EFFECTS OF INSPIRED AIR AND EXERCISE IN DIAGNOSING EXERCISE-INDUCED BRONCHOCONSTRICTION IN SWIMMERS

by

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Abstract

Competitive swimmers have high rates of exercise induced bronchoconstriction (EIB), which may be associated with repeated exposure to chlorinated pool water. The eucapnic voluntary hyperpnea (EVH) test is often used in a laboratory setting to provoke a reduction in lung function associated with EIB. Swimmers however, experience EIB symptoms in warm, humid and chlorinated environments. The relationship between EVH testing conditions and the development of EIB from swim exercise is unclear. **PURPOSE**: To compare the provoking effects of inspired air and high-intensity exercise in inducing EIB in swimmers to laboratory-based EVH method. **METHODS:** 15 collegiate swimmers (n=5 male, n=10 female; 21±2 years) completed three days of testing in random order. On day one, subjects performed an EVH test in a laboratory (EVH_L). On a separate day, swimmers performed a modified EVH test, while breathing chlorinated pool air (EVH_{Cl}). On a third day subjects completed a swimming challenge (Swim), performing consecutive 200 and 400 m freestyle efforts at 85% of their season's best time (average achieved 200 and 400 m time; 2:18.52±7.79 and 4:55.22±20.38, respectively) and age-predicted heart rate maximum. Lung function was measured at baseline, as well as 3-, 5-, 10-, 15-, and 20-minutes following EVH testing and swim exercise. **RESULTS:** EVH_L elicited a -9.7±6.4 % fall compared to the EVH_{CL} test, -6.6±9.2 % (p>0.05) and Swim, -3.0±7.5 % (p>0.05). A significant correlation in FEV_1 fall index between EVH_L vs. EVH_{Cl} (r =0.78, p<0.05) with no significant relationship between EVH_L vs. Swim (r =0.20, p>0.05) and EVH_{CL} vs. Swim (r =0.50, p>0.05). A greater reduction in forced expired flow between 25 and 75 % lung volume (FEF_{25-75}) was induced by the EVH_L (-16.6±8.7 %) compared to the EVH_{Cl} (-8.2±14.9 %) (p>0.05) and Swim test (-1.3±15.6 %) (p<0.05). **CONCLUSION**: The EVH_L elicits a greater bronchoconstrictive response, compared to EVH_{Cl}

and Swim tests. There is little relationship in reduction of lung function between the EVH_L test and Swim tests.

Lay Summary

Swimmers have a high prevalence of exercise-induced bronchoconstriction (EIB), commonly known as exercise-induced asthma. Swimmers are thought to develop EIB from chronic exposure to chlorine from treated pool water. A commonly used method for provoking EIB is the eucapnic voluntary hyperpnea (EVH) test, in which subjects hyperventilate compressed cool, dry air. The EVH test however, has little application to swimmers. The purpose of this thesis was to determine the effects of humid, chlorinated air and swim exercise on inducing exercise-induced bronchoconstriction in swimmers, compared to the EVH method. The results indicated the EVH test elicited a greater reduction in lung function, compared to the new modified version and swim exercise. Therefore, it can be speculated that condition-specific methods used in detecting EIB in swimmers are likely less sensitive than current standard methods.

Preface

The following thesis was developed and designed by myself, Michael Leahy, with the support of my supervisory committee and members of the Health and Integrative Physiology Lab. Scheduling, subject recruitment, and testing execution was completed by myself. Analysis of the research data was completed by myself, with interpretive assistance from members of the Health and Integrative Physiology Lab. All methods executed in this thesis was approved by The University of British Columbia's Research Ethics Board (H17-02535).

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List of Abbreviations

AHR	Airway Hyperresponsiveness
ANOVA	Analysis of Variance
ASL	Airway Surface Liquid
EIA	Exercise Induced Asthma
EIAO	Exercise Induced Airway Obstruction
EIB	Exercise Induced Bronchoconstriction
EVH	Eucapnic Voluntary Hyperpnea
EVH_L	EVH Test Completed in Lab
EVH_{Cl}	EVH Test Completed on Pool Deck
FEF_{25-75}	Forced Expired Flow Between 25 and 75% FVC
FEV_1	Forced Expired Volume in One Second
FVC	Forced Vital Capacity
HIAO	Hyperpnea Induced Airway Obstruction
IOC	International Olympic Committee
MIN	Minutes
PEF	Peak Expired Flow
S	Seconds
SR	Slope-Ratio

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Dedication

To Michael and Linda Leahy

Thank you

Chapter 1: Introduction

Exercise-induced bronchoconstriction (EIB) is a reversible phenomenon in which airways narrow upon onset of, or following intense exercise (1). The condition is managed with the use of inhaled $ß_2$-adrenoceptor agonists (IBAs) (2). An increase in therapeutic use exemptions of IBAs has been observed in Olympic competition; from 46% at the Atlanta Summer Games in 1996 to 87% at the Beijing Summer Games in 2008 (3). Specifically, competitive swimmers have high frequencies of EIB, with rates of up to 21% to 76% (4,5).

Increased rates of therapeutic use exemptions has lead the International Olympic Committee Medical Commission (IOC-MC) to required all athletes, including swimmers, to provide a positive test result within two weeks prior to Olympic competition in order to use high-dose prescribed IBAs up to 2010 (6). Currently, the 'gold standard' method of identifying EIB is the eucapnic voluntary hyperventilation (EVH) test (1). The test requires an individual to hyperventilate cool, dry air for 6 minutes and perform pre- and post-test spirometry maneuvers. Positive identification of EIB, according to the IOC-MC requires a fall-index of force expired volume in one second (FEV_1) of ≥10%, within 30 minutes following the test.

Bronchoconstriction in swimmers is provoked in a chlorinated, humid respiratory environment; however, the method by which they are tested is cool and dry. It is hypothesized that the EIB that develops in swimmers is due to chronic inhalation of chlorine metabolites resting above the surface of the water in indoor aquatic centres (7). Chronic exposure to biological biproducts can have adverse affects on airway responsiveness and remodeling (4). EIB occurs from changes in osmotic and thermal properties of the airways as well as mechanical stress occurred during high intensity ventilation.

EIB is also a condition that is identified using a single variable, FEV_1, using a test that is relatively sensitive (63%) and has poor short-term test-retest reproducibility (studies demonstrating correlation coefficient = 0.81, r^2 = 0.66) (1,8). There are alternative methods of identifying homogeneous lung emptying, such as Slope-Ratio (SR) Index (9). SR-Index is a method in which the slope of the forced expired curve is averaged between 20 and 80 % lung volume, and has been used to comparing lung emptying between healthy controls and individuals with COPD and asthma. Changes in homogeneous lung emptying following provocation could potentially be identified using Slope-Ratio (SR) Index (9).

Understanding the mechanisms in which chlorine provokes EIB in swimmers is still an area left to be investigated. Provocation of the upper airways using a voluntary hyperventilation method with chlorinated inspired air is unknown. The application of additional expired flow analysis such as SR-Index following a variety of bronchial provocation tests has yet to be observed in athletic populations.

1.1 REVIEW OF LITERATURE

Exercise-induced asthma (EIA) is the term used when observing or experiencing shortness of breath or severe dyspnea following exercise (10). Other terms such as exercise-induced airway obstruction (EIAO) and hyperpnoea-induced airway obstruction (HIAO) have also been used interchangeably throughout the literature (11,12). For the purposes of this review of literature and thesis, the term exercise-induced bronchoconstriction (EIB) will be used as it most appropriately outlines the phenomenon of reduced pulmonary function due to airway constriction during or following exercise (12–14).

EIB is typically identified as a reduction in forced expired volume in the first second of forced expiration. The sensation of obstruction is identified following exercise, however symptoms can be experienced throughout (12). The diagnosis of EIB has been a phenomenon on the rise in sport, therefore the literature surrounding the topic is broad. The condition affects many endurance athletes, although its prevalence differs between types of exercise.

The mechanisms of EIB have been heavily debated and relate to the drying of the airways and/or thermal compensation from increased blood flow to the upper respiratory tract with hyperpnoea. These debated mechanisms became the basis in developing appropriate tests to detect bronchoconstriction and its related symptoms from exercise (15). There are a number of investigations using a variety of provocation methods in various populations. This review will only highlight what would be considered the current provocation methods relevant to EIB: the eucapnic voluntary hyperventilation, methacholine and mannitol challenge, exercise challenge tests and sport specific field tests. There are several reviews that have highlighted the cost and benefits of the various provocation and mediation methods surrounding EIB. The purpose of this review is to provide background on; the prevalence of EIB in athletic populations and

specifically swimmers, the mechanisms that provoke EIB, and methods that have been used to measure and mediate EIB in clinical and applied settings.

1.1.1 **Prevalence of EIB**

1.1.1.1 Prevalence in the General Sporting Population

Exercise-induced bronchoconstriction and asthma share a strong relationship. Asthma is a condition typically diagnosed in children who experience respiratory dysfunction, 10% of whom will experience bronchospasm during and following exercise (16). Approximately 50-90% of all individuals who have been clinically diagnosed and treated with asthma are hyper-responsive to some form of exercise (17). Rates of EIB are observed at rates of up to 70-80% in untreated asthmatics, while others indicate rates as high of 80-90% in asthmatics. General cases of asthma predicts that up to 11% of the population experiences EIB (12).

The relationship between the status of the respiratory health of elite athletes and the supposed beneficial nature of high-intensity aerobic exercise is perplexing. It is possible that elite level athletes have an increased susceptibility for EIB relative to sedentary individuals (18). For example, prior to the 1984 Summer Olympics in Los Angeles, athletes were inducted into a screening program to detect for EIB. It was found that over 11% (67 of 596) of these summer athletes were positive for EIB, but more surprising was that only 39% (26 of 67) detected with EIB had previously been positively identified as having asthma (19).

During the 1996 Summer games, 17% (117 of 699) of United States Olympic Team Athletes reported having asthma or taking medications to mediate their asthma symptoms. Nearly 30% of swimmers who participated in the 1996 Summer games for the United States had asthma, with 26% (35 of 135) reportedly experiencing EIB. An prevalence of 30% is a considerably greater number of athletes than the 9% (16 of 188) of basketball, field hockey,

soccer, handball, water polo, badminton, beach volleyball, table tennis, and volleyball combined (19).

Weiler et al completed a similar investigation during the 1998 Winter Olympics and 22.4% (44 of 196) of US athletes who participated had asthma and took medications to alleviate their symptoms. In this case the majority who had asthma were where Nordic athletes. Of all US athletes competing at Nagano, 57% were Nordic athletes, and 60% of all Nordic athletes had asthma or a history of asthma, and took medications mediating their symptoms (20). It is clear rates of asthma and exercise-induced bronchoconstriction are high among aerobic athletes, in both aquatic setting and in cold dry settings.

1.1.1.2 Prevalence in Swimmers

As previously reported by Weiler et al (1998), swimmers have high incidences EIB compared to their summer sport counterparts (19). A study completed by Langdeau et al. (2000) found the high prevalence of EIB in swimming was more likely attributed to the contents of inspired air and not the nature of the exercise itself (21). During the 1976 and 1980 Summer Olympics games, 9.7% and 8.5% respectively, of Australian athletes reported having asthma, of which 10% were swimmers (22). During the 2004 and the 2008 Olympic games in Athens and Beijing, 17% of swimmers were approved to use some form of $ß_2$-Agonists, due to positive tests for exercise-induced bronchoconstriction. Previous reports in which 738 competitive swimmers were surveyed, 21% of the 165 international level swimmers were positive for experiencing asthma or exercise-induced bronchoconstriction (23). Other studies observed elite level swimmers to report high levels of symptomatic and asymptomatic hyper responsiveness and EIB, and their winter athlete counterparts reported prevalence of exercise-induced respiratory symptoms similar to that of controls (24). A number of the investigations observing the

prevalence of EIB in athletes are survey based, and did not directly measure lung athletes lung dysfunction.

Rates of EIB in swimmers seem to differ between survey-based investigations and those directly measuring for the condition. In a study completed by Castricum et al (2010), 33 elite level swimmers completed a variety of bronchial provocation tests. 18 swimmers (13%) presented positive for exercise-induced bronchoconstriction (25). Zwick et al (1999), 14 competitive swimmers where matched, controlling for age and sex, to those not exposed to chlorine. When completing a methacholine challenge, 5 of 14 controls tested positive for EIB, compared to 11 of 14 swimmers (79%)(26). Helenius et al (1998), reported that swimmers presented with the greatest odds ratio when undergoing skin prick tests in detecting airways hyperresponsiveness compared to control track athletes. Furthermore, when exposed to a dose of histamines, swimmers experienced a fall index of FEV_1, considerably greater than controls (27).

What remains clear is a discrepancy in which athletes report the incidences of EIB in swimmers, and the prevalence when they are directly tested.

1.1.2 **Provocation of the Airways**

1.1.2.1 Effects of osmotic and thermal conditions

During resting breathing in temperate conditions, inspired air is warmed and humidified via mucosa in the upper airways. Air is heated to body temperature, becomes saturated, and as such, there is a cooling and drying of the airways (28). There has been debate between whether the stimulus for EIB is respiratory heat loss, referring to the thermal hypothesis, or water loss, the so-called osmotic hypothesis. The osmotic hypothesis suggests that water loss experienced from hyperpnoea during exercise results in increased osmolarity of the upper airway surface liquid, thereby inducing bronchoconstriction (29). To support this hypothesis,

bronchoconstriction can be blunted through humidifying inspired air (30). Additionally, bronchoconstriction can be intensified with increased ventilation intensity and duration, leading to dehydration of airway surface liquids over a greater surface area (31).

Provocation of bronchoconstriction is amplified when inspired air is cooled, substantiating further heat loss and water loss of the airways (32). In concurrence with the osmotic hypothesis, evidence has been suggested the provocation of the airways is directly proportional to the "thermal load" on the airways (33). Since then, the thermal hypothesis has been considered secondary to osmotic effects (34). This is supported by studies that have indicated that not only does the inspiration of cool air not enhance an EIB response, but differences in temperature gradients are not achieved during exercise (15).

1.1.2.2 Effects of hyperpnea

Hyperpnoea plays an influential role in stimulating EIB. When investigating the effects of repetitive hyperpnoea on peripheral airway obstruction in ventilated dogs, repeated hyperpnoea induced airway obstruction and inflammation, not unlike that in human (35). Voluntary hyperpnoea alone is adequate in provoking a bronchial constrictive response (12). In early developments of the EVH test, asthmatics would hyperventilation temperate air and successfully induce a bronchoconstrictive response. Inspiration of cool, dry air coupled with high levels of minute ventilation (to simulate the hyperpnoea of high intensity exercise) is capable of inducing EIB (36). Small changes in exercise intensity, and ventilations at such intensities has an effect in inducing bronchoconstriction. Significant differences in EIB can be observed at 85 and 95% exercise intensity (37).

When provoking EIB, the importance of achieving sufficient ventilation in a clinical setting has been a measured. Subjects who can not reach predicted target ventilations in EVH

tests are less likely to demonstrate accurate fall indexes (38). The evidence indicates the physical stress inflicted on the airways during intense ventilations, either during exercise or that mimic during hyperpnea, has a significant impact on inducing EIB.

1.1.2.3 Effects of particulates

Pollutants increase the vulnerability for asthmatic patients to experience adverse respiratory effects (39). Chronic exposure to pollutants and mixed exhausts have negative effects on the upper respiratory tract. Acute exposure has been proven to decrease respiratory function in both healthy and asthmatic population during exercise (17). Multiple studies have shown potentially harmful effect on respiratory health in environmental settings such as youth athletic fields and indoor hockey arenas (40,41).

Swimmers are chronically exposed to specific particulates that have adverse effects on the upper airways. Unique to the indoor swimming environment, water is treated with sodium or calcium hypochlorite, otherwise known as chlorine, to treat microbiological pathogens (42). When these chemical treatments react with organic or inorganic matter, numerous oxidants and derivatives are produced which rest above the surface of the water, and become inhaled during high intensity exercise (43). Swimmers in the indoor environment are exposed to chlorine metabolites, which can cause asthma and airway hyperresponsiveness. The World Health Organization recommends chlorine levels should not exceed 3mg/L in public pools. At an average chlorinated-air concentration of 0.42 mg/m^3, swimmers are exposed to more chlorine during a standard two hour training sessions than is recommended for work place safety in the United States (8 hours at 4-7 mg) (27). Numerous studies have identified the negative effects of these by-products through biomarker research indicating these chemicals increased the prevalence of airway hyperresponsiveness (4,44).

It should be noted that airway dysfunction in swimmers is not entirely the result of chronic exposure to chlorine particulates. Bonsignore et al. (2003) completed an investigation with swimmers who exclusively trained in outdoor settings for 3 years, measuring airway cell counts and responsiveness following various 5 km swimming efforts. In the case of reduced chlorine exposure, the results indicated the negative status of their respiratory health was likely attributed to airway neutrophilia; the inflammation of the airways. Additionally, any acute changes observed in airway caliber were considered "modest" compared to that of running. Swimming compared to other forms of exercise in an outdoor environment exerts lesser inflammatory effects on the airways (45).

1.1.3 **Clinical Methods for Screening EIB**

The means of detecting exercise-induced bronchoconstriction via reported symptoms merits poor predictive value, therefore there are clinical and applied methods for provoking airway constriction and detecting the condition (46). In the following review, only three commonly recommended clinical methods will be discussed, however there are other mechanisms for detecting EIB.

1.1.3.1 Methacholine and Mannitol Challenge

The methacholine challenge test was developed to identify reversible airway obstruction that cannot be identified via spirometry. This method provides a means of bronchial provocation through inhalation of a short acting antigenic agent. Methacholine (acetyl-β-methacholine) is an aerosol agent that stimulates bronchial smooth muscle receptors that cause bronchoconstriction. Individuals who have asthma or EIB are more sensitive to methacholine than non-asthmatics and therefore, would experience bronchoconstriction (47). When diagnosing EIB via methacholine challenge, subjects perform baseline spirometry, followed by an inhalation of saline. This is then

followed by a doubling of concentration of methacholine (baseline 0.03-0.05mg/mL), inhaled during tidal breathing for consecutive 2-min intervals. FEV_1 is measured every 30 s and 90 s after every inhalation and every 2 minutes (48). The overall conclusion surrounding the methacholine challenge prove it to be reproducible, however in regards to testing athletes, the method lacks application (47).

The mannitol challenge, developed by Anderson and associates (1997) requires a inhalation of a provoking drug exposure and similar testing time intervals as the methacholine method. However, inhalation of mannitol does not require the use of saline solution. It is clear that a mannitol challenge test can provoke a bronchial constrictive response, using a dry powder combination in replacement of a wet aerosol interventions, however this test too lacks applicability with aerobic athletes (49,50).

1.1.3.2 Laboratory Exercise Challenge Tests

Investigations have used ‘standardized’ exercise challenge tests to either legitimize or validate the applicability of subjects EIB. Although this method is specific in detecting EID, its sensitivity is debated. Athletes are challenged to perform a maximal cycle ergometer or treadmill test (typically while fitted with a unidirectional mouthpiece to measuring metabolic parameters). Although there is a more applied nature using an exercise challenge test, subjects are often challenged to perform an unfamiliar exercise than they are challenged to maintain a high ventilatory demand. When subjects are subjected to completing unfamiliar exercise, they are likely unable achieve adequate rates of ventilation to induce provocation (51).

When comparing a sensitivity and specificity of laboratory exercise challenge test to other forms of provocation, studies have been completed. Participants completed a lab exercise challenge test to failure on a cycle ergometer, an EVH test and a methacholine challenge. The

laboratory challenge test, however, was less sensitive in eliciting a bronchoconstrictive response. During exercise on the cycle ergometer, subjects failed to achieve adequate minute ventilation, which conflicted with the standardization of exercise intensity (52). In study comparing a laboratory challenge and EVH tests, 11 of 23 subjects presented with EIB via the EVH method, and of those individuals only 5 subjects responded during a laboratory exercise challenge (53).

1.1.3.3 Eucapnic Voluntary Hyperpnea Challenge

The EVH test was originally designed and standardized by the United States military, to act as a substitute for exercise to detect airway dysfunction that could potentially impede performance (54). Currently the EVH test is commonly considered the 'Gold Standard' method in detecting EIB (1). What the EVH test requires a subject to perform baseline spirometry, in which the greatest FEV_1 values is multiplied by 30 and is set as a target ventilation. The subject will then hyperventilate at their target ventilation for a minimum of 6 minutes, in which inspired air is a cooled, dried compressed gas mixture of 5% CO_2 and 21% O_2 with a nitrogen balance. The subject then performs post test spirometry 3-, 5-, 10- and 15-minutes following the challenge (55). EIB is diagnosed if there is a greater than 10% fall index in $FEV_{1.}$ One of the benefits of the EVH test is that it allows for standardization of ventilation (36).

Although this method is sensitive and successful in detecting EIB, there are a number of critiques of the EVH test. Hull et al. presents an argument that the data available to permit claiming the EVH as the 'Gold Standard' is highly heterogeneous with respect to athletes, sex, age, etc. (2016). Additionally, issues are found when predicted target ventilation required for the test. Predicted ventilations that are purely based on FEV_1 are accurate for populations, but not for individuals. Evidence has been presented that non athletic individuals are challenged to reach even 60% of their predicted MVV during maximal exercise, and high performance athletes are

challenged to each 90% (38). Stickland et al (2006) also compared the EVH test and other methods to the exercise challenge test and found that many patients presented negative for EIB during an exercise challenge test, but presented positive during an EVH test. Arguably, the rates of false positive EVH tests are higher than acceptable, ranging from 25-71 %. The overall conclusion of the study indicated that more "higher-quality" studies need to be completed before EVH can be deemed an alternative test over exercise challenge tests (56).

The sensitivity of the EVH test and its ability to provoke EIB in wide variety of athletes is likely a primary reason for its regard as the 'Gold Standard Method'. Methacholine, mannitol, and exercise challenge tests are regularly used in detecting EIB in both athletic and non-athletic populations however lack individual application.

1.1.4 EVH Versus Field Tests

The development of the EVH test is beneficial as it requires little equipment, time, or risk for a subject. The EVH test provides the stresses associated with hyperpnoea, without the physical restraints of exercise. Methods detecting EIB in athletes; arguably, has little to no use if it cannot be applied. What point is there in detecting a condition specific dysfunction, under alternative conditions? There have been multiple developments in executing successful field tests and comparing them to the sensitivity of the EVH test.

Wilber and associates (2000) observed EIB in variety of winter sport disciplines. Athletes completed a normal warm-up and an "actual" or "simulated" competition for the presence of EIB. Following the performance, athletes completed spirometry 5-, 10- and 15-minutes post-exercise. Results indicated the prevalence of EIB across all sports was 23 %. Prevalence ranges from 0 % (biathletes) to 50 % (cross-country skiers) (57). A weakness to this study was the field and exercise challenge tests were not compared to a clinical or control method. Dickinson's group

(2006) produced a study in which 14 British winter sport athletes (short track speed skating and biathlon) completed a laboratory based exercise challenge, sport specific field test and an EVH test. Only three subjects were positive for EIB following both the EVH test and field test, with a total of 10 athletes testing positive for EIB following the EVH (58). Rundell and colleagues (2004) also compared field tests to the EVH test in which 38 athletes (figure skating, Nordic combined, canoe/kayak, cross-country skiing, and biathlon) completed a 6- to 8-minute exercise field test. The study identified the EVH test to be 74% accurate, with a positive predictive value of 53% and negative predictive value of 90% as identified by a field exercise challenge. Nine of the 38 athletes testing positive for EIB in both tests, with the majority not testing positive in either test. 11 subjects tested positive in the field, 9 of which produced positive results following the EVH test (38). Although these studies are successful in producing adequate field tests, they are mostly specific to winter sport athletes, not swimming.

Thirty-three elite level swimmers performed two different clinical provocation tests, an EVH and an 8-minute laboratory exercise challenge (cycle ergometer), along with an 8-minute swimming field challenge. The swimming field test required athletes to swim for 8 continuous minutes at the highest intensity sustainable, at >85% of age predicted heart rate maximum. 1 of the 33 swimmers presented with EIB following the field swim challenge, although 17 presented positive following the EVH test. One subject tested positive for EIB in all three provocation methods. Of note, two subjects tested negative for EIB in the field swim challenge but positive in the EVH with a mean FEV_1 fall index of 36±3% and the laboratory exercise challenge with a mean fall index of 17±6%. Castricum concluded that the results demonstrated not only the varying sensitivity of these methods, but the discrepancy that occurs when diagnosing EIB in swimmers (25). The low number of subjects who experienced EIB in the field could demonstrate

that chlorine is not required to induce bronchial constriction in swimmers, however it could be countered that exercise intensity wasn't high enough to provoke a response. Pederson and colleagues (2008) conducted a similar study in which 16 swimmers completed an EVH test, field swimming challenge, methacholine challenge and laboratory exercise test. The swimming challenge was completed in a race condition, in which the swimmer chose the distance of their choice, ranging from 200m to 800m. Eight swimmers (50%) presented with EIB, 4 of which presented in the field-based challenge, and of those, two also tested positive in the EVH test and one tested positive in the EVH and laboratory exercise challenge. Following the EVH test 2 subjects tested positive for EIB (59). A downside of this study is that swimmers were likely not subjected to a long enough exercise challenge in the pool to sustain high ventilations for an adequate time. As the swimming challenge was completed in a race setting, swimmers likely completed a warm-up prior to their swimming effort, causing a refractory effect, to be discussed later.

Field-based exercise challenge tests holds little between study standardization. The literature of field-based swimming challenge tests in provoking EIB is also sparse considering the prevalence of asthma and EIB in these athletes.

1.1.5 **Diagnosis of EIB Using Fall Index**

Currently the International Olympic Committee Medical Commission (IOC-MC), requires a fall index of ≥10% following a 6-minute EVH test (6). The current threshold was determined as previous studies concluded a fall index of FEV_1 ≥11.3% falls outside the 95% confidence limit for healthy individuals. The calculated confidence limit was also performed on subjects hyperventilating room air (36). Hurwtiz et al. completed a comparison study in which a fall index of 10% using an EVH test compared well to other commonly executed provocation

challenges (55). Reviews of interpreting the fall index have considered a 10% fall index to be too sensitive based on the wide standard deviation achieved by those with EIB (1). Conversely, it has been suggested that the threshold be lowered to 7%, based on the conclusion that EVH interpretation is not sensitive enough (53). Others have proposed a categorical method in which a fall index of between 10 and 19.9% would be considered mild, 20 and 29.9% would be moderate and greater than 30% would be considered severe (36).

There are between-study inconsistencies in what constitutes a positive test. The IOC-MC only requires a minimum 10% fall within 30 minutes of completing the EVH test, however literature measuring the validity and specificity of the EVH test and associated provocation methods are inconsistent (6). Previous investigations have been completed that do comply with the current IOC-MC standards; however, some studies require the greatest fall index to occur within the first 15 minutes following the EVH (53,60). Anderson et al (2001) required fall index to occur within 5 or 10 minutes following provocation (36). Inconsistencies in the literature are cause for misinterpretation of when comparing results, and are cause for potential false conclusions.

1.1.6 Methods Mediating EIB

There are various methods developed in mediating the negative affects EIB. Since the 1980s, athletes have been commonly prescribed some form of asthma medication. As asthma medications have been increasingly prescribed, rates of therapeutic use exceptions in Olympic competition has increased with; 46% in Atlanta 1996, 70% in Athens 2004, 77% in Torino 2006 and 87% in Beijing 2008 (3). A total of 117 athletes (17%) from the United States 1996 summer Olympic team reported using asthma medications, with the majority using short-acting β_2-agonists (68 of 117), followed by inhaled corticosteroids (31 of 117). More athletes reported

using short-acting β_2-agonists compared to long-acting, with 58% of asthma medications used were short acting and 7% were long acting (19). Similar trends were observed during the 1998 winter Olympics, as 44 athletes (22%) reported taking some form of asthma medications. The most of them using short-acting β_2-agonists (30 of 44) followed by inhaled corticosteroids (8 of 44). Furthermore, 68% of prescribed asthma medications were short acting β_2-agonists while 7% were long acting (20). Use of inhaled corticosteroids and β_2-agonists continued to increase from 2003-2008 (6). Since then, WADA has allowed to the free use of β_2-agonists and all other inhaled corticosteroids (maximum 1.6 mg), and its used has increased (61).

The use of short acting agonists provide protection against developing EIB-related symptoms during exercise. It is also further understood that dose tolerance does not occur from repeated use of short-acting agonists such as salmeterol (62). There are benefits in using asthma medications, although whether or not performance is benefitted or not is up for debate. The use of salbutamol can cause an increase in resting lung function, (i.e. FEV_1) in individuals who both test positive and negative for EIB; dyspnea, perceived leg exertion, mean power output are found to be unchanged (63).

What is recommended to athletes in addition to proper use of prescribed medication, is a vigorous warm-up. Various investigations have observed the phenomenon in which various methods of warm-up (prolonged submaximal, continuous, or high intensity interval) can induced a bronchoconstrictive reaction, creating a refractory period in which athletes can remain protected from future constraints. Studies have shown positive results, in which if a warm-up is performed properly, post-exercise FEV_1 fall indexes can remain less than 10% (64).

1.1.7 **Conclusion**

This review covered a fraction of the literature surrounding EIB and asthma. The onset of EIB is a combination of osmotic effects of water loss, rewarming of the airways and hyperpnoea. EIB occurs more so in swimmers due to the effects of particulates and chemical by-products that rest above the water's surface of indoor aquatic facilities. The chronic exposure to these metabolites is likely tied to the increased prevalence of EIB compared to their summer sport counterparts. The higher rate of EIB in swimmers is contrary to the understanding that swimming is considered a mode of exercise that does not inflict significant of respiratory stress. Methods for testing EIB is yet another topic of study that is widely investigated. Sport-specific applications and comparisons to clinical methods is a branch of research that continues to be sparsely referenced, innovated or standardized. Furthermore, more consistent comparisons can be made if standardization of field tests is implemented.

1.2 PURPOSE

Given the current body of research, the purpose this thesis was to determine the effects of humid, chlorinated air and swim exercise on inducing exercise-induced bronchoconstriction in swimmers, compared to the commonly conducted EVH method.

1.3 HYPOTHESES

The hypotheses for this thesis are as follows:

I. Swimmers who test positive for EIB following an EVH test will test positive following a chlorinated air provocation test and an exercise field test.

II. A similar number of swimmers will test positive for EIB in an aquatic EVH setting as the swimming field effort.

III. Analysis of SR-Index will indicate swimmers to experience some form obstruction, when a clinical EVH test has not.

Chapter 2: Body of Thesis

2.1 METHODS

2.1.1 Subjects

15 subjects (men=5, women=10) between the ages of 18-25 were recruited for this study. Subjects were free of respiratory or cardiovascular disease, with the exception of any previously diagnoses of asthma or exercise-induced bronchoconstriction. Subjects had at least 5 years of competitive swimming experience and trained a minimum of 10 hours a week in an indoor aquatic setting. Subjects were excluded from the study if they presented with any contraindications to exercise testing (as indicated upon completion of a PAR-Q+), smoked or had smoked more than 10 packs per year, had an acute shoulder injury, or were pregnant.

2.1.2 Experimental Overview

Testing took place at the University of British Columbia. Subjects completed three testing sessions, each separated by a minimum of 48 hrs (Averaged across16±19 days). On Day 1, participants reported to the lab, and on Day 2 and 3 reported to the UBC Aquatic Centre. Each day consisted of pre-provocation spirometry to establish baseline, a provocation, and post-provocation spirometry at 3-, 5-, 10-, 15-, and 20-min following each test. On Day 1 and 2, provocation was a standard EVH test (EVH_L) and a modified EVH test (EVH_{Cl}) respectively. Day 3 was a swimming field provocation (Swim). Days were completed in random order, with the only standardization that EVH_L proceed the EVH_{Cl} test, in order to control isocapnia. Pre-provocation spirometry was completed a number of times (on average 8±2) to ensure reproducibility of within 0.150 l, according to ATS Guidelines (65). Target ventilations for the EVH_L and EVH_{Cl}, were 30 x the best FEV_1 achieved during pre-provocation spirometry, which is approximately 85% of individuals maximal voluntary ventilation (38). A minimum of four

post-spirometry efforts were completed at each time point of 3-, 5-, 10-, 15- and 20-min following provocation.

2.1.3 **Procedures**

Prior to all testing, subjects were asked to refrain from taking short-acting bronchodilators (e.g. salbutamol, ventolin, etc.) for up to 8 hours, or inhaled corticosteroids (ICS) for up to 48 hours prior to testing. Subjects refrained from exercise for a minimum of 12 hours prior to testing. Subjects were asked to refrain from consuming caffeine the morning of or prior to testing. To avoid possible bronchodilation from a warm-up effect, subjects refrained from active transportation to each testing session.

2.1.3.1 EVH in a Clinical Setting

EVH_L test protocols were completed as outlined by Anderson et al. (2001). Subjects became familiar with the breathing required to achieve target ventilations during the testing, as well as common coaching cues. Subjects were fitted with a mouthpiece, in which a compressed gas mixture was connected to a Douglas bag that allowed inspired gas to decompress. Inspired air contained 21% oxygen (O_2), 4.9-5.1% carbon dioxide (CO_2), with the balance being nitrogen (N_2). End-tidal CO_2 was measured at rest and during provocation, in order to be replicated for the EVH_{Cl} provocation.

2.1.3.2 EVH in an Aquatic Setting

Subjects underwent an identical protocol as the EVH_L test. All procedures, including pre- and post-test spirometry, was completed on the pool deck.

Inspired air was sampled above the surface of pool water. Large Bohr tubing rested above the water surface connected to a mixing container which was then connected to a unidirectional

mouth piece. 100% CO_2 was titrated into the inspired mouthpiece, and end-tidal CO_2 (ET_{CO2}) was maintained to replicate achieved end-tidal as EVH_L.

2.1.3.3 Swimming Challenge Test

The Swim test took place at The University of British Columbia Aquatic Centre. Prior to exercise, subjects performed pre-exercise spirometry to establish baseline values, along with resting heart rate. Subjects were allowed to complete a static warm-up (i.e. arm-swings, quad stretches, etc.), however no aerobic warm-up was performed in or out of the water. Subjects then performed a 200m followed by a 400m freestyle effort at 85-95% intensity. Efforts were separated by 30-45 seconds. Intensity was determined by 85% of their season's best time, and 85% of age-predicted maximum heart-rate upon completing each effort. Post-exercise spirometry was completed 3-, 5-, 10-, 15- and 20-minutes following swim exercise.

2.1.4 **Measurements**

2.1.4.1 Resting Pulmonary Function

Forced vital capacity (FVC), forced expired volume in one second (FEV_1), percentage FEV_1 to FVC (FEV_1/FVC), peak expired flow (PEF), and forced expiratory flow between 25% and 75% (FEF_{25-75}) were measured using pneumotachographs. Measures were accustomed to ATS Standardization and compared to predicted, reference values (65,66).

2.1.4.2 Post Test Pulmonary Function

Post-provocation FVC, FEV_1, FEV_1/FVC, FEF_{25-75} and PEF were measured using the same pneumotachograph. Measures conformed to ATS Standards and compared to predicted, reference values (65,66). Measurements were repeated at 3-, 5-, 10-, and 20-minutes following EVH and exercise field tests.

2.1.4.3 End-Tidal CO_2, Flow & Volume

End-Tidal CO_2 was measured through a port at the mouthpiece connected to a calibrated CO_2 analyzer (model 17630, VacuMed, USA). Expiratory flow was continuously measured by having subjects breathe through a unidirectional T-Valve (Series 2600, Hans Rudolph Inc., USA) mouthpiece. Volume was integrated from expired flow.

2.1.4.4 Heart Rate

Heart rate was measured using a finger pulse transducer (TN1012/ST, ADInstruments, Colorado Springs, CO).

2.1.4.5 Data Sampling & Recording

End-tidal CO_2, tidal volume (V_T), minute ventilation (V_E), and expired flow were sampled at a rate of 200 Hz. All data were recorded using a PowerLab 16/30 analog-to-digital converter running LabChart Pro Version 7.3 software. Data were imported and analyzed using Microsoft Excel (Version: 15.19.1).

2.1.5 **Data Analysis**

2.1.5.1 Pulmonary Function

FVC, FEV_1, FEV_1/FVC, FEF_{25-75} and PEF were assessed every effort. Subjects' resting pulmonary function was compared to post-test pulmonary function. Post-provocation spirometry was compared to the best-achieved FEV_1 effort completed pre-provocation. A subject was considered positive for exercise-induced bronchoconstriction (EIB+) if a fall index greater than 10% was observed for two consecutive time points following provocation. If a subject failed to reach a fall index of 10%, they were considered negative for exercise-induced bronchoconstriction (EIB-).

2.1.5.2 Flow, Volume & End-Tidal CO_2

Expired flow was collected throughout both EVH tests. 30-sec average of achieved minute ventilation was measured at the first, third and sixth minute of hyperpnea efforts, and recorded to indicate sustained and adequate ventilation. End-tidal CO_2 was compared throughout hyperpnea efforts between EVH_L and EVH_{Cl} to ensure similar isocapnia.

2.1.5.3 Inspired Air Temperature

Inspired air temperature was measured via a temperature sensor (Thermistor Temperature Sensor, ADInstruments, Sydney, Australia) inserted 5 cm from the mouth, at the inspired valve of the mouth piece. Temperature differences were compared from the first minute of ventilation to the sixth minute of ventilation, during both EVH_L and EVH_{Cl}.

2.1.5.4 Slope Ratio Index

Raw expired flow traces were collected every effort. Traces were used to create subject flow-volume curves and measure slope ratio of expired flow. SR-Index is an average measure of slope between 20-80% lung volume. Average SR -Index completed pre-provocation was compared to average SA-Index achieved at the point of greatest lung function change and an overall post-provocation. An increased in SR-Index is indicative of possible or increased obstruction. A SR-Index of 1.0 is indicative of homogeneous lung emptying, however values outside the range of 0.5-2.5 are characteristics of nonhomogeneous lung emptying (67,68).

2.1.5.5 Statistical Analysis

Repeated measures analysis of variance (ANOVA) was used to compare the changes in spirometry data before and after tests. Specifically, One-Way ANOVA in comparing mean percentage fall indexes. Two-way, repeated measures ANOVA was used when observing absolute test and time interactions. Post-hoc Bonferroni tests were conducted to determine which

test and time conditions were considered significant. Planned unpaired t-tests were used to analyze the difference between positive and negative results for each method. Paired t-tests were used in comparing ventilation and temperature parameters observed during both hyperpnea tests. Pearson correlations were used in observing fall indexes between tests and relationship between lung size and temperature differences achieved during the hyperpnea challenge tests. The level of significance was set at $P \leq 0.05$ for all tests. Statistical analysis was performed using SigmaPlot Version 12.5.

2.2 RESULTS

2.2.1 Subject Characteristics

Subjects were competitive swimmers recruited from the University of British Columbia Varsity Swim Team. Subject characteristics can be seen in Table 1. Three subjects reported having been previously diagnosed with asthma by a physician, two of which consistently used bronchodilators. An additional subject reported use of a bronchodilator without an asthma diagnosis. Two of the three asthmatics, had been previously diagnosed with EIB. Individual results can be seen in Table 4, Appendix A.

Table 1. Subject Characteristics. Percentage predictive values are compared to values obtained in the lab setting. Individual spirometry values can be seen in Table 6. FVC, forced vital capacity, FEV_1, forced expired volume in one second, FEF_{25-75}, forced expired flow between 25 and 75 % lung volume. PEF, peak expired flow. Predictive values determined using predictive equations by Hankinson et al (2006).

	Women *n*=10	Men *n*=5
Age (*years*)	21±1	21±3
Height (*cm*)	175±8	184±3
Weight (*Kg*)	65±8	78±3
Predicted FVC (*l*)	4.41±0.39	6.01±0.75
Predicted FEV_1 (*l*)	3.80±0.30	4.97±0.58
Predicted FEV_1/FVC	0.86±0.00	0.83±0.01
Predicted FEF_{25-75} (*l/sec*)	4.20±0.18	5.14±0.48
Predicted PEF (*l/sec*)	7.63±0.51	10.67±0.97
% Predicted FVC (*%*)	112±14	106±13
% Predicted FEV_1 (*%*)	104±18	97±10
% Predicted FEV_1/FVC (*%*)	96±8	93±9
% Predicted FEF_{25-75} (*%*)	119±30	103±21
% PEF (*%*)	119±14	110±34

Table 2. Baseline resting spirometry values collected prior to testing three methods of provocation; EVH_L, EVH_{Cl}, and Swim tests. FVC, forced vital capacity, FEV_1, forced expired volume in one second, FEF_{25-75}, forced expired flow between 25 and 75 % lung volume, PEF, peak expired flow. All values are statistically similar to one another (p>0.05).

	EVH_L	EVH_{Cl}	Swim
FVC (*l*)	5.39±0.98	5.44±0.96	5.43±0.98
FEV_1 (*l*)	4.29±0.79	4.27±0.80	4.26±0.78
FEV_1/FVC	0.80±0.07	0.79±0.08	0.79±0.08
FEF_{25-75} (*l/sec*)	5.07±1.25	4.87±1.24	4.97±1.23
PEF (*l/sec*)	9.90±2.23	9.92±2.01	10.15±2.32

FVC (p=0.987), FEV_1 (p=0.996), FEF_{25-75} (p=0.905), and PEF (p=0.941) were similar across the three days (See Table 2).

2.2.2 Ambient and Pool Conditions

Ambient temperature was significantly lower during EVH_L (22.6±1.6 ºC) compared to EVH_{Cl} (24.5±0.7 ºC) and Swim (24.5±0.7 ºC) tests completed on the pool deck (both p<0.001). No significant differences were found between EVH_{Cl} and Swim (p=0.937). Ambient humidity was significantly lower during EVH_L (45.8±12.8 %) compared to EVH_{Cl} (67.5±7.8 %) and Swim (67.6±8.1 %) tests (both p<0.001). There were no significant differences between EVH_{Cl} and Swim (p=0.972). Barometric pressure was similar throughout all days of testing (756±2 vs. 758±3 vs. 758±4 mmHg, p=0.066).

Aquatic conditions testing during EVH_{Cl} and Swim tests were all statistically similar (all p>0.05). Average values can be seen in Table 3.

Table 3. Pool conditions during EVH_{Cl} and Swim tests. All values are statistically similar between days. Cl, chlorine, Ca, calcium, ORP, oxidation reduction potential.

	EVH_{Cl}	Swim
pH	7.5±0.1	7.4±0.1
Free Cl (*mg/L*)	2.8±0.7	2.9±1.0
Combined Cl (*mg/L*)	0.1±0.1	0.2±0.5
Total Chlorine (*mg/L*)	2.9±0.7	3.1±1.2
Total Alkaline (*ppm*)	72±18	72±21
Hard Ca (*ppm*)	247±18	247±21
ORP (*mV*)	835±7	836±11
Water Temperature (°*F*)	80±0	80±1

2.2.3 **Provocations**

Target ventilations were determined prior to each EVH_L and EVH_{Cl} test. The average target ventilations for EVH_L (128±23 l·min^{-1}) and EVH_{Cl} (127±23 l·min^{-1}) were similar (p=0.630). Subjects were not able to achieve their target V_E on nine occasions (7 subjects during EVH_L and 2 subjects during EVH_{Cl}). Subjects on average achieved a lower ventilation during the EVH_L (127±25 l·min^{-1}) compared to the EVH_{Cl} (138±26 l·min^{-1}, p=0.003). Individual results between hyperpnea challenges are presented in Figure 1. Relatively, subjects achieved -0.6±7.6 % less than their target ventilation during the EVH_L test and +8.6±9.3 % greater than their target ventilation during the EVH_{Cl} test (p=0.009).

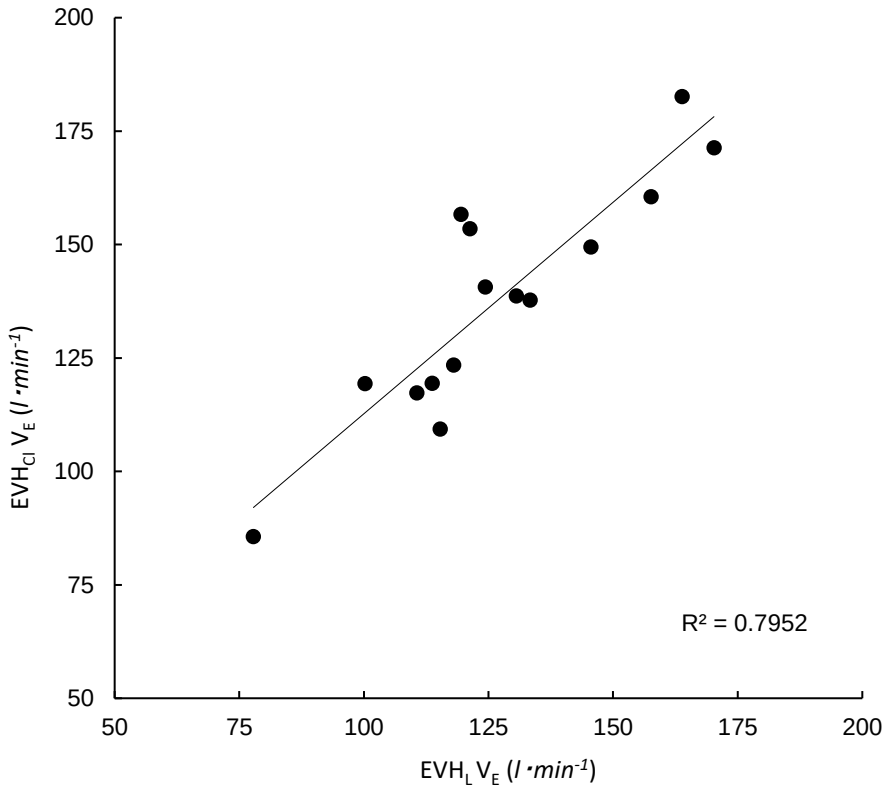

Figure 1. Minute ventilation achieved in the EVH_L test completed in the lab and EVH_{Cl} test completed on the pool deck. Ventilations achieved during EVH_{Cl} significantly greater than ventilations achieved during the EVH_L test (p<0.05).

Minute ventilation at the 1st, 3rd, and 6th minute of the EVH test were averaged over 30 seconds. During the 1st minute of provocation, ventilations were statistically similar between EVH_L and EVH_{Cl} (125±28 vs. 131±25 $l \cdot min^{-1}$, p=0.112). Ventilations at the 3rd (127±24 vs. 138±26, p=0.003) and 6th (130±26 vs. 141±27 $l \cdot min^{-1}$, p=0.023) minute of provocation were statistically greater during EVH_{Cl}. Ventilation variability was greater during EVH_L compared to EVH_{Cl} (21±5 vs. 16±5 $l \cdot min^{-1}$, p=0.001). Breathing frequency (63±9 vs. 66±9 bpm, p=0.136) and V_T (2.1±0.6 vs. 2.2±0.6 L, p=0.119) during EVH_L and EVH_{Cl} respectively, were similar.

End-tidal CO_2 was statistically similar throughout EVH_L and EVH_{Cl} (39±2 vs. 39±1 mmHg, p=0.565). Similarly, relative isocapnia was also observed at the 1st (36±3 vs. 34±5 mmHg, p=0.100), 3rd (40±2 vs. 40±2 mmHg, p=0.919) and 6th minute (41±2 vs. 39±3 mmHg, p=0.100) of provocation.

Subjects' self-reported season-best 200 and 400 m freestyle times were 2:05.00 (1:47.00-2:13.00) min and 4:27.60 (4:00.00-4:45.00) min, respectively. During the swimming provocation, subjects averaged 89% (2:18.52± 07.79 min) and 90% (4:55.22± 20.38 min) of their 200 and 400 m seasons best, respectfully. Upon completing the 200 and 400 m effort, heart rate was 163±15 and 181±9 bpm, respectively, which was 82±7 and 91±5 % of their age predicted heart rate maximum. Subjects achieved 97±9 (p=0.153) and 107±6 % (p<0.001) of their goal 85% age predicted heart rate maximum.

2.2.4 Inspired Air Temperature

Reduction in inspired air temperature during EVH_L (-0.12±0.39 ºC) was statically similar to EVH_{Cl} (-0.08±0.15 ºC) (p=0.729). Relatively, EVH_L (-0.5±1.7 %) elicited a slightly greater reduction in inspired air temperature during provocation compared to EVH_{Cl} (-0.3±0.6 %,

p=0.755). Relative to resting vital capacity, a non-significant relationship was observed between FVC and absolute reduction in inspired air temperature through EVH_L (r= -0.420, *p*=0.199). No relationship was found between FVC and absolute change in inspired air temperature during EVH_{Cl} (r= 0.144, *p*=0.608).

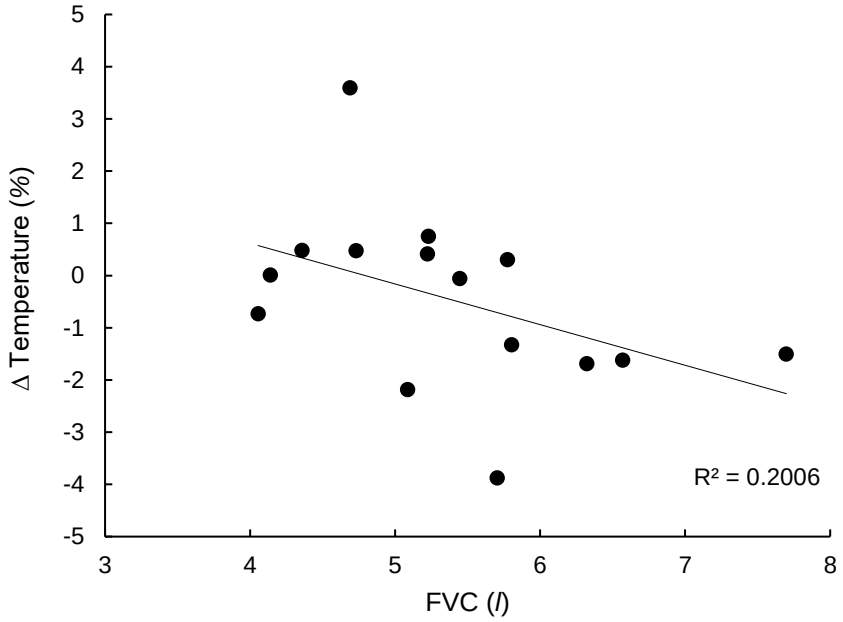

Figure 2. Change in inspired air temperature during EVH_L test relative to subject FVC, forced vital capacity (*p*>0.05)

As seen in Figure 2. There was a non-significant trend between absolute temperature difference and achieved minute ventilation (r= -0.444, *p*=0.097), and the relationship between percentage drop in inspired air temperature and achieved minute ventilation during the EVH_L test (r= -0.452, *p*=0.090).

2.2.5 **Fall Indices**

2.2.5.1 *FEV_1*

EVH_L elicited greater reductions in FEV_1 than EVH_{Cl} and Swim tests, as shown in Figure 3. No significant differences were detected across the three conditions (p=0.072). Individual trends can be observed in Figure 4. A significant correlation was observed between the greatest observed FEV_1 fall index between EVH_L and EVH_{Cl} (r= 0.78, p<0.001, Figure 4.A); however, there was not a significant correlation between EVH_L vs. Swim (r= 0.20, p=0.480, Figure 4.B), nor EVH_{Cl} vs. Swim (r= 0.50, p= 0.06, Figure 4.C).

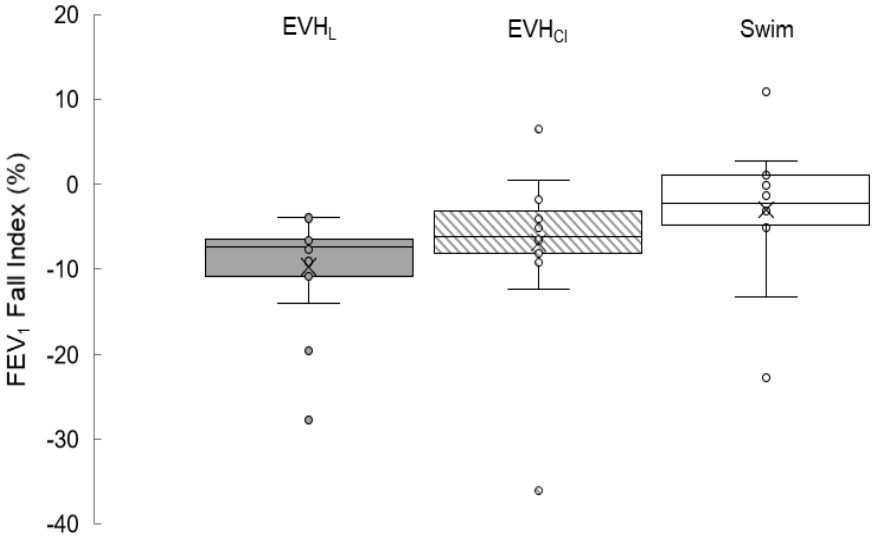

Figure 3. Mean greatest achieved FEV_1 fall index following provocation in each testing method. Greyed box represents EVH_L (-9.7±6.4 %) conducted in the lab, dashed box presents data following EVH_{Cl} (-6.9±9.2 %) conducted on the pool deck, and white box represents Swim data (-3.0±7.5 %) collected following swimming exercise. There were no statistically significant differences between the three groups (p>0.05).

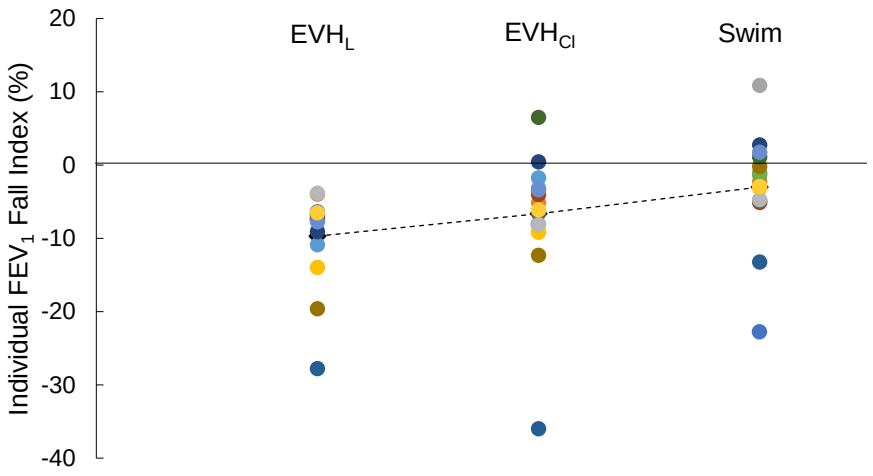

Figure 4. Individual trends of greatest achieved FEV_1 fall index following provocation in each testing method. Dashed line represents mean trend across all three tests (p>0.05).

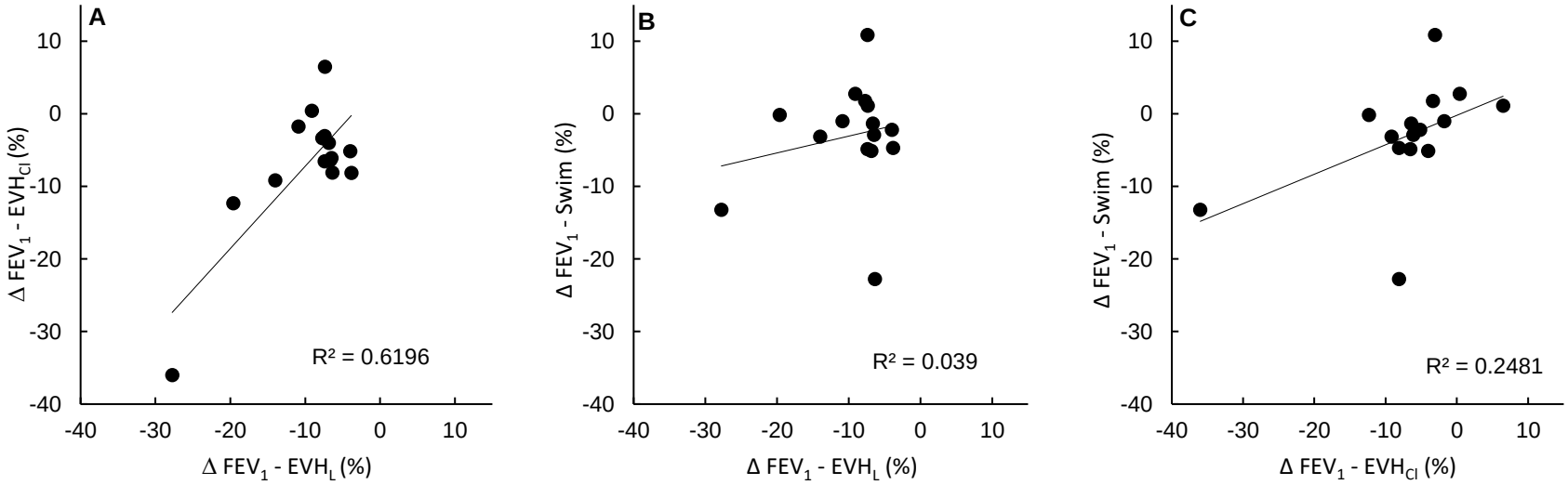

Figure 5. Pairwise relationships of greatest achieved fall indices of FEV_1 achieved following three provocation methods. A. Pairwise relationship between EVH_L and EVH_{Cl} tests ($p<0.05$). B. Pairwise relationship between EVH_L and Swim tests ($p>0.05$). C. Pairwise relationship between EVH_{Cl} and Swim tests ($p>0.05$).

When comparing absolute fall in FEV_1 over time between tests, there was no significant difference between EVH_L and EVH_{Cl} (p=0.261), EVH_L and Swim (p=0.097), or EVH_{Cl} and Swim tests (p=1.000). There was a significant test and time interaction (p<0.001).

Interactions between tests, within post-provocation time points can be seen in Figure 6. Within the EVH_L test, all post test procedures were significantly lower than baseline (all p<0.05). Within the EVH_{Cl} and Swim tests, all post-provocation FEV_1 values were statistically similar to baseline (all p>0.100). Additionally, within the EVH_{Cl} test, 3- and 5-minutes post-provocation compared to 10- (p=0.022 and p=0.002), 15- (p=0.010 and p<0.001), and 20-minutes post-provocation (p=0.013 and p=0.001, respectively), were significantly greater.

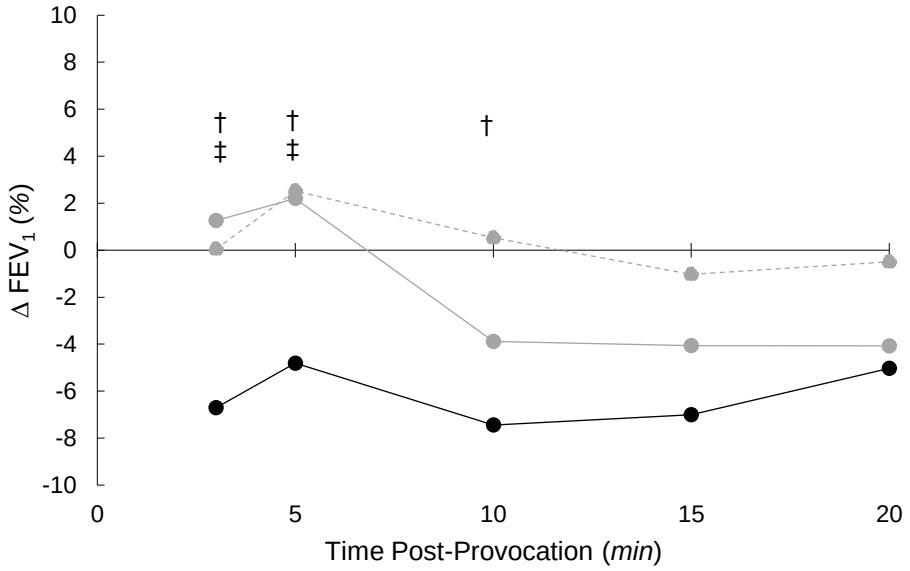

Figure 6. Average greatest fall index at 3-, 5-, 10-, 15- and 20-minutes following three provocation methods. Black line represents EVH_L completed in the lab. Solid grey line represents EVH_{Cl} completed on the pool deck, and dashed grey line represents Swim effort. †, p<0.05 between EVH_L and Swim tests. ‡, p<0.05 between EVH_L and EVH_{Cl}.

2.2.5.2 Mid-Flow

The reduction in FEF_{25-75} was greatest following EVH_L test (-16.6±8.8 %), followed by EVH_{Cl} (-8.2±14.9 %) and Swim tests (-1.3±15.6 %). The differences between the three tests were statistically significant (p=0.012). A pairwise comparison indicated reductions of FEF_{25-75} following EVH_L were significantly more reduced than following Swim tests (p=0.010) however, no significant differences were found between EVH_L and EVH_{Cl} (p=0.178), or EVH_{Cl} and Swim (p=0.165).

Observing absolute comparisons of FEF_{25-75} between tests, EVH_L was significantly lower than Swim tests (p=0.003), however no significant difference was observed between EVH_L and EVH_{Cl} (p=0.512), or EVH_{Cl} and Swim (p=0.086).

Interaction between tests, within points of time post-provocation can be observed in Figure 7.A. Within the EVH_L test, all post-provocation measurements were significantly lower from baseline (all p<0.005). Within the EVH_{Cl} test, all post-provocation measurements were not significantly lower from baseline (all p>0.05). A delayed reduction in FEF_{25-75} following EVH_{Cl} was observed as both 3- and 5-minutes post-test were similar to baseline (both p=1.000), however were significantly greater than 15-minutes post-test (p=0.010 and p=0.002, respectively). Following the Swim test, all post-provocation measurements were statistically similar to baseline (all p>0.05), with the exception of 5-minutes post provocation, which presented with an average increase of 0.49±0.46 L in FEF_{25-75} compared to baseline (p=0.010), indicating a bronchodilation effect.

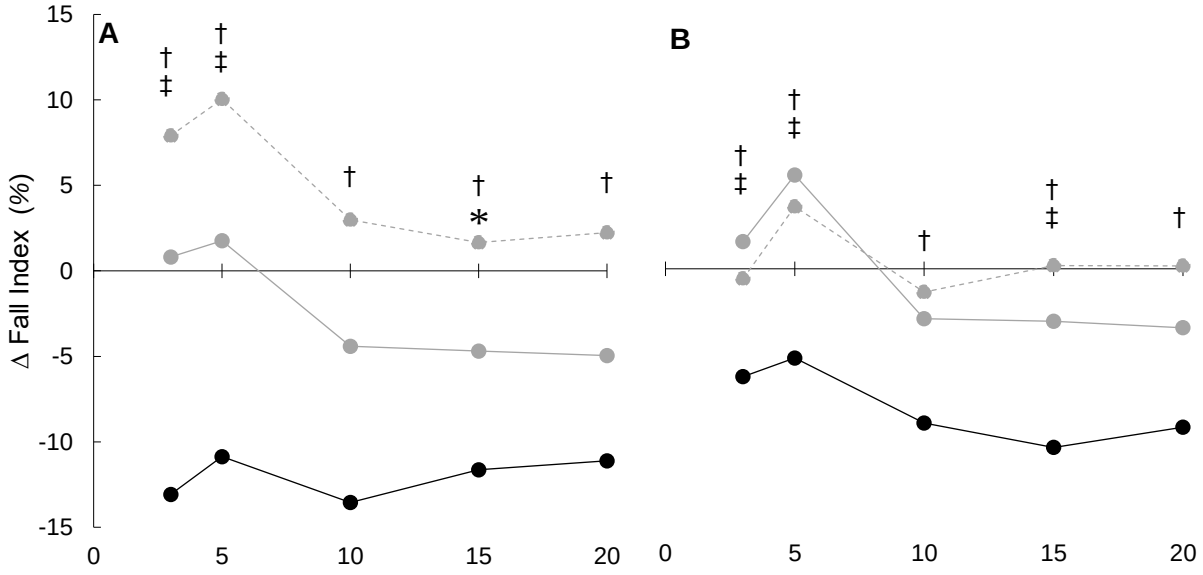

Figure 7. Average fall Index of resting spirometry values recorded over time, 3-, 5-, 10, 15-, and 20-minutes following provocation. Black line represents EVH_L test, grey line represents EVH_{Cl} test, and grey dashed line represents Swim tests. A. Fall Index of FEF_{25-75}. B. Fall Index of PEF following provocation. †, $p<0.05$ between EVH_L and Swim tests. ‡, $p<0.05$ between EVH_L and EVH_{Cl}. *, $p<0.05$ between EVH_{Cl} and Swim tests.

2.2.5.3 Peak Expired Flow

The greatest reduction in PEF following provocation was observed following EVH_L tests (-12.7±8.1 %), followed by the EVH_{Cl} (-6.3±10.5 %) and Swim Tests (-4.9±8.0 %). The differences in PEF Index between the three tests was statistically significant (p=0.047). In pairwise comparisons of PEF, there was no significant differences; EVH_L vs. EVH_{Cl} (p=0.110), EVH_L vs. Swim (p=0.060), or EVH_{Cl} vs. Swim (p=0.654).

There was a significant test, time interaction of PEF (p=0.003). When comparing the absolute decrease in PEF between tests, EVH_L was significantly reduced post-provocation compared to EVH_{Cl} (p=0.028) and Swim tests (p=0.002). EVH_{Cl} was not significantly lower than Swim test (p=0.874).

Differences of achieved PEF within time points, post-provocation can be seen in Figure 7.B. Within the EVH_L test, over time all PEF measurements were significantly lower than baseline (all $p<0.05$) with the exception of 5-minutes post provocation ($p=0.110$). Within the EVH_{Cl} test, all post-provocation measures of PEF were similar to baseline (all $p>0.100$). There was a delayed decrease in PEF, supported by significant differences from 5-minutes to 10- ($p=0.001$), 15- ($p<0.001$), and 20-minutes post-test ($p<0.001$). The only interaction observed with the Swim test was a decrease in PEF between 5- and 10-minutes post provocation ($p=0.034$).

2.2.6 **Slope-Ratio Index**

Average baseline slope-ratio indexes between EVH_L (1.05±0.31), EVH_{Cl} (1.15±0.33), and Swim (1.15±0.26) were similar ($p=0.617$). Average SR following provocation was greater following EVH_L (1.11±0.29, $p=0.024$), and similar in EVH_{Cl} (1.16±0.31, $p=0.737$) and Swim Tests (1.11±0.37, $p=0.611$). Post provocation SR-Index was statistically similar between the three tests ($p=0.918$). SR Index of observed in efforts indicating the greatest FEV_1 fall index were statistically similar to baseline in the EVH_L (1.07±0.34, $p=0.802$), EVH_{Cl} (1.21±0.38, $p=0.097$), and Swim tests (1.19±0.50, $p=0.729$), and were all similar to one another ($p=0.581$).

When comparing Delta SR-Index, between subjects who were classified EIB+ or EIB-, no significant trends were observed in the EVH_L (-0.17±0.46 vs. +0.08±0.10, $p=0.347$) or EVH_{Cl} (+0.27±0.24 vs. +0.04±0.12, $p=0.405$), however differences in Swim tests were statistically significantly different (+1.01±0.09 vs. -0.11±0.20, $p<0.001$).

When correlating fall index of FEV_1 and delta SR-Index, there were moderate, albeit inconsistent, correlations following the EVH_L (r= 0.76, $p=0.001$), EVH_{Cl} (r= -0.60, $p=0.017$), and Swim Tests (r= -0.67, $p= 0.005$).

Chapter 3: Discussion & Conclusion

3.1 DISCUSSION

The purpose of this thesis was to determine the effects of humid, chlorinated air and swim exercise in provoking exercise-induced bronchoconstriction in swimmers, compared to the commonly practiced EVH test. It was hypothesized individuals who tested EIB+ following the EVH_L test would also test EIB+ following the EVH_{Cl} test. Under the qualifying conditions, EIB is diagnosed if a greater than 10% fall in FEV_1 occurred; the hypothesis is rejected. Not all individuals who were classified as EIB+ during the EVH_L achieved the same result following the EVH_{Cl}. There was however a significant correlation between the fall index of FEV_1 following the EVH_L and EVH_{Cl} tests. The second hypothesis which predicted individuals who were classified as EIB+ following the EVH_{Cl} test would also test positive for EIB following the swimming field test, is also rejected. The final hypothesis estimating that SR-Index would indicate obstruction regardless of whether or not FEV_1 substantially changed, is also rejected. Although there were statistically significant results indicating a correlation of Δ FEV_1 and Δ SR-Index, the results are not consistent with one another, nor indicate similar trends in changed expired flow and reduced FEV_1.

The primary finding of this thesis is that the EVH_L test was the more sensitive provocation method. Using identical mechanisms, the EVH_L elicited a greater bronchoconstrictive response compared to the inhalation of the warm, humid and chlorinated air above the pool water surface during the EVH_L. Furthermore, the EVH_L test was significantly more sensitive in eliciting a bronchoconstrictive response compared to the Swim test. Increased bronchoconstriction following the EVH_L was seen in both FEF_{25-75}, and PEF, although was not

present in every subject. Additional trends such as increased resting lung function and decreased inspired air temperature during EVH_L were also observed.

3.1.1 EVH_L Versus EVH_{Cl}

During provocation, subjects achieved greater average minute ventilations during the EVH_{Cl} test compared to the EVH_L test. Subjects completed the EVH_L prior to the EVH_{Cl} test to achieve identical isocapnia, and this difference could be due to a possible order effect. Additionally, subjects could have found it easier to achieve greater ventilations in warm humid chlorinated air during the EVH_{Cl} test.

There was an observable trend in FEV_1 fall index between the three tests, however no statistically significant differences. A purpose in developing this thesis was based on the premise that swimmers are frequently observed to have increased rates of EIB or AHR from chronic exposure to chlorine. Previous studies had found signs of bronchial epithelial damage following repeated episodes of high ventilations in chlorinated pools, which contribute to increased remodeling of the airways and in turn increased AHR (35). Therefore, it was hypothesized swimmers would experience similar responses to hyperpnea following the EVH_L and EVH_{Cl} tests as acute chlorine exposure could trigger a hyperresponsive mechanism. Swimmers acutely exposed to high intensity hyperpnoea in chlorinated conditions in the current thesis did not present with significant bronchoconstriction. A study completed by Bougault and colleagues, (2009) found minimal to no inflammation of the airways in swimmers who had not exercised in the 12 hours prior to testing, with the exception of those who were considered AHR (44). The current thesis required athletes to abstain from exercise for a minimum of 12 hours; therefore, irritation and effect of airway inflammation caused by intense chlorine exposure could have been minimized due to time required between exercise and testing. Swimmers also did not experience

as significant reductions in lung function following the EVH_{Cl} test as the EVH_L test due to the ambient humidity experienced on the pool deck. A Bronchoconstrictive response following the EVH_{Cl} test could have potentially been attenuated as osmolar respiratory water loss would have been reduced in the humid environment (11,69).

A delayed bronchoconstrictive effect was observed following the EVH_{Cl} test and not following the EVH_L test. Presented in Figure 5., a significant trend was observed in which FEV_1 fall index at 10-, 15-, and 20-minutes post provocation was significantly lower than 3- and 5-minutes post provocation of the EVH_{Cl} test. Following the EVH_{Cl}, 100 % of the subjects presented the greatest reduction of FEV_1 at 10-, 15- or 20-minutes post-provocation, compared to the 73% following the EVH_L. Reasons for the delayed trend are not known. Previous reviews have indicated hyperpnea under warm conditions created less severe or delayed bronchoconstrictive response, and have displayed figures displaying the relayed response similar to the current study (15). It could be hypothesized the ambient humidity and temperature during the EVH_{Cl} test provides immediate protective qualities during hyperpnoea. Once subjects rest post-provocation, acute exposure to chlorinated air takes effect on the airways creating delayed airway hypersensitivity, and therefore bronchoconstriction. There is no evidence however, to support such conclusion. Studies observing significantly delayed bronchoconstrictive response are not under chlorinated conditions and typically observe delayed responses 3-12 hours post-provocation (70,71).

3.1.2 EVH_L Versus Swim Exercise

There was a significant difference in airway function following the EVH_L test and swim exercise. When comparing the EVH_L and Swim test, there were two primary contributors of EIB; the ambient conditions of the inspired air and the mechanism of hyperpnea. A breadth of

research has observed the effects of dry air in inducing EIB and the protective effects of humid air (11,30). Not measured in the current thesis, previous studies have observed the drying properties of the EVH from marked increases in biomarkers such as CC16 (72). CC16 is a plasma protein secreted during dehydration in terminal bronchioles, as a response to reduce airway inflammation. A study completed by Bolger et al (2011), found that regardless of a diagnosis of EIB, athletes presented with a significant increase in CC16 following an EVH test (72). Another study however, found the presence of CC16 was significantly greater in elite swimmers following a swim exercise challenge compared to a mannitol challenge (73). The physiological mechanism in inducing bronchoconstriction via mannitol challenge differs from the EVH test and swim exercise, but this still raised a significant question of the effects of dehydration experienced during context-specific testing. Chronic exposure to chlorinated water creates for hyper-reactive responses in swimmers' airways; however, there are still protective qualities of the aquatic environment that protect against bronchoconstriction during swim exercise. Studies have indicated body position, hydrostatic pressure, immersion and water loss associated with swimming can be 'ruled out' as sole mechanisms that protect against EIB, however they all contribute to some extent. (74,75).

The discrepancy in provoking a bronchoconstrictive response between clinical challenge tests and swim exercise is not novel. The thesis would agree with how Clearie et al (2010) outlined this inconsistency, as methods such as the mannitol challenge inflicts a 'traditional asthmatic' response, whereas swim challenge tests evoke swimming-specific bronchoconstriction (76).

3.1.3 Effectiveness of Swim Exercise Provocation

Two drawbacks occur when executing swimming provocation challenges. Swim challenge tests have been executed in a variety of protocols and frequently swimmers fail to achieve adequate ventilation or extended exercise intensity during swim exercise. The nature of swimming (with the exception of backstroke) suppresses the ability to achieve high ventilations based on stroke rate (77).

To compare a swimming challenge to an EVH test, achieving an adequate comparison of ventilation intensity is key. A previous study completed in our lab conducted graded $VO_{2\ MAX}$ tests of collegiate swimmers completing freestyle swim exercise. V_E at peak exercise (122±33 $l \cdot min^{-1}$) and 85% of age predicted HR_{MAX} (104±21 $l \cdot min^{-1}$) were lower than the hyperpnea ventilations achieved in the current thesis. Although achieved V_E was lower, inspired work of breathing at any given ventilation was greater during swimming compared to terrestrial exercise. Therefore, although the ventilation volume is reduced during freestyle swim exercise compared to terrestrial, the intensity at a given absolute ventilation during swimming is greater. Unless there is a direct measure of ventilation, a comparison of swim exercise and voluntary hyperpnea must be based on an estimated ventilation at a given exercise intensity. The current study required swimmers to achieve 85% of age predicted HR_{MAX} and at least 85% of their season's best time. The guideline was determined as target ventilations used in hyperpnea challenge tests are typically estimated from 85% MVV (52,53,78).

Swimming challenge tests have previously ranged from 600 to 800 m of freestyle efforts at 85 % of age predicted HR_{MAX} (25,76). Others have recorded spirometry before and after race simulations or full-length practices (59). The current study sought to have consecutive 200 and 400 m efforts for two reasons; the majority of swimmers are familiar with pacing high intensity

200 and 400 m swimming efforts opposed to 600 or 800 m efforts, and therefore achieved times during testing can be compared to season best times. Secondly, intensity of consecutive 200 and 400 m efforts can potentially be greater than a single, continuous 600 or 800 m effort. The author would argue, given the achieved heart rates and 200 and 400 m times, the exercise intensity achieved by the subjects was sufficient in achieving adequate and prolonged ventilations.

3.1.4 Resting Lung Size and Inspired Air Temperature

Inspired temperature and humidity are frequently indicated as primary variables related to bronchoconstriction. Trends between changes in inspired air temperature during hyperpnea and resting lung volumes or prescribed target ventilation was observed.

It is well understood that EVH tests are typically conducted with a compressed gas mixture of ~5% CO_2, ~21% O_2, and balance N_2. Boyles Law would indicate that decompression as the air exits the tanks will reduce the temperature compared to ambient conditions. Subjects with large resting lung volumes, typically have greater FEV_1 values and therefore greater target ventilations. When inspired ventilation is greater, compressed gas must be supplied at a greater rate. Greater inspiration of compressed gas likely decreases the temperature of the inspired air more in individuals with greater resting lung volumes. Although there are techniques available to allow for the control of inspired air temperature, many technicians do not have the resources to do so (36). In order to observe this possible trend, a Thermopod was placed 5 cm from the mouth, to measure inspired air temperature during hyperpnea.

The current study observed non-significant trends of greater resting lung volumes and therefore target ventilations indicating greater changes in inspired air temperature, as seen in Figure 2. A greater sample size could indicate a significant relationship. To the knowledge of the

author, there has not been a study to observe the relationship of resting lung volume, and the change in inspired air temperature experienced during an EVH test.

3.1.5 Variables Affected by Bronchoconstriction

The current study would argue the use of SR-Index is not a suitable measure in quantifying Although this thesis has a relatively small sample size, previous studies with greater than 50 subjects have also failed to show significant differences in SR-Index following hyperpnea in asthmatics (68). The applicability of SR-Index would be difficult for athletic populations. Immediate smoothing of expired flow traces is required in order to obtain 'clean' SR-Index values. Filtered expired flow traces, however, are not entirely feasible for practitioners, nor can it be applied in a timely manner.

There is a relationship between fall index of FEV_1 and $FEF_{25-7,}$ as seen in Figure 8. Mid flow has not been a variable that is typically taken into account when considering EIB diagnosis. There were significant differences in FEF_{25-75} between the three tests, indicating the conditions had differing effects on these variables. On multiple occasions, there would be a significant decrease (>10% fall) in FEF_{25-75} (14, 15, 25 %) however FEV_1 did not meet the same threshold (7, 7, and 9 %, respectively) (Table 4.) Mid-flow could be a variable taken into more consideration when observing respiratory dysfunction.

3.1.6 Individualization and Specification in Detecting EIB

The current study observed both significant and insignificant trends surrounding standard, modified and applied provocation methods. EIB or any other airways dysfunction an athlete might experience is always going to be individualized. How the phenomenon of exercise related bronchoconstriction should be detected and managed is through the mechanisms the athlete experiences such distress. The EVH test is a very efficient method in inducing EIB for

individuals who experience bronchoconstriction in cool, dry conditions. However, the current study observed two occasions in which EVH_L tests elicited a positive result (FEV_1 fall index= -14 and 11%) in swimmers who has been previously aware of their asthma condition, however no significant changes in lung function were detected following the EVH_{Cl} (FEV_1 fall index= -9 and 2 %, respectively) or Swim Tests (FEV_1 fall index= -3 and -1 %, respectively). Do these individuals require medications? Another case observed a subject reported no previous problems with asthma or EIB, tested EIB- following both the EVH_L (FEV_1 fall index= -6 %) and EVH_{Cl} (FEV_1 fall index= -8%) tests, however presented a significant reduction of FEV_1 the Swim exercise test (FEV_1 fall index= -23%). The subject potentially experiences bronchoconstriction specific to swim exercise and not hyperpnea. These examples justify the use of exercise-specific methods in induce exercise specific bronchoconstriction.

3.2 LIMITATIONS

Hypotheses of this thesis were established on the qualitative distinction of EIB, in which subjects either achieved a fall index of FEV_1 greater than 10 % or not. Comparing mechanisms of bronchoconstriction however is arguably not a qualitative comparison, but quantitative. Retrospectively, hypotheses should have been developed from a quantitative perspective.

The results of this study could have potentially been strengthened with a greater sample size. Testing for bronchoconstriction however, requires testing rested lungs. Testing swimmers prior to exercise is a difficult task. Swimmers have incredibly high morning training volumes, therefore testing a subject, three individual times requires flexibility. Adding three more relatively high intensity testing sessions to a training regimen is also substantial. Unfortunately, when testing a specific population such as this, there is rarely an answer to this solution without

altering training schedules. In relationship to sample size, having a greater number of swimmers with confirmed asthma or EIB could have added strength to comparing the three tests.

A measurement of ambient chloramines could potentially add strength to how much exposure airways were subjected to. Chlorine concentrations however, do slightly vary from day-to-day, and between aquatic centres. Concentration of airborne chemicals is not a controllable variable, nor is it controlled for in a training or competition setting. The aquatic center used in the current study was also a new facility with new methods in chlorine treatment and ventilation. Exposure to chlorine would likely differ in an older facility with out dated treatment methods and ventilations systems.

A pneumotachograph was used in obtaining all spirometry values in order to obtain raw data, and estimate SR-Index. The use of a pneumotachograph is not the most precise means in obtaining spirometry values. In order to detect expired flow, the threshold for minimum pressure required for a pneumotach greater than that used in portable turbine spirometers or filament spirometers. A portable spirometer which exports 200 Hz of raw expired flow traces would be the ideal method in measuring the subjects lunch function. Obtaining a full pulmonary profile before and after each tested could have provided greater insight in the changes that are occurring following each of the provocation methods.

Finally, the current study was only able to make a measurement of effort based off time, and heart rate upon completion of each of the 200 and 400 m freestyle efforts. A live measurement of ventilation and heart rate during swim exercise, all the while inspiring the same contents during natural swimming, would strengthen the comparison to voluntary hyperpnea. Ensuring the prescribed exercise challenge is sufficient in eliciting ventilations related to typical high-intensity swim exercise and the EVH tests is a consistent challenge.

3.3 CONCLUSION

The standard EVH test is more sensitive in eliciting a bronchoconstrictive response compared to hyperpnea with humid chlorinated air and swim exercise. Hyperpnea of the humid, chlorinated air during the EVH_{Cl} test elicited a delayed bronchoconstrictive response, for reasons that are not known; however, one can speculate the protective qualities of humid air protect against immediate bronchoconstriction and chlorinated bi-products elicit a delayed bronchoconstrictive response.

The use of SR-Index in monitoring changes in forced expired flow following provocation cannot be classified as a valid additional method in screening for EIB, following this study. Conclusions would require a greater sample size and observations of more subjects with EIB under a single condition.

Having developed an applied method provoking EIB in swimmers has assisted in understanding the phenomenon. These athletes experience EIB under much difference conditions than terrestrial or outdoor endurance athletes. The management and detection of EIB should mimic the means in which the phenomenon is experienced. If current methods do not apply to the athletes who use them, new mechanisms must be developed. The current thesis supports the premise that condition specific induced bronchoconstriction requires condition specific provocation and detection.

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Appendices

Appendix A – Raw Data

Table 4. Individual anthropometric data.

Subject	Sex	Age (*years*)	Height (*cm*)	Weight (*Kg*)	BMI (*Kg*/m^2)
S1	F	23	180	68.0	21.0
S2	F	21	174	75.5	24.9
S3	F	21	179	59.3	18.5
S4	F	20	176	61.1	19.8
S5	F	20	170	67.8	23.5
S6	F	21	168	55.6	19.7
S7	F	20	188	73.0	20.7
S8	F	18	161	51.7	20.1
S9	F	22	180	71.0	21.9
S10	F	20	175	68.2	22.3
S11	M	21	187	87.0	24.9
S12	M	20	182	78.9	23.8
S13	M	21	200	87.0	21.8
S14	M	19	178	71.2	22.5
S15	M	26	172	66.0	22.3

Table 5. Individual ventilation data collected from both EVH_L and EVH_{Cl} tests. B_f, breathing frequency, V_T, tidal volume, V_E, minute ventilation, V_E STDV, variation of achieved minute ventilation, End-Tidal CO_2, pressure of end-tidal carbon dioxide.

	EVH_L							EVH_{Cl}						
Subject	Target V_E ($l \cdot min^{-1}$)	B_f (bpm)	V_T (l)	V_E ($l \cdot min^{-1}$)	V_E STDV ($l \cdot min^{-1}$)	% Target V_E	End-Tidal CO_2 ($mmHg$)	Target V_E ($l \cdot min^{-1}$)	B_f (bpm)	V_T (l)	V_E ($l \cdot min^{-1}$)	V_E STDV ($l \cdot min^{-1}$)	% Target V_E	End-Tidal CO_2 ($mmHg$)
S1	151	61	2.61	158	23	4	39.0	143	68.3	2.43	161	20	6	39.7
S2	125	62	1.93	119	17	-4	42.2	130	67.4	2.33	157	11	21	38.1
S3	83	63	1.25	78	9	-6	43.0	80	59.4	1.45	86	8	7	39.4
S4	128	73	1.55	114	23	-11	39.9	121	76.0	1.61	119	18	-1	35.9
S5	143	56	2.41	133	25	-7	34.8	136	57.3	2.42	138	16	1	39.0
S6	108	73	1.52	111	13	2	36.2	115	77.1	1.53	117	9	2	37.6
S7	122	63	2.08	131	17	7	40.2	125	63.0	2.22	139	19	11	39.7
S8	114	65	1.57	100	22	-12	38.8	101	77.4	1.57	119	20	18	40.3
S9	108	59	2.02	118	18	9	40.3	114	62.8	1.97	123	9	8	38.9
S10	115	71	1.65	115	21	0	38.8	119	64.5	1.71	109	20	-8	40.7
S11	167	80	2.22	170	27	2	38.9	168	70.9	2.45	171	17	2	38.2
S12	135	61	2.12	124	24	-8	41.3	117	58.7	2.55	141	24	20	37.7
S13	172	65	2.54	164	21	-5	37.8	174	72.9	2.52	183	18	5	40.7
S14	114	57	2.27	121	30	6	39.3	122	73.7	2.10	153	18	26	38.5
S15	130	42	3.49	146	21	12	36.5	135	42.4	3.54	149	16	11	36.7

Table 6. Individual spirometry data and percentage predicted values. FVC, force vital capacity, FEV_1, forced expired volume in one second, FEF_{25-75}, forced expired flow between 25 and 75 % lung volume, FEF_{25}, forced expired flow at 25 % lung volume, FEF_{50}, forced expired flow at 50 % lung volume, FEF_{75}, forced expired flow at 75 % lung volume, PEF, peak expired flow. Predicted values were determined based on estimated equations from Hankinson et al (2006).

Subject	FVC (l)	FEV_1 (l)	FEV_1/FVC	FEF_{25-75} ($l \cdot sec^{-1}$)	FEF_{25} ($l \cdot sec^{-1}$)	FEF_{50} ($l \cdot sec^{-1}$)	FEF_{75} ($l \cdot sec^{-1}$)	PEF ($l \cdot sec^{-1}$)	% Pred FVC	%Pred FEV_1	%Pred FEV_1/FVC	% Pred FEF_{25-75}	% Pred PEF
S1	5.70	4.92	0.87	6.74	10.33	6.63	3.20	10.64	122	124	101	157	133
S2	5.23	4.18	0.80	4.60	6.60	4.74	2.52	8.46	120	111	92	110	112
S3	4.14	2.78	0.68	2.32	3.84	2.18	1.09	7.37	90	70	78	54	93
S4	5.09	4.10	0.87	4.99	7.40	5.11	2.87	9.59	115	107	100	118	126
S5	5.80	5.01	0.88	6.21	8.98	6.36	3.48	10.13	140	139	102	152	139
S6	4.06	3.66	0.90	4.97	6.34	5.32	2.71	8.60	100	104	104	124	120
S7	5.22	4.12	0.79	4.54	7.02	4.62	1.93	8.95	102	95	91	100	106
S8	4.36	3.80	0.88	5.77	8.28	5.94	2.75	8.49	119	116	101	149	128
S9	4.73	3.77	0.81	4.90	8.75	4.44	1.87	9.66	101	95	95	114	121
S10	7.70	5.57	0.76	5.98	9.83	6.45	3.21	14.83	124	109	90	114	136
S11	6.32	4.53	0.75	4.63	7.81	4.38	1.93	8.01	107	93	90	90	77
S12	6.57	5.85	0.92	7.50	9.96	7.53	4.85	10.41	92	100	109	130	85
S13	5.45	3.90	0.72	3.76	6.48	3.48	1.81	9.68	97	83	85	75	96
S14	4.69	3.78	0.84	4.52	6.04	4.58	2.73	8.65	107	99	97	107	114
S15	5.77	4.38	0.76	4.67	8.36	4.54	1.84	15.02	112	103	92	105	155

Table 7. Individual results of subjects completing three provocation methods. Subjects self-reported if a physician had previously indicated they had some form of asthma. Subjects self-reported if they took some form of short- or long-acting bronchodilator. Bolded FEV_1 values indicate if subjects exceeded a fall index of 10%. FEV_1, forced expired volume in one second, FEF_{25-75}, forced expired flow between 25 and 75 % lung volume, PEF, peak expired flow.

				EVH_L			EVH_{Cl}			Swim		
Subject	Sex	Previous Asthma	Prescribed ß-Agonist	ΔFEV_1 (%)	ΔFEF_{25-75} (%)	ΔPEF (%)	ΔFEV_1 (%)	ΔFEF_{25-75} (%)	ΔPEF (%)	ΔFEV_1 (%)	ΔFEF_{25-75} (%)	ΔPEF (%)
S1	F	×	×	-6.4	-8.1	-13.4	-8.1	-25.1	-3.8	**-22.8**	-46.6	-3.3
S2	F	×	×	-4.0	-7.1	-6.2	-5.2	-4.7	-6.0	-2.2	2.3	-7.7
S3	F	×	×	-7.4	-13.5	-11.1	-3.1	-6.0	-4.5	10.9	18.5	1.7
S4	F	Yes	Yes	**-14.0**	-20.9	-15.8	-9.2	-2.2	-13.2	-3.1	-2.1	-12.1
S5	F	Yes	×	**-10.9**	-18.0	-8.9	-1.8	1.2	-6.0	-1.0	6.5	-8.8
S6	F	×	×	-6.7	-14.9	-10.8	-6.4	-8.2	-2.4	-1.4	-2.7	-3.3
S7	F	×	Yes	-9.1	-25.7	-3.2	0.4	-4.3	-7.1	2.7	9.3	4.4
S8	F	×	×	-6.8	-12.4	-9.2	-4.0	-4.7	-1.2	-5.1	-7.0	0.6
S9	F	×	×	-7.4	-14.6	-18.8	-6.5	-3.0	-1.0	-4.9	14.3	-8.6
S10	F	×	×	-3.8	-5.3	-12.3	-4.4	-0.5	-7.2	-4.7	-3.5	-6.3
S11	M	×	×	**-19.6**	-28.4	-34.0	**-12.3**	-11.8	-1.8	-0.2	-1.3	13.7
S12	M	Yes	Yes	**-27.8**	-37.6	-25.1	**-36.0**	-53.5	-36.4	**-13.2**	-21.1	-9.2
S13	M	×	×	-7.4	-10.7	-5.5	6.5	10.7	14.6	1.1	6.5	-21.4
S14	M	×	×	-7.7	-20.5	-7.4	-3.3	2.6	-12.4	1.8	4.2	-8.3
S15	M	×	×	-6.5	-12.0	-9.3	-6.1	-13.5	-6.7	-2.9	3.9	-4.5

Appendix B – Additional Figures

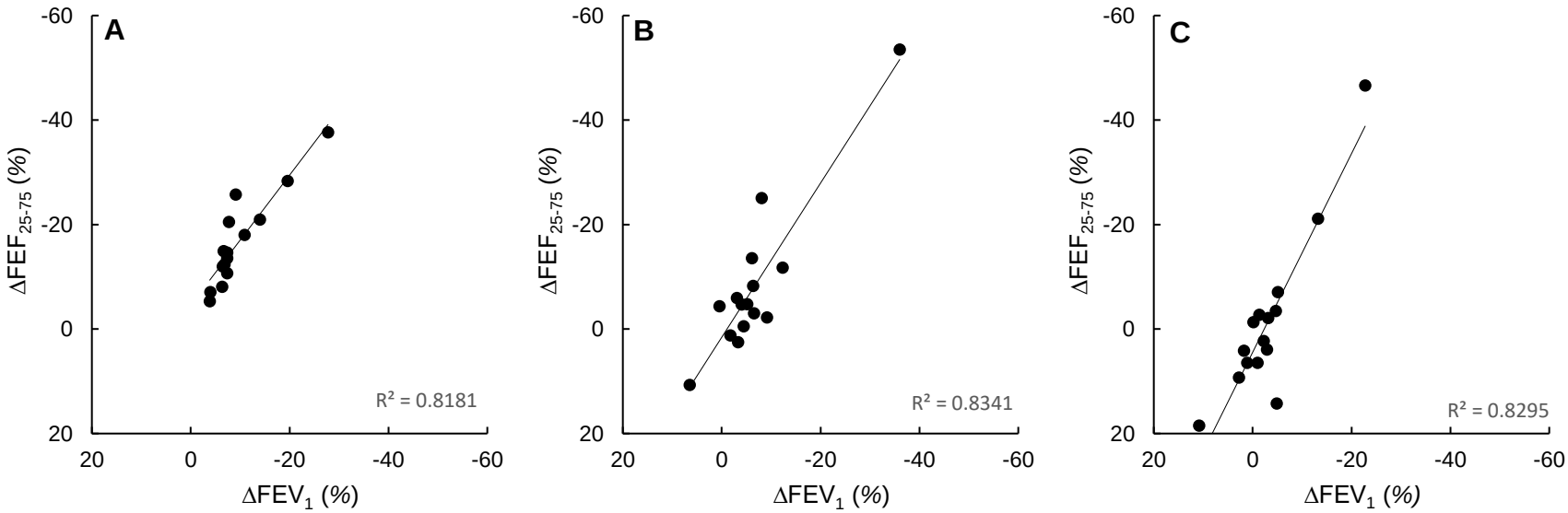

Figure 8. Relationship between greatest fall index of FEV_1 and FEF_{25-75} follow three provocation methods. The following figures present significant relationships for the; **A**, EVH_L (r=0.90, *p*<0.001), **B**, EVH_{Cl} (r= 0.91, *p*<0.001) and **C**, Swim tests (r= 0.91, *p*<0.001).

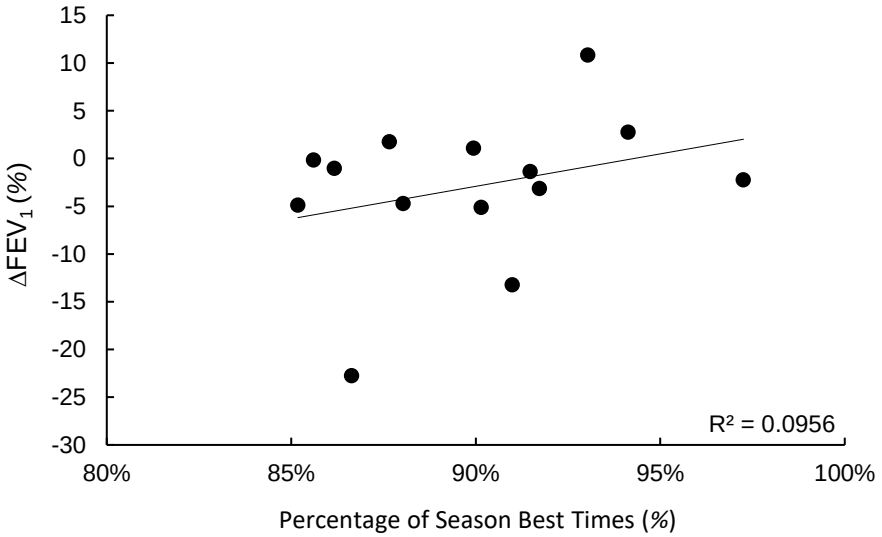

Figure 9. Relationship of FEV_1 fall index and percentage of combined seasons best 200 and 400 m freestyle efforts (r= 0.29, *p*=0.300).