INTRAPULMONARY SHUNTING IN ASTHMATIC ATHLETES

by

Jennifer Chung-chi Chao

B.Sc., Simon Fraser University, 2009

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Human Kinetics)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

June 2011

Abstract

This study investigated the presence of intrapulmonary shunt in asthmatic athletes during exercise. The effects of airway obstruction on the onset of intrapulmonary shunt were examined by comparing two conditions of airway obstruction: induced airway obstruction and reversed airway obstruction. The study also examined the effect of body position on intrapulmonary shunt recruitment by comparing three positions at rest: supine, head-down tilt and upright. We hypothesized that induced airway obstruction would trigger an earlier onset of intrapulmonary shunt during exercise when compared to reversed airway obstruction; we also hypothesized that the head-down tilt position will recruit intrapulmonary shunt at rest. Ten asthmatic, highly aerobically trained (VO_2max = 62.6 ± 6.7 ml/kg/min) males completed 3 resting stages and 6 stages of incremental exercise under both conditions of airway obstruction. Agitated saline contrast echocardiography was used to determine the presence of intrapulmonary shunt. Ten of ten subjects demonstrated intrapulmonary shunt during exercise; three of ten subjects demonstrated shunt at rest. Nine of ten subjects demonstrated shunt onset at the same workload for both conditions; one individual showed a delay in shunt onset with the reversed airway obstruction condition. Mean shunt onset under induced airway obstruction and reversed airway obstruction was 41.4 ± 7.9 %VO_2max and 42.5 ± 8.1 %VO_2max, respectively; no significant difference in mean shunt onset was found. Among the 3 subjects who shunted at rest, 2 subjects shunted at resting supine and 1 subject at both supine and head-down tilt. Conditions of airway obstruction did not have an influence at shunt onset at rest. Intrapulmonary shunting during exercise is evident in asthmatic athletes and appears to occur at low workloads but is not consistently influenced by acute conditions of airway obstruction or body position.

Preface

Collaborators and co-authors are the following:

- Dr. Donald C. McKenzie produced the research idea, assisted in developing the research design, provided advice and guidance in the writing of the thesis and ethics protocol, performed the agitated saline injections on data collection days, and helped coordinate committee members for meetings, thesis proposal and defence.
- Jennifer Chao developed the research design, conducted the literature review, recruited subjects, coordinated data collection days, collected, analysed and interpreted the data (excluding echocardiograms), and wrote all ethics and thesis documents.
- Dr. Michael Koehle assisted in developing the research design and performed the agitated saline injections on data collection days.
- Dr. William Sheel assisted in developing the research design and provided advice in constructing the agitated saline contrast echocardiography protocol.
- Dr. Shiroy Dadachanji performed the agitated saline injections on data collection days.
- Allen McLean collected the contrast echocardiography images.
- Dr. Jonathan Tang analyzed the contrast echocardiography images.
- Sarah Koch assisted in data collection.

No publications arising from the work presented in this thesis have been published to-date.

The study involved human subjects and received full board approval from the University of British Columbia Clinical Research Ethics Board (H10-01608).

Table of Contents

List of Tables

List of Figures

Acknowledgments

I would like to express my sincere gratitude to the following individuals who have not only helped make this thesis project possible but my experiences thoroughly enjoyable:

- Dr. Donald C. McKenzie, my supervisor, who has been instrumental in the completion of this thesis project and invaluable in my growth as a graduate student and as a person.
- Dr. Michael Koehle, who fostered great care, provided meaningful guidance to this thesis project as well as critical assistance in data collection.
- Dr. William Sheel, who provided extensive advice in the development of this thesis project.
- Allen McLean, who not only collected all the contrast echocardiography images but also gave significant consultation for data collection.
- Diana Jespersen, who has contributed so much to all that I have learned and gained in the last two years and provided endless support and care.
- Dr. Shiroy Dadachanji, who was dependable and extremely helpful with data collection.
- Dr. Jonathan Tang, who analysed the echocardiograms and provided meaningful advice to the thesis project.
- Sarah Koch, who was instrumental to data collection.
- Eric Carter, Lianne Dolan, Luisa Giles, Amy Kirkham, Meaghan MacNutt, Martin McInnis, and Sarah Neil, who are absolutely wonderful colleagues and friends.
- Sarah Dobrowolski, Taylor Drury, and Jill Kennedy, who helped make data collection possible.

Chapter 1: Introduction

1.1 Exercise-Induced Asthma

This review on exercise-induced asthma will be centred on its relevance to an aerobically fit athlete and thus will discuss its relationship with exercise. Firstly, the definition of exercise-induced asthma will be described, along with the prevalence and the different methods of diagnosis. Next, the pathophysiological topics of exercise-induced asthma will be discussed: how exercise-induced asthma may be triggered, how the disease may progress and develop as well as the possible functional limitations an asthmatic athlete may endure at rest and during exercise. Lastly, the different types of treatment and the issues regarding the effects of pharmaceutical interventions on an asthmatic athlete will be reviewed.

1.1.1 Definition

Asthma is a chronic inflammatory disease that affects the airways. Asthma can be described as a "complex disorder characterized by variable and recurring symptoms, airflow obstruction, airway hyperresponsiveness, and an underlying inflammation" (78). Asthma is a syndrome featured by a wide variety of clinical phenotypes (38). About 90% of individuals with chronic asthma have exercised-induced asthma (60). Exercise-induced asthma (EIA) can be described as the transient increase in airway resistance triggered by vigorous exercise in individuals with asthma (90). The term, EIA, is not to be confused with exercise-induced bronchoconstriction (EIB) – which describes the increase in airway resistance caused by bronchoconstriction in individuals are not asthmatic. The main difference between EIA and EIB is that EIB does not include the inflammatory features of EIA due to the absence of the effects of chronic asthma.

1.1.2 Prevalence

Exercise-induced asthma (EIA) is more prevalent in the athletic population than in the general population. This is intuitive as an athlete is more often exposed to his/her trigger – *i.e.,* exercise. In North America, 5-20% of the general population have EIA (38), while about 8-46% of athletes (92) suffer from this disease. The wide range in the reported rates of EIA in both populations can be attributed to the varying methods of diagnosing EIA, as well as the differences in demographic and anthropometric characteristics within the two populations. However, the variability shown in the athletic population can also be caused by the varying types of athletes. Each athlete can differ in endurance, strength, power, agility, speed, coordination, etc. EIA appears to be more prevalent among endurance athletes (*e.g.* long distance runners, cyclists, swimmers, cross-country skiers) than power athletes (*e.g*. wrestlers, weight-lifters, ice hockey players, gymnasts) (7, 21, 43, 117) and this difference is most likely due to their contrast in training regime (111, 117). Typically, an athlete who is more aerobically fit will train with higher ventilation rates for longer periods of time than a more anaerobically fit athlete. The development and trigger of EIA is associated to the cooling and drying of the airways due to evaporative water loss during hyperventilation (7). Please refer to Mechanisms (page 5) for more details.

1.1.3 Diagnosis

Diagnosis of EIA is recommended to include a pulmonary function assessment and a provocation challenge in combination with the athlete's history of symptoms (21). The most common symptoms of EIA are: coughing, wheezing, shortness of breath (dyspnoea), excess mucous production, and chest tightness. Unfortunately, asthma is commonly diagnosed solely

on self-reported symptoms alone in both the general and athletic population (88). Diagnosis based on self-reported symptoms has been shown to be neither specific nor sensitive to asthma (88).

A pulmonary function assessment requires the use of spirometry. Spirometry measures lung function by measuring an array of lung volumes and flow rates. The most common measurements assessed in EIA are: forced vital capacity (FVC), forced expiratory volume in 1 second ($FEV_{1.0}$), $FEV_{1.0}$/FVC, peak expiratory flow (PEF), and mean forced expiratory flow during the middle half of FVC (FEF_{25-75}). With airway obstruction that comes with EIA, the expiratory flow rates are most often affected (92). Typically, an asthmatic would have decreased $FEV_{1.0}$, PEF, and $FEF_{25-75.}$ Because airway obstruction should not change the volume of the lungs, FVC should not decrease significantly and thus the ratio $FEV_{1.0}$/FVC would also decrease (92). Spirometry is also used with provocation challenges in determining the severity of EIA (2).

There are two types of provocation challenges: direct and indirect. Direct challenges include inhalation of histamine or methacholine. These chemical challenges act to directly stimulate the smooth muscles of the airways. Direct challenges are often highly sensitive but have poor specificity due to the lack of involvement of the inflammatory features of the asthmatic airways (21). Furthermore, such challenges assess airway hyperresponsiveness independent of exercise or hyperventilation (8), and consequently may not be suitable in diagnosing an athlete with exercise-induced asthma. Indirect challenges include eucapnic voluntary hyperpnoea, exercise, or inhalation of hyperosmolar aerosols, mannitol, or adenosine monophosphate (AMP).

Eucapnic voluntary hyperpnoea (EVH) was developed in the 1980s as a surrogate for exercise to identify EIA among members of the United States Army (82). The EVH challenge is

centred on the concept that high ventilation increases the osmolarity of the airway epithelium due to evaporative water loss. Hyperosmolarity of the airways triggers an inflammatory response that leads to airway narrowing (90) – see Mechanisms (page 5) for more details. The EVH test measures the $FEV_{1.0}$ decline after an athlete hyperventilates at 60-85% of maximal voluntary ventilation for 6 minutes. Generally, this is estimated by multiplying the subject's best baseline $FEV_{1.0}$ by 30 (27, 90). The eucapnic air is comprised of 5% CO_2, 21% O_2, and balance N_2 from a commercially compressed gas cylinder. Thus, the air is dry which further acts to induce evaporative water loss at the airway epithelium. There are some variations that involve cooling the air as well to prompt a reactive hyperaemia response. The IOC considers an athlete to be positive for EIA if there is at least a 10% decline in $FEV_{1.0}$ within 30 minutes after the hyperpnoea challenge (21). The EVH test is currently the International Olympic Committee (IOC)-recommended challenge for EIA due to its higher rates of sensitivity and specificity when testing elite athletes (5, 27).

Based on its name, one may assume that exercise would best assess exercise-induced asthma. However, this is not always the case. The biggest limitation with using exercise to trigger a response is that there is a lack of standardization that exists for any particular challenge. As each sport shapes its own athlete, it is difficult to formulate a single exercise test that will be both sensitive and specific to detecting EIA in all types of athletes. For example, a field cycling test may be highly sensitive for cyclists, but not for rowers. Furthermore, in field exercise tests, minute ventilation, inspired gas humidity and temperature, and exercise intensity are often not measured (90).

Hyperosmolar aerosol (*i.e.* hypertonic saline) challenge is based on the osmotic hypothesis in that hyperosmolarity is the key trigger to airway obstruction in EIA (6). The

challenge involves inhalation of nebulised hypertonic saline (4.5%) with pre- and post-$FEV_{1.0}$ measures. Mannitol is a sugar alcohol and the challenge involves inhalation of dry powder mannitol. The AMP challenge is also another indirect challenge but is not yet accepted by the IOC.

1.1.4 Pathophysiology

1.1.4.1 Mechanisms

There are two established hypotheses that have been proposed to explain the mechanisms of EIA: the osmotic hypothesis by Anderson (4, 6) and the thermal hypothesis by McFadden (72). The osmotic hypothesis is centered on the dehydrating effects of high ventilation during exercise. The epithelial lining of the airways plays a critical role in preparing inspired air for gas exchange. For adequate gas exchange to occur, the humidity of air must increase from ambient levels of 30-60% to 100% by the time it reaches the alveoli-capillary interface (114). During exercise, an increase in workload prompts an increase in ventilation. At maximal workloads, minute ventilation can reach as high as 200L/min in elite athletes. The capacity of the epithelium to sufficiently humidify the inspired air becomes quickly challenged as workload increases. Thus, exposure to high volumes of dry air causes evaporative water loss which leads to an increase in osmolarity of the airway epithelium. This response appears to be augmented among asthmatics (4). Hyperosmolarity triggers the release of mediators from a wide variety of cells resulting in bronchoconstriction, excessive mucous production and increased vascular permeability (4, 6). These effects all contribute to the marked airway obstruction demonstrated in EIA.

The thermal hypothesis is based on the cooling effects of high ventilation during exercise as evaporation leads to a loss in heat energy. Heat loss within the airways during high ventilation causes a reactive hyperaemia response by the bronchial circulation (72). The bronchial circulation is systemic and acts to warm the airways. An increase in bronchial blood flow can be detrimental as airway microvasculature can become leaky and hyperreactive. Vascular engorgement with or without oedema or bronchoconstriction can result in airway obstruction (70). Through great debate, the osmotic hypothesis appears to be favoured as studies have shown that severe airway obstruction can be produced without changes in temperature in the airways (1, 4, 10). Airway cooling is a contributing but not necessarily essential factor to airway obstruction in exercise-induced asthma (7).

1.1.4.2 Pathogenesis

The severity of asthma is associated with the severity of airway inflammation and airway hyperresponsiveness (AHR). Airway inflammation increases airway resistance through the release and action of inflammatory mediators that cause excess mucous production, increased vascular permeability, thickening of the airway walls, and indirect bronchial smooth muscle contraction (11). Elevated counts of mast cells, T-lymphocytes, eosinophils, macrophages and neutrophils have been demonstrated in asthmatics (43, 89). There are also higher levels of inflammatory mediators (*e.g*. prostaglandins, leukotrienes and histamine) present in body fluids of asthmatics following exercise. Sputum and urine are common fluids used to assess the severity of inflammation in asthmatics (19, 89). On the other hand, AHR increases airway resistance predominantly through direct bronchial smooth muscle contraction (11). AHR is most often triggered by allergens in the environment or pharmacologic agents (7). However, AHR can

also be augmented by airway inflammation (57). The release of inflammatory mediators by the mast cells and other associated inflammatory cells can further trigger AHR. Leukotrienes, prostaglandins, and histamine release lead to bronchoconstriction, increased vascular permeability and mucous production (92).

For endurance athletes who train with high ventilation rates for extended periods of time, the airways of these athletes are exposed to repeated bouts of airway dehydration and cooling. Furthermore, if an athlete trains in high-allergenic environments such as indoor swimming pools, ice rinks or in cities with high pollution and pollen, the lack of air quality can also play contributing roles in damaging the airways (31). These bouts act as small, acute injuries to the respiratory epithelium with each injury leading to exudation of bulk plasma and release of inflammatory cells and the eventual remodelling of the epithelial and surrounding smooth muscle cells of the airways (7). Although, classical asthma is often associated with childhood onset, it is not uncommon for elite athletes to develop exercise-induced asthma during their adult years (117). Moreover, the severity of exercise-induced asthma can also be dependent on the athlete's current level of endurance training (44).

1.1.4.3 Functional Limitations

Exercise-induced asthma (EIA) is the transient narrowing of the airways that occurs with intense exercise in asthmatics. Airway obstruction caused by inflammatory mediator release, bronchial smooth muscle contraction, mucosal oedema, and thickening of the airway wall can all contribute to the increase in airway resistance. Although this increase in airway resistance is more commonly observed *after* exercise as exercise is also a potent stimulus for bronchodilation, a number of studies have shown airway resistance to be higher *during* exercise in asthmatics than

non-asthmatics (17, 41, 59, 102). Exercise-induced bronchodilation appears to be attenuated in asthmatics (16, 75, 102) which further compounds the increase in airway resistance. Increased airway resistance causes an increase in expiratory flow limitation. Excessive expiratory flow limitation can lead to hyperinflation of the lungs, marked by an increased end-expiratory lung volume (55). Lung hyperinflation disrupts the ventilatory responses required for adequate hyperventilation during heavy exercise (71). Expiratory flow limitation has been shown to prevent exercise-induced hyperventilation in asthmatics (40, 41). Inadequate hyperventilation can lead to exercise-induced arterial hypoxemia (EIAH) which is a decrease in arterial blood oxygen (26).

The detrimental effect of airway obstruction during exercise may go beyond the ventilatory responses to exercise. Although there is scarce literature on pulmonary gas exchange in asthmatics, it has been shown that airway obstruction can also interfere with pulmonary gas exchange (12, 40-42, 112). Pulmonary gas exchange impairment appears to be more severe in asthmatics than non-asthmatics (41, 112). The leading candidate for the impaired pulmonary gas exchange in asthmatics appears to be ventilation-perfusion (V_A/Q) mismatch (12, 112). As airway obstruction is not evenly distributed throughout the airways, heterogeneous airway obstruction results in non-uniform V_A/Q ratios across the lungs and consequently, worsens V_A/Q mismatch (112).

Hypoxia is a known potent vasoconstrictor of the pulmonary arteries. Hypoxic vasoconstriction acts to maintain adequate V_A/Q distribution by deterring pulmonary blood flow to poorly ventilated airways. Would hypoxic vasoconstriction also be present in an individual with obstructed airways? If so, would such a response result in changes in pressure within the pulmonary vasculature? Hypoxic vasoconstriction has been suggested to be involved in limiting

V_A/Q mismatch in asthmatics as an attempt to limit perfusion to low V_A/Q (*i.e*. poorly ventilated) lung units. When 100% oxygen is given too severe asthmatics, there is an increase in blood flow to low V_A/Q lung units and the worsening of V_A/Q mismatch ensues (12). Furthermore, it is well-documented that individuals with chronic obstructive pulmonary disease often develop secondary pulmonary hypertension (13). Interestingly, there are lacking data on the hemodynamic responses of the pulmonary system to changes in airway resistance in asthmatics. As there is scant research on pulmonary gas exchange impairment in asthmatics – other contributors to widening of $AaDO_2$, such as the intrapulmonary shunt may have been overlooked.

1.1.5 Treatment

Previous philosophy for treating individuals with EIA was to exclude them from exercise entirely – children were excused from gym class by physicians because of their intolerance to exercise. Now with the improvement in understanding EIA, the treatment of EIA is based around controlling the symptoms rather than entirely removing the stimulus (89). Underlying inflammation will unlikely be completely eliminated with intervention but minimizing airway obstruction is critical. Participation in physical activity is now encouraged for children and adults with EIA, once their asthma is controlled (33).

Airway hyperresponsiveness (AHR) and airway inflammation play critical roles in EIA and thus, these two features are the primary goals for treatment. The treatment of AHR and airway inflammation use separate approaches: treating airway inflammation involves “control therapy” while treating AHR uses “relief therapy” (11, 92, 101).

1.1.5.1 Control Therapy and Relief Therapy

Control therapy is long-term, applied daily, and usually involves the use of inhaled corticosteroids (ICS). ICS are anti-inflammatory and seem to reduce the decline in lung function ($FEV_{1.0}$) by at least 50% (45, 108). Treatment of airway inflammation in asthmatics has been found to improve arterial oxygenation during exercise by improving airway function and pulmonary gas exchange efficiency (42). The use of ICS appears to treat airway obstruction by decreasing bronchoconstriction and mucosal edema, and preventing luminal secretions and thickening of the airway wall (14). According to the Global Initiative for Asthma guidelines (33), an individual with daily asthmatic symptoms should be treated with an ICS (20). Elite athletes with EIA fit under this description as they train almost every day under asthmogenic conditions. Treatment with ICS is considered the most effective intervention for controlling asthmatic symptoms (22, 33).

Relief therapy is typically used prior to an exercise bout and consists of administration of an inhaled beta-2 agonist (IBA) (11). There are two types of IBA currently used: short-acting beta-2 agonist (SABA) and long-acting beta-2 agonist (LABA). SABAs relieve AHR by relaxing bronchial smooth muscle, reducing vascular permeability and preventing mediator release. SABAs, as the name suggests, last only about 2-4 hours with its peak effect within 60 minutes post-administration (46, 92). Common SABAs are: salbutamol, terbutaline, fenoterol, and levalbuterol. LABAs have the same bronchodilatory effects as SABAs but last up to 12 hours (17, 46). Common LABAs are: salmeterol, formoterol, and clenbuterol. There are a number of problems with the excessive use of IBA. Overuse of IBA results in increased sensitivity of the airways to bronchoconstrictive stimuli (31). Furthermore, frequent use of IBA

leads to tolerance of the drug which can occur as quickly as within a few days (37, 39) Thus, it is recommended for athletes to use IBA sparingly and only when needed (22, 31).

1.1.5.2 Physiological Effects of Salbutamol During Exercise

Salbutamol is the most common relief treatment for asthma among athletes (30). Salbutamol is a short-acting beta-2 agonist and its target – beta-2 adrenergic receptors – are found nearly everywhere in the body. They are found in cardiac muscle, smooth muscles of the respiratory and gastrointestinal tract, skeletal muscle, and adipose tissue. With systemic stimulation, the beta-2 adrenergic response causes increased cardiac output, bronchodilation, vasodilation, and lipolysis. From an athlete's perspective, these effects are desirable for performance. Increased cardiac output will increase systemic blood delivery, bronchodilation will decrease airway resistance, vasodilation at the skeletal muscles will increase muscle perfusion, and lipolysis can increase efficiency in aerobic energy metabolism (5).

Due to the ubiquity of beta-2 adrenergic receptors across the body, the mode of drug administration dictates the degree of beta-2 adrenergic stimulation. There are three common modes of salbutamol administration: oral, inhaled and intravenous. Oral and intravenous intake of salbutamol can both cause a systemic adrenergic response as the drug enters the bloodstream. Consequently, such intake of any SABA or LABA has been banned from sport for over 20 years (30) as they have also shown to have anabolic effects on skeletal muscle (107). On the other hand, inhaled salbutamol acts to exclusively stimulate the beta-2 adrenergic receptors in the airway. Although inhaled salbutamol can effectively increase lung function, its use has failed to demonstrate ergogenic effects on parameters such as time trials (81), time to exhaustion (93), peak power (96, 97), and maximal oxygen consumption (97). Numerous review articles have yet

to find unequivocal evidence of performance enhancement through inhaled salbutamol (22, 56, 106).

1.1.5.3 Salbutamol and Pulmonary Gas Exchange

There have been few studies on the effects of salbutamol on pulmonary gas exchange in asthmatic subjects. Most of the studies looking at salbutamol and pulmonary gas exchange have been done at rest in individuals with severe asthma. Intravenous salbutamol given during an acute attack in severe asthmatics has been shown to be detrimental to pulmonary gas exchange – V_A/Q mismatch worsens due to the conflicting beta-2 adrenergic responses occurring the bronchioles and pulmonary vasculature (12, 87). Inhaled salbutamol does not appear to have the same deleterious effect on V_A/Q mismatch (12). There is scarce research on pulmonary gas exchange during exercise in asthmatics.

1.2 Pulmonary Gas Exchange

The three major contributors to pulmonary gas exchange impairment: ventilation-perfusion (V_A/Q) mismatch, diffusion limitation, and shunt will be briefly reviewed.

1.2.1 Definition

Pulmonary gas exchange maintains arterial oxygen partial pressure (PaO_2) and thus serves a critical role in oxygen delivery. Pulmonary gas exchange occurs between the alveolar oxygen and pulmonary capillary blood. During exercise, an increase in oxygen demand is met by increases in cardiac output and oxygen extraction; both these responses challenge pulmonary gas exchange. Pulmonary gas exchange is indicated by the difference in partial pressure of

alveolar oxygen and arterial oxygen ($AaDO_2$). In healthy, untrained individuals, $AaDO_2$ is kept minimal at 5-10 mmHg at rest but often increases to 15-25 mmHg during maximal exercise (115). In highly trained athletes, $AaDO_2$ can sometimes exceed 35-40mmHg (26). Excessive pulmonary gas exchange impairment leads to the decline in PaO_2 and arterial oxygen saturation (SaO_2) – known as "exercise-induced arterial hypoxemia" (EIAH). EIAH impairs oxygen transport (26), oxygen consumption (83), and ultimately performance (58). In healthy males, EIAH appears to correlate with aerobic fitness as measured by their maximal oxygen consumption (VO_2max) (26). Elite endurance athletes often have VO_2max 150-200% of the predicted normal values. It is estimated that EIAH occurs in as many as 50% of male elite endurance athletes (83). It is still unclear why these highly trained individuals experience such pulmonary limitations during exercise.

The widening of $AaDO_2$ can be caused by: ventilation-perfusion mismatch, diffusion limitation, and shunt (26). For an athlete exercising at sea level, ventilation-perfusion (V_A/Q) mismatch and diffusion limitation are currently believed to be the leading players in impairing pulmonary gas exchange (26, 49). However, V_A/Q mismatch and diffusion limitation alone do not always add up to the widening of $AaDO_2$ during exercise (94). Recent studies on intrapulmonary shunting during exercise suggest that shunt may have a greater role in influencing pulmonary gas exchange than previously perceived (28, 67, 99).

1.2.2 Ventilation-Perfusion Mismatch

Ventilation-perfusion (V_A/Q) mismatch occurs when there is a discrepancy in lung units ventilated and lung units perfused. V_A/Q mismatch increases with increasing exercise intensity (36, 52) but unlike EIAH, there is no relationship between V_A/Q mismatch severity and aerobic

fitness (*i.e.* VO_2max) (51). In healthy individuals, V_A/Q mismatch accounts for all of $AaDO_2$ at rest (111). V_A/Q mismatch seems to occur during exercise regardless of an individual's susceptibility to EIAH (49). In individuals with minimal pulmonary gas exchange impairment, V_A/Q mismatch nearly accounts for all of the increase in $AaDO_2$ (49). However, in individuals who do experience significant gas exchange limitations, V_A/Q mismatch appears to only contribute for about one half of the increase in $AaDO_2$ during exercise (34, 105, 111).

The mechanisms by which V_A/Q mismatch worsens are unclear (26). There appear to be four main candidates: 1) differences in modulation of airway and vascular tone; 2) mild pulmonary edema; 3) excess secretions in the airways; and 4) bronchoconstriction. The leading candidate to increasing V_A/Q mismatch during exercise appears to be mild pulmonary edema (26, 49). During exercise, increase in cardiac output results in increases in pulmonary blood flow and pulmonary vascular pressures. These changes will increase capillary filtration and cuffing of the airways which alters the perfusion distribution to the ventilated lung units, ultimately worsening V_A/Q mismatch (49)

1.2.3 Diffusion Limitation

The lungs are well adapted such that oxygen equilibration between the alveolar air and capillary blood occurs well before the erythrocyte leaves the alveolar-capillary interface (114). At rest, diffusion limitation unlikely contributes to any existing $AaDO_2$ (23, 113). However during heavy exercise, diffusion limitation becomes a significant factor to pulmonary gas exchange impairment (105, 111). It has been shown that as exercise intensity increases, the contribution of diffusion limitation to the widening of $AaDO_2$ increases where at maximal exercise about two thirds of the total $AaDO_2$ is caused by diffusion limitation (105, 111).

Diffusion limitation is affected by capillary transit time and diffusing capacity. With increase in cardiac output, pulmonary blood flow increases which decreases capillary transit time (50). The leading theory to diffusion limitation during heavy exercise is the shortening of capillary transit time (26, 49). Diffusion limitation due to decreased diffusing capacity caused by the thickening of the blood-gas barrier has yet to be clearly demonstrated. Although it has been shown that prolonged and heavy exercise can result in mild pulmonary edema (74) and reduced diffusing capacity (73, 99), the relationship between pulmonary edema and reduced diffusion capacity is still unclear.

1.2.4 Shunt

The term "shunt" is used to define any blood entering the systemic arterial circulation without traversing ventilated units of the lung (114). Excluding the intracardiac shunt, which is most often caused by an atrial septal defect (*i.e*. patent foramen ovale), there are essentially two forms of shunt that occur outside of the cardiac chambers: extrapulmonary and intrapulmonary.

Extrapulmonary shunts are naturally occurring and take place outside of the pulmonary circulation. Extrapulmonary shunts involve the thebesian circulation – where some coronary venous blood drains directly into the left heart, and the bronchial circulation – where some bronchial venous blood drains into the pulmonary veins (69). Although only about 1% of the total resting cardiac output is contributed by extrapulmonary shunt, as cardiac output increases with increase in exercise intensity, extrapulmonary shunt can become a minor but significant contributor to pulmonary gas exchange impairment (34).

Intrapulmonary shunt occurs within the lungs as the shunt bypasses gas exchange at the pulmonary capillaries despite traversing from the pulmonary arteries to the pulmonary veins.

There are two ways an intrapulmonary shunt can occur: 1) physiologically through an underventilated lung unit; or 2) anatomically through a separate vessel (64). The former may be a result of atelectasis which is most often caused by the collapse of the alveoli. This form of intrapulmonary shunt usually arises from a post-surgical complication and thus is rarely present in otherwise healthy individuals (64). The intrapulmonary shunt that is present in health is the anatomical intrapulmonary shunt (28, 67, 99). This type of shunt is suggested to emerge from distinct arteriovenous vessels and has been termed "intrapulmonary arteriovenous shunting" (28).

1.3 Intrapulmonary Arteriovenous Shunting

What is intrapulmonary arteriovenous shunting? The definition and brief history of this type of shunting in health will be described. The different methods of measuring intrapulmonary arteriovenous shunting and their effectiveness will be discussed. The significance of intrapulmonary shunting is still unclear. The different hypotheses of its effect in health and performance will be presented. Lastly, a brief review on the likely mechanisms on how the intrapulmonary shunt is recruited will also be presented.

1.3.1 Definition

Intrapulmonary arteriovenous shunting occurs in healthy individuals and is believed to arise from vessels known as "arteriovenous anastomoses". These vessels conduct flow from the pulmonary arteries directly to the pulmonary veins and thus bypass gas exchange at the capillaries. Arteriovenous anastomoses seem to be relatively dormant at rest but open with stresses to the cardiopulmonary system – most notably, exercise (28, 67, 99, 100, 116) and

hypoxia (62, 67). The notion of intrapulmonary arteriovenous shunting in health may be fairly recent but the existence of arteriovenous anastomoses is not a novel finding. Evidence of such anatomical arteriovenous shunts date as far back as the 1950s – these vessels have been well-documented in foetal (76, 118) and adult human lungs (70, 103, 104), as well as in rabbits, dogs, birds, and baboons (70, 79, 85, 86, 98).

1.3.2 Measurement

The biggest barrier hindering the understanding of intrapulmonary shunt is the available techniques used to measure the intrapulmonary arteriovenous shunt. Current methods are not able to safely and effectively measure both shunt path and shunt fraction in humans at rest and during exercise (69). There are essentially two ways of measuring shunt: gas exchange-independent methods and gas exchange-dependent methods. Gas exchange-independent methods include agitated saline contrast echocardiography and microsphere injection, while gas exchange-dependent methods include multiple inert gas elimination technique (MIGET) and 100% O_2 breathing.

1.3.2.1 Gas Exchange-Independent Methods

Agitated saline contrast echocardiography is a standard clinical method used to detect intrapulmonary shunting (15, 35, 47), and is a technique that can be used at rest and/or exercise. The method consists of injecting small amounts (5-7 ml) of agitated saline solution (*i.e.* bubbles) intravenously and observing the appearance of bubbles in the heart through a four-chamber apical view (28). Agitated saline contrast echocardiography detects intrapulmonary shunting through the timing and presence of bubbles from the right side of the heart to the left. The

presence of at least 3 bubbles in the left side of the heart upon the delay of at least 3-5 cardiac cycles indicates an intrapulmonary shunt occurred (28).

Considerable criticism has been made regarding the validity of agitated saline contrast echocardiography to detect the intrapulmonary arteriovenous shunt. Firstly, the problem with depending on the sole appearance of bubbles is that the path taken by the bubbles to get to the heart is unknown. During exercise, pulmonary vascular pressures increase and thus it can be argued that the bubbles may not be traversing across arteriovenous vessels but through distended capillaries (28). Secondly, it has been argued that changes in F_iO_2 could alter *in vivo* bubble dynamics and thus facilitate the passage of bubbles through the pulmonary capillaries and to the left side of the heart (53). Thirdly, as the bubbles are created via manual agitation, the true bubble size is also unknown making it difficult to assess the size of these arteriovenous vessels. Lastly and perhaps most importantly, agitated saline contrast echocardiography is based on qualitative imaging and thus is unable to measure shunt fraction. Without shunt fraction, the actual contribution to pulmonary gas exchange efficiency is unknown (53).

Proponents of using agitated saline contrast echocardiography to detect intrapulmonary arteriovenous shunting argue that the path of the bubbles is unlikely to come through distended capillaries (28, 64, 67). The pulmonary capillaries are no larger than 10 µm in diameter at rest and 13 µm during exercise (32). Bubbles smaller than 8 µm have survival time of <190 ms, and thus collapse well before entering the left heart after 3 cardiac cycles (67). At a maximal heart rate of 180 beats per min, the transit time of 3 cardiac cycles will be a full second. Furthermore, *in vivo* bubble dynamics may not be as influenced by changes in gas compositions as proposed. Elliott tested the influence of ambient gases on bubble stability and found no difference in bubble scores between various gas compositions (29).

Agitated saline contrast echocardiography can be used to determine the presence of intrapulmonary arteriovenous shunting. The technique is relatively non-invasive and thus is more convenient to do during exercise. Although it is unable to measure shunt fraction, it is effective in determining the presence of shunt (28, 67, 99).

Microsphere injection is an extremely invasive technique and consists of injecting large diameter polymer microspheres intravenously and detecting the presence of microspheres in tissue samples. Microsphere methodology is able to assess shunt fraction as well as provide insight on the size of the shunt as the microspheres are of a known fixed diameter.

The existence of exercise-induced intrapulmonary arteriovenous shunting has been strengthened through the injection of solid polymer microspheres by Stickland and colleagues (98). 25 µm isotope-labelled neutron-activated microspheres were injected in dogs at rest and during exercise (98). Considering the maximal physiological diameter of capillaries in mammals to be 13 µm (32), the transpulmonary passage of 25 µm microspheres provided strong indication of intrapulmonary shunt. They found that no shunting occurred at rest but measured a shunt fraction of 1.42% of total cardiac output during exercise (98).

The polymer microsphere technique requires highly invasive surgery and thus is not performed on humans *in vivo*. An alternative technique that is used on humans involves injecting technetium-99m labelled macroaggregated albumin microspheres and measuring the uptake of the microspheres into tissues through gamma imaging. The mean size of each microsphere is approximately 45 µm. Using this technique to compare intrapulmonary shunting in individuals with pulmonary arteriovenous malformations versus controls (*i.e.* healthy individuals), Whyte found that 5 of 5 control subjects demonstrated intrapulmonary shunting and had a mean increase in shunt fraction of 2.2% from rest to exercise (116). Lovering also used

this technique and found that 6 of 7 subjects presented intrapulmonary shunt during incremental exercise and calculated that the shunt fraction increased from 0.4% at rest to 1.7% at maximal exercise (65).

Because this technique indirectly measures shunt fraction through body imaging, the technological limitation of image resolution causes a number of sources of error in estimating and calculating the transpulmonary passage of the microspheres (65). Furthermore, as this method is highly invasive, there have only been a dozen human subjects tested in total – a larger sample size will be needed to assess a more representative shunt fraction during exercise. Nevertheless, more use of the microsphere technique on human subjects will only improve the understanding of intrapulmonary arteriovenous shunting due to its ability to assess shunt size and estimate shunt fraction.

1.3.2.3 Gas Exchange-Dependent Methods

The MIGET is based on the mass balance relationship between the addition of a dissolved gas in the venous circulation and its elimination at a lung unit (110). This relationship is dependent on the solubility of the gas and ventilation-perfusion ratio of the lung unit. The MIGET uses the intravenous infusion of trace amounts of six inert gases each with a known solubility in blood and measures their retention in arterial circulation. As the solubility of gases remains constant, the retention will be dependent of the elimination of the gases at the lung units that is dictated by the efficiency of pulmonary gas exchange. Ventilation-perfusion ratios can be calculated with distinct ratios indicating a particular cause of pulmonary gas exchange impairment. Extreme ratios (*i.e.* < 0.005 or > 100) indicate no gas exchange has occurred and that a shunt is present (110). The 100% O_2 breathing technique uses the similar concepts as

MIGET but compares the disappearance of alveolar oxygen at a lung unit with its appearance in arterial blood. Breathing 100% O_2 eliminates diffusion limitation and ventilation-perfusion mismatch as causes of pulmonary gas exchange impairment and thus singles out shunt as the culprit to increases in $AaDO_2$ (109, 114).

Using gas exchange-dependent methods, intrapulmonary shunting appears to have minimal contribution to pulmonary gas exchange impairment during exercise. Studies using MIGET found that intrapulmonary shunting accounted for no more than 1% of the total cardiac output during exercise (52, 111), while studies using 100% O_2 breathing technique measured shunt fraction of less than 2% of the total cardiac output during exercise (109, 111).

However, some may argue that gas exchange-dependent methods inaccurately measure shunt fraction (69, 99). It appears that the effect of pre-capillary gas exchange can significantly underestimate shunt fraction detected by MIGET and 100% O_2 breathing. Pre-capillary gas exchange was demonstrated over 40 years ago, (54) and it was shown that alveolar oxygen can directly diffuse across pulmonary arteries up to 2 mm in diameter. Conhaim and Staub found that precapillary vessels as large as 500 µm can be fully equilibrated between alveolar oxygen and pulmonary arterial blood (24). Both MIGET and 100% O_2 breathing assume that all gas exchange takes place at the capillaries – thus, calculated shunt fractions may actually be lower than true shunt fractions. Furthermore, both MIGET and 100% O_2 breathing are unable to distinguish between the different types of shunt as it assumes that zero gas exchange is caused by a sole shunt and as previously described, there are multiple types of shunts.

Measurement of intrapulmonary shunt that is both accurate and valid is lacking. More work is needed to assess intrapulmonary shunt's contribution to pulmonary gas exchange

impairment. Future use of comparing results of gas exchange-dependent methods versus gas exchange-independent methods may prove to be more useful.

1.3.3 Intrapulmonary Shunt in Health

Evidence of intrapulmonary arteriovenous shunting in healthy individuals is fairly recent as the first notable study was conducted by Eldridge in 2004 (28). Using agitated saline contrast echocardiography, Eldridge showed that 21 of 23 healthy individuals showed indication of intrapulmonary arteriovenous shunting during exercise on a upright cycle ergometer (28). Starting from a 65W baseline with 30W gains every 2 minutes until volitional fatigue, 11 of 13 men and 10 of 10 women shunted at some point during the exercise bout. The mean shunt onset was at 59% (SD = 20) of their maximal oxygen consumption (%VO_2max). The difference in mean shunt onset between sexes was found to be insignificant – 61%VO_2max (SD = 18) in men and 56% VO_2max (SD = 23) in women. Eldridge suggested that intrapulmonary arteriovenous shunting may play a more influential role in pulmonary gas exchange impairment during exercise than conventionally deemed (28).

Stickland took on a more extensive investigation on the effects intrapulmonary shunting on pulmonary gas exchange during exercise (99). Changes in pulmonary arterial pressure, cardiac output, and alveolar-arterial oxygen difference with exercise were also measured and compared with the presence of intrapulmonary shunting. Using agitated saline contrast echocardiography, they found 7 of 8 healthy, fit (mean VO_2max = 54.7 ml/kg/min) males demonstrated intrapulmonary shunting during exercise. It was found that alveolar-arterial oxygen difference ($AaDO_2$), an indicator of pulmonary gas exchange inefficiency, increased with increase in exercise intensity. The increase in $AaDO_2$ was also found to be significantly

correlated with increases in cardiac output (Q) and pulmonary arterial pressure (PAP). Using within-subject point bi-serial correlations, Stickland demonstrated that in the 7 subjects who developed shunt, shunt presence was correlated to $AaDO_2$ ($r = 0.68$), Q ($r = 0.76$), and PAP ($r = 0.73$). These findings prompted the notion that the recruitment of intrapulmonary shunt were perhaps pressure or flow-regulated; that these arteriovenous vessels open in response to the gains in pressure or flow during exercise. Interestingly, the one subject who did not present shunt had the highest pulmonary arterial pressure measurements of the total 8 subjects (99).

1.3.4 Mechanisms of Intrapulmonary Shunt

The understanding of how the intrapulmonary shunt is recruited has yet to be elucidated. As demonstrated by Stickland's study, changes in pressure and flow during exercise appear to have some impact on shunt recruitment (99). Qualitatively, bubble presence does appear to be more prominent with increase in workload (28). Considering the fundamental concept of Poiseuille's law, where the change in pressure within a tube is inversely related to its cross-sectional area, are the arteriovenous vessels recruited via size in a stepwise manner? If so, increases in pulmonary vascular pressure should lead to earlier onset of the intrapulmonary shunt through the initial recruitment of the smaller sized arteriovenous vessels.

Stickland investigated the effect of pulmonary vascular pressures on shunt recruitment through the manipulation of lower body positive pressures (100). It was found that small acute increases in pulmonary arterial pressure (3.7 mmHg) and pulmonary artery wedge pressure (4.0 mmHg) led 1/7 subjects to shunt at rest but overall, there was an inconsistent impact on the onset of intrapulmonary shunting and pulmonary gas inefficiency at rest or during exercise. Nevertheless, shunt regulation via pressure and/or flow was not ruled out – Stickland suggested

the small increases in pulmonary vascular pressures via lower body positive pressure may have not been high enough to trigger shunt opening (100).

Lovering used an alternative route to alter pulmonary conditions by controlling inspired oxygen tension (67). Hypoxia is a potent pulmonary vasoconstrictor as well as a trigger for increase in cardiac output which respectively can lead to gains in pulmonary vascular pressure and flow. Lovering found that hypoxia (F_iO_2 = 12%) had both acute and persistent effects on intrapulmonary shunting. Hypoxia induced 3 of 9 subjects to shunt at rest while no subjects showed any indication of shunt under normoxia (F_iO_2 = 21%). Hypoxia also triggered 4 of 9 subjects to shunt at a lower workload during exercise in comparison to normoxic conditions. All 9 subjects continued to shunt during the 3 min recovery stage (post-exercise) under hypoxia while only 5 subjects shunted during normoxic recovery. Lovering suggested that these arteriovenous vessels may not behave like conventional pulmonary vessels which vasoconstrict in response to a hypoxic stimulus. Instead, these vessels may respond to changes in oxygen tension directly and vasodilate under hypoxia (67).

Laurie found that 30 minutes of breathing 10% oxygen at rest triggered intrapulmonary arteriovenous shunting in 12 of 12 subjects and also demonstrated decreasing FiO_2 led to an earlier onset (*i.e.* time) of intrapulmonary shunting (62). Pulmonary artery systolic pressure (PASP) was measured using continuous wave Doppler imaging but no significant difference was found between PASP during normoxic and hypoxic breathing despite an increase in shunt presence.

Lovering showed breathing hyperoxic air (F_iO_2 = 100%) substantially decreased the onset of intrapulmonary shunting during exercise in comparison with normoxic exercise (68). Furthermore, using a bubble score where the amount of "shunting" was qualitatively assessed by

the numbers of bubbles present in the image, Lovering was able to show that the bubble grade also decreased at each workload during hyperoxia when compared with normoxia (68). Lovering also demonstrated acute responses of intrapulmonary arteriovenous shunting to hyperoxia: breathing hyperoxia for 120 s eliminated shunting at the same workload at which shunting occurred during normoxia (68).

It appears that the recruitment of intrapulmonary shunting can be regulated through changes in pressure and oxygen tension. The effect of oxygen tension contributes to changes in pulmonary pressure but whether oxygen tension has a direct role in vasodilation of these arteriovenous vessels remains to be elucidated. As it appears that once these vessels are open, they remain open until the cessation of the stimulus. It is unclear whether these vessels open in an "all-or-none" manner or that they open progressively with smaller ones being triggered first.

1.3.5 Significance of Intrapulmonary Shunt

The arteriovenous vessels responsible for intrapulmonary shunting have been hypothesized as to be remnant foetal vessels that played an *in utero* role in shunting blood away from the pulmonary circulation (66). These vessels seem to be present in late gestation and early neonatal mammalians but close off in adult development (76). As these vessels can be recruited during exercise or hypoxic conditions, it is likely that these vessels do not fully seal and can open with changes in pressure and/or oxygen tension. The role of the arteriovenous intrapulmonary shunt remains controversial (53, 66, 109).

Physiologically, intrapulmonary shunting could explain the proportion of pulmonary gas exchange inefficiency that is unaccounted for during exercise (28). Though the presence of intrapulmonary shunt has been found to be significantly correlated with the widening of $AaDO_2$

(67), the direct quantification of intrapulmonary shunt to the widening of $AaDO_2$ has not been investigated. Furthermore, in order to rule out the other causes of pulmonary gas exchange impairment, there has yet to be a study that has compared V_A/Q mismatch or diffusion limitation with intrapulmonary shunt in their relation to the widening of $AaDO_2$. However, with respect to the only study that quantified the intrapulmonary shunt with the use of technetium-99m labelled albumin microspheres (65), there appears to be a ~1-2% increase in shunt fraction from baseline to maximal exercise. Though it may seem minor and insignificant, a 2% shunt fraction of cardiac output is adequate enough to contribute to two-thirds of the widening of $AaDO_2$ during exercise (28, 34).

From a pathological standpoint, intrapulmonary shunting has also been suggested to be involved in paradoxical embolizations that result in neurological insults such as strokes, migraines, and transient ischemic attacks and post-operative neurological sequelae such as delirium and cognitive dysfunction (53, 66). Although the intracardiac shunt (*e.g.* patent foramen ovale) can account for a portion of these events, up to 54% of patients who have experienced a cryptogenic stroke did not have a PFO (61). Emboli are harmless in venous circulation but can cause serious injury once entering the arterial systemic circulation. Without the presence of an intracardiac shunt, one possible route for emboli to bypass the 8-10 µm sieve provided by the pulmonary circulation could be via these arteriovenous shunts.

1.4 Conclusion: Asthma and Shunt

Exercise puts stress on the respiratory and cardiovascular systems. An increase in oxygen demand is supplied by increases in ventilation and cardiac output. The increase in ventilation results in greater flow within the airways. When ventilation is high, the airways

become exposed to unfavourable conditions. Asthma is a disease that affects airway function. In exercise-induced asthma, the increase in ventilation dries and cools the airways resulting in a hyperactive narrowing response. This increase in airway resistance can be detrimental to an exercising individual as the delivery of oxygen to working tissues is challenged which is further compounded by the increase in work of breathing.

An increase in cardiac output puts stress onto the cardiovascular system with the gains in pressure and flow within the pulmonary and systemic vasculature. It is this increase in pulmonary pressure and flow that appears to trigger the opening of the intrapulmonary arteriovenous shunts. The physiological consequence of intrapulmonary arteriovenous shunting is still not well established but it appears to compromise oxygen delivery by impairing pulmonary gas exchange. There is very little known about the mechanism of the intrapulmonary shunt.

Hypoxic vasoconstriction is a protective mechanism that limits perfusion to hypoxic or poorly ventilated lung units. An asthmatic airway can become obstructed and thus poorly ventilated. If ventilation in the airways influences its perfusion, is it possible that the changes in airway resistance in an asthmatic airway could influence blood flow through the intrapulmonary shunts? If so, an exercising asthmatic individual could potentially be slowed by two deterrents of oxygen delivery – airway obstruction and intrapulmonary shunting.

1.5 Research Question

1.5.1 Purpose

There are three objectives for this thesis. The primary objective is to investigate the presence of intrapulmonary shunting in asthmatic athletes. The secondary objective is to

examine the effects of airway obstruction on intrapulmonary shunt recruitment at rest and during exercise. Two conditions will be compared: induced airway obstruction and reduced airway obstruction. The study will induce airway obstruction in fit asthmatics with the eucapnic voluntary hyperpnoea test and will reduce airway obstruction through the administration of 200 µg of inhaled salbutamol. The last objective is to examine the effects of gravity on intrapulmonary shunt recruitment at rest. Three body positions will be used to examine the effect of gravity on the lungs: supine, head-down tilt and upright. The presence of intrapulmonary shunting will be determined by the technique of agitated saline contrast echocardiography.

1.5.2 Hypotheses

We hypothesize that asthmatic athletes will demonstrate intrapulmonary shunting at submaximal exercise. We also hypothesize that induced airway obstruction will result in an earlier onset of intrapulmonary shunting during incremental exercise than the reversed airway obstruction condition. Lastly, we hypothesize that the head-down tilt will trigger intrapulmonary shunting at rest.

Chapter 2: Body of thesis

2.1 Introduction

Asthma is a chronic inflammatory disease that affects the airways. Approximately 90% of individuals with chronic asthma have exercise-induced asthma (EIA) (60). EIA is described as the transient increase in airway resistance triggered by vigorous exercise in individuals with asthma (92). The prevalence of EIA is higher among the athletic population than the general population. It is estimated between 5-20% of the general population have EIA (38), while up to 50% of elite endurance athletes have EIA (113).

The hallmark features of asthma are airway hyperresponsiveness, airway inflammation, and variable airway obstruction (75). Airway hyperresponsiveness leads to bronchoconstriction and in combination with airway inflammation, the asthmatic airway narrows and there is an increase in airway resistance (75). These changes in the airways contribute to the common symptoms associated with asthma: coughing, wheezing, shortness of breath (dyspnoea), and excessive mucous production. One of the most common prophylactic treatments for bronchoconstriction is the use of inhaled salbutamol. Salbutamol is a short-acting beta-2 adrenergic agonist that acts to relieve symptoms via inducing bronchodilation, preventing mediator release and reducing vascular permeability (92). Such effects are desirable ventilatory features for exercise and have fuelled controversy over the last 30 years regarding the potential ergogenic effects of inhaled beta-2 adrenergic agonists (30).

Airway obstruction, caused by bronchoconstriction, inflammatory mediator release, mucosal edema, and thickening of the airway wall, can also interfere with pulmonary gas exchange (19). There are few studies that have evaluated pulmonary gas exchange in asthmatics;

however, it appears that pulmonary gas exchange impairment is more severe in asthmatics than non-asthmatics (39, 97).

The alveolar-arterial oxygen difference ($AaDO_2$) is indicative of abnormalities in pulmonary gas exchange and excessive widening of $AaDO_2$ during exercise can lead to the decline in arterial oxygen saturation, known as "exercise-induced arterial hypoxemia" (EIAH) (25, 26). EIAH impairs oxygen transport (25, 26), oxygen consumption (84), and ultimately performance (58). In healthy individuals, the development of EIAH is related to aerobic fitness (*i.e.* maximum oxygen consumption or VO_2max) (25). Elite endurance athletes often have VO_2max 150-200% of the predicted normal values and it is estimated that EIAH occurs in as many as 50% of elite endurance athletes (83).

Increased $AaDO_2$ can be caused by ventilation-perfusion (V_A/Q) mismatch, diffusion limitation, and shunt (26). V_A/Q mismatch and diffusion limitation have long been advocated as the primary factors to pulmonary gas exchange impairment during exercise (26). In asthmatics, V_A/Q mismatch is further worsened due to non-uniform airway obstruction (112). However, the understanding of pulmonary gas exchange impairment is far from complete. Recent work on gas exchange during exercise has shed new light on the role of the intrapulmonary shunt (28, 67, 99). Exercise-induced intrapulmonary shunt has been demonstrated in the healthy, fit population and is suggested to occur via anatomical arteriovenous vessels (28). Shunt recruitment appears to be significantly correlated with increasing cardiac output, increasing pulmonary vascular pressures, and widening of $AaDO_2$ (99). Modulation of oxygen tension also seems to be involved in shunt recruitment: breathing hypoxic gas induces the recruitment of the intrapulmonary shunt at rest (62, 67) while breathing hyperoxic gas results in the delay in onset of intrapulmonary shunting during exercise (68). Lastly, body position also appears to have an effect on intrapulmonary

shunt recruitment. Intrapulmonary shunting appears to be present in some individuals lying supine at rest (99, 100). However, the mechanism by which shunting occurs has yet to be elucidated (53).

During exercise, asthmatic athletes have two potential sources of pulmonary gas exchange impairment: exercise and airway obstruction. There is scant research on pulmonary gas exchange during exercise in asthmatic athletes. To our knowledge, exercise-induced intrapulmonary shunting has not been demonstrated in asthmatic athletes. More research on this unique sub-population is needed, particularly the effects of airway obstruction and intrapulmonary shunting during exercise.

The study will have three objectives: the primary objective of the study is to investigate the presence of intrapulmonary shunting in asthmatic athletes at rest and during exercise. The secondary objective is to see if an acute change in airway obstruction has an effect on the recruitment of the intrapulmonary shunt. The subjects will be examined under two experimental conditions: induced airway obstruction via an eucapnic voluntary hyperpnoea test (Condition A); and reversed airway obstruction via an inhalation of salbutamol (Condition B). We hypothesize that all asthmatic athletes will present intrapulmonary shunting during exercise and that induced airway obstruction will trigger an earlier onset of intrapulmonary shunting compared to reversed airway obstruction. Lastly, the tertiary objective is to investigate the effects of body position on the recruitment of the intrapulmonary shunt. The subjects will be examined under three positions at rest: supine, head-down tilt, and upright. We hypothesize that the head-down tilt position will trigger the opening of the intrapulmonary shunt at rest. Studying the intrapulmonary shunt during exercise within the asthmatic, highly aerobically trained population will have relevance to their asthma, health and performance.

2.2 Methods

The study received approval from the University of British Columbia Clinical Research Ethics Board. Each subject was given a detailed description of the study and permitted to participate after giving a written, informed consent.

2.2.1 Subjects

The inclusion criteria for the study comprised of two elements: asthma and high aerobic fitness. To participate in the study, the subject had a positive test for EIA (the eucapnic voluntary hyperpnoea test) and a VO_2max of at least 55ml/kg/min (or 4.2 L/min) determined by a maximal exercise test. Subjects with atrial or ventricular septal defects (*i.e*. intracardiac shunt) were excluded. 14 male, competitive athletes aged 19-40 years of age were recruited from the Point Grey University of British Columbia (UBC) campus and the Greater Vancouver triathlon and cycling clubs. A resting echocardiogram revealed a previously unrecognized patent foramen ovale in 1 subject who was then excluded from the study. 3 subjects were excluded due to technical reasons. The remaining 10 subjects composed of: 2 recreational cyclists, 5 competitive cyclists, 1 competitive triathlete, 1 professional triathlete, 1 varsity soccer player and 1 varsity track athlete.

2.2.2 Study Design

All data collection took place at the Exercise Physiology Lab at the Allan McGavin Sports Medicine Centre at UBC. The subjects came to the laboratory on 3 occasions with each visit separated by at least 72 hours. The first visit served as a screening day where the subject performed the EVH test and the maximal exercise test. Upon meeting the inclusion criteria, the

subject returned for two more visits. Two experimental conditions were randomly assigned to one visit each: induced airway obstruction (Condition A) and reversed airway obstruction (Condition B). The overview of the study design is outlined in Appendix A.

Prior to Day 1 and the day Condition B was applied, subjects were asked to withhold the use of long- and short-acting bronchodilator medication at least 48 hours and 8 hours, respectively. Regular use of inhaled corticosteroids was also refrained for 72 hours prior to the aforementioned visits. Any other asthmatic treatments were permitted but recorded. Consumption of caffeine was withheld at least 8 hours as well as vigorous exercise on the day of each visit.

2.2.3 Pulmonary Function

Pulmonary function was measured using spirometry. Spirometry was performed according to the American Thoracic Society guidelines (2). Each spirometry test included: 3 tidal breathing flow-volume curves proceeded by a maximal expiratory flow-volume (MEFV) loop. Calibration via a 3-L calibration syringe (Hans Rudolph Inc., Kansas City, MO) was done prior to data collection. The flow-volume loops were obtained from True One 2400, Parvo Medics (Salt Lake City, UT) with pulmonary function capabilities. From the flow-volume loops, the following variables were measured and calculated: forced vital capacity (FVC), forced expiratory volume in 1 second ($FEV_{1.0}$), $FEV_{1.0}$/FVC ratio, peak expiratory flow (PEF), and mean forced expiratory flow of midexpiratory volume (FEF_{25-75}).

2.2.4 Eucapnic Voluntary Hyperpnoea Test

The eucapnic voluntary hyperpnoea (EVH) test used to diagnose exercise-induced asthma closely followed the protocol used by numerous sporting bodies (*e.g.* the International Olympic Committee) on elite athletes (3, 90). Baseline pulmonary function was measured 3 times in the standing position. The subject was seated onto a designated chair positioned in front of a monitor that displayed the subject's current ventilation rate. The subject was instructed to ventilate through a mouthpiece, with nose-clip on, at a target ventilation rate for 6 minutes. The target ventilation rate was calculated as 30 x $FEV_{1.0}$ (3). The mouthpiece was connected to a two-way nonrebreathing valve (Hans Rudolph 2700 Series, Kansas City, MO). The inspired gas, comprised of 5.0% CO_2, 21.0% O_2, and balance N_2 at room temperature, was delivered to a 150L Douglas Bag which was kept inflated and monitored throughout the testing. Ventilation was measured with a heated pneumotach (True One 2400, Parvo Medics, Salt Lake City, UT). Subjects were encouraged to meet and maintain the target ventilation rate. Pulmonary function was measured twice at 3-min, 5-min, 10-min, 15-min, and 20-min post-hyperventilation. The subject was confirmed positive for EIA if the decline in $FEV_{1.0}$ from baseline was greater than 10% (27, 90). The greatest percent decline in $FEV_{1.0}$ following EVH was used to determine the severity of the subject's EIA.

2.2.5 Maximal Exercise Test

Each subject's aerobic fitness was determined by the measurement of the maximum oxygen consumption (VO_2max) during a maximal exercise test. The subject was seated on a Velotron cycle ergometer (RacerMate Inc., Seattle, WA) with a nose-clip and mouthpiece on. The gas analyzer was calibrated using standard gases and the pneumotach was calibrated with

the 3-L calibration syringe. Expired gases were collected and analyzed with the data recorded in 15-s intervals (True One 2400, Parvo Medics, Salt Lake City, UT).

The subject completed the EVH test at least one hour before the start of the maximal exercise test. Considering bronchodilator treatment was withheld prior to the EVH test, all subjects were given 200 µg of inhaled salbutamol 15 minutes prior to the maximal exercise test – this ensured that any airway obstruction was reversed. Spirometry was performed to confirm full recovery of lung function. The subjects were given a self-selected warm-up for 5 minutes. At the start of the maximal exercise test, the workload was reset to 0W and a constant ramp of 30W per minute was applied until volitional fatigue. The subject's VO_2max was indicated when 3 of the 4 criteria were met: > 90% of age-predicted maximum heart rate, respiratory exchange ratio (RER) > 1.15, plateau of VO_2 with increase in workload, and volitional exhaustion. The value of VO_2max was calculated as the 4 highest consecutive 15-second VO_2 measures.

Arterial oxygen saturation (SaO_2) was estimated through pulse oximetry (SpO_2) (Ohmeda Biox 3740, BOC Health Care Inc., Louisville, CO) and heart rate was measured via telemetry (Polar Vantage XL, Kempele, Finland). SpO_2 and heart rate was measured continuously and averaged every 15 seconds. The pulse oximeter was taken at the ear lobe and a vasodilator gel (Finalgon, Boehringer Ingelheim, Burlington, ON) was applied to improve perfusion to the ear. The pulse oximeter was internally calibrated prior to each test. Oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory exchange ratio (RER), minute ventilation (V_E), breathing frequency (B_f), and tidal volume (V_T) was obtained every 15 seconds from the metabolic cart (True One 2400, Parvo Medics, Salt Lake City, UT). A fan was provided to prevent the subject from over-heating. Verbal encouragement was used to help push the subjects to perform the best of their capabilities and was kept consistent for all subjects.

2.2.6 Condition A: Induced Airway Obstruction

Airway obstruction was induced in each subject via the same 6 minute hyperventilation procedure used in the EVH test. The EVH test induces airway obstruction due to the cooling and drying effects of hyperventilation (4). High ventilation during exercise (due to evaporative heat loss) leads to increasing osmolarity of the airway epithelium and decreasing temperature within the airways, which both in turn cause bronchoconstriction, vascular engorgement, inflammatory mediator release, and ultimately, airway obstruction. Pulmonary function was measured three times before the start of the EVH and 5 minutes after to ensure that airway obstruction had been induced. The change in pulmonary function was assessed by the percent change in $FEV_{1.0}$, $FEV_{1.0}$/FVC, PEF and FEF_{25-75}. Following spirometry, the subjects were instrumented and prepared for the incremental exercise protocol with agitated saline contrast echocardiography. An average of 27 (±10) minutes elapsed between the end of the EVH test and the start of the initial agitated saline injection.

2.2.7 Condition B: Reversed Airway Obstruction

The reversal of airway obstruction was done by the administration of salbutamol prior to exercise. Following the measurement of baseline pulmonary function, subjects were given a therapeutic dose (200 µg) of inhaled salbutamol. Proper instructions were given prior to use and an Aerochamber spacer (Aerochamber, Boehringer Ingelheim, Burlington, ON) was used to standardize and optimize delivery of the drug. Pulmonary function was measured 5 minutes following the inhalation of salbutamol. The change in pulmonary function was assessed by the percent change in $FEV_{1.0}$, $FEV_{1.0}$/FVC, PEF and FEF_{25-75}. Following spirometry, the subjects were instrumented and prepared for the incremental exercise protocol with agitated saline

contrast echocardiography. An average of 27 (±14) minutes elapsed between the inhalation of salbutamol and the start of the initial agitated saline injection.

2.2.8 Body Positions and Incremental Exercise

On visits 2 and 3, subjects performed incremental exercise while agitated saline contrast echocardiography was applied. There were a total of 9 stages. The first 3 were resting stages: supine, head-down tilt and upright, followed by 6 exercise stages – EI, EII, EIII, EIV, EV, and EVI. The subject laid on a clinical examination table for the resting supine and head-down tilt stages. The head-down tilt was made by propping the subject's leg end of the table upwards which allowed gravity to increase blood flow towards the lungs; the angle of deviation from horizontal was measured to be 17°. Each position was maintained for at least 1 minute prior to testing for shunt.

Each exercise stage increased in power output and was designed individually based on the results of each subject's VO_2max test. Considering the shunt onset appears to be at approximately 60%VO_2max in healthy fit individuals (28), we aimed to start at a lower intensity and gradually increase in a stepwise fashion to increase the sensitivity in determining shunt onset. The power output assigned at each stage was set to target the subject's VO_2max at: 40% VO_2max (EI), 50% VO_2max (EII), 60% VO_2max (EIII), 75%VO_2max (EIV), 85% VO_2max (EV), and 95% VO_2max (EVI). A fan was provided during exercise to prevent the subject from overheating.

During exercise, heart rate was measured through a polar heart rate monitor (Polar Vantage XL, Kempele, Finland); peripheral oxygen saturation was measured at the ear lobe through a pulse oximeter (Ohmeda Biox 3740, BOC Health Care Inc., Louisville, CO). Heart

rate and peripheral oxygen saturation were recorded prior to agitated saline injection. Power output was measured through the cycle ergometer software (Velotron 1.5CS, RacerWatt, Seattle, WA). Borg's Category Ratio scale (0-10) was used to determine each subject's rating of s perceived exertion and perceived dyspnoea (see Appendix B). The definition of "exertion" and "dyspnoea" was explained to the subject during the intravenous line preparation. "Exertion" was described as "how hard is your whole body working at this particular time point." "Dyspnoea" was described as "how hard is your breathing at this particular time point." Ratings of perceived exertion and dyspnoea were taken every mid-point of each of the six exercise stages.

Oxygen consumption (VO_2) was not measured during incremental exercise. To estimate the subject's oxygen consumption at a given workload, we took the prescribed power output and located its point on the VO_2max test, then averaged two neighbouring VO_2 measurements at that particular power output. For example: if 150W was our target power output, we looked at the 5:00 (min:sec) mark in the VO_2max report – which at 30W/min will equate 150W – then took the average of two consecutive VO_2 measures (*i.e.* at 5:00 and 5:15).

2.2.9 Agitated Saline Contrast Echocardiography

Agitated saline contrast echocardiography was used to detect intrapulmonary shunting during incremental exercise on Day 2 and 3. Prior to the start of the protocol, a resting echocardiogram was performed to exclude intracardiac shunting. Agitated saline contrast echocardiography is a standard clinical method used to detect intrapulmonary shunting at rest (2, 8). An apical four-chamber view with harmonic imaging (Sonos 5500, Agilent Phillips Ultrasound, Andover, MA) was taken at each of the 9 stages of the resting and incremental exercise protocol. A three-lead electrocardiogram was used to count the cardiac cycles. A 20-

guage intravenous catheter with a saline solution lock was placed into one of the subject's forearm veins. A three-way stopcock was attached to the lock with two 5ml syringes connected to the other two ports. One syringe contained <1 ml of air and the other with 4-5 ml of sterile saline solution. The contrast bubbles were manually created by forcefully flushing the two syringes. The agitated saline solution was injected manually at the 1:30 min mark of each of the 2-min stages. Video Home System (VHS) recordings of the echocardiogram were made during the last 45 seconds of the 2-min stages. All VHS recordings were analyzed by an echocardiologist from Vancouver General Hospital who was blinded to the experimental conditions. The timing of appearance of bubbles on the echocardiogram was used to indicate the presence of intrapulmonary shunt. The presence of intrapulmonary shunt was defined as the appearance of bubbles in the left side of the heart after at least 3 cardiac cycles following the appearance of bubbles in the right side of the heart (28).

2.2.10 Shunt Onset During Exercise

Shunt onset was defined as the initial presence of intrapulmonary shunt during the 6 exercise stages. Nine variables were compared at shunt onset between Condition A and Condition B: power output, percent of maximum power output (%Max P), estimated VO_2, percent of maximum oxygen consumption (%VO_2max), heart rate, percent of maximum heart rate (%Max HR), oxygen saturation (SpO_2), perceived exertion, and perceived dyspnoea.

2.2.11 Data Analyses

Paired t-tests were used to compare within subject means between Condition A and Condition B (SPSS Statistics 17.0, IBM Cooperation, Endicott, New York). Relations between

variables were analysed using Pearson's correlation coefficients. Statistical significance was set at $P < 0.05$. Unless otherwise stated, data were reported as means ± SD. All echocardiograms were recorded onto a total of 2 VHS cassettes. A copy of both cassettes were made and sent to the echocardiologist at the Vancouver General Hospital for analysis of shunt presence at each stage of the resting and incremental exercise protocol.

2.3 Results

2.3.1 Subject Characteristics

Anthropometric data, aerobic fitness, baseline pulmonary function and severity of exercise-induced asthma are shown on Table 2.1. The VO_2max value was used to indicate aerobic fitness. The highest decline in $FEV_{1.0}$ following EVH was used to assess severity of exercise-induced asthma. Based on the mean VO_2max and decline in $FEV_{1.0}$, these subjects can be described as highly aerobic-trained athletes with mild to moderate exercise-induced asthma. Individual characteristics are listed in Table C.1 in Appendix C.

Table 2.1: Subject Characteristics

N	10
Age (years)	26.7 ± 6.4
Height (cm)	179.1 ± 5.7
Weight (kg)	72.2 ± 8.2
VO_2max (ml/kg/min)	62.6 ± 6.7 (136 ± 18.2)
VO_2max (L/min)	4.5 ± 0.4
Max P (Watts)	410.4 ± 40.4
FVC (L)	6.21 ± 0.55 (112.3 ± 11.4)
$FEV_{1.0}$ (L)	4.52 ± 0.41 (98.5 ± 9.0)
$FEV_{1.0}$/FVC	0.73 ± 0.07
PEF(L/s)	10.34 ± 1.31
FEF_{25-75} (L/s)	3.69 ± 0.79 (75.7 ± 16.2)
$FEV_{1.0}$´ (%)	19.2 ± 7.9

Values are means ± SD. Values in parentheses are percent predicted. VO_2max, maximum oxygen consumption; Max P, maximum power output; FVC, baseline forced vital capacity; $FEV_{1.0}$, baseline forced expiratory volume in 1 second; PEF, baseline peak expiratory flow;

FEF_{25-75}, baseline forced expiratory flow of midexpiratory volume; $FEV_{1.0}$´, highest percent decline in $FEV_{1.0}$ after the screening eucapnic voluntary hyperpnoea test.

2.3.2 Pulmonary Function Change Under Condition A and B

The individual and mean changes in pulmonary function under Condition A and Condition B are described in Table 2.2. Pulmonary function was measured after the intervention was applied. Changes in $FEV_{1.0}$, $FEV_{1.0}$/FVC, PEF, and FEF_{25-75} between Condition A and Condition B within subjects were found to be significantly different ($P < 0.05$). More individual data on pulmonary function change under Condition A and Condition B are listed in Table C.2 and Table C.3 in Appendix C, respectively.

Table 2.2: Pulmonary Function Change

	$FEV_{1.0}$ (%)		$FEV_{1.0}$/FVC (%)		PEF (%)		FEF_{25-75} (%)	
Subject	A	B	A	B	A	B	A	B
1	-12.6	8.6	-12.0	6.8	-17.4	14.0	-29.4	20.4
2	-11.5	7.4	-1.3	10.3	-23.9	6.2	-4.7	25.4
3	-4.9	6.2	-1.8	0.2	-19.7	13.9	-4.7	6.7
4	-17.5	3.5	-11.0	4.8	-13.2	5.7	-27.0	5.7
5	-17.6	1.2	-11.9	4.8	-23.6	-2.8	-33.9	13.1
6	-19.8	2.0	-9.5	4.1	-15.4	-0.17	-28.8	11.6
7	-22.9	4.0	-17.3	3.3	-26.3	6.7	-41.2	11.0
8	-11.5	5.8	-7.6	9.2	-13.9	1.8	-24.2	10.0
9	-14.3	5.8	-7.0	7.8	-18.7	3.1	-19.6	19.0
10	-18.3	-0.21	-13.9	-0.03	-15.6	0.15	-42.4	0.92
M ± SD	-15.1 ± 5.2	4.4* ± 2.8	-8.8 ± 5.7	5.11* ± 3.47	-18.77 ± 4.54	4.9* ± 5.7	-26.3 ± 12.0	12.4* ± 7.4

All values are percent change; $FEV_{1.0}$, forced expiratory volume in one second; FVC, forced vital capacity; PEF, peak expiratory flow; FEF_{25-75}, forced expiratory flow of midexpiratory volume; A, Condition A: induced airway obstruction; B, Condition B: reversed airway obstruction; M ± SD, mean ± standard deviation; *, significantly different from Condition A ($P < 0.05$).

2.3.3 Workloads During Incremental Exercise

The mean assigned power output for each exercise stage (EI-EVI), the respective estimated oxygen consumptions (~VO_2), percent of maximum power output (%MaxP) and

percent of maximum oxygen consumption (%VO_2max) are described on Table 2.3. Mean %MaxP and %VO_2max at each exercise stage correlated significantly ($R^2 = 0.999$, $P < 0.05$); see Figure D.1 in Appendix D. The assigned power outputs at each exercise stage (EI-EVI) for each subject and their respective estimated oxygen consumptions are listed in Tables C.4-C.13 in Appendix C.

Table 2.3: Workloads During Incremental Exercise

	EI	EII	EIII	EIV	EV	EVI
Power Output (W)	112.5± 13.2	162.5 ± 13.2	225 ± 26.4	278 ± 27.8	327 ± 29.1	378 ± 31.5
%Max P	27.4 ± 1.6	39.9 ± 2.0	55.0 ± 3.2	67.8 ± 2.5	79.8 ± 2.4	92.2 ± 2.1
~VO_2 (ml/kg/min)	23.8 ± 2.4	30.4 ± 2.9	39.5 ± 4.4	46.7 ± 5.8	54.6 ± 5.9	61.2 ± 7.0
%VO_2max	38.2 ± 3.9	48.7 ± 3.3	63.1 ± 2.2	74.5 ± 4.1	87.2 ± 2.0	97.7 ± 1.5

Values are means ± SD. EI-EVI, exercise stage I-VI; %Max P, percent of maximum power output; ~VO_2, estimated oxygen consumption at given workload (ml/kg/min); %VO_2max, percent of maximum oxygen consumption.

2.3.4 Intrapulmonary Shunt via Agitated Saline Contrast Echocardiography

Intrapulmonary shunt was demonstrated in all subjects included in the study. Three of ten subjects shunted at rest: 1 subject demonstrated shunt at supine under Condition B, 1 subject at supine under Condition A, and 1 subject at supine under Condition B as well as at head-down tilt under both Condition A and B. Ten of ten subjects shunted during exercise; once a subject demonstrated the onset of intrapulmonary shunt at a particular exercise stage, the shunt remained recruited through all subsequent stages. Under Condition A, 8 subjects demonstrated shunt at the first workload (EI), 1 subject shunted at the second workload (EII) and 1 subject at the third workload (EII). Under Condition B, 9/10 subjects demonstrated shunt at the same workload as in Condition A. There was a delay in shunt onset in 1 subject; this individual shunted at EI in Condition A and at EII in Condition B. Individual data taken while determining presence of shunt are listed in Tables C.4-C.13 in Appendix C.

Figures 2.1, 2.2 and 2.3 were obtained from a 24 year-old male (%predicted VO_2max = 160) and the images were taken from an apical 4-chamber view. The echocardiogram images taken for all 9 stages on all 10 subjects under both experimental conditions were clear and a decision of intrapulmonary shunt presence for each stage was able to be made. Figure 2.1 depicts an echocardiogram of the subject sitting upright on the Velotron; the image is taken after at least 3 cardiac cycles following appearance of bubbles in the right heart. At this point, both the right atrium and right ventricle are densely opacified with contrast bubbles, while the left atrium and left ventricle remain free of bubbles – this indicates the trapping of bubbles at the pulmonary capillaries and thus, no indication of intrapulmonary shunt. Figure 2.2 depicts an echocardiogram the subject cycling upright at 125 W; the image is taken at least 3 cardiac cycles following appearance of bubbles in the right heart. Again, the right side of the heart is full of contrast bubbles but there are clearly bubbles present in the left side of the heart which indicates the presence of intrapulmonary shunting. Figure 2.3 depicts an echocardiogram of the subject cycling at 300 W; at least 3 cardiac cycles following appearance of bubbles in the right heart, the right heart is full of contrast bubbles while the left heart has a cloud of contrast bubbles as well – this shows a prominent presence of intrapulmonary shunting. Although we are unable to quantify the shunt fraction, the degree of contrast bubble presence in the left side of the heart between figure 2.1, 2.2, and 2.3 can qualitatively provide some insight on the likelihood of the increase in intrapulmonary shunt presence as workload increases.

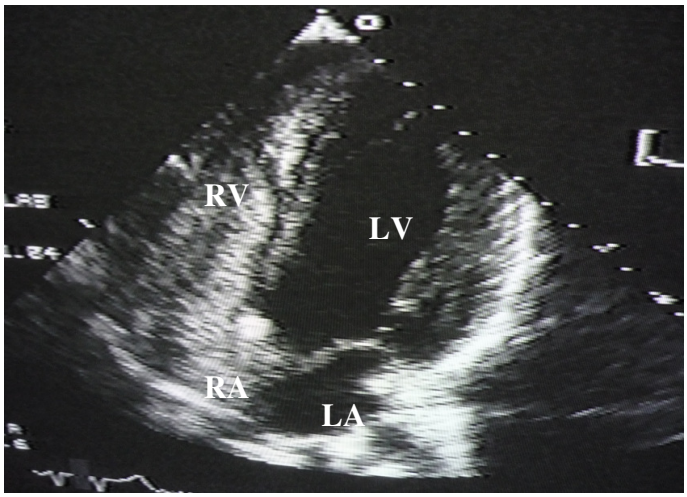

Figure 2.1: Contrast Echocardiogram of 24-year-old Male at Resting Upright. Image depicts the subject's heart >3 cardiac cycles following appearance of bubbles in right heart. Note: no presence of bubbles in left side of heart; RA, right atrium; RV, right ventricle; LA, left atrium; LV, left ventricle.

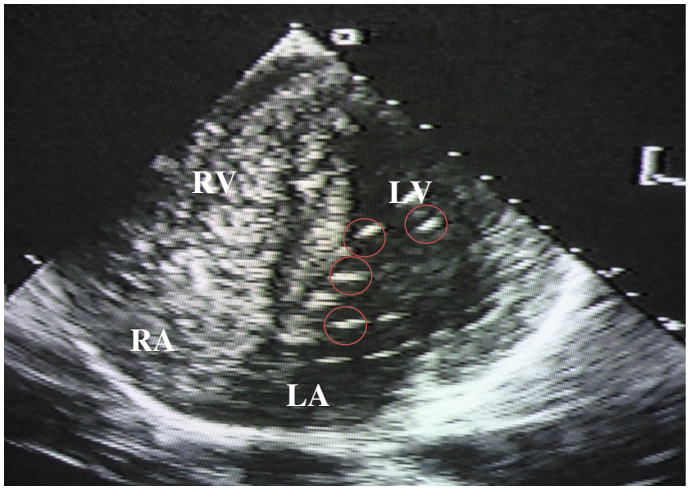

Figure 2.2: Contrast Echocardiogram of 24-year-old Male Cycling Upright at 125 W. Image depicts the subject's heart >3 cardiac cycles following appearance of bubbles in the right heart. Note: the presence of bubbles highlighted by the circles in the left side of the heart; RA, right atrium; RV, right ventricle; LA, left atrium; LV, left ventricle.

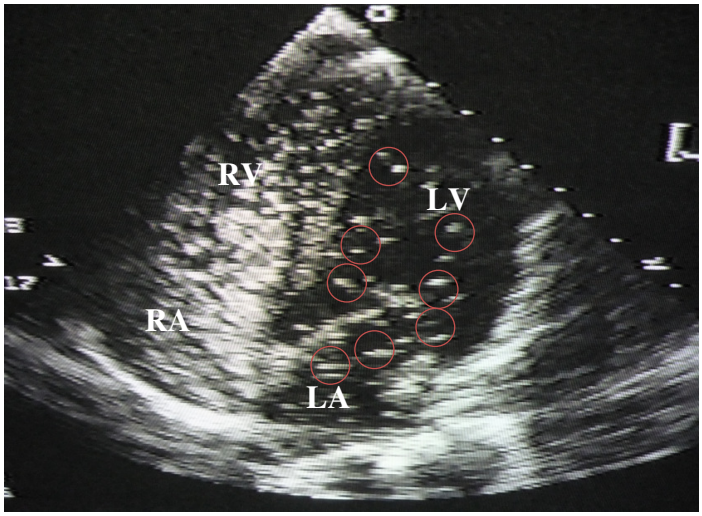

Figure 2.3: Contrast Echocardiogram of 24-year-old Male Cycling Upright at 300 W. Image depicts the subject's heart >3 cardiac cycles following appearance of bubbles in the right heart. Note: the clouds of bubbles highlighted by the circles in the left side of heart; RA, right atrium; RV, right ventricle; LA, left atrium; LV, left ventricle.

2.3.5 Physiological Data on the Onset of Intrapulmonary Shunt

Individual power output (P), %Max P, estimated VO_2, %VO_2max, heart rate (HR), %Max HR, SpO_2, perceived exertion, and perceived dyspnoea at the onset of intrapulmonary shunt under Condition A and Condition B are listed on Table 2.4 and 2.5, respectively. Mean P, %Max P, estimated VO_2, %VO_2max, HR (bpm), %Max HR, SpO_2 (%), perceived exertion, and perceived dyspnoea are listed on Table 2.6. There was no significant difference found between mean shunt onset under Condition A and Condition B. Shunt onset between Condition A and Condition B in %Max P and %VO_2max are depicted in Figure 2.4 and Figure 2.5, respectively.

Table 2.4: Physiological Data at Onset of Intrapulmonary Shunt Under Condition A

S	P (W)	%MaxP	~VO_2 (ml/kg/min)	%VO_2max	HR (bpm)	%MaxHR	SpO_2 (%)	E	D
1	200	52.2	38.5	60.3	131	74.4	97	5	4
2	150	37.3	30.4	46.1	136	69.7	98	3	4
3	100	28.7	23.5	40.3	113	60.4	98	2	2
4	125	29.4	25.8	34.2	108	61.4	97	2	1
5	125	29.4	23.5	34.9	108	60.0	98	1	1
6	100	26.6	19.6	37.9	102	56.4	98	2	2
7	100	26.7	21.8	34.9	112	58.0	96	3	2
8	125	26.9	27.2	40.9	101	53.7	96	0.5	0.5
9	125	26.6	26.2	46.2	118	61.1	95	2	1
10	125	28.7	22.7	38.8	115	62.5	98	1	1

S, subject; P, power output (watts); %MaxP, percent of maximum power output; ~VO_2, estimated oxygen consumption at given workload (ml/kg/min); %VO_2max, percent of maximum oxygen consumption; HR, heart rate (beats per minute); %MaxHR, percent of maximum heart rate; SpO_2, peripheral oxygen saturation (%); E, perceived exertion (0-10); D, perceived dyspnoea (0-10).

Table 2.5: Physiological Data at Onset of Intrapulmonary Shunt Under Condition B

S	P (W)	%MaxP	~VO_2 (ml/kg/min)	%VO_2max	HR (bpm)	%MaxHR	SpO_2 (%)	E	D
1	200	52.2	38.5	60.3	132	60.2	97	2	2
2	150	37.3	30.4	46.1	122	54.9	98	0.5	0.5
3	100	28.7	23.5	40.3	121	64.7	97	2	0.5
4	125	29.4	25.8	34.2	108	61.4	97	2	2
5	125	29.4	23.5	34.9	105	58.3	97	1	2
6	150	39.9	25.3	48.8	113	62.4	97	2	1
7	100	26.7	21.8	34.9	112	68.9	97	1	1
8	125	26.9	27.2	40.9	110	58.5	95	0	0
9	125	26.1	26.2	46.2	111	66.8	97	1	1
10	125	28.7	22.7	38.8	126	68.5	98	3	3

S, subject; P, power output (watts); %MaxP, percent of maximum power output; ~VO_2, estimated oxygen consumption at given workload; %VO_2max, percent of maximum oxygen consumption; HR, heart rate (beats per minute); %MaxHR, percent of maximum heart rate; SpO_2, oxygen saturation (%); E, perceived exertion (0-10); D, perceived dyspnoea (0-10).

Table 2.6: Mean Physiological Data on the Onset of Intrapulmonary Shunt

	Condition A	Condition B
Power Output (W)	127.5 ± 29.9	132.5 ± 29.0
%Max P	31.3 ± 8.0	32.5 ± 8.3
Estimated VO_2 (ml/kg/min)	25.9 ± 5.4	26.5 ± 4.9
%VO_2max	41.4 ± 7.9	42.5 ± 8.1
Heart Rate (bpm)	114.4 ± 11.4	116.0 ± 8.7
%Max HR	61.8 ± 6.1	62.7 ± 5.5
SpO_2 (%)	97.3 ± 1.0	97.0 ± 0.8
Perceived Exertion (0-10)	2.2 ± 1.3	1.5 ± 0.9
Perceived Dyspnoea (0-10)	1.9 ± 1.2	1.3 ± 0.9

Values are means ± SD. Condition A, induced airway obstruction; Condition B, reversed airway obstruction; %Max P, percent of maximum power output; VO_2, oxygen consumption; %VO_2max, percent of maximum oxygen consumption; Heart rate (beats per minute); %Max HR, percent of maximum heart rate; SpO_2, oxygen saturation (%).

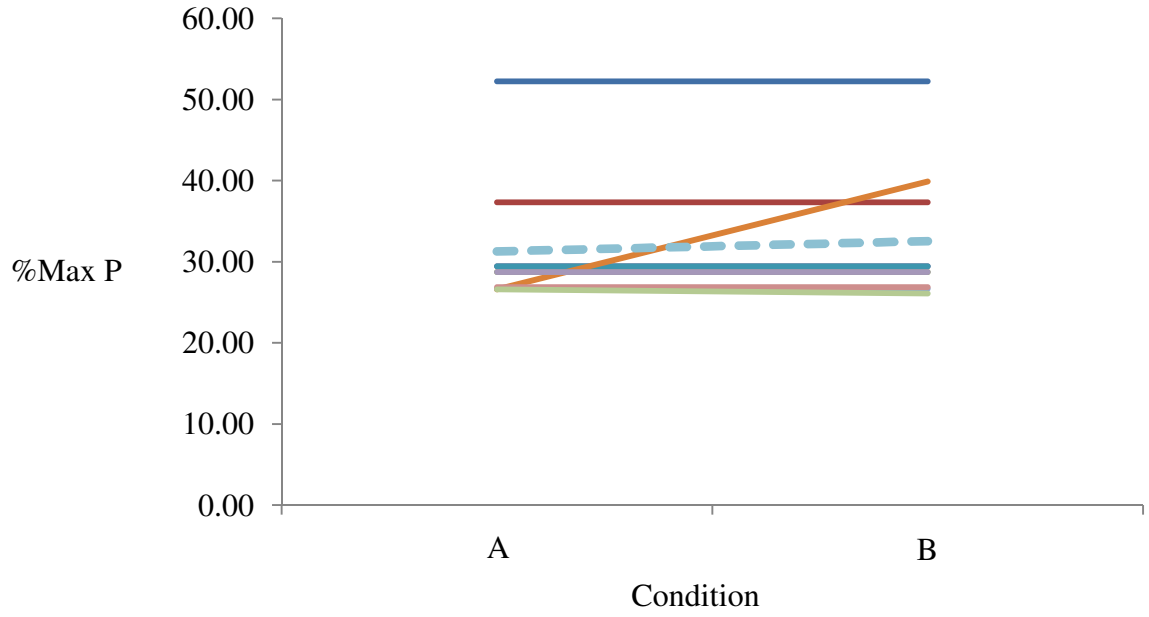

Figure 2.4: Comparison of %MaxP at Shunt Onset Between Condition A *vs*. Condition B. %Max P, percent of maximum power output; Condition A, induced airway obstruction; Condition B, reversed airway obstruction; solid lines depict individual data; dotted line depicts mean.

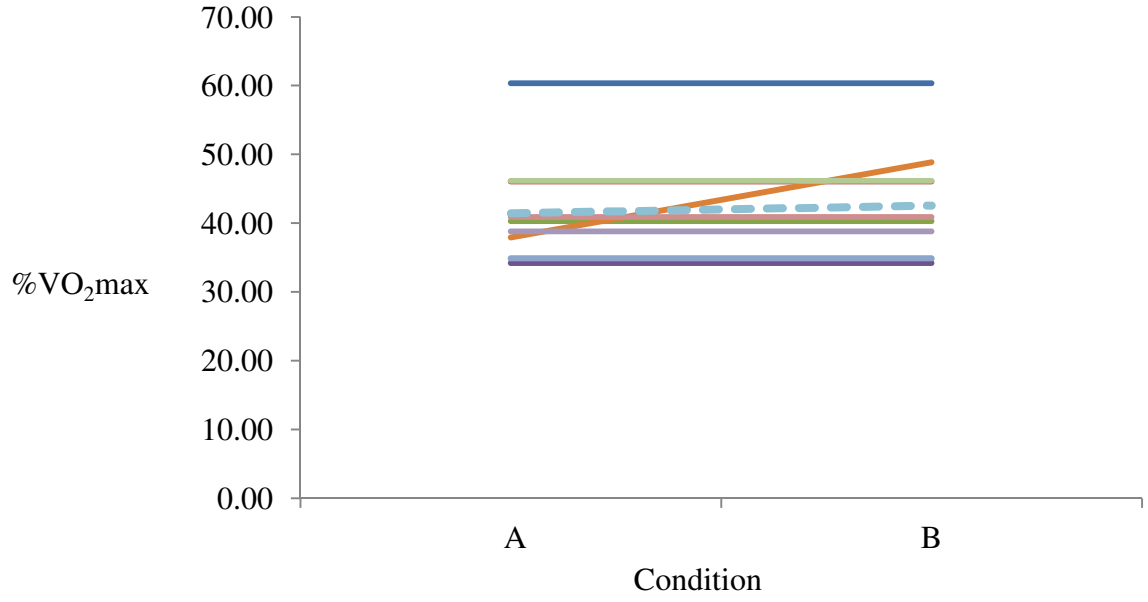

Figure 2.5: Comparison of %VO_2max at Shunt Onset Between Condition A vs. Condition B. %VO_2max, percent of maximum oxygen consumption; Condition A, induced airway obstruction; Condition B, reversed airway obstruction; solid lines depict individual data; dotted line depicts mean.

2.4 Discussion

The objectives of this study were to investigate the presence of intrapulmonary shunting in asthmatic athletes at rest and during exercise, and to determine the effects of airway obstruction and body position on shunt recruitment. Using agitated saline contrast echocardiography, we found the presence of intrapulmonary shunting during exercise in all subjects tested. Transpulmonary passage of agitated saline occurred at rest in 3/10 subjects and at submaximal exercise in 10/10 subjects. Among the 3 subjects who shunted at rest, 2 subjects demonstrated shunt at the supine position and 1 from both the supine and head-down tilt position. Responses to Condition A and Condition B at rest was inconsistent. During exercise, once a subject demonstrated shunt onset, the shunt persisted throughout each subsequent, progressive exercise stage. Mean shunt onset under induced airway obstruction and reversed airway obstruction was 41.4 ± 7.9 %VO_2max and 42.5 ± 8.1 %VO_2max, respectively; no significant difference was found. The recruitment of intrapulmonary shunt does not appear to be significantly influenced by acute changes in airway obstruction nor body position.

2.4.1 Methodological Considerations

Agitated saline contrast echocardiography is a standard clinical method used to detect intrapulmonary shunting at rest (15, 35, 47) and is also more recently used during exercise (28, 67, 68, 99, 100). In spite of its extensive use, there are a few aspects of this technique that need to be addressed. Firstly, the path taken by the bubbles to get to the heart is unknown. During exercise, pulmonary vascular pressures increase and thus it is possible the bubbles may not be traversing across arteriovenous vessels but through distended capillaries (28). However, the likeliness of transpulmonary passage of the bubbles through the distended capillaries is slim (28,

64, 67). The pulmonary capillaries are no larger than 10 µm in diameter at rest and 13 µm during exercise (32). Bubbles smaller than 8 µm have survival time of <190 ms, and thus collapse well before entering the left heart after 3 cardiac cycles (67). Even at a maximal heart rate of 180 beats per min, the transit time of 3 cardiac cycles will be a full second. Thus, the "smaller" bubbles that may pass through the capillaries are unlikely to survive by the time they reach the left side of the heart (28). Furthermore, the passage of bubbles larger than the pulmonary capillaries through the pulmonary microcirculation would require a driving pressure of 300 mmHg and pulmonary vascular pressures, even during maximal exercise, rarely exceed 30 mmHg (28). Next, agitated saline contrast echocardiography is based on qualitative imaging and thus is unable to measure shunt fraction. Without shunt fraction, the actual contribution to pulmonary gas exchange efficiency is unknown (53). With respect to this limitation, this study is descriptive in nature and conclusions made are based on qualitative findings. When strictly determining the presence of intrapulmonary shunting, agitated saline contrast echocardiography was an effective tool to use for this study.

Shunt onset is defined in this study as the initial presence of intrapulmonary shunt. To assess the changes in shunt onset with the two conditions of airway obstruction, the mean differences in power output, %Max P, heart rate, %Max HR, estimated VO_2, %VO_2max, SpO_2, perceived exertion, and perceived dyspnoea were compared. As oxygen consumption was not directly measured during shunt data collection, there are a few limitations in regards to its use in determining mean shunt onset. Oxygen consumption at each workload was estimated by the power output performed, cross-referenced with the same workload during the maximal exercise test (where VO_2 was measured). Considering the subject performed the exercise on the same cycle ergometer and that the subjects were competitive endurance athletes who maintained their

aerobic conditioning, the variability within subjects in regards to VO_2 and workload should not be very significant. Furthermore, basic exercise physiology dictates that workload and VO_2 are linearly related. In spite of the estimation, the relationship between workload and VO_2 was strong as we found the correlation of %Max P and $\%VO_2max$ at each exercise stage to have a $R^2 = 0.999$ ($P < 0.05$), see Figure D.1 in Appendix D.

2.4.2 Intrapulmonary Shunt Recruitment

In this study, it was hypothesized that the induced airway obstruction condition would trigger an earlier onset of the intrapulmonary shunt during exercise. Although we did not find significant differences between mean shunt onset between Condition A and Condition B, the effect of airway obstruction cannot necessarily be ruled out. 8/10 subjects demonstrated presence of shunt immediately at the first workload under Condition A and 1 individual showed a delay in shunt recruitment when airway obstruction was reversed (*i.e.* under Condition B). Furthermore, when comparing mean shunt onset of the asthmatic athletes of this study to that of the non-asthmatic fit population in a separate study (28), it appears that asthmatic athletes shunt at a lower workload than non-asthmatics. Eldridge in 2004 found the mean shunt onset of 11 healthy males ($VO_2max = 51.9 \pm 10.1$ ml/kg/min) to be $61 \pm 18\%VO_2max$ while we found the mean shunt onset of 10 asthmatic, aerobically males ($VO_2max = 62.6 \pm 6.7$ ml/kg/min) under Condition A to be $41.4 \pm 7.9\%VO_2max$. This difference is significant and leads us to wonder if there is an inherent difference in the pulmonary hemodynamic responses to exercise in asthmatics vs. non-asthmatics due to their differences within the airways.

The pulmonary and systemic vasculatures have a unique relationship in that they are directly connected in series. Cardiac output from the right heart passes through the pulmonary

circulation, back to the left heart, and out to the systemic circulation. Thus during exercise, an increase in cardiac output leads to an increase in pulmonary vascular pressure. However, the pulmonary vascular bed is delicate and unlike the systemic vasculature, it is unable to withstand high pressures. Under normal resting conditions, the mean pulmonary artery pressure (mPAP) is approximately 12-16 mmHg while mean arterial pressure (MAP) is usually about 70-90 mmHg. During exercise, mPAP rises up to 25 mmHg and it is this increase in pulmonary vascular pressure that appears to be involved in the recruitment of the intrapulmonary shunt (100).

Hypoxia, a potent vasoconstrictor of the pulmonary vasculature, has consistently been shown to trigger intrapulmonary shunting at rest and during exercise in healthy humans (62, 67). As alluded to earlier, there appears to be difference in shunt onset between asthmatics and non-asthmatics during exercise. We suggest that hypoxic pulmonary vasoconstriction may have a role in the earlier recruitment of intrapulmonary shunt in asthmatics. Although the literature on gas exchange during exercise in asthmatics is scarce, it has been found that asthmatics suffer greater pulmonary gas exchange impairment than non-asthmatics during exercise (41). This difference appears to be caused by non-uniform airway obstruction leading to worsened V_A/Q mismatch (41, 112). However, we propose that airway obstruction may also play another role in pulmonary gas exchange impairment: airway obstruction can lead to poorly ventilated airways which may become hypoxic and in turn, result in hypoxic pulmonary vasoconstriction. Hypoxic pulmonary vasoconstriction increases pulmonary vascular pressure and thus, an asthmatic may more frequently work under higher pulmonary pressures than non-asthmatics and consequently possess a more sensitive trigger to the opening of the intrapulmonary shunt. This suggestion is speculative as there are no studies to date that have investigated changes in pulmonary vascular

pressures during airway obstruction. However, the findings of this study should warrant future research on pulmonary hemodynamic responses to exercise in asthmatics.

2.4.3 Physiological Effects of Intrapulmonary Shunting During Exercise

In healthy individuals at rest, pulmonary gas exchange is adequate as $AaDO_2$ is kept relatively low at 5-10 mmHg. During exercise, $AaDO_2$ can rise to 25-35 mmHg at maximal workloads in aerobically fit athletes (115). Pulmonary gas exchange impairment can lead to the decline in arterial oxygenation and consequently limit performance (58). Increase in $AaDO_2$ can arise from V_A/Q mismatch, diffusion limitation and intrapulmonary shunting. In this study, we studied a group of highly aerobically trained (VO_2max = 62.6 ± 6.7 ml/kg/min), asthmatic males who demonstrated intrapulmonary shunting at low workloads (41-43%VO_2max). In both Condition A and B at the last exercise stage, where the subjects were cycling at maximal workloads (95%VO_2max), SpO_2 was measured to be above 95% in 7/10 subjects while 3/10 subjects went below 93%. By definition, these 3/10 subjects experienced EIAH. However, shunt fraction and arterial blood gases were not measured in this study. Thus, it is difficult to assess the influence of intrapulmonary shunting on the decline in SpO_2 during exercise in these subjects. V_A/Q mismatch and diffusion limitation are considered to be the main contributors to pulmonary gas exchange impairment despite being unable to fully account for the rises in $AaDO_2$ during exercise (94). The presence of intrapulmonary shunting has shown to be involved in the decline in pulmonary gas exchange efficiency during (99) and thus its role in gas exchange limitation may be overlooked. The role of intrapulmonary shunt in pulmonary gas exchange needs further investigation.

The prevalence of intrapulmonary shunting during normoxic exercise appears to be 90% in healthy, fit population (28, 67, 99, 100). In the one study that measured pulmonary vascular pressures during exercise and compared the changes in pressure with the presence of intrapulmonary shunting, the single individual who did not shunt had the highest mean pulmonary artery pressure among the 7 others who developed intrapulmonary shunt during exercise (99). High pressures within the pulmonary vascular beds can result in pulmonary edema or haemorrhage as this is known to occur at high altitude (64, 114) and during maximal exercise in elite athletes (74). It is possible that the arteriovenous vessels responsible for the intrapulmonary shunt have a protective role in the pulmonary circulation and serve as "pop-off" valves to relieve increases in pulmonary vascular pressures. Future studies should investigate the presence of intrapulmonary shunting in individuals susceptible to high altitude pulmonary edema or exercise-induced pulmonary haemorrhage.

2.4.4 Effects of Airway Obstruction During Exercise

Under the two conditions of airway obstruction, all but one subject were able to complete the exercise stages. Subject 4 was unable to complete the final exercise stage under the condition of induced airway obstruction. Measurement of airway obstruction during exercise was not made; however, perceived exertion and perceived dyspnoea were used as surrogates to assess the subject's perception of overall workload and work of breathing. It is interesting to find that during maximal exercise, there appears to be a difference in the perception of work between Condition A and Condition B. At the final exercise stage (*i.e*. EVI), the mean perceived exertion of Condition A and Condition B were 9.3 ± 1.1 and 8.0 ± 1.5 (P = 0.056) and the mean perceived dyspnoea of Condition A and Condition B were 9.1 ± 1.4 and 7.3 ± 2.0 (P = 0.05).

The ratings of perceived exertion and perceived dyspnoea under Condition A and Condition B during the exercise stages are depicted in Figure D.2 and Figure D.3 of Appendix D, respectively. Whether their airways were obstructed or reversed, it appears that asthmatic athletes were able to perform under the same workloads in spite of the differences in their perception of work. The perception of work by the mind and the body can be different – future studies should examine how this difference may influence performance.

2.4.5 Summary

Using agitated saline contrast echocardiography we have demonstrated the presence of intrapulmonary shunting in 3/10 subjects at rest and in all 10 subjects during exercise. Our subjects were male, asthmatic and highly aerobically trained. No significant difference in mean shunt onset was found between induced airway obstruction and reversed airway obstruction. However, we did find that reversed airway obstruction delayed shunt onset in 1 subject when compared to induced airway obstruction. Furthermore, shunt onset during exercise in asthmatics appears to occur at a lower workload than non-asthmatics. The relationship between airway obstruction and intrapulmonary shunting remains unclear, however, this study suggests that the long-term airway obstruction asthmatics experience may have some influence on their intrapulmonary shunt recruitment. We propose that hypoxic pulmonary vasoconstriction, brought on by airway obstruction, is involved in intrapulmonary shunt recruitment via changes in pulmonary vascular pressure. Airway obstruction also appears to have an effect on an asthmatic's perception of work and breathlessness during exercise. This study also reinforces the need for asthmatic athletes to have their condition properly treated.

Chapter 3: Conclusion

This study investigated a unique group of individuals who were highly aerobically trained despite having exercise-induced asthma. During exercise, asthmatic athletes deal with pulmonary challenges that their non-asthmatic counterparts are not faced with. Recent studies investigating the intrapulmonary shunt have shifted the dogma of pulmonary gas exchange during exercise. Intrapulmonary shunting occurs at submaximal exercise in 90% of the healthy population (28, 67, 99, 100). This study is the first to demonstrate intrapulmonary shunting in the asthmatic population and is also the first to specifically investigate its presence in highly aerobically trained individuals. All 10 subjects tested presented the intrapulmonary shunt at submaximal workloads (41-43%VO_2max).

Airway obstruction is a hallmark feature of asthma. When comparing two conditions of airway obstruction: induced airway obstruction and reversed airway obstruction, no difference in mean shunt onset was found. However, there are two findings of the study that indicate the effects of airway obstruction on an asthmatic during exercise: First, asthmatics appear to shunt at lower workload than non-asthmatics (41.4 ± 7.9% VO_2max *vs*. 61 ± 18% VO_2max) (28). It is also interesting to find that 1 subject demonstrated a delay in shunt onset when airway obstruction was reversed. Second, at higher workloads, perceived exertion and perceived dyspnoea were greater in the induced airway obstruction condition than the reversed airway condition. The subjective awareness of having to work harder at a given workload may deter an asthmatics ability to perform.

The recruitment of the intrapulmonary shunt has been shown to be influenced by pulmonary vascular pressures (99, 100), body position (99, 100), and hypoxia (62, 67). In this study, we also investigated the effect of gravity and body position on shunt recruitment. We

examined three body positions at rest: supine, head-down tilt, and upright. We found 3/10 subjects shunted at rest: 2 subjects at the supine position and 1 subject at both the supine and head-down tilt position. Changes in body positions did not appear to have a consistent impact on intrapulmonary shunt recruitment.

3.1 Application to Exercise Physiology

Exercise puts stress to nearly all systems of the body. The heart and lung work harder and faster to supply working tissues with adequate oxygen while removing metabolic waste. This increase in power generated causes an increase in pressures within the systemic and pulmonary vasculature. The body must now balance functional supply and demand with structural limitations. Intrapulmonary shunting epitomizes this see-saw battle. Shunting is suggested to help relieve the increases in pulmonary vascular pressures during exercise but also appears to contribute to pulmonary gas exchange impairment (28, 69, 99).

An asthmatic faces limitations of air flow within the airways. In exercise-induced asthma, it is the air itself that harms the airways (4). The intricate relationship between the heart and lungs extends to the blood-gas interface between the pulmonary capillaries and alveoli. Ventilation to the alveoli and perfusion to the capillaries is necessary for adequate pulmonary exchange to occur. In asthma, increased airway resistance caused by airway obstruction can lead to poor ventilation of the affected airways and this may result in V_A/Q mismatch, which is detrimental to pulmonary gas exchange (112). It has been shown that asthmatics experience greater impairment of pulmonary gas exchange than non-asthmatics (41, 112), though V_A/Q mismatch may not be the sole contributor to the rise in $AaDO_2$. We found a group of fit asthmatic males to have a shunt onset significantly lower than a group of non-asthmatic males

(28). Frequent exposures to airway obstruction during exercise may be involved in the recruitment of intrapulmonary shunt and thus, may be more sensitive to trigger in asthmatics than individuals without such a condition.

3.2 Conclusions Regarding Thesis Hypotheses

The purpose of this study was three-fold: our primary objective was to demonstrate the presence of intrapulmonary shunting in asthmatic athletes. Our secondary objective was to investigate the effects of airway obstruction on the onset of intrapulmonary shunting during exercise. 10/10 subjects demonstrated shunt during exercise. Using two conditions of airway obstruction, induced airway obstruction vs. reversed airway obstruction, we found that no significant difference in mean shunt onset between the two conditions. Our last objective was to examine if body position had an influence on shunt recruitment. We compared three body positions at rest: supine, head-down tilt and upright. Three of ten subjects demonstrated shunt at rest: 2 subjects at the supine position and 1 subject at both the supine and head-down tilt position. We conclude that asthmatic athletes shunt like 90% of their non-asthmatic counterparts (28, 67, 99, 100) during submaximal exercise but appear to have an earlier onset of shunt during exercise than non-asthmatics (41.4 ± 7.9%VO_2max *vs*. 61 ± 18%VO_2max) (28). Acute changes in airway obstruction and body position do not appear to have a consistent effect on intrapulmonary shunt recruitment.

3.3 Strengths and Limitations

The difference in mean shunt onset between Condition A and Condition B was not found to be significant; this could have been contributed by a number of things: first, following the

EVH test, the subjects may have experienced a refractory period where the airways fail to narrow following a subsequent attack – this may have prevented the induction of airway obstruction during the exercise stages. Because pulmonary function was not measured during the resting and exercise stages, the actual degree of airway obstruction is unknown; second, the appearance of bubbles are limited by the time constraints of the last 30 seconds of imaging during each 2-min stage and the 2-dimensional view of the echocardiograms – there is a chance of the shunt being triggered but we were unable to "see" the bubbles. Furthermore, as there was one subject who did demonstrate a difference in shunt onset between Condition A and Condition B, the sample size of 10 may have been too small to accurately assess the significance of this difference.

This study is descriptive and all conclusions made from intrapulmonary shunting were based on the qualitative imaging provided by agitated saline contrast echocardiography. Shunt fraction, blood gases and pulmonary vascular pressures were not measured. Arterial oxygen saturation was estimated using pulse oximetry (SpO_2). Thus, the relationship between airway obstruction and pulmonary gas exchange impairment during exercise was not accurately assessed.

However, the objectives of the study were centred on investigating the presence of intrapulmonary shunt. From this perspective, obtaining shunt fraction, though useful, was not the primary objective. There are few studies that have investigated pulmonary gas exchange in asthmatics during exercise (41, 42, 112). Though pulmonary gas exchange was not directly measured, intrapulmonary shunt is still a contributor to pulmonary gas exchange (26, 99, 114). We demonstrated the presence of intrapulmonary shunting in a population that has not been extensively investigated. Asthmatic athletes are a unique sub-population and are sometimes able to out-perform their non-asthmatic counterpart despite being limited by their condition (75). In

this study, we were able to provide evidence for airway obstruction may be involved in intrapulmonary shunt recruitment. Understanding how this shunt is triggered will provide insight on its mechanism and effect on the body.

3.4 Future Directions

Intrapulmonary shunting occurs at submaximal exercise and is persistent throughout exercise. Furthermore, it appears to be more prominent as workload increases. More work is needed to assess the changes in shunt fraction during incremental exercise and its effect on pulmonary gas exchange. As the intrapulmonary shunt appears to be recruited by increases in pulmonary vascular pressures, it would be instrumental to investigate the presence of intrapulmonary shunting in individuals susceptible to high altitude or exercise-induced pulmonary edema. It would be also interesting to examine the changes in pulmonary gas exchange and pulmonary vascular pressures between individuals who shunt and don't shunt.

To further evaluate the effects of airway obstruction on pulmonary hemodynamics, future studies should investigate the changes in pulmonary vascular pressure caused by airway obstruction. It is shocking that there is scarce literature on the physiological responses to airway obstruction in spite of the high prevalence of asthma in the trained and un-trained population. In this study, we found our group of asthmatics to have a mean shunt onset that is at much lower workload than non-asthmatics. However, it is noteworthy to mention the difference in aerobic fitness between the two samples: the 11 healthy males from Eldridge's study (28) had a mean VO_2max of 51.9 ± 10.1 ml/kg/min while this study collected 10 asthmatic males with a mean VO_2max of 62.6 ± 6.7 ml/kg/min. To reinforce our findings, a follow-up study could compare the effects of airway obstruction on intrapulmonary shunting between non-asthmatics *vs*.

asthmatics that are equally fit. Future studies should also compare the hemodynamic responses to exercise between asthmatics and non-asthmatics.

References

1. Aitken ML, Marini JJ. Effect of heat delivery and extraction on airway conductance in normal and in asthmatic subjects. *Am Rev Respir Dis* 131: 367-361, 1985.

2. American Thoracic Society. Standardization of spirometry – 1994 update. *Am J Respir Crit Care Med* 152: 1107-1136, 1995.

3. American Thoracic Society. Guidelines for methacholine and exercise challenge testing – 1999, *Am J Respir Crit Care Med* 161: 309-329, 2000.

4. Anderson SD. Is there a unifying hypothesis for exercise-induced asthma? *J Allergy Clin Immun* 73: 660-665, 1984.

5. Anderson SD, Caillaud C, Brennan JD. Beta2-agonists and exercise-induced asthma. *Clin Rev Allergy Immu* 31: 163-180, 2006.

6. Anderson SD, Daviskas E. The mechanism of exercise-induced asthma is... *J Allergy Clin Immun* 106: 453-459, 2000.

7. Anderson SD, Kippelen P. Exercise-induced bronchoconstriction: Pathogenesis. *Cur Allergy Asthma Rep* 5: 116-202, 2005.

8. Anderson SD, Kippelen P. Airway injury as a mechanism for exercise-induced bronchoconstriction in elite athletes. *J Allergy Clin Immun* 122: 225-235, 2008.

9. Anderson SD, Schoeffel RE, Black JL, Daviskas E. Airway cooling as the stimulus to exercise-induced asthma – a re-evaluation. *Eur J Respir Dis* 67: 20-30, 1985.

10. Argyros GJ, Phillips YY, Rayburn DB, Rosenthal RR, Jaeger JJ. Water loss without heat flux in exercise-induced bronchospasm. *Am Rev Respir Dis* 147: 1419-1424, 1993.

11. Backer V, Lung T, Pedersen L. Pharmaceutical treatment of asthma symptoms in elite athletes – doping or therapy? *Scand J Med Sci Sports* 17: 615-622, 2007.

12. Ballester E, Reyes A, Roca J, Guitart R, Wagner PD, Rodriguez-Roisin R. Ventilation-perfusion mismatching in acute severe asthma: effects of salbutamol and 100% oxygen. *Thorax* 44: 258-267, 1989.

13. Barbera JA, Peinado VI, Santos S. Pulmonary hypertension in chronic obstructive pulmonary disease. *Eur Respir J* 21: 892-905, 2003.

14. Barnes NC, Burke CM, Poulter LW, Schleimer RP. The anti-inflammatory profile of inhaled corticosteroids: biopsy studies in asthmatic patients. *Respir Med* 94: S16-21, 2000.

15. Barzilai B, Waggoner AD, Spessert C, Picus D, Goodenberger D. 2-Dimensional contrast echocardiography in the detection and follow-up of congenital pulmonary arteriovenous malformations. *Amer J Cardiol* 68: 1507-1510, 1991.

16. Beck KC, Offord KP, Scanlon PD. Bronchoconstriction occurring during exercise in asthmatic subjects. *Am J Respir Crit Care Med* 149: 352-357, 1994.

17. Becker AB, Simons FER. Formoterol, a new long-acting selective beta-2-agonist, decreases airway responsiveness in children with asthma. *Lung* 168: 99-102, 1990.

18. Berk J, Hagen J, Roo R, Nomoto S, Rupright M. Pulmonary arteriovenous shunting due to epinephrine. *Horm Metab Res* 5: 65, 1973.

19. Bonsignore MR, Morici G, Vignola AM, Riccobono L, Bonanno A, Profita M, *et al*. Increased airway inflammatory cells in endurance athletes: what do they mean? *Clin Exp Allergy* 33: 14-21, 2003.

20. Bousquet J. Global Initiative for Asthma (GINA) and its objectives. *Clin Exp Allerg*. 31: 2-5, 2000.

21. Carlsen KH, Anderson SD, Bjermer L, Bonini S, Brusasco V, *et al*. Exercise-induced asthma, respiratory and allergic disorders in elite athletes: epidemiology, mechanisms, and diagnosis: part I of the report from the Joint Task Force of the European Respiratory Society and the European Academy of Allergy and Clinical Immunology in cooperation with GA2LEN. *Allergy* 63: 387-403, 2008.

22. Carlsen KH, Anderson SD, Bjermer L, Bonini S, Brusasco V, *et al* Treatment of exercise-induced asthma, respiratory and allergic disorders in sports and the relationship to doping: Part II of the report from the Joint Task Force of the European Respiratory Society and the European Academy of Allergy and Clinical Immunology in cooperation with GA2LEN. *Allergy* 63: 492-505, 2008.

23. Cohen R, Overfield EM, Johannes AK. Diffusion component of alveolar-arterial oxygen pressure difference in man. *J Appl Physiol* 31: 223-226, 1971.

24. Conhaim RL, Staub NC. Reflection spectrophotometric measurement of O2 uptake in pulmonary arterioles of cats. *J Appl Physiol* 48: 848-856, 1980.

25. Dempsey JA, Hanson PC, Henderson KS. Exercise-induced arterial hypoxemia in healthy human subjects at sea level. *J Physiol Lond* 355: 161-175, 1984.

26. Dempsey JA, Wagner PD. Exercise-induced arterial hypoxemia. *J Appl Physiol*. 87: 1997-2006, 1999.

27. Dickinson JW, Whyte GP, McConnell AK, Harries MG. Screening elite winter athletes for exercise-induced asthma: a comparision of three challenge methods. *Brit J Sport Med* 40: 179-182, 2006.

28. Eldridge MW, Dempsey JA, Haverkamp HC, Lovering AT, Hokanson JS. Exercise-induced intrapulmonary arteriovenous shunting in healthy humans. *J Appl Physiol* 97: 797-805, 2004.

29. Elliott JE, Choi Y, Laurie SS, Yang X, Gladstone IM, Lovering AT. Effect of initial gas bubble composition on detection of inducible intrapulmonary arteriovenous shunt during exercise in normoxia, hypoxia or hyperoxia. *J Appl Physiol* 110: 35-45, 2011.

30. Fitch KD. B2-agonists at the Olympic Games. *Clin Rev Allerg Immu* 31: 259-268, 2006.

31. Fitch KD, Sue-Chu M, Anderson SD, Boulet LP, Hancox LP, McKenzie DC, *et al*. Asthma and the elite athlete: summary of the International Olympic Committee's consensus conference, Lausanne, Switzerland, January 22-24, 2008. *J Allergy Clin Immun* 122: 254-260, 2008.

32. Glazier JB, Hughes JM, Maloney JE, West JB. Measurements of capillary dimensions and blood volume in rapidly frozen lungs. *J Appl Physiol* 26: 65-76, 1969.

33. Global Initiative for Asthma. Pocket guide for asthma management and prevention – 2009.

34. Gledhill N, Froese AB, Dempsey JA. Ventilation to perfusion distribution during exercise in health. In: *Muscular Exercise and the Lung,* edited by Dempsey JA and Reed CE. Madison, WI: University. Of Wisconsin Press, 1977, p. 325-344.

35. Gudavalli A, Kalaria VG, Chen X, Schwarz KQ. Intrapulmonary arteriovenous shunt: diagnosis by saline contrast bubbles in the pulmonary veins. *J Am Soc Echocardiogr* 15: 1012-1014, 2002.

36. Hammond MD, Gale GE, Kapitan KS, Ries A, Wagner PD. Pulmonary gas exchange in humans during exercise at sea level. *J Appl Physiol* 60: 1590-1598, 1986.

37. Hancox RJ, Subbarao P, Kamada D, Watson RM, Hargreave FE, Inman MD. B2-agonist tolerance and exercise-induced bronchospasm. *Am J Resp Crit Care* 165: 1068-1070, 2002.

38. Handoyo S, Rosenwasser LJ. Asthma Phenotypes. *Curr Allerg Asthma Rep*. 9: 439-445, 2009.

39. Haney S, Hancox RJ. Recovery from bronchoconstriction and bronchodilator tolerance. *Clin Rev Allerg Immu*. 31: 181-196, 2006.

40. Haverkamp HC, Dempsey JA, Miller JD, Romer LM, Pegelow DF, Lovering AT, Eldridge MW. Repeat exercise normalizes the gas-exchange impairment induced by a previous exercise bout in asthmatic subjects. *J Appl Physiol* 99: 1843-1852, 2005.

41. Haverkamp HC, Dempsey JA, Miller JD, Romer LM, Pegelow DF, Rodman JR, Eldridge MW. Gas exchange during exercise in habitually active asthmatic subjects. *J Appl Physiol* 99: 1938-1950, 2005.

42. Haverkamp HC, Dempsey JA, Pegelow DF, Miller JD, Romer LM, Santana M, Eldridge MW. Treatment of airway inflammation improves exercise pulmonary gas exchange and performance in asthmatic subjects. *J Allergy Clin Immunol* 120: 39-47, 2007.

43. Helenius I, Lumme A, Haahtela T. Asthma, airway inflammation and treatment in elite athletes. *Sports Med* 35: 565: 574, 2005.

44. Helenius I, Rytila P, Sarna S, Lumme A, Helenius M, Remes V, Haahtela T. Effect of continuing or finishing high-level sports on airway inflammation, bronchial hyperresponsiveness, and asthma: A 5-year prospective follow-up study of 42 highly trained swimmers. *J Allergy Clin Immunol* 109: 962-968, 2002.

45. Henriksen JM. Effect of inhalation of corticosteroids on exercise induced asthma – randomized double-blind crossover study of budesonide in asthmatic children. *Brit Med J* 291: 248-249, 1985.

46. Henriksen JM, Agertoft L, Pedersen S. Protective effect and duration of action of inhaled formoterol and salbutamol on exercise-induced asthma in children. *J Allerg Clin Immu* 89: 1176-1182, 1992.

47. Hernandez A, Strauss AW, McKnight R, Hartmann AF, Jr. Diagnosis of pulmonary arteriovenous fistula by contrast echocardiography. *J Pediatr* 93: 258-261, 1978.

48. Ho V. Current concepts in the management of hepatopulmonary syndrome. *Vasc Health Risk Manag* 4: 1035-1041, 2008.

49. Hopkins SR. Exercise-induced arterial hypoxemia: the role of ventilation-perfusion inequality and pulmonary diffusion limitation. *Adv Exp Med Biol* 588: 17-30, 2006.

50. Hopkins SR, Belzberg AS, Wiggs BR, McKenzie DC. Pulmonary transit time and diffusion limitation during heavy exercise in athletes. *Respir Physiol* 103: 67-73, 1996.

51. Hopkins SR, Harms CA. Gender and pulmonary gas exchange during exercise. *Exerc Sport Sci Rev* 32: 50-56, 2004.

52. Hopkins SR, McKenzie DC, Schoene RB, Glenny RW, Robertson HT. Pulmonary gas exchange during exercise in athletes. I. Ventilation-perfusion mismatch and diffusion limitation. *J Appl Physiol* 77: 912:917, 1994.

53. Hopkins SR, Olfert IM, Wagner PD, Lovering AT, Eldridge MW, Stickland MK. Point-counterpoint: Exercise-induced intrapulmonary shunting is imaginary vs. real. *J Appl Physiol*. 107: 993-1001, 2009.

54. Jameson AG. Diffusion of gases from alveolus to precapillary arteries. *Science* 139: 826-828, 1964.

55. Johnson BD, Scanlon PD, Beck KC. Regulation of ventilatory capacity during exercise in asthmatics. *J Appl Physiol* 79: 892-901, 1995.

56. Kindermann W. Do Inhaled β2-Agonists have an ergogenic potential in non-asthmatic competitive athletes? *Sports Med* 37: 96-102, 2007.

57. Kirby JG, Hargreave FE, Gleich GJ, O'Byrne PM. Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects. *Am Rev Respir Dis* 136: 379-383, 1987.

58. Koskolou MD, McKenzie DC. Arterial hypoxemia and performance during intense exercise. *Eur J Appl Physiol* 68: 80-86, 1994.

59. Kosmas EN, Milic-Emili J, Polychronaki A, Dimitroulis I, Retsou S, Gaga M, Koutsoukou A, Roussos C, Koulouris NG. Exercise-induced flow limitation, dynamic hyperinflation and exercise capacity in patients with bronchial asthma. *Eur Respir J* 24: 378-384, 2004.

60. Lacroix VJ. Exercise-induced asthma. *Phys Sports Med*. 27: 75-92, 1999.

61. Lamy C, Giannesini C, Zuber M, Arquizan C, Meder JF, Trystram D, Coste J, Mas JL. Clinical and imaging findings in cryptogenic stroke patients with and without patent foramen ovale: the PFO-ASA Study. Atrial Septal Aneurysm. *Stroke* 33: 706-711, 2002.

62. Laurie SS, Yang X, Elliott JE, Beasley KM, Lovering AT. Hypoxia-induced intrapulmonary arteriovenous shunting at rest in healthy humans. *J Appl Physiol* 109: 1072-1079, 2010.

63. Lee WL, Graham AF, Pugash RA, Hutchison SJ, Grande P, Hyland RH, Faughman ME. Contrast echocardiography remains positive after treatment of pulmonary arteriovenous malformations. *Chest* 123: 351-358, 2003.

64. Lovering AT, Haverkamp HC, Eldridge MW. Responses and limitations of the respiratory system to exercise. *Clin Chest Med* 26: 439-457, 2005.

65. Lovering AT, Haverkamp HC, Romer LM, Hokanson JS, Eldridge MW. Transpulmonary passage of 99mTc macroaggregated albumin in healthy humans at rest and during maximal exercise. *J Appl Physiol* 106: 1986-1992, 2009.

66. Lovering AT, Pulmonary pathways and mechanisms regulating transpulmonary shunting into the general circulation: An update. *Injury* 41: S16-23, 2010.

67. Lovering AT, Romer LM, Haverkamp HC, Pegelow DF, Hokanson JS, Eldridge MW. Intrapulmonary shunting and pulmonary gas exchange during normoxic and hypoxic exercise in healthy humans. *J Appl Physiol* 104: 1418-1425, 2008.

68. Lovering AT, Stickland MK, Amann M, Murphy JC, O'Brien MJ, Hokanson JS, Eldridge MW. Hyperoxia prevents exercise-induced intrapulmonary arteriovenous shunt in healthy humans. *J Physiol* 586: 4559-4565, 2008.

69. Lovering AT, Stickland MK, Eldridge MW. Intrapulmonary shunt during normoxic and hypoxic exercise in healthy humans. *Adv Exp Med Biol* 588: 31-45, 2006.

70. Lovering AT, Stickland MK, Kelso AJ, Eldridge MW. Direct demonstration of 25- and 50-µm arteriovenous pathways in healthy human and baboon lungs. *Am J Physiol Heart CircPhysiol* 292: H1777-1781, 2007.

71. McClaran SR, Wetter TJ, Pegelow DF, Dempsey JA. Role of expiratory flow limitation in determining lung volumes and ventilation during exercise. *J Appl Physiol* 86: 1357-1366, 1999.

72. McFadden ER, Jr. Hypothesis: exercise-induced asthma as a vascular phenomenon. *Lancet* 335: 880-883, 1990.

73. McKenzie DC, Lama IL, Potts JE, Sheel AW, Coutts KD. The effect of repeat exercise on pulmonary diffusing capacity and EIH in trained athletes. *Med Sci Sports Exerc* 31: 99-104, 1999.

74. McKenzie DC, O'Hare TJ, Mayo J. The effect of sustained heavy exercise on the development of pulmonary edema in trained male cyclists. *Respir Physiol Neurobiol* 145: 209-218, 2005.

75. McKenzie DC, Stewart IB, Fitch KD. The asthmatic athlete, inhaled beta agonists, and performance. *Clin J Sport Med* 12: 225-228, 2002

76. McMullen DM, Hanley FL, Cohen GA, Portman MA, Riemer RK. Pulmonary arteriovenous shunting in the normal fetal lung. *J Am Coll Cardiol* 44: 1497-1500, 2004.

77. Milanese M, Saporiti R, Bartolini S, Pellegrino R, Baroffio M, Brusasco V, Crimi E. Bronchodilator effects of exercise hyperpnea and albuterol in mild-to-moderate asthma. *J Appl Physiol* 107: 494-499, 2009.

78. National Institute of Health. Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma – August 28, 2007.

79. Niden AH, Aviado DM Jr. Effects of pulmonary embolism on the pulmonary circulation with special reference to arteriovenous shunts in the lung. *Crit Res* 4: 67-73, 1956.

80. Nomoto S, Berk JL, Hagen JF, Koo R. Pulmonary anatomic arteriovenous shunting caused by epinephrine. *Arch Surg* 108: 201-204, 1974.

81. Norris SR, Petersen SR, Jones RL. The effect of salbutamol on performance in endurance cyclists. *Eur J Appl Physiol* 73: 364-368, 1996.

82. Phillips YY, Jaegar JJ, Laube BL, Rosenthal RR. Eucapnic voluntary hyperventilation of compressed gas-mixture – a simple system for bronchial challenge by respiratory heat-loss. *Am Rev Respir Dis* 131: 31-35, 1985.

83. Powers SK, Dodd S, Lawler J, Landry G, Kirtley M, McKnight T, Grinton S. Incidence of exercise-induced hypoxemia in elite endurance athletes at sea level. *Eur J Appl Physiol* 58: 298-302, 1988.

84. Powers SK, Lawler J, Dempsey JA, Dodd S, Landry G. Effects of incomplete pulmonary gas exchange on VO2max. *J Appl Physiol* 66: 2491-2495, 1989.

85. Prinzmetal M, Ornitz ME, Simkin B, Bergman HC. Arterio-venous anastomoses in liver, spleen and lungs. *Am J Physiol* 152: 48-52, 1948.

86. Rahn H, Stroud RC, Tobin CE. Visualization of arterio-venous shunts in by cineflurography in the lungs of normal dogs. *Proc Soc Exp Biol* 80: 239, 1952.

87. Rodriguez-Roisin R. Gas exchange abnormalities in asthma. *Lung* S: 599-605, 1990.

88. Rundell KW, Im J, Mayers LB, Wilber RL, Szmedra L, Schmitz HR. Self-reported symptoms and exercise-induced asthma in the elite athlete. *Med Sci Sports Exerc* 33: 208-213, 2001.

89. Rundell KW, Jenkinson DM. Exercise-induced bronchospasm in the elite athlete. *Sports Med* 32: 583-600, 2002.

90. Rundell KW, Slee JB. Exercise and other indirect challenges to demonstrate asthma or exercise-induced bronchoconstriction in athletes. *J Allergy Clin Immun* 122: 238-246, 2008.

91. Rundell KW, Spiering BA, Evans TM, Baumann JM. Baseline lung function, exercise-induced bronchoconstriction, and asthma-like symptoms in elite women ice hockey players. *Med Sci Sports Exerc* 36: 405-410, 2004.

92. Rundell KW, Wilber RL, Lemanske RF. *Exercise-induced asthma: pathophysiology and treatment.* Champaign, IL: Human Kinetics, 2002.

93. Sandsund M, Sue-Chu M, Helgerud J, Reinertsen RE, Bjermer L. Effect of cold exposure (-15°C) and salbutamol treatment on physical performance in elite nonasthmatic cross-country skiers. *Eur J Appl Physiol* 77: 292-304, 1998.

94. Schaffartzik W, Poole DC, Derion I, Tsukimoto K, Hogan MC, Arcos JP, Bebout DE, Wagner PD. V_A/Q distribution during heavy exercise and recovery in humans: implications for pulmonary edema. *J Appl Physiol* 72: 1657-1667, 1992.

95. Sheel AW, Road J, McKenzie DC. Exhaled nitric oxide during exercise. *Sports Med* 28: 83-90, 1999.

96. Signorile JF, Kaplan TA, Applegate B, Perry AC. Effects of acute inhalation of the bronchodilator, albuterol, on power output. *Med Sci Sports Exerc* 24: 638-642, 1992.

97. Stewart IB, Labreche JM, McKenzie DC. Acute formoterol administration has ergogenic effect in nonasthmatic athletes. *Med Sci Sports Exerc* 34: 213-217, 2002.

98. Stickland MK, Lovering AT, Eldridge MW. Exercise-induced arteriovenous intrapulmonary shunting in dogs. *Am J Respir Crit Care Med* 176: 300-305, 2007.

99. Stickland MK, Welsh RC, Haykowsky MJ, Peterson SR, Anderson WD, Taylor DA, Bouffard M, Jones RL. Intrapulmonary shunt and pulmonary gas exchange during exercise in humans. *J Physiol* 561:321-329, 2004.

100. Stickland MK, Welsh RC, Haykowsky MJ, Peterson SR, Anderson WD, Taylor DA, Bouffard, Jones RL. Effect of acute increases in pulmonary vascular pressures on exercise pulmonary gas exchange. *J Appl Physiol.* 100: 1910-1917, 2006.

101. Sue-Chu M, Henriksen AH, Bjermer L. Non-invasive evaluation of lower airway inflammation in hyper-responsive elite cross-country skiers and asthmatics. *Respir Med* 93: 719-725, 1999.

102. Suman OE, Babcock MA, Pegelow DF, Jarjour NN, Reddan WG. Airway obstruction during exercise in asthma. *Am J Respir Crit Care Med* 152: 24-31, 1995.

103. Tobin CE. Arteriovenous shunts in the peripheral pulmonary circulation in the human lung. *Thorax* 21: 197-204, 1966.

104. Tobin CE, Zariquiey MO. Arteriovenous shunts in the human lung. *Proc Soc Exp Biol Med* 75: 827-829, 1950.

105. Torre-Bueno JR, Wagner PD, Saltzman HA, Gale GE, Moon RE. Diffusion limitation in normal humans during exercise at sea level and simulated altitude. *J Appl Physiol* 58: 989-995, 1985.

106. Van Baak MA, de Hon OM, Hartgens F, Kuipers H. Inhaled salbutamol and endurance cycling performance in non-asthmatic athletes. *Int J Sports Med* 25: 533-538, 2004.

107. Van Baak MA, Mayer LHJ, Kempinski RES, Hartgens F. Effect of salbutamol on muscle strength and endurance performance in nonasthmatic men. *Med Sci Sports Exerc* 32: 1300-1306, 2000.

108. Vathenen AS, Knox AJ, Wisniewski A, Tattersfield AE. Time course of change in bronchial reactivity with an inhaled corticosteroid in asthma. *Am Rev Respir Dis* 143: 1317-1321, 1991.

109. Vogiatzis I, Zakynthinos S, Boushel R, Athanasopoulos D, Guenette JA, Wagner H, Roussos C, Wagner PD. The contribution of intrapulmonary shunts to the alveolar-to-arterial oxygen difference during exercise is very small. *J Physiol* 586: 2381-2391, 2008.

110. Wagner PD. The multiple inert gas elimination technique (MIGET). *Intens Care Med* 34: 994-1001, 2008.

111. Wagner PD, Gale GE, Moon RE, Torre-Bueno JR, Stolp BW, Saltzman HA. Pulmonary gas exchange in humans exercising at sea level and simulated altitude. *J Appl Physiol* 61: 260-270, 1986.

112. Wagner PD, Hedenstierna G, Rodgriguez-Roisin R. Gas exchange, expiratory flow obstruction and the clinical spectrum of asthma. *Eur Respir J* 9: 1278-1282, 1996.

113. Wagner PD, Saltzman HA, West JB. Measurement of continuous distributions of ventilation-perfusion ratios:theory. *J Appl Physiol*. 36: 588-599, 1974.

114. West JB. *Respiratory Physiology: The essentials.* Baltimore, MD: Lippincott, Williams & Wilkins, 2005

115. Whipp BJ, Wasserma K. Alveolar-arterial gas tension differences during graded exercise. *J Appl Physiol* 27: 361, 1969.

116. Whyte MK, Peters AM, Hughes JM, Henderson BL, Bellingan GJ, Jackson JE, Chilvers ER. Quantification of right to left shunt at rest and during exercise in patients with pulmonary arteriovenous malformations. *Thorax* 47: 790-796, 1992.

117. Wilber RL, Rundell KW, Szmedra L, Jenkinson DM, Im J, Drake SD. Incidence of exercise-induced broncho-spasm in Olympic winter sport athletes. *Med Sci Sports Exerc* 32: 732-737, 2000.

118. Wilkinson MJ, Fagan DG. Postmortem demonstration of intrapulmonary arteriovenous shunting. *Arch Dis Child* 65: 435-437, 1990

119. World Anti-doping Agency. 2010 Prohibited List – Summary of major modifications, September 19, 2009.

Appendices

Appendix A: Overview of Study Design

Overview of Study Design

Screening	Condition A*	Condition B*
• Explanation of study, consent form, PAR-Q • Anthropometric data: Age, Height, Weight • Restrictions check → pass / fail? • Baseline pulmonary function • EVH • Post-EVH pulmonary function → asthma yes / no • Training and Asthma questionnaires • Rest: change clothes, drink water, set-up bike • Inhalation of salbutamol • Warm-up • Pre-exercise pulmonary function → airways recovered? • VO_2max test • Cool-down • TOTAL TIME: 1.5 – 2 hours	• Restrictions check • Re-explanation of agitated saline procedure • Baseline pulmonary function • Bike set-up • EVH • Post-EVH pulmonary function • IV line insertion • Resting echo → Intracardiac shunt? • Resting supine • Resting headtilt • Resting upright • EI • EII • EIII • EIV • EV • EVI • Cool-down • Remove IV line • TOTAL TIME: 60-75 min	• Restrictions check • Re-explanation of agitated saline procedure • Baseline pulmonary function • Bike set-up • SABA • Post-SABA pulmonary function • IV line insertion • Resting echo → Intracardiac shunt? • Resting supine • Resting headtilt • Resting upright • EI • EII • EIII • EIV • EV • EVI • Cool-down • Remove IV line TOTAL TIME: 60 min

Each column describes one visit; Condition A, induced airway obstruction; Condition B, reduced airway obstruction; *, Condition A and Condition B were randomly assigned; PAR-Q, Physical Activity Readiness Questionnaire; EVH, Eucapnic Voluntary Hyperpnoea; VO_2max, maximal oxygen consumption; IV, intravenous; EI-VI, Exercise Stages I-VI; SABA, inhaled salbutamol.

Appendix B: Borg's Category Scale

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

Appendix C: Individual Raw Data

Table C.1: Individual Characteristics										
Subject	1	2	3	4	5	6	7	8	9	10
Age (years)	23	22	19	24	40	21	21	26	31	32
Heights (cm)	180	172	183	179	176	180	169	179	188	185
Weight (kg)	65.6	64.0	75.7	69.6	68.8	82.0	61.8	68.0	83.7	83.0
VO_2max (ml/kg/min)	63.6 (134)	66.0 (137)	58.3 (118)	75.4 (160)	67.4 (169)	51.7 (107)	62.3 (129)	66.4 (144)	56.7 (129)	58.4 (134)
VO_2max (L/min)	4.19	4.22	4.41	5.25	4.64	4.24	3.85	4.52	4.75	4.85
Max P (Watts)	383	402	348	425	425	376	375	465	470	435
FVC (L)	5.85 (104)	6.83 (131)	6.64 (112)	6.61 (118)	5.55 (110)	6.98 (122)	6.14 (122)	6.08 (110)	6.07 (102)	5.32 (92)
$FEV_{1.0}$ (L)	4.47 (95)	4.35 (99)	5.16 (104)	4.04 (87)	4.35 (106)	5.26 (111)	4.68 (109)	4.21 (92)	4.12 (85)	4.53 (97)
$FEV_{1.0}$/FVC	0.76	0.64	0.78	0.61	0.78	0.75	0.76	0.69	0.68	0.85
PEF (L/s)	10.97	9.65	8.87	8.35	10.57	10.65	10.93	9.64	10.70	13.04
FEF_{25-75} (L/s)	3.65 (74)	2.90 (60)	4.51 (81)	2.58 (53)	3.92 (91)	4.53 (87)	3.98 (81)	3.30 (65)	2.76 (58)	4.77 (101)
$FEV_{1.0}$´ (%)	32.6	17.4	10.9	17.1	17.2	21.7	33.5	12.6	12.9	16.3
Medication	ICS	none	SAB, ICS	SAB	SAB, ICS	SAB, ICS	SAB	LAB, ICS	none	SAB

Values in parentheses are percent predicted. VO_2max, maximum oxygen consumption; Max P, maximal power output; FVC, baseline forced vital capacity; $FEV_{1.0}$, baseline forced expiratory volume in 1 second; PEF, baseline peak expiratory flow; FEF_{25-75}, baseline forced expiratory flow of midexpiratory volume; $FEV_{1.0}$´, greatest percent decline in $FEV_{1.0}$ after eucapnic voluntary hyperpnoea test; SAB, short-acting beta-2 agonist; LAB, long-acting beta-2 agonist; ICS, inhaled corticosteroid.

Table C.2: Pulmonary Function Under Condition A

	FVC (L)		$FEV_{1.0}$ (L)		$FEV_{1.0}$/FVC		PEF (L/s)		FEF_{25-75} (L/s)	
Subject	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	6.06	5.93	4.67	4.08	0.77	0.69	10.88	8.99	3.98	2.81
2	6.56	5.88	4.60	4.07	0.70	0.69	9.22	7.02	3.42	3.03
3	6.83	6.38	5.12	4.87	0.75	0.76	10.30	8.27	4.24	4.04
4	6.72	6.23	4.41	3.64	0.66	0.58	8.42	7.31	3.11	2.27
5	5.29	4.95	4.04	3.33	0.76	0.67	9.71	7.42	3.33	2.20
6	6.63	5.88	5.16	4.14	0.78	0.70	10.33	8.74	4.34	3.09
7	6.10	5.69	4.77	3.68	0.78	0.65	10.82	7.97	4.18	2.46
8	5.86	5.61	4.16	3.68	0.71	0.66	9.4	8.09	3.23	2.45
9	5.97	5.50	3.77	3.23	0.63	0.59	10.36	8.42	2.35	1.89
10	5.42	5.14	4.70	3.84	0.87	0.75	12.93	10.91	5.1	2.94
M ± SD	6.14 ± 0.54	5.72 ± 0.44	4.54 ± 0.45	3.86 ± 0.47	0.74 ± 0.07	0.67 ± 0.06	10.24 ± 1.22	8.31 ± 1.11	3.73 ± 0.79	2.72 ± 0.61

$FEV_{1.0}$, forced expiratory volume in one second; PEF, peak expiratory flow; FEF_{25-75}, forced expiratory flow of midexpiratory volume; M ± SD, mean ± standard deviation

Table C.3: Pulmonary Function Under Condition B

	FVC (L)		$FEV_{1.0}$ (L)		$FEV_{1.0}$/FVC		PEF (L/s)		FEF_{25-75} (L/s)	
Subject	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	5.18	5.27	3.72	4.04	0.72	0.77	9.85	11.23	2.74	3.30
2	6.56	6.39	4.60	4.94	0.70	0.77	9.22	9.79	3.42	4.29
3	6.64	7.04	5.16	5.48	0.78	0.78	8.87	10.10	4.51	4.81
4	6.59	6.51	4.26	4.41	0.65	0.68	8.58	9.07	3.00	3.17
5	5.54	5.35	4.29	4.34	0.77	0.81	10.51	10.22	3.67	4.15
6	6.83	6.69	5.56	5.67	0.81	0.85	11.61	11.59	5.36	5.98
7	5.99	6.03	4.50	4.68	0.75	0.78	10.29	10.98	3.64	4.04
8	6.08	5.90	4.21	4.46	0.68	0.76	9.64	9.81	3.30	3.63
9	5.84	5.73	3.81	4.03	0.65	0.70	10.45	10.77	2.42	2.88
10	5.43	5.42	4.77	4.76	0.88	0.88	13.07	13.09	5.46	5.51
M ± SD	6.07 ± 0.57	6.03 ± 0.61	4.49 ± 0.57	4.68 ± 0.55	0.74 ± 0.07	0.78 ± 0.06	10.21 ± 1.34	10.67 ± 1.14	3.75 ± 1.04	4.18 ± 1.01

$FEV_{1.0}$, forced expiratory volume in one second; PEF, peak expiratory flow; FEF_{25-7}5, forced expiratory flow of midexpiratory volume; M ± SD, mean ± standard deviation

Table C.4: Exercise Data, Subject 1

Stage	Power	%MaxP	~VO_2	%VO_2max	Shunt	HR	%MaxHR	SpO_2	E	D
Condition A										
RS	--	--	--	--	N	52	29.6	98	--	--
RH	--	--	--	--	N	52	29.6	98	--	--
RU	--	--	--	--	N	62	35.2	98	--	--
EI	100	26.1	26.0	40.8	N	102	58.0	97	2	2
EII	150	39.2	29.4	46.1	N	111	63.1	97	3	3
EIII	200	52.2	38.5	60.3	Y	131	74.4	97	5	4
EIV	250	65.3	43.7	68.5	Y	144	81.8	96	6	6
EV	300	78.3	54.7	85.7	Y	161	91.5	96	9	9
EVI	350	91.4	62.9	98.6	Y	168	94.5	96	10	10
Condition B										
RS	--	--	--	--	N	65	36.9	99	--	--
RH	--	--	--	--	N	64	36.4	99	--	--
RU	--	--	--	--	N	70	39.8	99	--	--
EI	100	26.1	26.0	40.8	N	106	60.2	98	0.5	0.5
EII	150	39.2	29.4	46.1	N	113	64.2	98	0.5	0.5
EIII	200	52.2	38.5	60.3	Y	132	75.0	97	2	2
EIV	250	65.3	43.7	68.5	Y	150	85.2	97	3	2
EV	300	78.3	54.7	85.7	Y	162	92.1	96	4	3
EVI	350	91.4	62.9	98.6	Y	174	98.9	95	6	5

RS, resting supine; RH, resting head-down tilt; RU, resting upright; EI-EVI, exercise stages I-VI; %MaxP, percent of maximum power output; (%); ~VO_2, estimated oxygen consumption; %VO_2max, percent of maximum oxygen consumption (ml/kg/min); Shunt, presence of intrapulmonary (N=no; Y=yes); HR, heart rate (beats per minute); %MaxHR, percent of maximum heart rate; SpO_2, oxygen saturation; E, perceived exertion (0-10); D, perceived dyspnoea (0-10).

Table C.5: Exercise Data, Subject 2

Stage	Power	%MaxP	~VO_2	%VO_2max	Shunt	HR	%MaxHR	SpO_2	E	D
Condition A										
RS	--	--	--	--	N	72	36.9	98	--	--
RH	--	--	--	--	N	74	38.0	98	--	--
RU	--	--	--	--	N	78	40.0	98	--	--
EI	100	24.9	22.3	33.7	N	125	64.1	98	3	3
EII	150	37.3	30.4	46.1	Y	136	69.7	98	3	4
EIII	200	49.8	40.5	61.3	Y	144	73.9	98	5	4
EIV	260	64.7	49.8	75.5	Y	159	81.5	98	6	5
EV	310	77.1	57.8	87.5	Y	171	87.7	97	8	7
EVI	370	92.0	65.8	99.6	Y	188	96.4	97	9	9
Condition B										
RS	--	--	--	--	N	70	35.9	99	--	--
RH	--	--	--	--	N	70	35.9	99	--	--
RU	--	--	--	--	N	70	35.9	99	--	--
EI	100	24.9	22.3	33.7	N	107	54.9	98	0.5	0.5
EII	150	37.3	30.4	46.1	Y	122	62.6	98	0.5	0.5
EIII	200	49.8	40.5	61.3	Y	144	73.9	97	2	2
EIV	260	64.7	49.8	75.5	Y	159	81.5	97	3	2
EV	310	77.1	57.8	87.5	Y	177	90.8	97	4	3
EVI	370	92.0	65.8	99.6	Y	191	98.0	96	6	5

RS, resting supine; RH, resting head-down tilt; RU, resting upright; EI-EVI, exercise stages I-VI; %MaxP, percent of maximum power output; (%); ~VO_2, estimated oxygen consumption; %VO_2max, percent of maximum oxygen consumption (ml/kg/min); Shunt, presence of intrapulmonary (N=no; Y=yes); HR, heart rate (beats per minute); %MaxHR, percent of maximum heart rate; SpO_2, oxygen saturation; E, perceived exertion (0-10); D, perceived dyspnoea (0-10).

Table C.6: Exercise Data, Subject 3

Stage	Power	%MaxP	~VO_2	%VO_2max	Shunt	HR	%MaxHR	SpO_2	E	D
Condition A										
RS	--	--	--	--	N	72	38.5	98	--	--
RH	--	--	--	--	N	60	32.1	98	--	--
RU	--	--	--	--	N	62	33.2	98	--	--
EI	100	28.7	23.5	40.3	Y	113	60.4	97	2	2
EII	150	43.1	32.2	55.2	Y	124	66.3	97	3	3
EIII	200	57.5	36.5	62.6	Y	141	75.4	97	5	4
EIV	250	71.8	43.7	75.0	Y	157	84.0	96	6	6
EV	290	83.3	50.9	87.3	Y	173	92.5	96	9	9
EVI	330	94.8	57.2	98.1	Y	184	98.4	95	10	10
Condition B										
RS	--	--	--	--	N	68	36.4	97	--	--
RH	--	--	--	--	N	64	34.2	97	--	--
RU	--	--	--	--	N	75	40.1	97	--	--
EI	100	28.7	23.5	40.3	Y	121	64.7	97	2	0.5
EII	150	43.1	32.2	55.2	Y	137	73.3	97	2	1
EIII	200	57.5	36.5	62.6	Y	156	83.4	96	4	3
EIV	250	71.8	43.7	75.0	Y	169	90.4	96	5.5	4
EV	290	83.3	50.9	87.3	Y	182	97.3	96	5.5	4
EVI	330	94.8	57.2	98.1	Y	187	100.0	95	7	5

RS, resting supine; RH, resting head-down tilt; RU, resting upright; EI-EVI, exercise stages I-VI; %MaxP, percent of maximum power output; (%); ~VO_2, estimated oxygen consumption; %VO_2max, percent of maximum oxygen consumption (ml/kg/min); Shunt, presence of intrapulmonary (N=no; Y=yes); HR, heart rate (beats per minute); %MaxHR, percent of maximum heart rate; SpO_2, oxygen saturation; E, perceived exertion (0-10); D, perceived dyspnoea (0-10).

Table C.7: Exercise Data, Subject 4

Stage	Power	%MaxP	~VO_2	%VO_2max	Shunt	HR	%MaxHR	SpO_2	E	D
Condition A										
RS	--	--	--	--	N	47	26.7	98	--	--
RH	--	--	--	--	N	43	24.4	98	--	--
RU	--	--	--	--	N	54	30.7	98	--	--
EI	125	29.4	25.8	34.2	Y	108	61.4	98	2	1
EII	175	41.2	34.0	45.0	Y	119	67.6	98	3	2
EIII	250	58.8	47.6	63.1	Y	139	79.0	97	4	3
EIV	300	70.6	55.9	74.1	Y	151	85.8	97	7	6
EV	350	82.4	65.2	86.4	Y	158	89.8	95	10	10
EVI	400	--	--	--	--	--	--	--	--	--
Condition B										
RS	--	--	--	--	N	50	28.4	97	--	--
RH	--	--	--	--	N	43	24.4	97	--	--
RU	--	--	--	--	N	56	31.8	97	--	--
EI	125	29.4	25.8	34.2	Y	108	61.4	97	2	2
EII	175	41.2	34.0	45.0	Y	111	63.1	97	3	3
EIII	250	58.8	47.6	63.1	Y	133	75.6	97	5	5
EIV	300	70.6	55.9	74.1	Y	156	88.6	96	7	8
EV	350	82.4	65.2	86.4	Y	166	94.3	96	9	9
EVI	400	94.1	74.6	98.9	Y	170	96.6	95	10	10

RS, resting supine; RH, resting head-down tilt; RU, resting upright; EI-EVI, exercise stages I-VI; %MaxP, percent of maximum power output; (%); ~VO_2, estimated oxygen consumption; %VO_2max, percent of maximum oxygen consumption (ml/kg/min); Shunt, presence of intrapulmonary (N=no; Y=yes); HR, heart rate (beats per minute); %MaxHR, percent of maximum heart rate; SpO_2, oxygen saturation; E, perceived exertion (0-10); D, perceived dyspnoea (0-10).

Table C.8: Exercise Data, Subject 5

Stage	Power	%MaxP	~VO_2	%VO_2max	Shunt	HR	%MaxHR	SpO_2	E	D
Condition A										
RS	--	--	--	--	N	55	30.6	98	--	--
RH	--	--	--	--	N	56	31.1	98	--	--
RU	--	--	--	--	N	58	32.2	98	--	--
EI	125	29.4	23.5	34.9	Y	108	60.0	97	1	1
EII	175	41.2	33.4	49.5	Y	113	62.8	96	2	2
EIII	250	58.8	42.2	62.6	Y	136	75.6	96	5	4
EIV	300	70.6	51.6	76.5	Y	151	83.9	95	8	7
EV	350	82.4	59.9	88.8	Y	167	92.8	92	9	8
EVI	400	94.1	65.0	96.3	Y	173	96.1	91	10	10
Condition B										
RS	--	--	--	--	N	62	34.4	99	--	--
RH	--	--	--	--	N	57	31.7	99	--	--
RU	--	--	--	--	N	62	34.4	99	--	--
EI	125	29.4	23.5	34.9	Y	105	58.3	97	2.5	2
EII	175	41.2	33.4	49.5	Y	119	66.1	96	3	3
EIII	250	58.8	42.2	62.6	Y	135	75.0	95	4	4
EIV	300	70.6	51.6	76.5	Y	153	85.0	94	7	6
EV	350	82.4	59.9	88.8	Y	166	92.2	92	8	7
EVI	400	94.1	65.0	96.3	Y	174	96.7	91	9	8

RS, resting supine; RH, resting head-down tilt; RU, resting upright; EI-EVI, exercise stages I-VI; %MaxP, percent of maximum power output; (%); ~VO_2, estimated oxygen consumption; %VO_2max, percent of maximum oxygen consumption (ml/kg/min); Shunt, presence of intrapulmonary (N=no; Y=yes); HR, heart rate (beats per minute); %MaxHR, percent of maximum heart rate; SpO_2, peripheral oxygen saturation; E, perceived exertion (0-10); D, perceived dyspnoea (0-10).

Table C.9: Exercise Data, Subject 6

Stage	Power	%MaxP	~VO_2	%VO_2max	Shunt	HR	%MaxHR	SpO_2	E	D
Condition A										
RS	--	--	--	--	N	52	28.7	99	--	--
RH	--	--	--	--	N	52	28.7	99	--	--
RU	--	--	--	--	N	62	34.3	99	--	--
EI	100	26.6	19.6	37.9	Y	102	56.4	98	2	2
EII	150	39.9	25.3	48.8	Y	111	61.3	98	3	3
EIII	200	53.2	31.2	60.3	Y	131	72.4	97	5	4
EIV	250	66.5	35.1	67.8	Y	144	79.6	97	6	6
EV	300	79.8	44.0	85.1	Y	161	89.0	97	9	9
EVI	350	93.1	49.1	94.9	Y	168	92.8	96	10	10
Condition B										
RS	--	--	--	--	N	59	32.6	97	--	--
RH	--	--	--	--	N	64	35.4	97	--	--
RU	--	--	--	--	N	60	33.2	97	--	--
EI	100	26.6	19.6	37.9	N	95	52.5	97	0	0
EII	150	39.9	25.3	48.8	Y	113	62.4	97	2	1
EIII	200	53.2	31.2	60.3	Y	131	72.4	97	3	2
EIV	250	66.5	35.1	67.8	Y	149	82.3	96	4	2
EV	300	79.8	44.0	85.1	Y	161	89.0	96	4	3
EVI	350	93.1	49.1	94.9	Y	181	100.0	95	6.5	5.5

RS, resting supine; RH, resting head-down tilt; RU, resting upright; EI-EVI, Exercise Stages I-VI; %MaxP, percent of maximum power output; (%); ~VO_2, estimated oxygen consumption; %VO_2max, percent of maximum oxygen consumption (ml/kg/min); Shunt, presence of intrapulmonary (N=no; Y=yes); HR, heart rate (beats per minute); %MaxHR, Percent of maximum heart rate; SpO_2, oxygen saturation; E, perceived exertion (0-10); D, perceived dyspnoea (0-10).

Table C.10: Exercise Data, Subject 7

Stage	Power	%MaxP	~VO_2	%VO_2max	Shunt	HR	%MaxHR	SpO_2	E	D
Condition A										
RS	--	--	--	--	N	58	30.1	99	--	--
RH	--	--	--	--	N	64	33.2	99	--	--
RU	--	--	--	--	N	69	35.8	99	--	--
EI	100	26.7	21.8	34.9	Y	112	58.0	98	3	2
EII	150	40.0	29.5	47.3	Y	126	65.3	98	3	3
EIII	200	53.3	39.2	62.8	Y	150	77.7	97	4	4.5
EIV	250	66.7	45.6	73.2	Y	164	85.0	97	7	7
EV	300	80.0	52.9	84.9	Y	179	92.8	97	10	9
EVI	350	93.3	61.2	98.2	Y	188	97.4	96	10	10
Condition B										
RS	--	--	--	--	N	62	32.1	97	--	--
RH	--	--	--	--	N	61	31.6	97	--	--
RU	--	--	--	--	N	65	33.7	97	--	--
EI	100	26.7	21.8	34.9	Y	112	58.0	97	1	1
EII	150	40.0	29.5	47.3	Y	133	68.9	97	3	3
EIII	200	53.3	39.2	62.8	Y	158	81.9	98	5	5
EIV	250	66.7	45.6	73.2	Y	176	91.2	97	7	8
EV	300	80.0	52.9	84.9	Y	185	95.9	96	9	9
EVI	350	93.3	61.2	98.2	Y	193	100.0	96	10	10

RS, resting supine; RH, resting head-down tilt; RU, resting upright; EI-EVI, Exercise Stages I-VI; %MaxP, percent of maximum power output; (%); ~VO_2, estimated oxygen consumption; %VO_2max, percent of maximum oxygen consumption (ml/kg/min); Shunt, presence of intrapulmonary (N=no; Y=yes); HR, heart rate (beats per minute); %MaxHR, Percent of maximum heart rate; SpO_2, oxygen saturation; E, perceived exertion (0-10); D, perceived dyspnoea (0-10).

Table C.11: Exercise Data, Subject 8

Stage	Power	%MaxP	~VO_2	%VO_2max	Shunt	HR	%MaxHR	SpO_2	E	D
Condition A										
RS	--	--	--	--	N	53	28.2	98	--	--
RH	--	--	--	--	N	51	27.1	98	--	--
RU	--	--	--	--	N	59	31.4	98	--	--
EI	125	26.9	27.2	40.9	Y	101	53.7	96	0.5	0.5
EII	175	37.6	33.6	50.6	Y	117	62.2	95	0.5	1
EIII	250	53.8	43.1	64.8	Y	146	77.7	94	3	3
EIV	310	66.7	50.6	76.2	Y	164	87.2	93	5	4
EV	360	77.4	57.9	87.2	Y	174	92.6	91	7	6
EVI	415	89.3	64.1	96.5	Y	188	100.0	91	9	8
Condition B										
RS	--	--	--	--	Y	63	33.51	97	--	--
RH	--	--	--	--	N	54	28.72	97	--	--
RU	--	--	--	--	N	63	33.51	98	--	--
EI	125	26.9	27.2	40.9	Y	110	58.51	95	0	0
EII	175	37.6	33.6	50.6	Y	123	65.43	97	0.5	0.5
EIII	250	53.8	43.1	64.8	Y	148	78.72	93	3	3
EIV	310	66.7	50.6	76.2	Y	164	87.23	93	4	3
EV	360	77.4	57.9	87.2	Y	176	93.62	93	7	6
EVI	415	89.3	64.1	96.5	Y	183	97.34	90	8	8

RS, resting supine; RH, resting head-down tilt; RU, resting upright; EI-EVI, Exercise Stages I-VI; %MaxP, percent of maximum power output; (%); ~VO_2, estimated oxygen consumption; %VO_2max, percent of maximum oxygen consumption (ml/kg/min); Shunt, presence of intrapulmonary (N=no; Y=yes); HR, heart rate (beats per minute); %MaxHR, Percent of maximum heart rate; SpO_2, oxygen saturation; E, perceived exertion (0-10); D, perceived dyspnoea (0-10).

Table C.12: Exercise Data, Subject 9

Stage	Power	%MaxP	~VO_2	%VO_2max	Shunt	HR	%MaxHR	SpO_2	E	D
Condition A										
RS	--	--	--	--	Y	73	37.8	97	--	--
RH	--	--	--	--	N	75	38.9	97	--	--
RU	--	--	--	--	N	80	41.5	97	--	--
EI	125	26.6	26.2	46.2	Y	118	61.1	95	2	1
EII	175	37.2	29.9	52.7	Y	130	67.4	95	2	2
EIII	250	53.2	37.4	65.9	Y	152	78.8	94	3	3
EIV	310	66.0	43.3	76.4	Y	171	88.6	94	5	4
EV	360	76.6	49.5	87.3	Y	184	95.3	93	7	7
EVI	415	88.3	55.0	97.0	Y	190	98.5	92	8	8
Condition B										
RS	--	--	--	--	N	61	31.61	100	--	--
RH	--	--	--	--	N	65	33.68	100	--	--
RU	--	--	--	--	N	67	34.72	100	--	--
EI	125	26.6	26.2	46.2	Y	111	57.51	97	1	1
EII	175	37.2	29.9	52.7	Y	129	66.84	96	2	2
EIII	250	53.2	37.4	65.9	Y	152	78.76	95	4	3
EIV	310	66.0	43.3	76.4	Y	169	87.56	94	5	4
EV	360	76.6	49.5	87.3	Y	180	93.26	94	7	7
EVI	415	88.3	55.0	97.0	Y	189	97.93	92	8	8

RS, resting supine; RH, resting head-down tilt; RU, resting upright; EI-EVI, Exercise Stages I-VI; %MaxP, percent of maximum power output; (%); ~VO_2, estimated oxygen consumption; %VO_2max, percent of maximum oxygen consumption (ml/kg/min); Shunt, presence of intrapulmonary (N=no; Y=yes); HR, heart rate (beats per minute); %MaxHR, Percent of maximum heart rate; SpO_2, oxygen saturation; E, perceived exertion (0-10); D, perceived dyspnoea (0-10).

Table C.13: Exercise Data, Subject 10

Stage	Power	%MaxP	~VO_2	%VO_2max	Shunt	HR	%MaxHR	SpO_2	E	D
Condition A										
RS	--	--	--	--	N	67	36.4	99	--	--
RH	--	--	--	--	Y	70	38.0	99	--	--
RU	--	--	--	--	N	69	37.5	99	--	--
EI	125	28.7	22.7	38.8	Y	115	62.5	98	1	1
EII	175	40.2	27.0	46.2	Y	127	69.0	97	2	1
EIII	250	57.5	39.0	66.8	Y	142	77.2	97	3	2
EIV	300	69.0	47.9	82.0	Y	156	84.8	96	4	3
EV	350	80.5	53.7	91.9	Y	167	90.8	95	5	4
EVI	400	92.0	57.7	98.7	Y	176	95.7	95	7	6
Condition B										
RS	--	--	--	--	Y	73	39.7	98	--	--
RH	--	--	--	--	Y	68	37.0	98	--	--
RU	--	--	--	--	N	66	35.9	98	--	--
EI	125	28.7	22.7	38.8	Y	126	68.5	98	3	3
EII	175	40.2	27.0	46.2	Y	136	73.9	98	3	3
EIII	250	57.5	39.0	66.8	Y	152	82.6	96	4	4
EIV	300	69.0	47.9	82.0	Y	162	88.0	95	5	4
EV	350	80.5	53.7	91.9	Y	174	94.6	95	6	5
EVI	400	92.0	57.7	98.7	Y	184	100.0	94	9	8

RS, resting supine; RH, resting head-down tilt; RU, resting upright; EI-EVI, Exercise Stages I-VI; %MaxP, percent of maximum power output; (%); ~VO_2, estimated oxygen consumption; %VO_2max, percent of maximum oxygen consumption (ml/kg/min); Shunt, presence of intrapulmonary (N=no; Y=yes); HR, heart rate (beats per minute); %MaxHR, Percent of maximum heart rate; SpO_2, oxygen saturation; E, perceived exertion (0-10); D, perceived dyspnoea (0-10).

Appendix D: Supplementary Figures

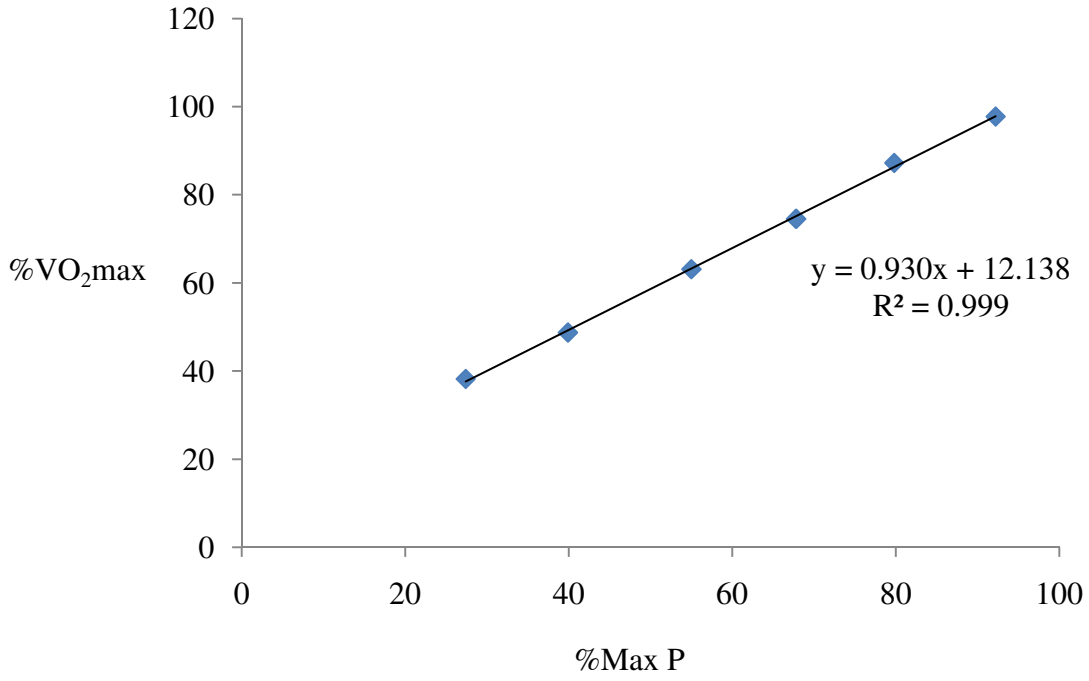

Figure D.1: Correlation Between %Max P *vs.* %VO_2max. %Max P, percent of maximum power output; %VO2max, percent of maximum oxygen consumption; Equation represents linear regression; ($P < 0.05$).

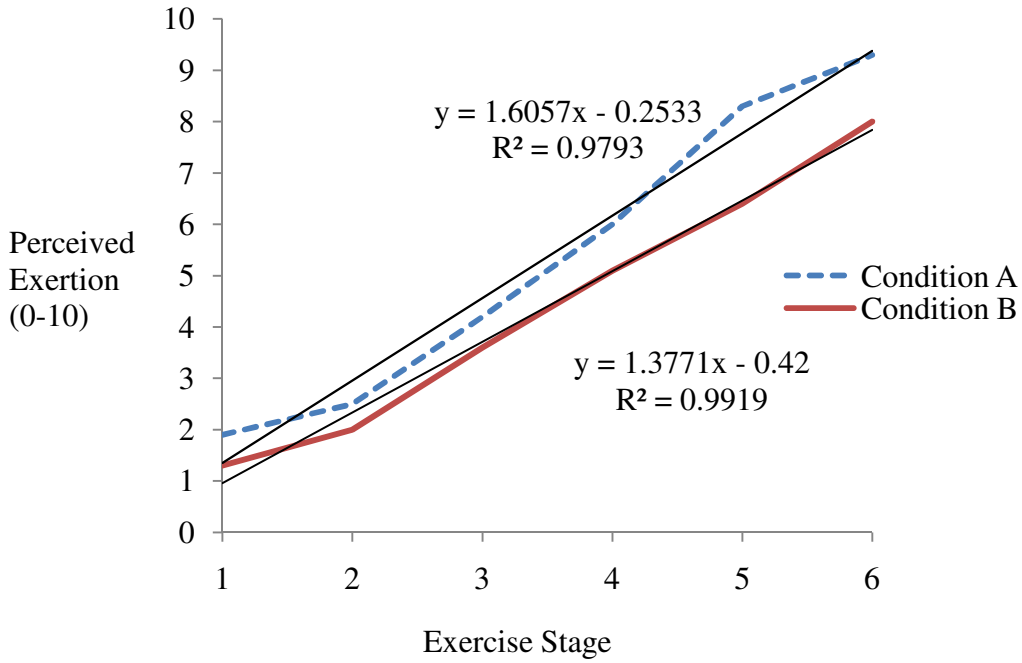

Figure D.2: Comparison of Perceived Exertion Under Condition A *vs*. Condition B. Condition A, induced airway obstruction (dotted line); Condition B, reversed airway obstruction (solid line); straight lines represent the line of best fit for each condition; equation describes the linear regression value for each condition; ($P < 0.05$).

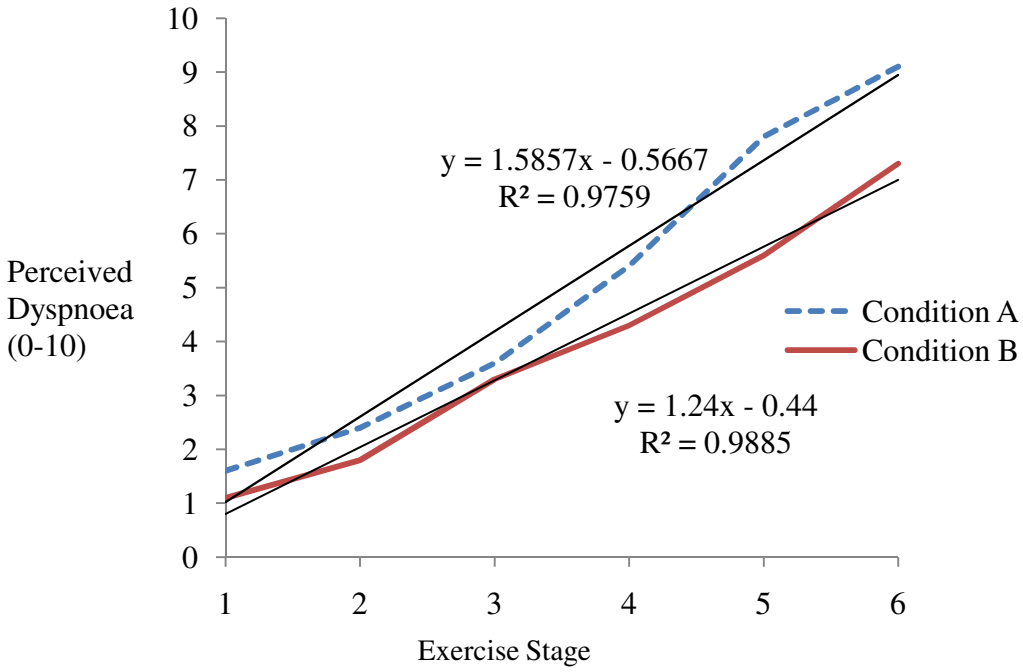

Figure D.3: Comparison of Perceived Dyspnoea Under Condition A vs. Condition B. Condition A, induced airway obstruction (dotted line); Condition B, reversed airway obstruction (solid line); straight lines represent the line of best fit for each condition; equation describes the linear regression value for each condition; ($P < 0.05$).