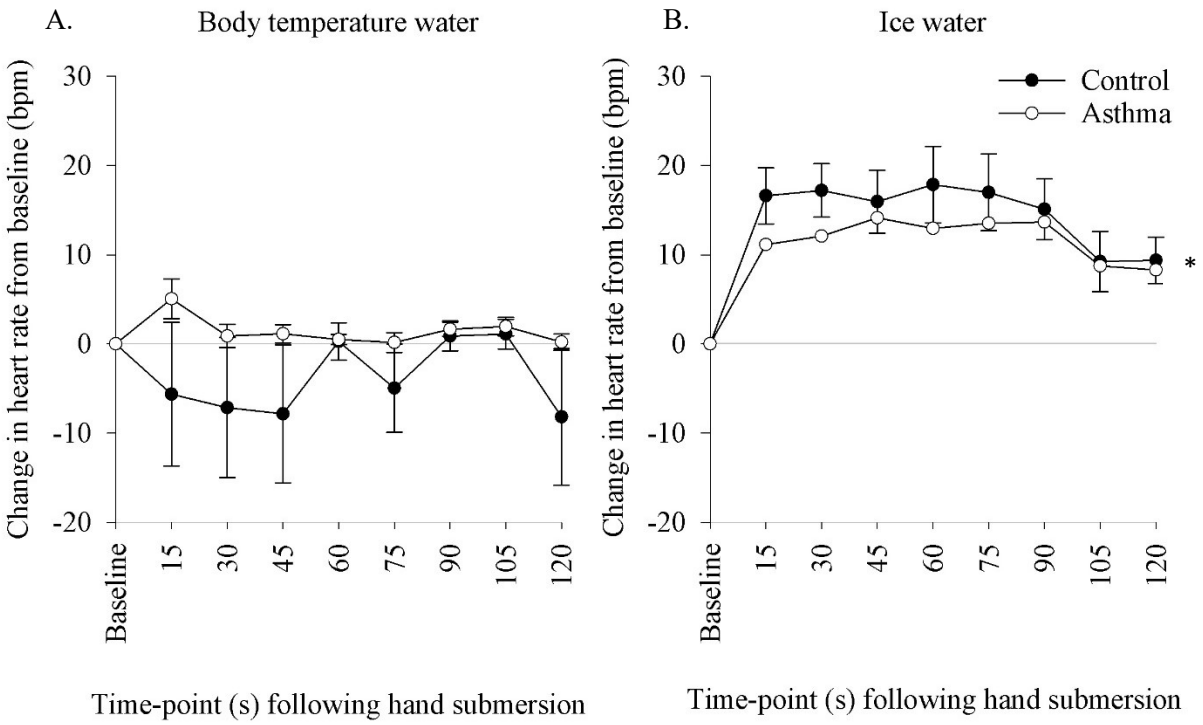

Figure 6.2. Heart rate responses to body temperature water (A) and ice water (B) hand submersion in control and asthma.

*$p<0.05$ for main effect for time independent of disease condition.

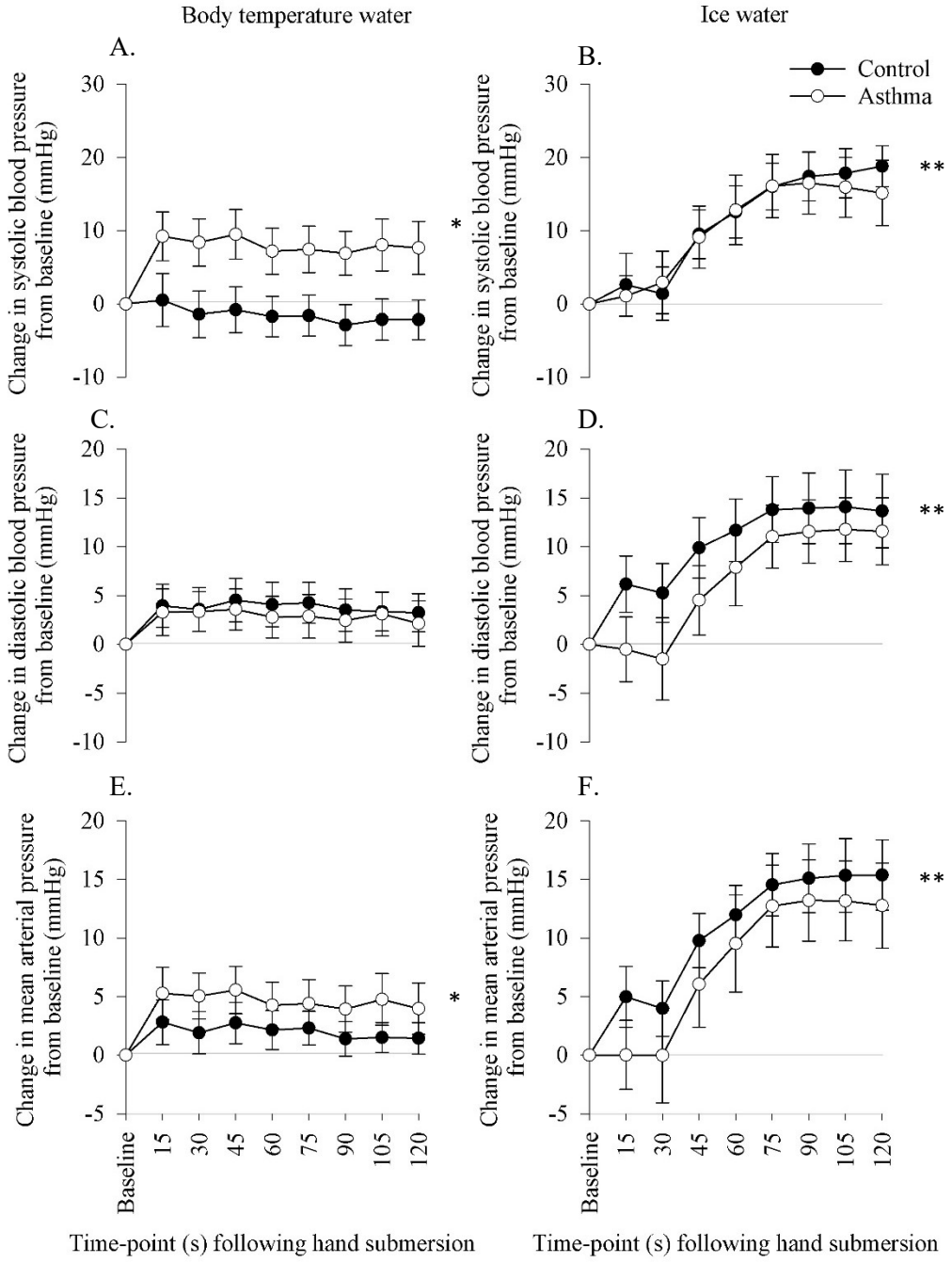

Figure 6.3. Hemodynamic responses during body temperature (BT) hand submersion (control condition (left panel) and the CPT (right panel). Data displayed as mean ± SEM.

*p<0.05 for main group effect, **p<0.05 for main time effect.

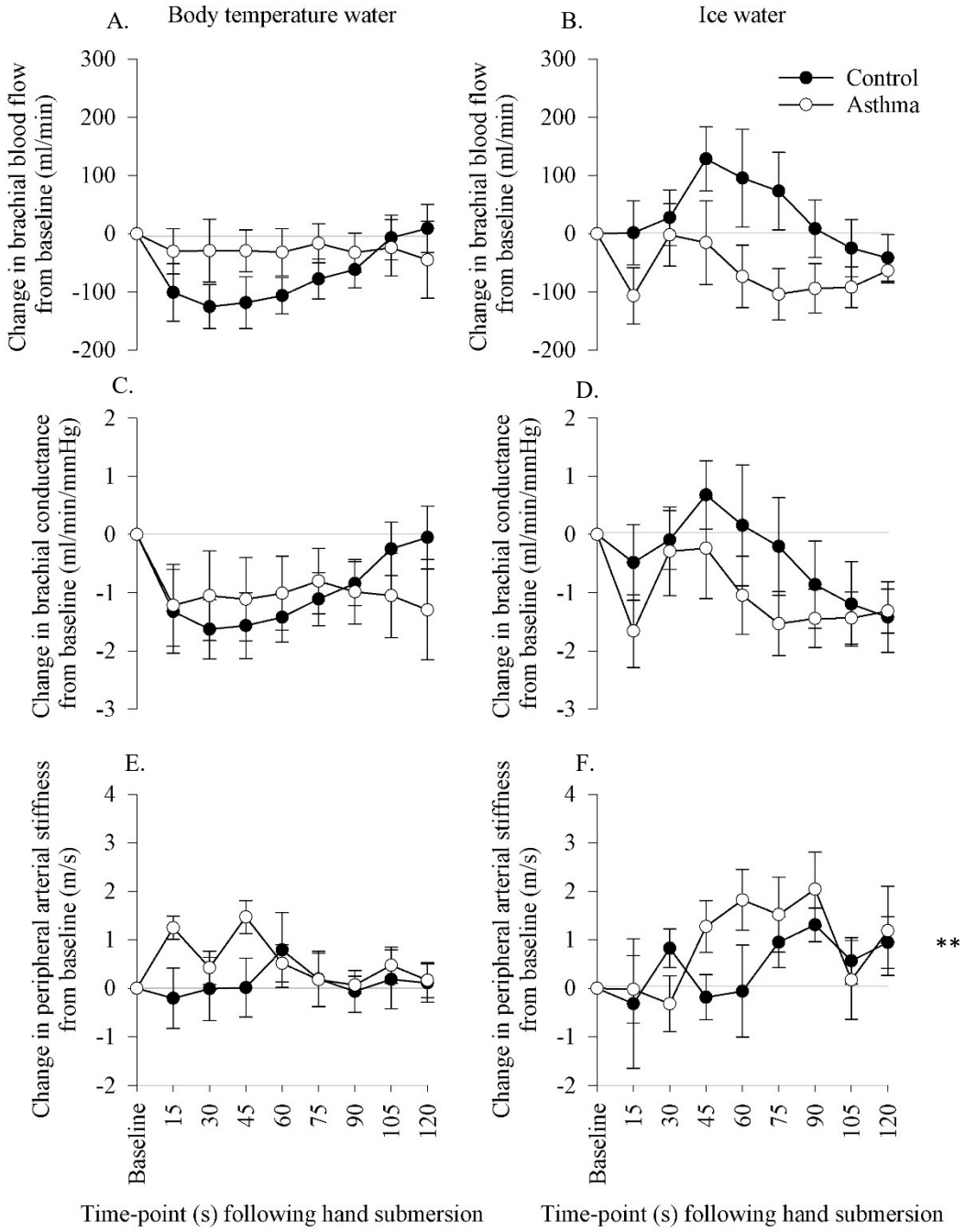

Figure 6.4. Brachial responses during body temperature (BT) hand submersion (left panel) and the CPT (right panel). Data displayed as mean ± SEM.

**p<0.05 for main time effect.

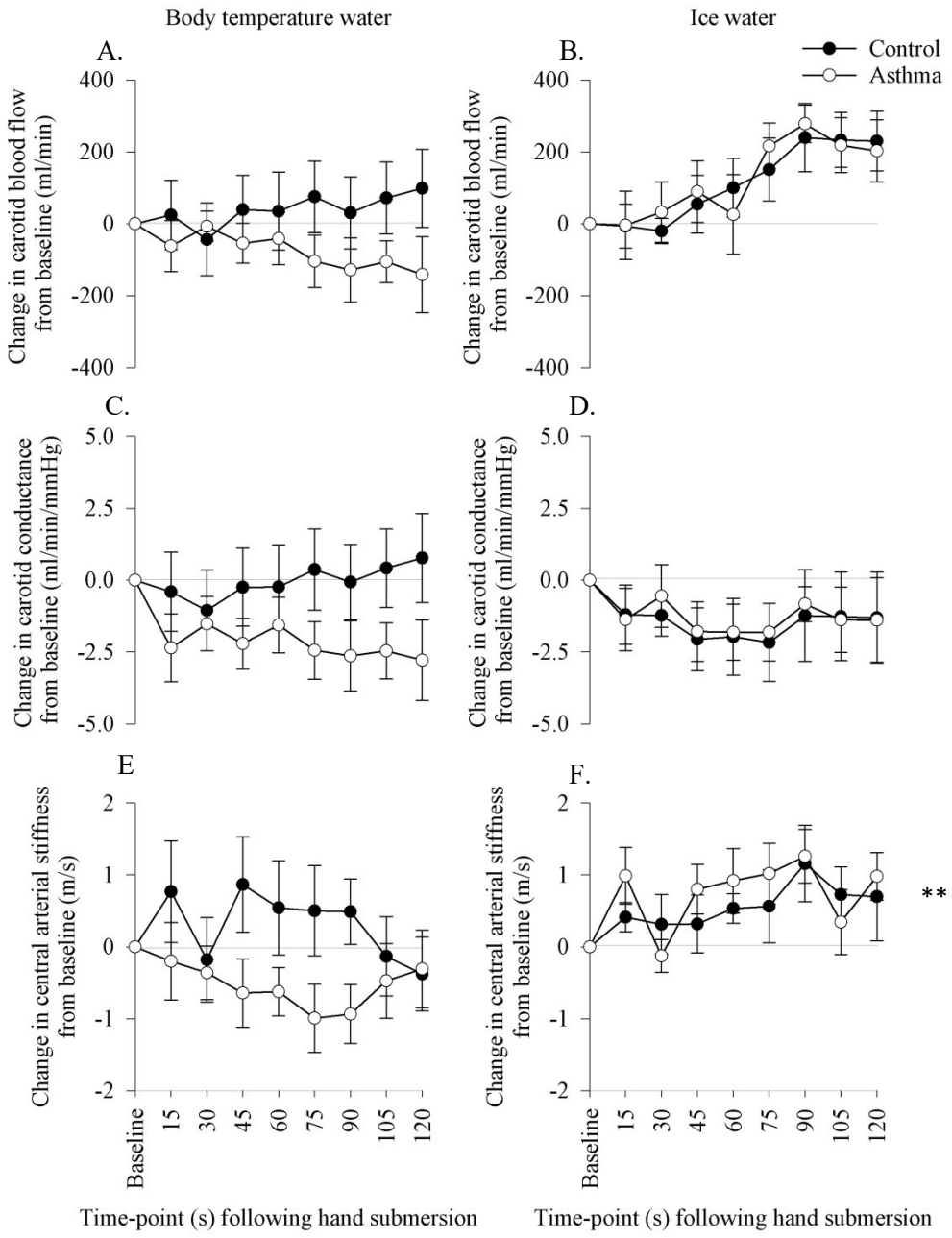

Figure 6.5. Carotid responses during body temperature (BT) hand submersion (left panel) and the CPT (right panel).

**p<0.05 for main time effect.

7 CHAPTER VII

General Discussion

7.1 Dissertation overview

This dissertation was undertaken to expand on the knowledge of the physiology underlying the increased risk of CV diseases such as coronary heart disease, cerebrovascular disease, and heart failure seen in asthma[1-9]. Three primary areas which may influence CV risk in asthma were identified: physical inactivity; medication usage; and asthma exacerbations (see Flowchart, Figure 7.1). To bridge the knowledge-gap in these three areas, project one (Chapter III) examined the impact of physical activity and fitness on markers of CV disease in controlled asthma. Further, project two (Chapter IV) investigated the physiological reasons to why asthmatics experience elevated exertional dyspnea, which has been identified as one of the main barriers to physical activity in asthma[10, 11]. Project three (Chapter V) and project four (Chapter VI) of this dissertation evaluated two distinct aspects of what happens during an asthma exacerbation; the impact of elevated pulmonary inflammation and bronchoconstriction on CV health, and CV side-effects of the commonly prescribed short-acting beta-2(β_2)-agonists reliever medication salbutamol.

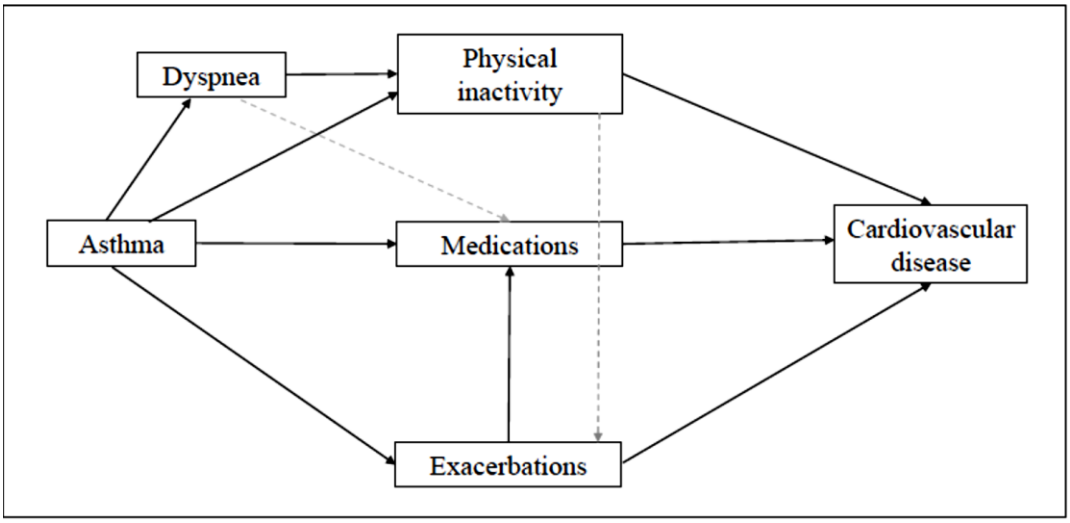

Figure 7.1. Flowchart of asthma-CV disease pathways.

7.2 Physical activity avoidance in asthma – its physiological causes, and effects on CV health

Chapter III of this dissertation evaluated the influence of physical activity and fitness levels on CV health in controlled asthma. Inactivity and low cardiopulmonary fitness are both known to be harmful for CV health[12, 13]. Asthmatics report being less physically active than their age-matched counterparts[14, 15], and it has thus been unknown if the increased CV risk seen in asthma is due to low-physical activity-lifestyle choices or the asthma itself. The results from Chapter III suggests that physical inactivity and reduced fitness levels do indeed account for some, but not all, of the increased CV risk in asthma. Endothelial function evaluated as FMD and normalized for shear stress (Appendix A), microvascular function (VTI) (Appendix A), and arterial stiffness (PWV) (Appendix A) have all been shown to be predictive of future risk of CV disease in otherwise healthy populations[16-18], and were used to evaluate future CV risk in this project. When fitness and physical activity-matched asthmatics and non-asthmatics were compared, there were no differences in FMD or VTI; however, PWV was elevated by 1.4 m/s in the asthmatics compared to controls (Figure 3.1, Chapter III). Research looking at the predictive value of PWV suggest each increment of 1m/s in PWV to correspond to a 15% increase in CV risk[19]. According to the results from Chapter III, people with mild and controlled asthma are at 21% increased risk of CV disease compared to non-asthmatics matched for fitness and physical activity levels.

The discrepancy in markers of CV prediction between the measures of vascular function and arterial stiffness seen in Chapter III may be explained by the difference in the type of vascular evaluation, and how they are regulated. While endothelial function and microvascular function both evaluate the *function* of the blood vessel, arterial stiffness is used to evaluate vascular *structure*[20]. The function of a blood vessel depends on the regulation and diffusion of nitric oxide

from the endothelium to the vascular smooth muscles, while the structure of the arteries reflects both short-term sympathetic regulation of vascular tone, and chronic adaptations of the elastin-collagen composition of the vascular wall, and in sections of bifurcations, the development of atherosclerotic plaque-buildup. The elevated PWV in asthmatics could be reflective of long-term changes following years of varying degrees of asthma control and/or medication usage. Furthermore, the CV benefits stemming from physical activity and fitness levels may lay in their ability to reduce systemic inflammation[21], which is a known modulator of endothelial function[22, 23]. At the time of measurement, the asthmatics did not show signs of elevated systemic inflammation compared to physical activity and fitness-matched controls, and with no downstream differences in endothelial function. Furthermore, as levels of systemic inflammation tend to be higher in more severe asthma[24, 25], it is also possible that the well-controlled asthmatics sampled in Chapter III had normal systemic inflammation, and subsequently normal vascular function. Had a sample of uncontrolled asthmatics been tested, larger baseline discrepancies in systemic inflammation may have existed, and potentially larger downstream differences in vascular function and CV risk. Thus, to fully understand the influence of fitness and physical activity on long-term CV risk in asthma, future research should examine uncontrolled asthmatics.

Dyspnea is believed to be one of the main reasons asthmatics avoid moderate and high intensity physical activity[10, 11], which is a contributor to overall lower physical activity levels among asthmatics than their age-matched counterparts[14, 15]. Resting studies have found that the sensation of dyspnea during bronchoconstriction in asthma to be related to IC and inspiratory muscle effort[26], but the physiological reason for exertional dyspnea in asthma has been unknown until now. In Chapter IV of this dissertation, the degree of dyspnea was evaluated during incremental exercise in asthmatics and controls, and plotted against different measures of operating

lung volumes. Asthmatics were breathing at higher lung volumes at rest and throughout exercise than controls, placing the inspiratory reserve volume closer to the total lung capacity at any given workload. With high operating lung volumes, asthmatics likely had a shift in the relationship between neural drive and lung volume expansion, causing greater dyspnea. A secondary purpose of Chapter IV was to assess the impact of the commonly prescribed β_2-agonist salbutamol on the sensation of dyspnea in asthma; however, no differences in either dyspnea or operating lung volumes during exercise were observed following β_2-agonist. To minimize breathing discomfort associated with dyspnea, and thus removing a major barrier to physical activity, future research is encouraged to evaluate how IRV can be normalized during exercise in asthma.

7.3 Asthma exacerbations and medication usage

Vascular and inflammatory markers predict elevated CV risk in severe asthma[24, 25, 27-30]. Thus, Chapter V and Chapter VI of this dissertation evaluated the CV impact of two distinct features stemming from reduced asthma control; the impact of pulmonary inflammation induced by the mannitol challenge and bronchoconstriction stemming from the methacholine challenge on CV health, and CV side-effects of the β_2-agonist salbutamol. Previous animal studies have shown that in mice-models of allergic asthma, systemic vascular function is compromised following pulmonary allergic challenges[31]. Additionally, when exposing mice to inhaled small particulate matter, both pulmonary and systemic inflammation increases, and systemic vascular function is impaired[32]. However, it is unknown whether a similar link exists between pulmonary inflammation, systemic inflammation, and vascular dysfunction in humans. In Chapter V of this dissertation, asthmatics were subjected to mannitol and methacholine inhalation challenges at a

minimum of one week apart, and the impact of induced by the mannitol challenge and bronchoconstriction induced by the methacholine challenge on the systemic inflammatory marker CRP, endothelial function, and arterial stiffness were assessed. While the degree of bronchoconstriction was larger following the methacholine challenges (average maximal drop in FEV_1 of 19.3 vs. 11.5 %baseline for methacholine and mannitol, respectively, $p<0.05$), only the mannitol challenge led to increases in systemic inflammation (change in CRP from baseline following mannitol: 60.4%, and methacholine: -20.6%, $p<0.05$). Two previous studies have looked at how increased systemic inflammation changes vascular function[22] and arterial stiffness[33] in otherwise healthy people. Both studies used salmonella typhi vaccine to induce systemic inflammation, and an increase in systemic inflammation was noted at eight hours following vaccination in both studies. At the same time-point, endothelial function was reduced and arterial stiffness increased, and strong correlations were seen between PWV and changes in both CRP and IL-6. In Chapter V of this dissertation, a greater increase in systemic CRP levels was seen following mannitol inhalation than what was seen in previous vaccine-studies, and it is thus surprising no change in either FMD, peripheral PWV, or central PWV were observed. The time-point of measurements was however different between the studies. While the previous studies looked at changes that occurred at eight and 32 hours post-vaccination, we evaluated systemic responses immediately (within one hour) following the mannitol challenge. Hence, it is possible that a longer time-course is required for the interaction between CRP and PWV to cause detectable changes.

During naturally occurring asthma exacerbations, the increase in serum CRP is substantially larger than what was seen both in healthy people following salmonella typhi vaccine and following the mannitol inhalation challenge in asthmatics in Chapter V of this dissertation.

Mak et al.,[24] reported mean plasma CRP values to be approximately 6 mg/L during asthma exacerbations, which is three times as high as during remission. Based on the link seen in Chapter V between pulmonary inflammation and systemic changes in CRP, and the vascular changes previously seen in otherwise healthy people in response to similar increases in systemic inflammation[22, 33], it is plausible that a large attenuation of vascular function and increases in arterial stiffness can be seen during naturally occurring asthma exacerbations, but the inflammatory insult in Chapter V was inadequate to inflict changes of this magnitude.

Increased CV risk in more severe asthma may stem from a side-effect of commonly taken asthma medications[2, 34]. One of the most commonly prescribed reliever medication for patients with asthma is the short-acting β_2-agonist salbutamol[35]. While no vascular changes were seen with acutely reduced asthma control in Chapter V, the results from Chapter VI suggest that salbutamol increases arterial stiffness and impairs endothelial function acutely after inhalation in asthmatics. This seems to occur in the absence of differences in neurovascular transduction between asthmatics and controls, which indicates that salbutamol likely increases muscle sympathetic nerve activity in people with asthma to a greater extent than in non-asthmatics.

As mentioned above, an increase in PWV of 1 m/s is predicted to increase CV disease risk by 15% in a linear manner across the physiological range[19]. Similarly, 1% reduction in FMD corresponds to 7-12% increased risk of CV disease[18]. Based on the data from Chapter VI, which shows an acute increase of 0.66 m/s in PWV and 3% baseline reduction in FMD, asthmatics who take salbutamol to reduce their asthma symptoms would have a substantial acute increase in CV risk. Furthermore, reviewing the results from Chapter V, asthmatics with uncontrolled asthma are already likely at increased risk of CV disease based on the acute increases in systemic

inflammation, and adding additional strain on the CV system with the use of salbutamol may explain why β_2-agonists have been suggested to contribute to CV events in asthma[34]. While it has been argued that respiratory infections themselves are associated with increased risk of CV events[36] and β_2-agonist usage would simply be an indicator of less controlled asthma, the combined results from Chapter V and Chapter VI demonstrate that CV risk in asthma likely stems from a combination of both changes in asthma control and as a CV side-effects of salbutamol usage.

7.4 Clinical implications and future research

The results from the first part of this dissertation suggest that people with controlled asthma have similar vascular function as their fitness and physical activity-matched counterparts; thus, remaining active is thus of foremost importance for CV health in asthma. Additionally, research studies have found that exercise interventions reduce pulmonary inflammation and improves asthma control[37, 38]. The results from Chapter V indicate that there is a link between the and systemic inflammation, which is known to be detrimental for CV health[23, 33, 39, 40]. Thus, increased levels of physical activity may, over time, translate to a significant reduction in CV risk in asthma due to both the overall anti-inflammatory effects of physical activity[21] as well as the specific beneficial influences of physical activity on pulmonary inflammation and asthma control. Further research is needed to evaluate the long-term influences of physical activity and exercise training on CV health in asthma, and the importance of physical activity should be further enhanced in asthma management guidelines.

One of the main barriers to physical activity in asthma is the fear of exercise-induced bronchoconstriction, which is perceived as an intensified sensation of dyspnea. While the risk of

severe asthma exacerbations during exercise has been shown to be minimal with proper disease management and pre-exercise warmup routines[41, 42], the findings from Chapter IV of this dissertation revealed that asthmatics do indeed experience intensified dyspnea at a given submaximal workload, and that there are important physiological differences in operating lung volumes during exercise between asthmatics and non-asthmatics. The sensation of dyspnea in asthma seems to be due to *inspiratory limitations* such as reduced IRV, which is secondary to an upward shift in operating lung volumes during exercise in asthma. To reduce exertional dyspnea, and remove an important barrier to physical activity in asthma, and enhance CV health, research should further explore how operating lung volumes can be normalized in asthma during physical activity.

Numerous studies indicate that maintained baseline asthma control is of foremost importance for CV health[3, 7, 24, 25, 27-30, 36], which is strongly supported by the results from Chapter V and Chapter VI of this dissertation. While current asthma management guidelines do indeed specify asthma control to be of importance[35], extra caution is recommended in the liberal prescription and usage of β_2-agonists. Other kinds of medications, such as inhaled corticosteroids, have also been linked to increased risk of CV disease in asthma[5], and further research is needed to evaluate CV side-effects of such medications.

7.5 Summary and conclusion

People with asthma are at increased risk of developing CV diseases such as coronary heart disease, cerebrovascular disease, and heart failure compared to their age, sex, and BMI-matched counterparts[1-9], but the reasons for this are unknown. Thus, three fundamental areas important for

CV health in asthma were identified: physical inactivity, medication usage, and asthma exacerbations (Figure 7.1).

People with asthma are generally less physically active than their age-matched counterparts[14, 15], which we in Chapter III found to explain some, but not all, of the increased risk of CV disease in asthma. Furthermore, one of the main reasons asthmatics avoid moderate and intense physical activity is a fear of intensified asthma symptoms such as dyspnea[10, 11]. In Chapter IV, we found that the reason people with asthma experience intensified exertional dyspnea is due to an upward shift in operating lung volume, resulting in a high IRV which explained the intensified dyspnea.

As markers of CV risk are elevated in severe and uncontrolled asthma[25, 27-30, 34], the second part of this dissertation was designed to look at the physiological reasons for this, and examining the impact of the commonly prescribed β_2-agonist salbutamol. In Chapter V, mannitol and methacholine were used to induce pulmonary inflammation and bronchoconstriction, respectively, and systemic CRP levels, vascular function, and arterial stiffness were evaluated at baseline and within an hour of the end of each challenge. While the methacholine challenge led to a larger mean reduction in lung function, only the mannitol challenge led to an increase in systemic CRP. This indicates that pulmonary inflammation induced by the mannitol challenge, but not bronchoconstriction alone, may cause systemic inflammation. Neither challenges resulted in changes in measured vascular parameters compared to a placebo challenge; however, the magnitude the increase in systemic CRP induced with mannitol was smaller than what has been seen previously during naturally occurring asthma exacerbations[24], where a larger impact on CV health would be expected. Additionally, the result from Chapter VI suggest that asthmatics who

use salbutamol to reduce asthma symptoms significantly elevate their arterial stiffness, and reduce their endothelial function, which may add strain to an already stressed CV system.

The results from this dissertation suggest the combined importance of a physically active lifestyle and maintained asthma control. Our results suggest that asthmatics do experience intensified dyspnea during exercise, which appears to be secondary to increased operating lung volumes. However, the risk of severe exercise induced bronchoconstriction is low with proper warm-up routines[41, 42], and physical activity is beneficial both for reducing pulmonary inflammation and stabilizing asthma control[37, 38]. To reduce pulmonary and systemic inflammation, and subsequently the prevalence of asthma exacerbations and the need to use medications containing β_2-agonists, the importance of physical activity should be further encouraged in asthma management as a mean to maintain CV health.

7.6 References

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9 Appendix A: Detailed Methods

Confirmation of asthma

Evaluation of reversibility – All spirometry testing was done at the Pulmonary Function Laboratory at Edmonton Clinic. Prior to the test, the system used for spirometry testing (Vmax, CareFusion, Yorba Linda, CA, USA) was calibrated for flow and volume according to standard calibration instructions provided by the manufacture. The subjects were instructed on how the test was performed and were then connected to the Vmax system by a mouthpiece. Nasal flow was occluded using a nose clip. Extra precaution was made to confirm that there was no air leakage between the subject and the system, and to make sure the subjects tongue did not block the air flow going through the mouthpiece. All subjects were asked to withhold short-acting asthma medications for a minimum of 4 hours, and long acting bronchodilators for a minimum of 24 hours, prior to testing[1].

After breathing normally into the mouthpiece for a few breaths, the subject was instructed to take a deep inhalation to fill the lungs until reaching total lung capacity (TLC). When TLC was reached (based on the subjects' inability to inspire more air), the subject exhaled forcefully through the mouthpiece for $\geq$ 6 seconds or until residual volume (RV; when the subject cannot expire further) had been reached. Forced vital capacity (FVC) is the total volume of exhaled air that can be forcefully exhaled following a full inspiration, and the one-second forced expiratory volume (FEV_1) was determined by the volume expired within first second of exhalation.

For an acceptable maneuver, the following criteria had to be met: 1) no signs of hesitation during the start of the exhalation, 2) a sharp peak achieved in peak expiratory flow (PEF), 3) no evidence of a second breath within the maneuver, 4) no coughing or Valsalva maneuver, 5) no leak or obstruction in the equipment, and 6) the exhalation must last for $\geq$ 6 seconds[1]. A minimum of 3 satisfactory maneuvers were required for a test to be acceptable. Additionally, the difference

between the largest and the second largest value in FVC had to be ≤ 0.150 L and the difference in FEV_1 ≤ 150 L in order for a test to be considered acceptable. In case the subject felt dizziness during the test, the maneuver was stopped and the subject allowed to rest until being able to proceed with the test without discomfort[1].

Airway reversibility was evaluated following the completion of 3 acceptable spirometry maneuvers by the use of a short-acting β-agonist; the subject inhaled a total of 400µg (4 x 100µg delivered in 30 seconds intervals), using a spacer during a deep inhalation. Immediately following each inhalation, the subject held his/her breath for 10 seconds before exhalation. Another set of three FVC maneuvers was acquired between 10 to 15 minutes following the last inhalation of the β-agonist[1, 2].

For baseline values, the largest values in FVC and FEV_1 were chosen among all acceptable maneuvers before inhalation of β-agonist. Where FVC and the vital capacity (VC) were not compatible, the highest values were reported and used to calculate the FEV_1/FVC or FEV_1/VC ratio. Lung volumes (i.e. FEV_1, FVC and VC) were expressed in both absolute values (L) and as a percentage of reference value (% ref.). The FEV_1/FVC or FEV_1/VC ratio was reported as a percentage (%) of age-predicted normative data[2]. FVC, VC, and FEV_1 values obtained following inhalation of the short-acting β-agonist were compared to baseline values, and airway reversibility was defined as ≥ 12 % and ≥ 200 mL improvement in FEV_1 post bronchodilator[2, 3].

Airway hyperresponsiveness - The methacholine challenge has been shown to be a valid approach to test airway hyperresponsiveness[4] and is widely used as a diagnostic tool when asthma is considered[5]. In cases where there were strong clinical indications of asthma, but airway

reversibility testing failed to yield positive results, subjects were asked to come in on a separate day for assessment of airway hyperresponsiveness. The specificity of methacholine challenges has been shown to be high[4], thus, in order to confirm the absence of airway hyperresponsiveness in non-asthmatic control subjects, in Chapter III, all control subjects did a standard methacholine challenge on a separate day from the spirometry reversibility test.

Before each test, the subjects were asked to withhold all short-acting asthma medications for a minimum of 8 hours, and long acting medications for a minimum of 48 hours, as well as caffeine, chocolate, and nicotine. Contraindications for methacholine challenge are CV disease, hypertension, low baseline FEV_1 (<1.5 L or <70 % ref. based on previous reversibility test) and pregnancy and/or breast feeding[5].

Baseline FEV_1 values were obtained to calculate the targeted minimal provocative concentration for a positive result yielding a reduction in FEV_1 of >20% (PC_{20}), according to previously described protocol for baseline spirometry FEV_1 values. The percent change in FEV_1 required for PC_{20} were calculated accordingly:

$$20\% \text{ reduction in FEV1} = \frac{Highest\ baseline\ FEV1 - Highest\ post\ mechacholine\ FEV1}{Highest\ baseline\ FEV1} * 100$$

The subject breathed normally for 2 minutes through a mouthpiece while the nose was occluded with a nose clip. Methacholine powder, diluted in sterile saline at concentrations of 0.031, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, and 16 mg/mL, was then administered with a nebulizer according to a 2-minute tidal breathing protocol[5] where the subject performed a FEV_1 test 30 and 90 seconds after each 2 minutes of methacholine at incremental concentrations. The test ended when PC_{20} was

reached or when the highest concentration of methacholine (16 mg/mL) was given. In the event of bronchoconstriction, a bronchodilator was being administered. The exact PC_{20} was estimated according to the following equation[5]:

$$\text{PC20} = \text{antilog}[\log C1 + \frac{(\log C2 - \log C1)(20 - R1)}{R2 - R1}]$$

where C1 is the known methacholine concentration inhaled before PC_{20} was obtained, C2 is the known concentration of methacholine at with PC_{20} has been reached, R1 represent the percent fall in FEV_1 at C1, and R2 the percent the fall in FEV_1 after C2. A spirometry test was performed 10 minutes following the end of the methacholine challenge to ensure proper restoration of spirometry. The result from the methacholine challenge was considered positive if PC_{20} is <4 mg/mL. PC_{20} values between 4 and 16 mg/mL were considered borderline indicative of asthma. If PC_{20} had not occurred before the end of the test, PC_{20} was reported as PC_{20}>16 mg/mL and the result considered negative[6].

Exercise challenge - An exercise challenge was only performed in order to confirm asthma among subjects where exercised induced bronchoconstriction (EIB) had been suggested, but documentation confirming EIB is older than 1 year, and the reversibility test and methacholine challenge failed to confirm the diagnosis of asthma. Non-asthmatic control subjects, defined as absence of reversibility and PC_{20} > 16 mg/mL during previous testing, were *not* be asked to perform an exercise challenge unless there were specific indications suggesting potential EIB.

Prior to the exercise challenge, height and weight of the subject were measured, and the subject was informed on how to perform the test. Baseline 12-lead ECG reading, pulse oximetry,

and brachial blood pressure were evaluated to screen for cardiac contraindications to exercise challenge testing. Baseline spirometry was performed and baseline FEV_1 values was used as a reference for the spirometry tests following the exercise protocol.

The exercise challenge was performed on an electronically braked cycle ergometer (Ergoselect 200, Ergoline, Bitz, Germany) at a pedaling cadence of 60-70 revolutions per minute. The subject was connected to the metabolic system (Vmax 2400, CareFusion, Yorba Linda, CA, USA) through a mouthpiece and recordings for expired oxygen, carbon dioxide, and ventilation were obtained at baseline and throughout the test. The workload was adjusted so 85-90% of predicted maximum heart rate (estimation based on 220 - age in years[5]) was reached within 2-4 minutes. Once the target heart rate was reached, the workload remained constant for at least 4 minutes, allowing for a maximum exercise time of 6-8 minutes[5].

Following the exercise protocol, the subject performed spirometry at 3, 5, 10, 15 and 20 minutes. If a significant ($\geq$ 10 %) reduction in FEV_1 was seen, the subject was given a bronchodilator and the test ended. Spirometry was performed again 15 minutes after the administration of bronchodilator to ensure the lung function was restored to within 5% of baseline values before the subject was allowed to leave. EIB was confirmed based on asthmatic symptoms and $\geq$ 10% reduction in FEV_1 within 20 minutes post-exercise[5, 6].

Arterial stiffness

Increased arterial stiffness, measured by pulse wave velocity (PWV), has been shown to be a strong independent risk factor for CVD[7], and has previously been shown to correlate to lung function in

asthmatics[8]. Arterial stiffness is indicative of *arterial structure*, and is hence closely related to vascular function[9].

PWV was expressed in m/s and calculated based on the distances between measuring sites and the changes in time between recorded pulse waves. The gold standard for assessing arterial stiffness using PWV has been the time of the up-stroke from the pulse wave between carotid and the femoral arteries[10]. Carotid-radial PWV has the advantages over the carotid-femoral PWV in the sense that it is more easily accessible and does not cause discomfort to the subject. Therefore, carotid-radial PWV was used to assess arterial stiffness for the purpose of the project in Chapter III. In Chapter V and Chapter VI, both carotid-femoral PWV and carotid-radial PWV was evaluated.

PWV was recorded following 10 minutes of supine rest, using applanation tonometry. The carotid, femoral, and radial pulse were obtained, whereby PWV was recorded. Approximately 30 waveforms were recorded from each subject. Out of the PWV waveforms recorded, 10 consecutive waveforms were selected for analysis (LabChart version 7.3.5 ADInstruments). Selection of events were based on the up-strokes of the waveforms recorded at the carotid, femoral, and radial arteries. To minimize potential errors occurring during the analysis, each waveform was controlled manually to ensure the set threshold correctly enabled measurements of the preferred time-point during the up-stroke. A timestamp for each waveform was added to the data pad within LabChart, and all values were then exported to excel (Microsoft Office 2010). The distance between measuring points was divided by the average difference in time between corresponding carotid-femoral and carotid-radial waveforms, and used for assessment of arterial stiffness[10].

Endothelial function

For the purpose of this dissertation, brachial FMD was assessed by longitudinal B-mode ultrasound imagining (8L-RS 4.0-13.0MHz probe, Vivid q, GE Healthcare, Mississauga, ON) and analyzed using FDA approved software available from Medical Imaging Applications, LLC (Coralville, IA, USA). Resting baseline images of the brachial artery diameter were captured after 10 minutes of supine rest. RH was stimulated by 5 minutes of supra-arterial BP occlusion of the forearm. In healthy subjects, peak brachial dilation after 5 minutes of forearm occlusion occurs within 1 minute[11]; however, it has been reported that in subjects with vascular disease there can be a delay in peak dilatory response[12]. Hence, ultrasound images of the brachial artery diameter were recorded for 3 minutes following RH and analyzed using FDA approved software available from Medical Imaging Applications, LLC (Coralville, IA, USA) for assessment of FMD. FMD was calculated accordingly:

$$\text{FMD} = \frac{Dpeak - Dbaseline}{Dbaseline} * 100,$$

where *Dpeak* was the greatest measured increase in brachial artery diameters following cuff inflation, and *Dbaseline* the brachial artery diameter measured before occlusion[13-15]. Peak hyperemic brachial arterial velocity were measured using Doppler ultrasound immediately after the release of 5 minutes of forearm occlusion. The velocity time integral (VTI) is important in vascular-based prediction of CVD[16]. For this study, VTI was measured from of the first waveform following cuff inflation, and used to calculate VRH accordingly[16]:

$$\text{VRH} = \frac{VTI * heart\ rate}{60}$$

where 60 is a constant used for conversion of seconds to minutes (mL/min). Since SSRH has been shown to be important as a regulator of FMD response[18] as well as a marker of cardiovascular risk[16], SSRH was evaluated and calculated accordingly:

$$\text{SSRH} = \frac{8 * 0.035 * VRH}{baseline\ diameter/10}$$

While FMD is a measurement of conduit artery function in response to SSRH, VRH and VTI are indicators of microvascular dilation[17]; a healthy microvasculature will respond to brachial artery occlusion by vasodilation, leading to decreased vascular resistance. Hence, increased flow during reactive hyperemia (*e.g.* high VTI and/or VRH) is indicative of a properly functioning microvasculature.

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10 Appendix B: Individual data, Chapter VI

Acute effects of salbutamol on systemic vascular function in asthma

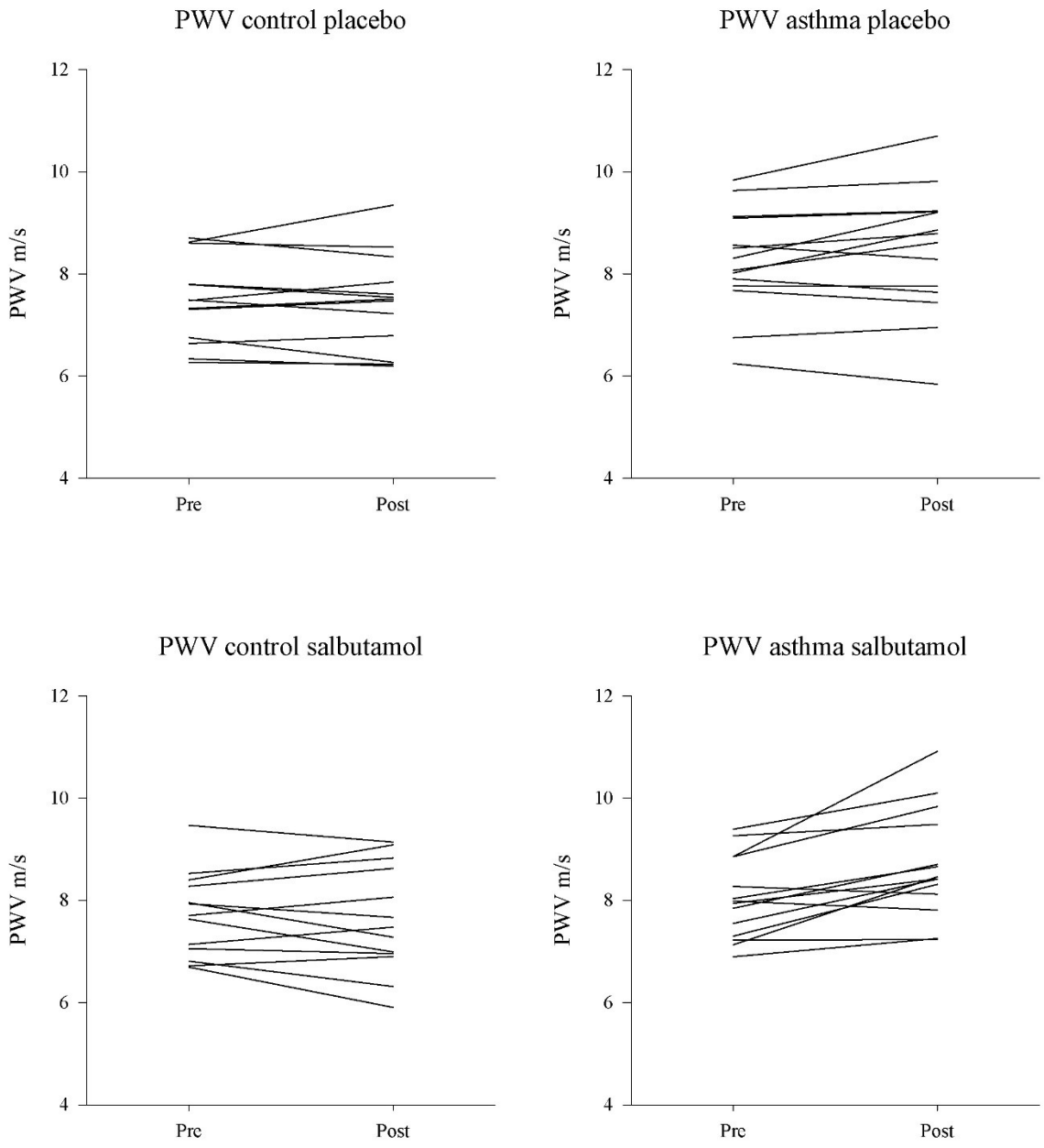

Figure B1. Individual changes in pulse wave velocity (PWV) in controls (left) and asthma (right) before and after placebo (top) and 400 mcg salbutamol (bottom).

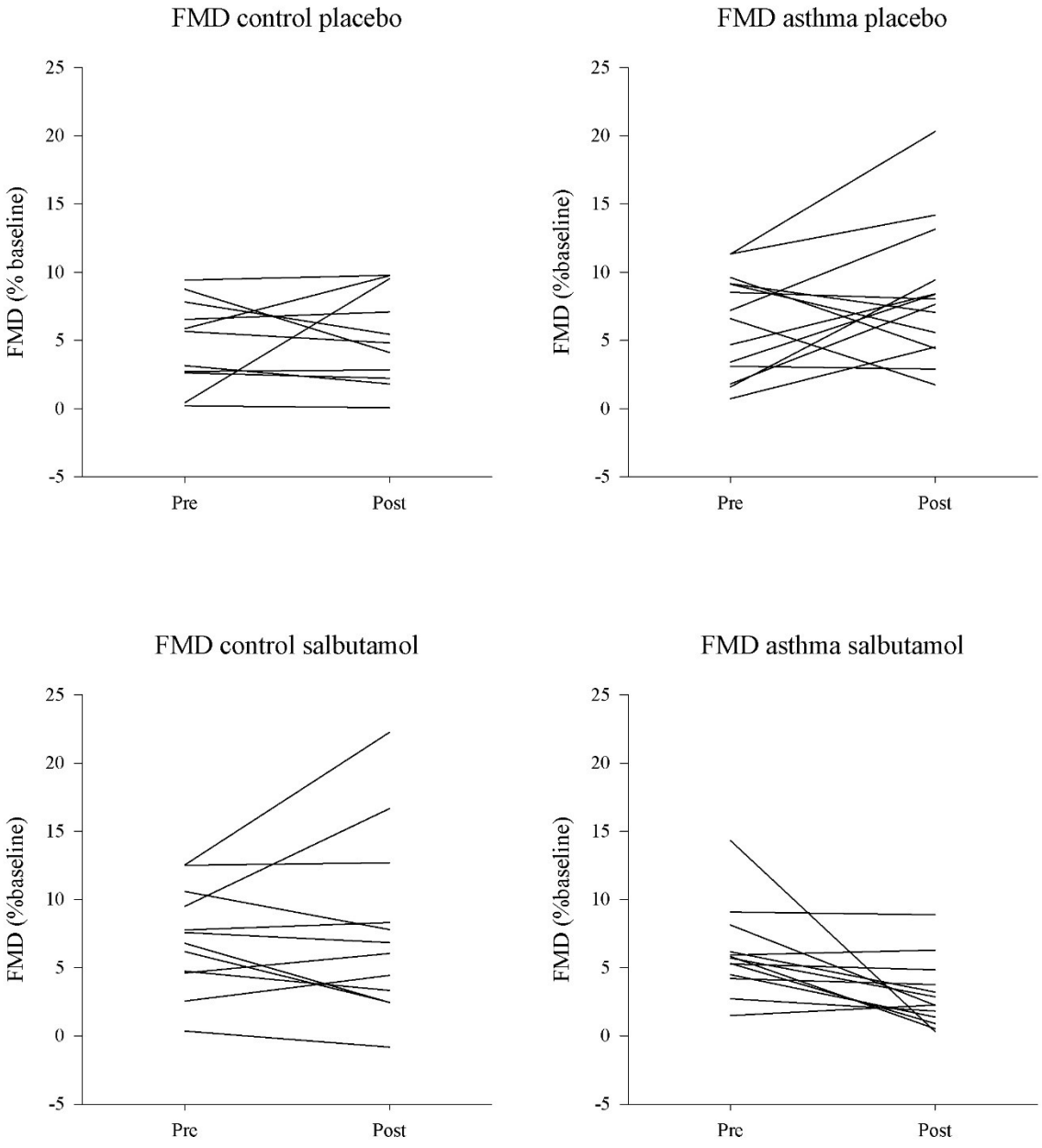

Figure B2. Individual changes in flow-mediated dilation (FMD) in controls (left) and asthma (right) before and after placebo (top) and 400 mcg salbutamol (bottom). Data-points are not normalized for sheer stress.

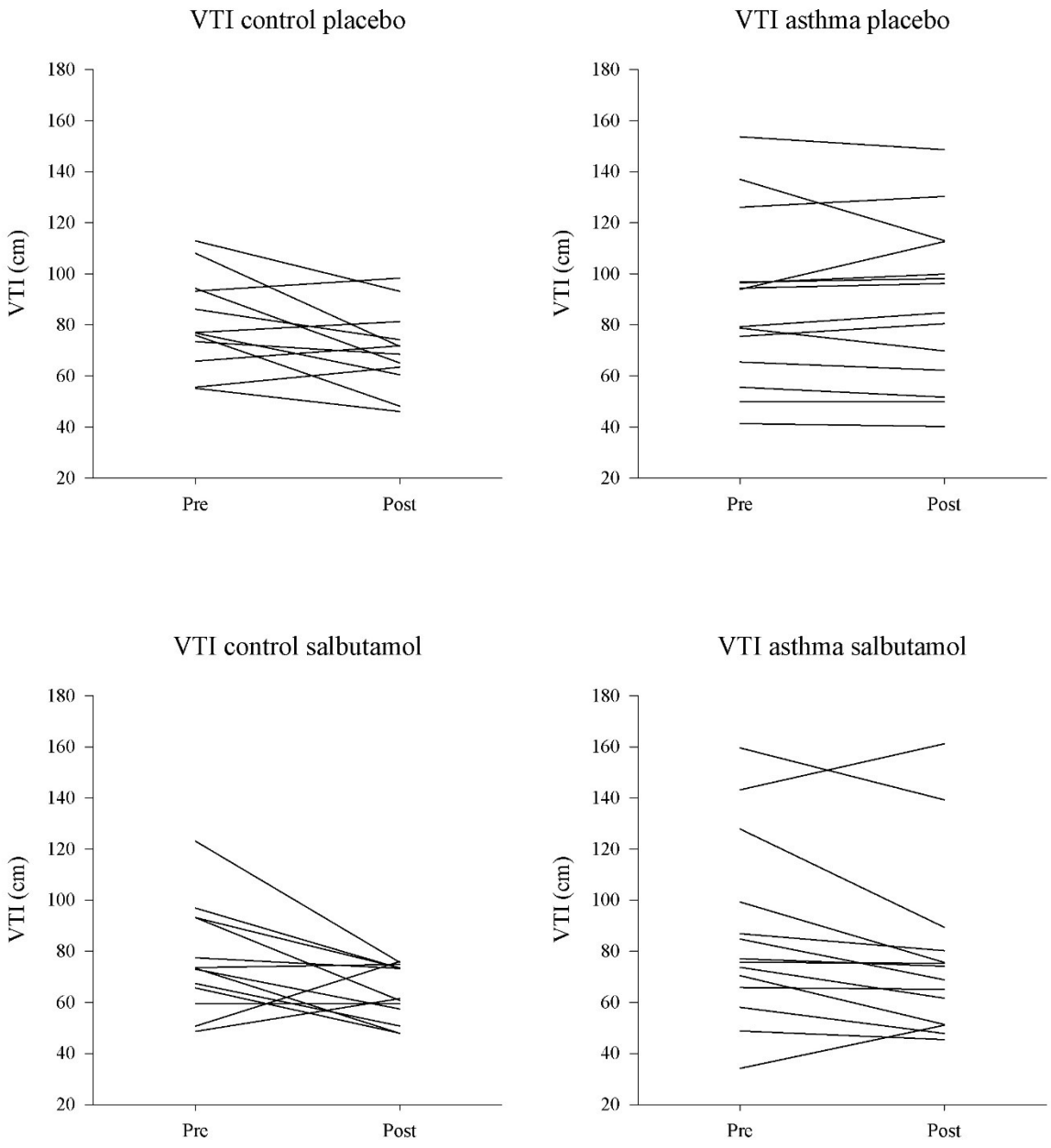

Figure B3. Individual changes in velocity time integral (VTI) in controls (left) and asthma (right) before and after placebo (top) and 400 mcg salbutamol (bottom).

11 Appendix C: Supplementary figures, Chapter VI

Acute effects of salbutamol on systemic vascular function in asthma

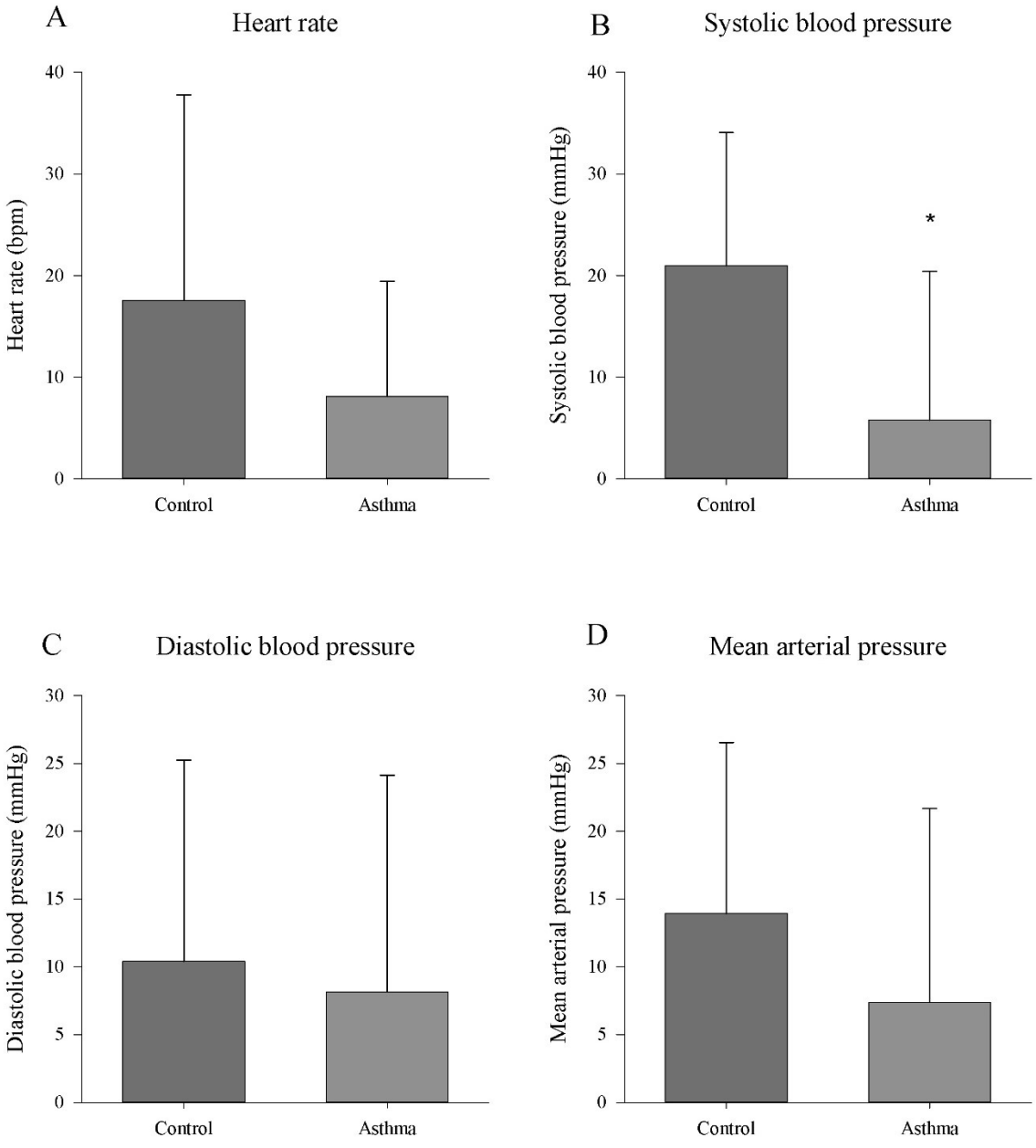

Figure C1. Differences in changes in A. heart rate ($p=0.185$), B. systolic blood pressure (*$p<0.05$), C. diastolic blood pressure ($p=0.980$), and D. mean arterial pressure ($p=0.416$) in controls and asthmatics following 2 minutes of body temperature vs. coldpressor test hand submersion. Graphs are displaying means and standard deviations.

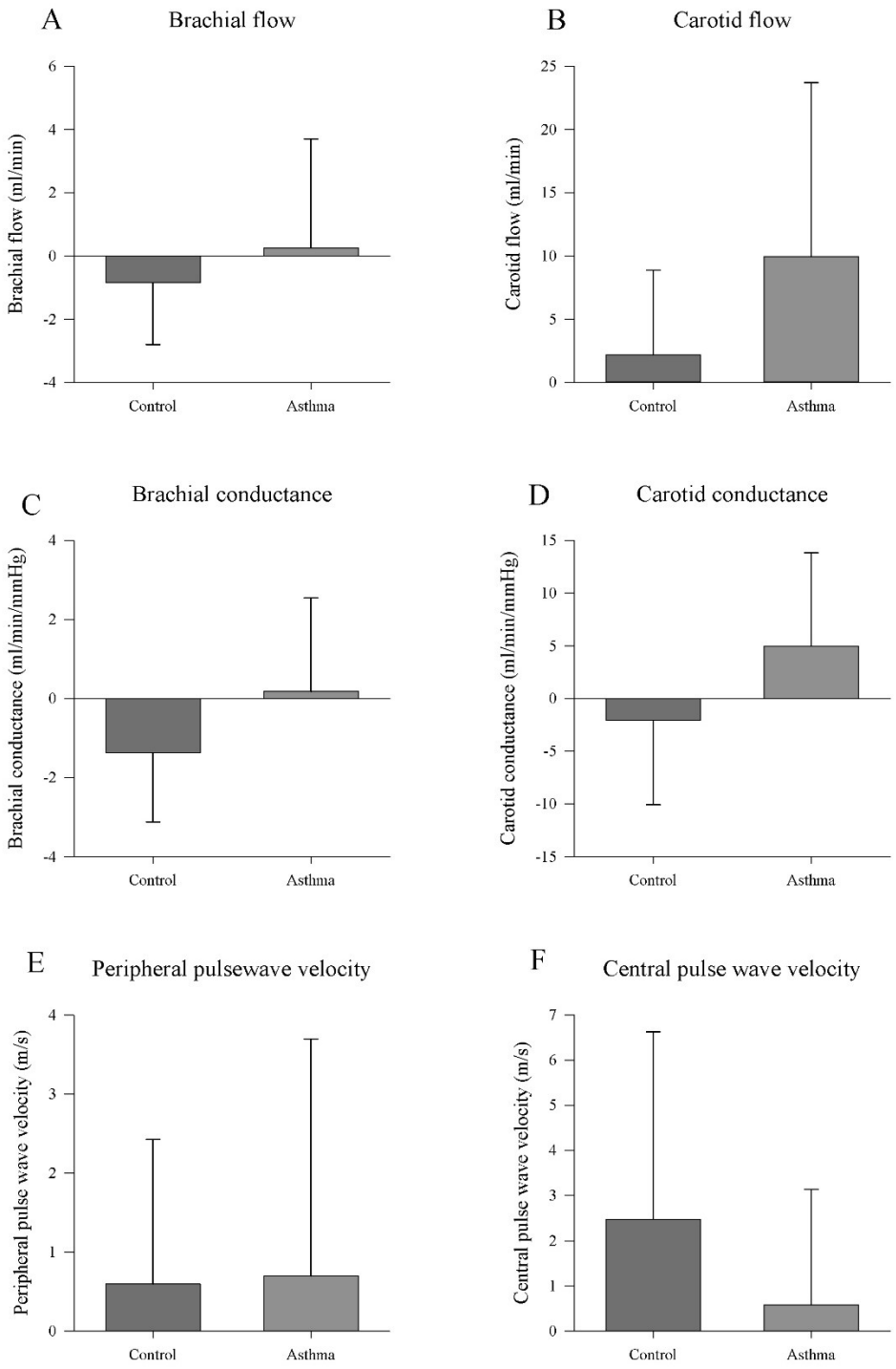

Figure C2. Differences in changes in A. brachial flow (p=0.354), B. carotid flow (p=0.182), C. brachial conductance (p=0.119), D. carotid conductance (p=0.154), E. peripheral pulse wave velocity (p=976), and F. central pulse wave velocity (p=0.215) in controls and asthmatics following 2 minutes of body temperature vs. coldpressor test hand submersion. Graphs are displaying means and standard deviations.