# INDICES OF AIRWAY FUNCTION IN IN-SEASON COLLEGIATE SWIMMERS OVER EIGHT WEEKS

A Thesis by HANNAH SNYDER

Submitted to the Graduate School at Appalachian State University in partial fulfillment of the requirements for the degree of Master of Exercise Science

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#### Abstract

# INDICES OF AIRWAY FUNCTION IN IN-SEASON COLLEGIATE SWIMMERS OVER EIGHT WEEKS

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The repeated exposure to disinfectant by-products in swimming pool environments may worsen pulmonary function and contribute to symptoms of exercise induced bronchoconstriction (EIB) in swimmers. The purpose of this study was to comprehensively examine whether spirometric indicators of pulmonary function change over an indoor swim season in competitive collegiate swimmers and to perform a pilot investigation on the efficacy of fish-oil supplementation in swimmers with EIB over the course of 8 weeks. Competitive swimmers (n=13, 18-25 years of age) were recruited for participation in the study. Swimmers underwent pulmonary function and submaximal exercise testing (if part of the EIB portion of the study) before and after an eight-week period. Additionally, pulmonary function was assessed at 3 and 6 weeks. Data were analyzed using a one-way ANOVA and t-test in the SPSS data software. Researchers observed no significant changes in pulmonary function or EIB over the course of an 8week swim season. It is important to understand the physiological impact inhalation of chemical by-products may have on swimmers throughout their lifetime. Looking at this impact may help to ultimately improve the swimmers performance over the course of his/her swimming career. A better understanding of treatments for asthma and EIB symptoms is needed to help abate long term respiratory limitations that may occur due to pool environment exposure. It is also important to further exam pool quality standards to provide a safe and healthy pool environment for these athletes.

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### Foreword

This thesis will form the basis of a manuscript to be submitted to the *International Journal of Exercise Science*; it has been formatted according to the style guide for that journal.

# List of Abbreviations and Symbols

ANOVA	analysis of variance
EIA	exercise-induced asthma
EIB	exercise-induced bronchoconstriction
FEF	forced expiratory flow
FEV <sub>1</sub>	forced expiratory volume in one second
FVC	forced vital capacity
kg	kilogram
L	liters
L∙min <sup>-1</sup>	liters per minute
L·s <sup>-1</sup>	liters per second
min	minutes
mL·kg <sup>-1</sup> ·min <sup>-1</sup>	milliliters per kilogram per minute
PFT	pulmonary function test
RV	residual volume
S	seconds
SD	standard deviation
TLC	total lung capacity
$\dot{\mathbf{V}}_{\mathrm{E}}$	minute ventilation
<sup>İ</sup> VO <sub>2</sub>	oxygen consumption
<b>VO</b> <sub>2max</sub>	maximal oxygen consumption
VT	tidal volume

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## Dedication

I dedicate this thesis to my parents and sister. Without their patience, understanding, support, and most of all love, the completion of this work would not have been possible.

#### Chapter 1

#### INTRODUCTION

It is widely recognized that competitive swimmers spend a large amount of time training to increase their performance. Researchers have found swimmers have greater measures of pulmonary function when compared to control groups of the same age, weight, and stature as other athletes (12, 15, 57). When compared to athletes engaged in other activities (i.e., running, basketball, canoeing, and rowing) and sedentary controls, swimmers had superior forced expiratory volume (FEV<sub>1</sub>), an indicator of airway function, independent of stature and age (12, 15). Thus, from a superficial perspective, it appears that swimmers exhibit greater pulmonary and airway function compared with other athletes.

Public swimming pools are required by law to use disinfectants in order to kill and prevent the spread of bacteria. Pool water disinfectants include chlorine (gas, calcium/sodium hypochlorite, or chlorine dioxide), bromine (bromochlorodimethylhydantoin), ozone, and/or ultraviolet radiation, with chlorine being the most common (18, (Ondolo, 2009 #32, 49)). Yet, during swim exercise, chemical disinfectants react with biological materials (i.e. sweat, skin oils, urine, etc.) introduced to the pool environment. As a result of this reaction, disinfectant by-products are produced, whereby the greatest concentration accumulates at the water's surface. Therefore, the air that swimmers breathe during exercise, regardless of stroke style/technique, contains the greatest proportion of toxic chemicals (i.e., chemical disinfectant by-products) known to impair respiratory health (18, 29, 49). The amount of time and ventilatory volume during exercise are determinants of exposure to disinfectant by-products. Individuals who swim for long periods of time and at moderate to vigorous activity levels, such as competitive swimmers, are exposed to more by-products than those who do not. On a daily basis, a competitive swimmer may be exposed to disinfectant by-products for two to five hours. The effect of the amount of swimming pool environment exposure on pulmonary function has not been thoroughly investigated. Studies have been conducted examining the effects of swimming pool environments on respiratory health of pool attendants and leisure swimmers, however, research using competitive swimmers is lacking.

Though swimmers may have larger respiratory muscle mass and generally greater pulmonary function measures than the general population, the pool environment may still have an effect on swimmers' lung function. Swimmers have shown increased sputum eosinophilia and higher exhaled breath temperatures after training (13). Rhino-sinusual disorders, external otitis, dermatitis, conjunctivitis, and upper respiratory tract infections are common disorders of competitive swimmers (49). Ferrari et al. found participants who visited indoor pools more frequently developed new onset asthma at a significantly higher rate than those who did not (17, 49). Nordberg et al. (46) also found a significant decrease in FEV<sub>1</sub> after two hours of exposure to swimming pool environments.

Traditionally, swimming exercise is regarded as one of the best modes of exercise for individuals with exercise-induced bronchoconstriction (EIB) (39). EIB is a transient narrowing of the bronchial airways during (6) or after (37) vigorous exercise, which results in airflow limitation. Yet, despite this "favorable" environment, the prevalence of EIB among swimmers is greater than in athletes of all summer sports and most winter sports (39). EIB is estimated to affect approximately 90% of asthmatic individuals and 35-40% of those suffering from allergic rhinitis (30). Additionally, a high prevalence of

airway hyperresponsivness has been reported in many elite level athletes (36 to 79%), including cross country skiers, long distance runners, figure skaters, and swimmers (21). Thus, this observation leads one to wonder, if swimming is truly the best (e.g., most recommended) mode of exercise for individuals with asthma and EIB, why is the prevalence of EIB so great among competitive swimmers (21)?

While the underlying mechanism of EIB is not completely known, it likely involves loss of heat and water from the conducting airways during the ventilatory process of conditioning the inspired air. In addition to common medical treatment (e.g., beta agonist), supplementation with dietary nutrients/compounds (i.e., ascorbic acid, vitamin E, omega-3 fatty acids, and zinc) has been shown to effectively abate the 1) reduction in lung function and 2) the inflammatory response typically observed in individuals with EIB (7). Little research has been done on the effects of indoor swimming pool environments on swimmer lung functions over the course of a swimming season. There is a need for future research to study the effects of swimming pool environments on pulmonary function of swimmers over the course of a season to understand if antioxidant supplements help to decrease change in pulmonary function.

#### **Statement of the Problem**

The purpose of this study was twofold: 1) to comprehensively examine whether or not spirometric indicators of pulmonary function change over an 8-week portion of the indoor swim season in competitive collegiate swimmers and 2) to perform a pilot investigation on the efficacy of fish-oil supplementation in swimmers with EIB over the course of 8 weeks.

#### Hypotheses

The following hypotheses will be tested:

- Pulmonary function, characterized by FEV<sub>1</sub>, will decrease from week 0 to week 8 in competitive swimmers.
- The change in pulmonary function, characterized by a change in FEV<sub>1</sub>, in competitive swimmers with EIB in response to an exercise challenge will be reduced following 8 weeks of fish oil supplementation compared with a control.
- 3. Markers of airway bronchoconstriction, characterized by the presence of cysteinyl leukotrienes in the urine in response to an exercise challenge, in competitive swimmers with EIB will be reduced at the conclusion of an indoor swim season following fish oil supplementation compared with a control.

#### Delimitations

The study will be delimited by the following factors:

- The participants selected will be male or female competitive swimmers, aged 18 to 25 years.
- Swimmers who have injuries or illnesses that exclude them from practice or meets for more than one week will not participate in the study.
- Diet will be monitored at Weeks 0 and 8 using a Food Frequency Questionnaire (FFQ) to ensure the potential change in pulmonary function is a result of the treatment only.
- 4. Bronchodilator usage will be monitored using a Medication Log to ensure potential change in pulmonary function is result of treatment only.

5.

#### Limitations

The following limitations will be considered when interpreting the results of the research:

- 1. The effort displayed by the swimmer will be assumed to be the best ability of the participant.
- 2. The supplements given to participants are assumed to be pure.
- 3. All of the swimmers will reside in the same geographical area.
- 4. Allergens in the air due to location potentially may have an effect on airway hyperresponsivenes that cannot be controlled.
- 5. The chemical by-product concentrations at the water's surface will not be assessed.
- 6. The pool environment for all participants will be the same.
- 7. The yardage and pool exposure for all participants will be the same.

#### **Definition of Terms**

For the purpose of the study, the following definitions will be utilized:

*Airway Hyperresponsiveness (AHR)*: A characteristic of asthma and EIB which consists of an increased sensitivity of the airways to constrictor agonists (47).

*Eucapnic Voluntary Hyperventilation (EVH) Test:* A provocative indirect stimulus test used to diagnose exercise-induced asthma or exercise-induced bronchospasm (1).

*Exercise Induced Asthma (EIA):* An exacerbation of asthma symptoms during exercise in individuals diagnosed with asthma (5).

*Exercise Induced Bronchoconstriction (EIB):* A participant who shows evidence of asthma symptoms during or after exercise (26).

*Forced Expiratory Volume:* A measurement of how much air a participant can exhale during a forced breath (44).

*Forced Expiratory Volume in 1 second (FEV<sub>1</sub>)*: The maximum volume of air that can be forced out in one second started from a level of total lung capacity, an important measure of pulmonary function (20).

*Forced Vital Capacity (FVC)*: The volume of air expelled by a forced maximal expiration to residual volume after a full inspiration (20).

*Forced Expiratory Flow 25-75 % (FEF*<sub>25-75 %</sub>): The average expiratory flow over the middle half of the FVC (20).

*Pulmonary Function Test (PFT):* A gauge of how the lungs are expanding and contracting and measures the efficiency of the exchange of oxygen and carbon dioxide between the blood and the air within the lungs (44).

*Leukotriene C4/D4/E4 (LTC4, LTD4, LTE4), Cysteinyl Leukotrienes*: Category of leukotrienes released by mast cells and eosinophils which have been shown to accompany exercise bronchial reactivity by increased concentrations in urine (50).

#### Chapter 2

#### **REVIEW OF LITERATURE**

The literature related to the effect of chlorinated, indoor swimming pools on swimmers and the effects of fish oil supplementation on respiratory system health are presented in this chapter. The following review of literature will discuss (a) pulmonary function in swimmers, (b) respiratory health in swimmers and pool attendance, (c) the swimming pool environment, (d) EIB, and (e) the potential treatments for EIB.

#### **Pulmonary Function in Competitive Swimmers**

Researchers have found swimmers have greater measures of pulmonary function when compared to control groups of the same age, weight, and stature and other athletes (12, 15, 57). In a study performed on children and preadolescents with at least 3 years of swimming training, results indicated children who train to swim competitively have cardiorespiratory capacities which are greater than one would expect to see in untrained youth of similar ages (15, 57). Vaccaro et al. found measures of total lung capacity (TLC), forced vital capacity (FVC), and forced expiratory capacity in one second (FEV<sub>1</sub>) in child and preadolescent swimmers were approximately 10-16% above normal after 3 years of training (41, 57). When compared to land based activities (runners, basketball, canoeing, and rowing) and sedentary controls, swimmers had superior FEV<sub>1</sub> independent of stature and age (12, 15).

#### **Respiratory Health in Swimmers and Pool Attendance**

In swimming, individuals experience increased load of the water pressure against the chest wall and elevated airway resistance as the result of immersion. Researchers found that the elevated pressure could comprise a conditioning stimulus to influence a positive impact on swimmers lung volumes (15, 33). Training factors, such as breath holding and rhythmic breathing, did not influence the lung volumes of swimmers but did have a positive impact on lung ventilatory functions (33). Though swimmers may have larger respiratory muscle mass and generally greater pulmonary function measures than the general population, respiratory limitations seem to still have an effect on swimmers' lung function.

Swimmers lung function becomes limited over time due to the inflammation and edema of the mucous membranes of the lungs (29). It is believed swimmers exposure to the chlorine by-products at the surface of the water causes this effect on their lung function. During a two-hour period of swimming, swimmers may be exposed to amounts of chlorine gas exceeding the United States recommended exposure limit for an eighthour chemical factory worker (21).

Swimmers have shown increased sputum eosinophilia and higher exhaled breath temperatures after training (13). An analysis of induced sputum in elite swimmers who did not have asthma showed increased inflammatory cells compared to healthy nonswimmers (17). Two factors that contribute to these types of mediators of bronchoconstriction in competitive swimmers are hyperventilation associated with endurance training and chronic exposure to chlorine derivatives (9, 22). Rhino-sinusual disorders, external otitis, dermatitis, conjunctivitis, and upper respiratory tract infections are common disorders of competitive swimmers (49). In a study conducted on 20 elite swimmers, 83% reported respiratory symptoms and 65% had at least one positive bronchial hyperresponsive provocation test (55).

Prevalence of asthma in swimmers increases by 45% with a mean nine years of training (13). Ferrari found participants who visited indoor pools more frequently developed new onset asthma at a significantly higher rate than those who did not (17). Nordberg et al. found a statistically significant relationship between the number of hours spent in an indoor swimming pool environment and the age of acute asthma symptoms (i.e. dyspnea, cough, nose, throat, and eye irritation) (46). They also found a significant decrease in FEV<sub>1</sub> after two hours of exposure to swimming pool environments (46).

Increased attendance at swimming pools is correlated with higher input of organic and minerals pollutants introduced by swimmers in the swimming pool water (18). In a study conducted by Florentin et al. elite swimmers individually secreted around 20-80 mL of urine and produce 0.1-1.0 L of sweat in 2 hours (18). The mineral nitrogen compounds found in the urine react with free chlorine to form chloramines. The presence of chloramines in the air of swimming pools was associated with an increased prevalence of allergic symptoms (i.e. Rhino-sinusal disorders, external otitis, dermatitis, conjunctivitis, and upper respiratory tract infections) and asthma in elite swimmers training in indoor swimming pool environments (18, 45, 49). An increase in pathological conditions when swimming in indoor pools for more than 30 hours/week was also found (49).

Daily or intermediate exposure to "normal" indoor pool atmospheres have been shown to be as pungent and irritating for the eyes and upper airways as some industrial environments (45). Concentrations of chloramines are generally higher in pools with recreational activities, especially slides and whirl pools (29). The first complaints of

irritation were registered at around 0.5 mg m<sup>-3</sup> of chloramines and all participants complained at concentrations of 0.7 mg m<sup>-3</sup> (29).

A statistically significant relationship was found between the number of hours spent in swimming pool environment and the acute symptoms (i.e. dyspnea, cough, nose irritation, throat irritation, eye irritation) (46). A significant decrease in pool attendee FEV<sub>1</sub> were found after 2 hours of exposure in a study conducted by Nordberg et al. (46). Considerable lung function changes and patterns were found in swimming pool instructors (29).

#### **The Swimming Pool Environment**

Chlorine is a chemical element and is the second lightest of the halogens. Chlorine is a toxic gas that attacks the respiratory system, eyes, and skin (52). Because it is denser than air, it tends to accumulate at the bottom of poorly ventilated spaces. Chlorine gas is a strong oxidizer, which may react with flammable materials (52).

Chlorine is the most frequently used disinfectant in swimming pools (29, 45). Disinfection by-products, such as chloramines, are created from organic matter (i.e. sweat, urine, and skin). The most volatile and easily released of these chloramines are trichloramines (29) Trichloramines are the most likely contaminant suspected to cause irritated respiratory symptoms among swimmers and workers (29). The recommended level of trichloramines is <0.5 mg-m<sup>-3</sup> (41). Most pools averaged 0.67 mg-m<sup>-3</sup> (29). Production of chloramines depends on degree of water chlorination, contamination of water by nitrogen compounds, water temperature, water circulation, and ventilation. Researchers have found a significant association between chloramine levels and the number of swimmers, free chlorine concentration in pool, and ceiling height (29). An increase of 50 bathers was associated with a .40 mg-m<sup>-3</sup> increase in trichloramine level (29) and ventilation has been known to lead to lower air contamination (34).

Visiting chlorinated pools strongly relates to prevalence of asthma and positive exercise-induced bronchoconstriction and long-term exposure had a significant association with upper respiratory symptoms (29). Researchers have found that levels of chloramines were somewhat lower in leisure pools compared to competition pools. Currently, there are no regulations that exist for air quality in indoor swimming pools (29).

#### **Exercise-Induced Bronchoconstriction**

EIB is "a transient narrowing of the airways that occurs during or after exercise" (26). Upon hyperventilation during high intensity exercise, mouth breathing promotes the inhalation of cooler, drier air compared with water content of air and tissue existent in the lung (28). Currently, there are two dominating theories about the underlying mechanism responsible for EIB. The first theory states vasodilation of the bronchiolar blood vessels results after exercise as heat passes down its thermal gradient to rewarm the airway. This results in vascular hyperemia and pulmonary edema, which results in airflow limitation (40). The second theory states water is lost from the bronchial epithelium as it humidifies the drier inhaled air. The subsequent water loss increases tissue osmolality, which activates the release of histamine and pro-inflammatory mediators causing bronchoconstriction (3). Although the actual mechanism is probably some combination of the two, the role and release of inflammatory mediators has been well documented (31). Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 (IL-1) are released in response to airway trauma, which initiates the inflammatory response. Arachidonic metabolites,

including cysteinyl 4-series leukotrienes (LTC<sub>4</sub>, LTD<sub>4</sub>, LTE<sub>4</sub>) and prostaglandin (PG) D<sub>2</sub> are potent mediators causing bronchoconstriction (48) (**Figure 1**).

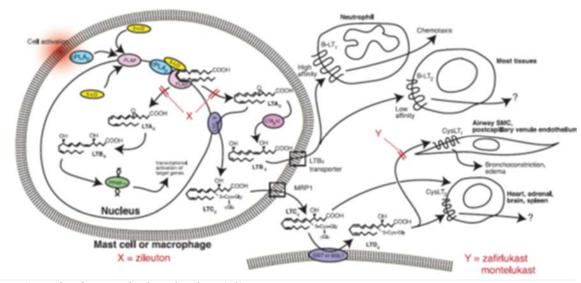


Figure 1. Leukotriene synthesis and actions (19)

The bronchoconstrictive effect results in lung function deterioration characterized by at least a 10% decrease in post-exercise forced expiratory volume in one second (FEV<sub>1</sub>) compared to resting baseline values, with the greatest decreases occurring 5-10 minutes after the cessation of exercise (16). Mean mid-expiratory flow (FEF<sub>25-75 %</sub>) decreases of 15-25 % have also been accepted as a positive diagnosis of EIB (53). Breathlessness, cough, and increased mucus production are reported frequently in athletes with EIB (31).

#### **Prevalence of EIB in Swimmers**

Mediators of bronchoconstriction seems to affect swimmers, ice hockey players, and cross country skiers more than any other type of athlete (22). "Olympic level swimmers are more likely to have asthma, airway hyperresponsiveness, positive skin prick tests, and allergic rhinoconjunctivitis than any other group of athletes" (22). In a study done by Haahtela et al., asthma in swimmers was increased nearly six-fold and the risk of asthma is 96 fold compared to the controlled participants (21).

In a study conducted by Holmer , trained swimmers, cyclists, and runners have approximately the same  $VO_{2max}$  (27). The difference between the three groups was maximum ventilation. Swimmers have a lower ventilation rate for the same  $V\dot{O}_{2max}$  when compared to trained runners and cyclists (**Figure 2**).

Figure 2. Pulmonary ventilation, maximum oxygen uptake, and heart rate during maximal running,

	Max Vo2 , 1 min <sup>-1</sup>				Max VE , 1 min⁻¹			Max Heart Rate, beats min <sup>-1</sup>			
Subj	Run	Cycl	of run	Swim	% of run	Run	Cycl	Swim	Run	Cycl	Swim
Elite (I)											
PF*	5.59	5.11	91.4	5.08	90.9	192.5	195.2	136.7	190	187	185
LI†	3.61	3.24	89.8	3.63	100.6	132.0	139.0	133.5	182	186	182
Swim trained (II),	4.48	4.18	93.3	4.26	95.1	148.4	139.9	127.5	203	200	185
n = 3†	4.20-4.76	3.99-4.45		3.94-4.57		128.5-165.7	127.8-162.9	111.3-133.1	194–211	192-211	179-191
Not swim trained (III),	3.76	3.38	89.9	3.03	80.6	129.2	114.1	108.4	190	186	181
$n = 4\ddagger$	2.51-4.93	2.46-4.28		2.28-3.70		100.6-153.8	100.6-122.2	85.8-121.8	181-206	176-200	170-191
Mean, $n = 9$	4.19	3.82	91.2	3.73	89.0	143.0	134.5	120.7	193	191	183

\* Back crawl. † Breaststroke. ‡ Two subjects back crawl and two subjects breaststroke.

cycling, and swimming (27).

Why is it that, of these three types of athletes (swimmers, runners, and cyclists), swimmers are more likely to have EIB? It is believed that the exposure to the chlorine by-products causes inflammation and edema of the mucous membranes of the lungs (29). During a two-hour period of swimming, swimmers may be exposed to amounts of chlorine gas exceeding the United States recommended exposure limit for an eight-hour chemical factory worker (21).

Individuals who exercise in indoor swimming pools for five or more hours per week increase their risk of developing asthma or EIB by 5% yearly (17). Competitive swimmers are at a higher risk of developing bronchial hyper-responsiveness than other summer athletes (17). Nordberg found a statistically significant relationship between the hours spent in a swimming pool environment and the age of acute symptoms (i.e. dyspnea, cough, nose irritation, throat irritation, and eye irritation) (46). The researchers reported a significant decrease in FEV<sub>1</sub> after two hours of exposure to indoor swimming pool environments.

Haahtela found that athletes who train indoors report more exercise-induced respiratory symptoms than outdoor training (21). This effect was also seen in athletes training outdoors at subzero temperatures. A study conducted at the Olympic Games in Athens, Greece in 2004 suggests that environment and training, such as indoor pool environments and endurance training, contribute to the incidents of asthma in swimmers (22). Competitive swimmers breathe the air floating just above the surface of the water encouraging them to inhale water droplets and chemicals (22). The presence of chloramines in the air of swimming pools was associated with an increased prevalence of EIA and EIB in elite swimmers (18, 33, 41). Due to the daily exposure of chloramines, swimmers' lungs created an inflammatory response to heal damage; however, repair was delayed because of frequent exposure (13).

#### **Treatment Methods**

Treatment methods currently used to limit EIB exacerbations are bronchodilators. According to Liesker, the main purpose of a bronchodilator is "to decrease airflow limitation in the airways and, as a consequence, improve dyspnea and exercise tolerance (36)." A bronchodilator consists of some form of beta agonist (51). Stimulation of beta adrenergic receptors can result in vasodilation of blood vessels and tachycardia (37). In addition, it has been found chronic exposure to beta agonist drugs leads to reduced responsiveness and a decrease in the number of receptors to the agonist (61). In a study conducted by Wraight, the effects of tolerance increased as use of bronchodilator increased (61). The results of the Wraight study demonstrated a decrease in response to bronchodilators acutely administered to patients who have continuous exposure to inhaled beta agonist (61). The increasing tolerance to bronchodilators linearly correlated with bronchoconstriction (61). In four studies conducted by Hancox, Wahedna, Vathenen, and Van Schayck, researchers found discontinuation of short acting beta agonists decreased FEV<sub>1</sub> and increased bronchial responsiveness (23, (Wahedna I, 1993 #66), (59), (58)).

Currently, bronchodilators are the main source of treating asthma and bronchoconstriction. The problem to be faced with this method of treatment is the impact of tolerance to the beta agonists. Antioxidant treatment may offer an alternative treatment to EIB that avoids the tolerance issue because antioxidant act directly on the inflammatory mediator pathways.

#### **Omega-3 Fatty Acids and EIB**

A fatty acid is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated. Fatty acids are usually derived from triglycerides or phospholipids. Fatty acids are important dietary sources of fuel for animals because, when metabolized, they yield large quantities of adenosine triphosphate (ATP). Fatty acids that are required by the human body but cannot be made in sufficient quantity from other substrates, and therefore must be obtained from food, are called essential fatty acids. Two essential fatty acids are omega-3 and omega-6 fatty acids.

The human body breaks down omega- fatty acids into useful and more important long-chain omega-3 fatty acids, EPA and DHA, which help with fetal development and healthy aging (25). Consumption of omega-3 fatty acids from marine sources has also shown lowers markers of inflammation in the blood (35). Fish oil has been shown to play a role in shaping and regulating inflammatory processes and responses (43). The research may suggest that the omega-3 fatty acids might be important in determining the development and severity of inflammatory diseases (43). The anti-inflammatory actions of omega-3 fatty acids has led to the idea that supplementation of the diet of patients with inflammatory diseases may be of clinical benefit (43). In a study conducted by Calder, "fish oil supplementation in healthy human participants demonstrated a decrease in chemotaxis of neutrophils and monocytes towards various chemo-attractants including LTB4 (10)."

Since 1930, leukotrienes have been known to play an important role in immunology, specifically in anaphylaxis (8). During the break down of arachidonic acid, leukotrienes are produced by the enzyme 5-lipoxygenase (5-LO) (8). The 5-LO pathway products have been detected in body fluids (i.e. blood and urine) after experimentally induced bronchoconstriction and during spontaneous asthma attacks (28). Leukotrienes produce mast cells, eosinophils, basophils, macrophages, and monocytes, all of which have been linked to cases of EIB (8). Although leukotrienes are rapidly cleared from the blood, concentrations of these inflammatory mediators have been found specifically in bronchoalveolar lavage fluids take from patients with asthma. Research has shown that leukotriene levels will return to baseline after 3 to 7 hours post exercise (38).

The most potent chemotactic compound is Leukotriene B4 (LTB4) (32). LTB4 plays a significant role in inflammatory and allergic reactions" (32). In a study conducted by Kumar, the formation of LTB4 from LTA4 was measured by incubating

lung microsomes from normal and exercised rats (32). Production of LTB4 in lung microsomes almost doubled after exhaustive exercise in the form of swimming (32). The results of the Kumar study suggest LTB4 plays a role in the involvement of lung tissue damage during exercise-induced oxidative stress (32).

#### **Additional Dietary Strategies and EIB**

Physical exertion generates oxidative stress in the respiratory system (26). Oxidant stress can initiate apoptosis (death of cells) which can be prevented by antioxidants. (60) Sridhar found that antioxidant such as vitamin C, vitamin E, betacarotene, vitamin A, fatty acids and some minerals play a protective role against lung inflammation. A high intake of antioxidants in the diet can reduce the risk of respiratory disorders and increase FEV and FVC (54). Deficiencies in dietary antioxidants and fatty acids may contribute to the prevalence of asthma in the United States and other countries (54). High intakes of fruits and vegetables could reduce the risk of lung cancer in participants by close to 78% over the course of 12 years (54).

The effects on the severity of upper and lower respiratory tract infections and the common cold show the importance of vitamin C on the respiratory system (26). Vitamin C supplementation significantly improved pulmonary function, decreased mediators of inflammation, and provided a protective effect against airway narrowing caused by exercise (7). In a study performed by Hemila, vitamin C supplementation reduced the postexercise FEV decline by 48% (26). Vissers found vitamin C and E are able to protect against oxidative stress and provide specific protection against the apoptotic process (60). Tecklenburg found that adding an ascorbic acid supplement to participant's diets significantly improved post-exercise FEV<sub>1</sub> and reduced EIB (56). They found an absence

of significant difference in  $FEV_1$  between any of their treatment protocols, which suggests that the differences in treatments were due to changes in diet (56). The maximum fall in  $FEV_1$  was halved on the ascorbic acid supplementation diet.

Like vitamin C and E, zinc plays a protective role in the lung and prevents pulmonary epithelial damage (7). Biltagi found zinc decreased total sputum white blood cell count as well as other mediators of inflammation (7). Zinc blocked the binding of white blood cells to endothelial cells and inhibited the release of mediators from mast cells, basophils, and eosinophils. Biltagi states "zinc greatly reduces airway hyperresponsiveness and inflammation (7)."

Omega-3 fatty acids primarily found in fish oil are essential to the human diet. Mickleborough found dietary fish oil supplements have a protective effect in suppressing EIB in elite athletes (42). Omega-3 fatty acids improve lung functions, decrease the severity of bronchial asthma, and may make asthma easier to control (7). Fish oil reduces the generation of proinflammatories and the production of cytokines from inflammatory cells. Mickleborough and Rundell found that after three weeks of fish oil supplementation, the severity of EIB was reduced and significantly suppressed several proinflammatory mediators in elite athletes who demonstrated symptoms of EIB (53). Athletes who had EIB and were taking the fish oil supplement reduced the fall of FEV by 80% 15 minutes post-exercise and the use of bronchodilators (i.e. inhalers) by 20%. Mickleborough found that a diet high in omega fatty acids suppressed urinary inflammatory mediator (9 $\alpha$  and 11 $\beta$ -PGF) concentrations after exercise in elite athletes with EIB (42).

#### **Summary of Findings**

Though researchers have found swimmers to have larger VC, FVC, and FEV<sub>1</sub> than normal healthy individuals, the presence of chloramines in the air of swimming pools was associated with changes in pulmonary function in elite swimmers (18, 33, 41). The changes in pulmonary function include increased allergic symptoms and asthma (18, 45, 49). No research has been conducted serially examining pulmonary function of competitive swimmers over 8 weeks during the competitive indoor season. Thus, there is a need for future research to study the effects of swimming pool environments on pulmonary function of swimmers over the course of a season. EIB is especially prevalent in swimmers and potentially affects exercise performance (21). Future research is needed specifically in order to gain an understanding of how better pulmonary function can allow swimmers to perform at an optimal level.

#### Chapter 3

#### **METHODS**

The research study was designed to investigate pulmonary function over the course of 8 weeks during an indoor season in competitive swimmers. Additionally, a pilot investigation was conducted to assess the physiologic effects of a polyunsaturated fatty acid, specifically an omega-3 fatty acid, on pulmonary function in swimmers with EIB.

### Participants

Approval from the Institutional Review Board was provided prior to initiation of the project. Participants consisted of 13 competitive swimmers (12 female) ages 18-25 years. Data was collected during the months of September to December. Prior to the study, an e-mail was sent to competitive collegiate swimmers within the region asking for participation. Informed consent was obtained prior to enrolling in the study. All participants were medically cleared to participate in the study and swim training by the college medical staff. Participants were excluded from participation if they had any injury or significant illness preventing them from practicing or competing during the swim season.

#### Study design and protocols

Following the consent process, all swimmers completed pulmonary function testing at study entry. Further, questionnaires were administered asking whether or not participants exhibited symptoms of or had previously be informed that they had EIB or exercise-induced asthma (EIA) (4, 16). If the participants answered positively, an exercise challenge test was used to confirm the presence of EIB, which was indicated by a more than 10% decrease in FEV<sub>1</sub> following the exercise challenge compared with

before. Participants with EIB were administered a log sheet to record their use of all medications throughout the course of the study. Upon entry into and completion of the study, participants with EIB completed a food frequency questionnaire (FFQ) to monitor dietary habits. Subsequently, participants with EIB were provided with a fish oil supplement to consume daily over the following 8 weeks. After the initial visit, all participants performed pulmonary function testing at weeks 3, 6 and 8. The participants with EIB also completed another exercise challenge test at week 8 in order to examine the potential effect of fish oil supplementation on EIB.

#### **Pulmonary Function**

All participants performed pulmonary function tests at Weeks 0, 3, 6, and 8. PFTs were generally performed at least 4 hours after exercise and between the times of 10am and 8pm. Eight weeks has been supported as a sufficient amount of time to see changes in pulmonary function measures in previous research (1). Participants performed the spirometry (2001-2NP, EasyOne, Bohemia, NY) procedures in a seated position while breathing room air, with nasal breathing occluded by a nose clip. The procedure for all spirometry tests was 1) three normal tidal volume breaths, 2) maximal inhalation, 3) forced maximal exhalation, and 4) maximal inhalation. This procedure required each participant to perform three acceptable spirograms. If any pulmonary function measurement was technically unacceptable, the measurement was repeated. FVC and FEV<sub>1</sub> were collected at weeks 0, 3, 6, and 8. Forced mid expiratory flow rate (FEF<sub>25-75%</sub>) and peak expiratory flow rate (PEF) were reported at weeks 0 and 8.

#### **Exercise Challenge Test**

To elicit symptoms of EIB, a target ventilation ( $\dot{V}_E$ ) was required. Due to the relationship between  $V_E$  and  $\dot{V}O_2$ , a target  $\dot{V}O_2$  was calculated to elicit the target  $V_E$ . The target workload for each subject was calculated using the subject's predicted  $\dot{V}O_2$ . Target workload was chosen in order to achieve ventilation between 50 and 60% of predicted maximum in the last 4 min of the exercise challenge test (2). Speed and grade were calculated using the ACSM treadmill running equation required to elicit the target  $\dot{V}O_2$ . The protocol used for the 8-minute treadmill exercise challenge test is below:

*Minute 1*: 60 % of target  $\dot{V}O_2$ *Minute 2*: 70 % of  $\dot{V}O_2$ *Minute 3*: 90 % of  $\dot{V}O_2$ *Minutes 4-8*: 100 % of  $\dot{V}O_2$ 

Heart rate was monitored using a F1 Polar Heart Rate Monitor (Polar, Helsinki, Finland). A mouthpiece and nose clip were worn for the full duration of the test.

#### **Identification of EIB**

A subset of participants performed pulmonary function tests before and after an exercise challenge test at Weeks 0 and 8. Spirometry was assessed immediately before (baseline) and at 1, 5, 10, 15, and 20 following an exercise challenge test. The percent decline in FEV<sub>1</sub> at each time point from the baseline value was calculated using the following equation: % decline = (highest pre-exercise challenge test FEV<sub>1</sub> – lowest post-exercise challenge test FEV<sub>1</sub> at each time point)/ (highest pre-exercise challenge test FEV<sub>1</sub> = following test FEV<sub>1</sub> at each time point). Participants who demonstrated a decrease in FEV<sub>1</sub> greater than 10% were

identified as having EIB. The maximum percent decline in  $FEV_1$  was determined using the largest value obtained at Weeks 0 and 8.

#### Supplementation

Participants with EIB (n=2) consumed 2g omega-3 fish oil (Ultimate Omega, Nordic Naturals, city state) two times daily, at breakfast and dinner. This dose of 4g per day has previously been shown to reduce inflammation in the respiratory system in elite and active participants (7). Adherence to the treatment regimen was monitored by asking the participants to document the dose of capsules consumed daily and to return any unused capsules.

#### **Urinary Analysis of Bronchoconstrictive Mediators**

Urine samples were collected before and 90 min after the exercise challenge test during laboratory visits at week 0 and week 8 in participants with EIB. Urine samples were immediately stored in a freezer (-18°C). Samples were transported to the laboratory using a cooler filled with ice and stored at -80°C until analysis. Urine concentrations of cysteinyl leukotrienes (LTC4-LTE4) were measured using enzyme linked immunosorbent assay (ELISA; Cayman Chemical, Ann Arbor, MI) in triplicate. All assay wells were washed with ultrapure water and a buffer. An ELISA standard was added to particular cells and then the samples were added. Once incubated overnight at 4°C, the assay was developed using a reagent. Assay wells were emptied and washed with a buffer 5 times before being filled with a reagent and incubated for 90 more minutes at room temperature. The assay plate was read at a wavelength between 405 and 420 mm and the absorbance was checked at a range of 0.3-1.5 AU. Cysteinyl leukotrienes and creatinine were analyzed using a database provided by Cayman Chemical. Cysteine leukotriene values were normalized for urinary creatinine levels to minimize effect of excessive dilution in the urine (11).

#### **Statistical Analysis**

Statistical Package for the Social Science (SPSS; Cary, NC) was used for analysis of data. One-way analyses of variance (ANOVA) were completed to determine the progression of pulmonary function (FVC, FEV<sub>1</sub>, and FEV<sub>1</sub>/FVC) over time. Paired samples t-test was used to determine change in PEF, FEF<sub>25-75%</sub>. The lack of subjects precluded statistical analysis of change in leukotriene concentration at week 0 and week 8. Statistical significance was set at < 0.05. Data are expressed as mean ± standard deviation (SD).

#### Chapter 4

#### RESULTS

Topics discussed in this chapter include results regarding the following: (a) participant characteristics, (b) PFT results for participants without EIB, (c) PFT results for participants with EIB, (d) urinary analysis measures in EIB participants, (e) diet and bronchodilator usage, (f) average yardage per university, and (g) average pool chemical content per university.

#### **Participant Characteristics**

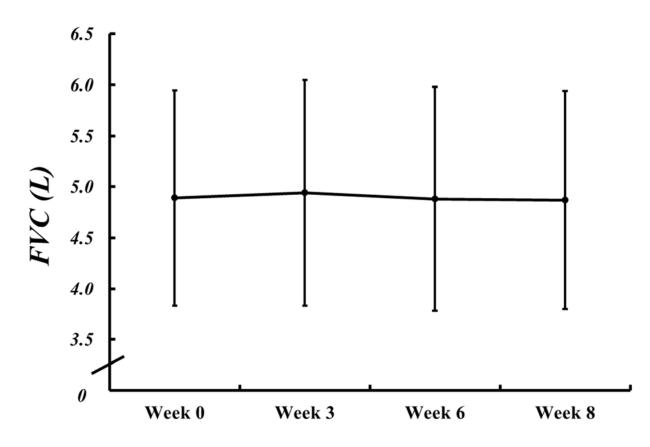
A total of thirteen participants (N=13; 12 females) consented to participate in the study. Anthropometric data for participants are displayed in **Table 2**. No participants reported any history of cardiovascular, respiratory, or metabolic diseases. All participants were never-smokers. Participants were involved in swimming training at the time of the investigation.

**Table 2.** Pulmonary function and EIB participant characteristics (mean±SD).

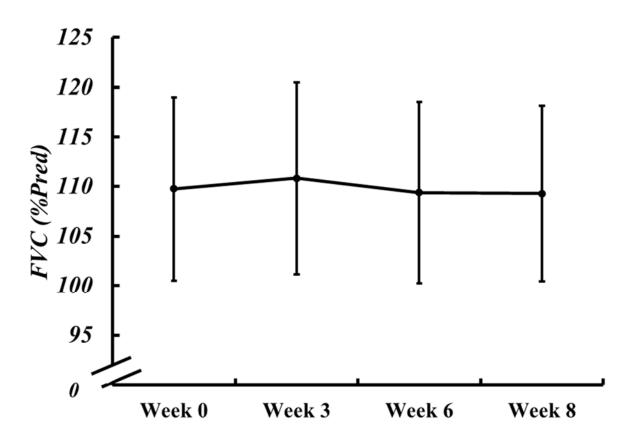
Table 2. I dimonary interior and Lib participant endracteristics (mean=5D).							
	Age (y)	Height (cm)	Weight (kg)	BMI (kg·m <sup>-2</sup> )			
Pulmonary	20.3±0.5	173.4±5.5	73.7±12.0	24.4±2.9			
Function (n=11) EIB (N=2)	20.5±2.1	166.4±5.4	61.6±6.8	22.2±1.0			

#### **Pulmonary Function**

In participants without EIB (N=11), there were no significant changes in pulmonary function measures from week 0 to week 8. FVC as an absolute value and as a percent of predicted (24) at weeks 3, 6, and 8 were similar to that at study entry (**Figures 3 and 4**).

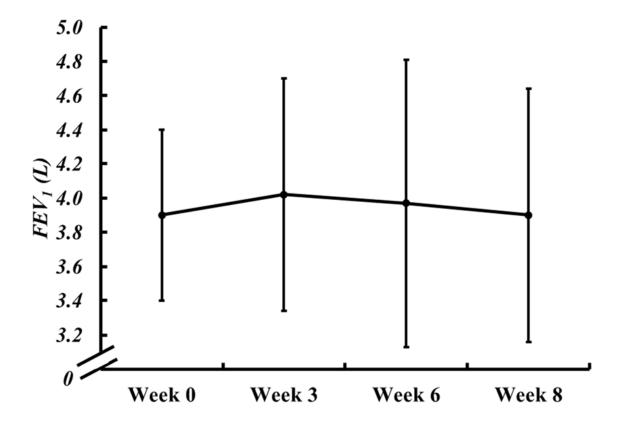


**Figure 3**. Forced vital capacity (FVC) at weeks 0, 3, 6, and 8 of a competitive indoor swim season in participants without exercise-induced bronchoconstriction (mean±SD).

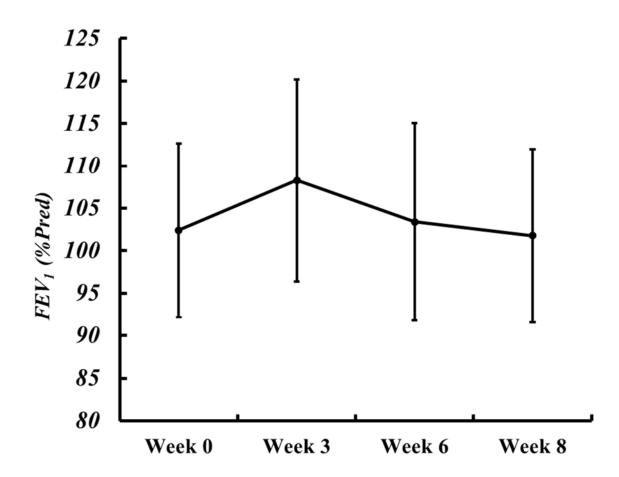


**Figure 4**. Forced vital capacity (FVC) as a percent of predicted at weeks 0, 3, 6, and 8 of a competitive indoor swim season in participants without exercise-induced bronchoconstriction (mean±SD).

FEV<sub>1</sub> as an absolute value and as a percent of predicted (24) at weeks 3, 6, and 8 were similar to that at study entry (**Figures 5 and 6**).

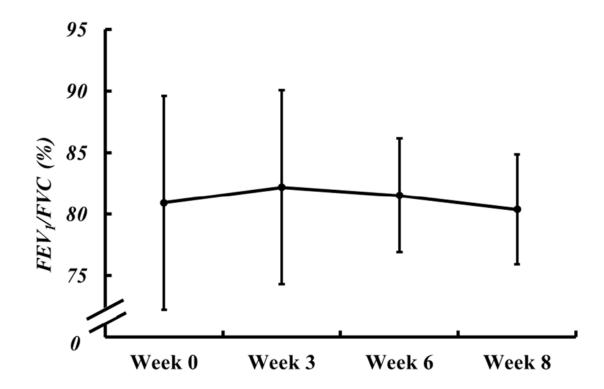


**Figure 5**. Forced expiratory volume in the first second (FEV<sub>1</sub>) from week 0 to week 8 of a competitive indoor swim season in participants without exercise-induced bronchoconstriction (mean±SD).



**Figure 6**. Forced expiratory volume in the first second (FEV<sub>1</sub>) as a percent of predicted at weeks 0, 3, 6 and 8 of a competitive indoor swim season in participants without exercise-induced bronchoconstriction (mean±SD).

FVC % predicted, PEF, and FEF<sub>25-75%</sub> as absolute values at weeks 3, 6, and 8 were similar to that at study entry (**Figure 7-9**).



**Figure 7**. Forced expiratory volume in the first second divided by forced vital capacity (FEV<sub>1</sub>/FVC) at weeks 0, 3, 6, and 8 of a competitive indoor swim season in participants without exercise-induced bronchoconstriction (mean±SD).

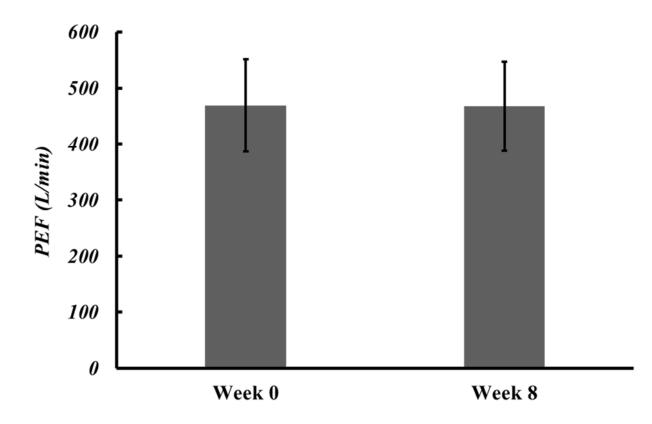
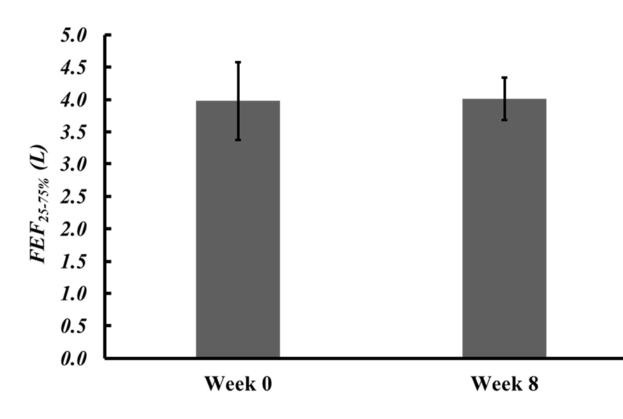


Figure 8. Peak expiratory flow (PEF) for participants without EIB at weeks 0 and 8 (mean±SD).



**Figure 9.** Forced expiratory flow 25–75% (FEF<sub>25-75%</sub>) for participants without EIB at weeks 0 and 8 (mean±SD).

#### **Pulmonary Function of EIB Fish Oil Participants**

Participants with EIB (n=2) exhibited a decrease in  $FEV_1$  greater than or equal to 10% (**Figure 10 and 11**). Subject 1 experienced a 34.2% decrease in  $FEV_1$  at week 0 and a 19.5% decrease in  $FEV_1$  at week 8. Subject 2 experienced a 11.6% decrease in  $FEV_1$  at week 0 and a 11.9% decrease in  $FEV_1$  at week 8,

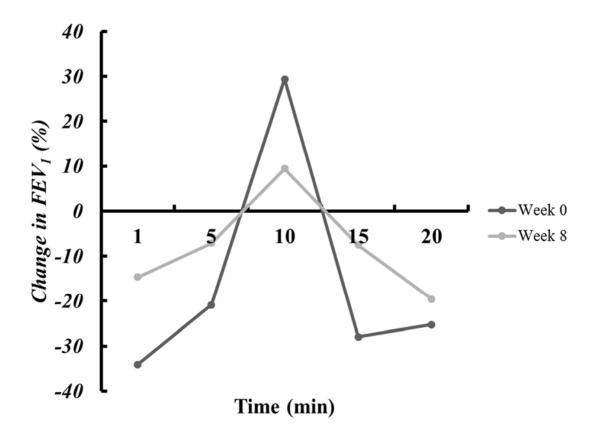


Figure 10. Change in forced expiratory volume in the first second ( $FEV_1$ ) from before to after the exercise challenge tests in Subject 1.

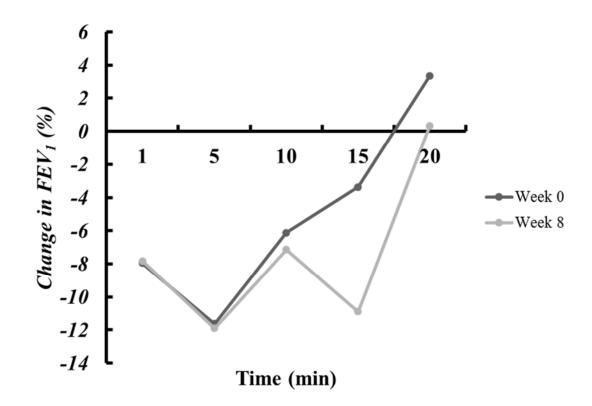


Figure 11. Change in forced expiratory volume in the first second ( $FEV_1$ ) from before to after the exercise challenge tests in Subject 2.

To the exercise challenge test was set to elicit 60% of the target  $\dot{V}O_2$  for the 1st minute, then 70%, 90%, and 100% for the 2nd, 3rd, and 4<sup>th</sup> through 8th minutes, respectively. Percent target VO<sub>2</sub> reached by subjects 1 and 2 are displayed in **Figure 12 and 13**.

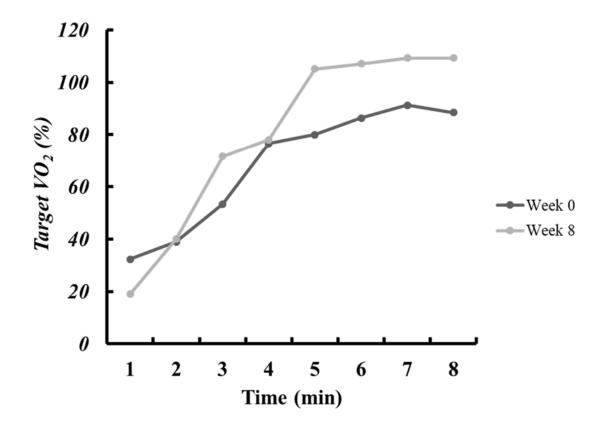


Figure 12. Percent target VO<sub>2</sub> reached during exercise challenge tests at week 0 and week 8 for Subject 1.

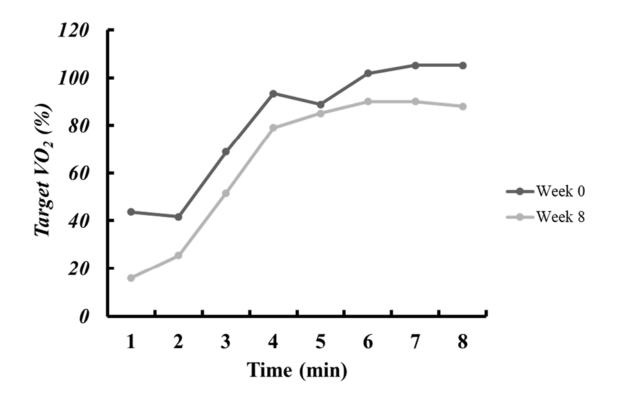


Figure 13. Percent target  $\dot{V}O_2$  reached during exercise challenge tests at week 0 and week 8 for Subject 2.

Ventilation achieved at each minute during the exercise test for subjects 1 and 2 are displayed in **Figure 14 and 15**. Subject 1's target Ve was 64.3 L/min at week 0 and 60.3L/min at week 8. These values were achieved at both weeks. Subject 2's target Ve was 61.8 L/min at week 0 and 59.6 L/min. These values were achieved at both weeks.

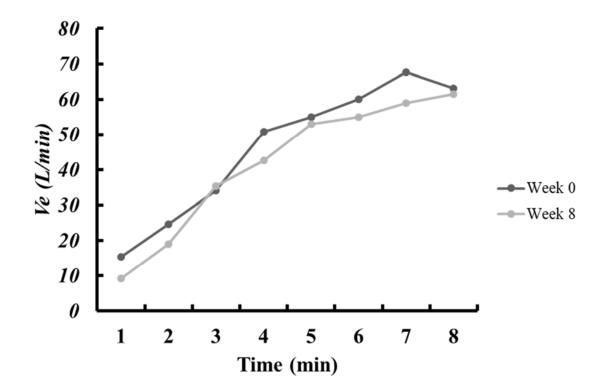


Figure 14. Ventilation (Ve) reached during exercise challenge tests at week 0 and week 8 for Subject 1.

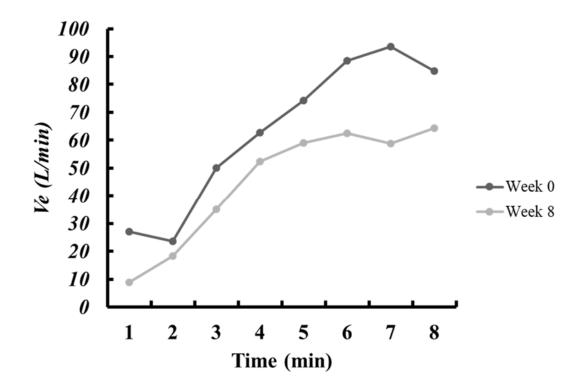
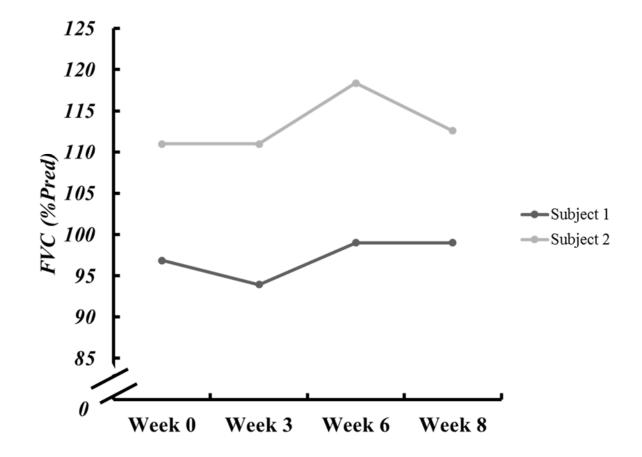
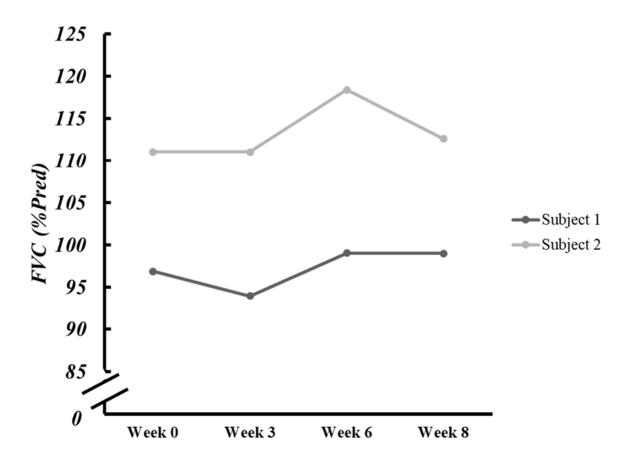


Figure 15. Ventilation (Ve) reached during exercise challenge tests at week 0 and week 8 for Subject 2.

FVC as an absolute value and as a percent of predicted (24) at weeks 3, 6, and 8 were similar to that at study entry. (Figure 16 and 17)



**Figure 16.** Forced vital capacity (FVC) at weeks 0, 3, 6, and 8 of a competitive indoor swim season in participants with exercise-induced bronchoconstriction.



**Figure 17**. Forced vital capacity (FVC) percent predicted at weeks 0, 3, 6, and 8 of a competitive indoor swim season in participants with exercise-induced bronchoconstriction.

FEV<sub>1</sub> as an absolute value and as a percent of predicted (24) at weeks 3, 6, and 8 were similar to that at study entry. (Figure 18 and 19).

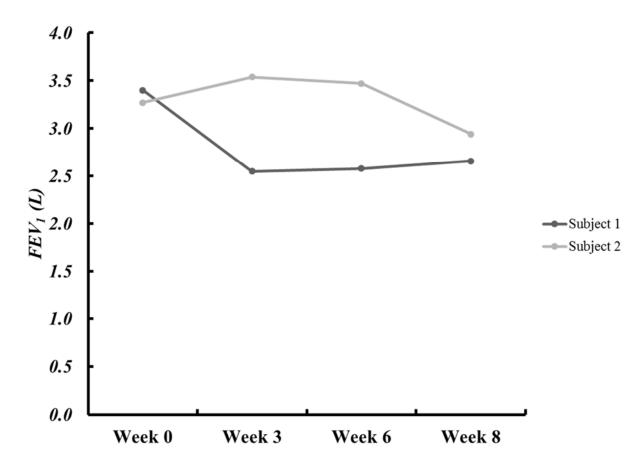
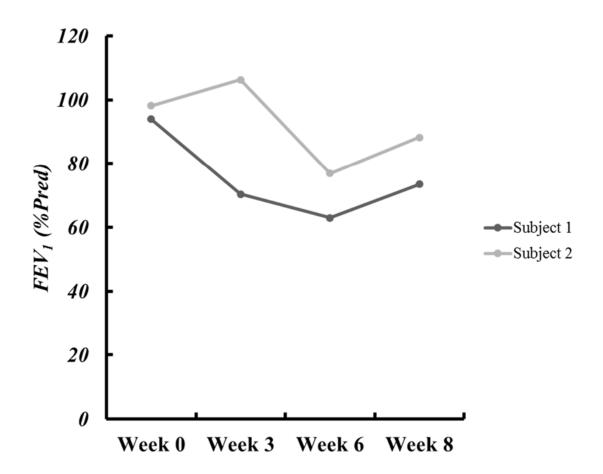
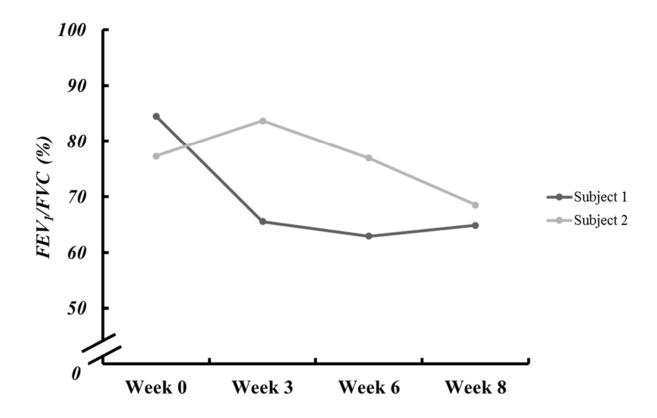


Figure 18. Forced expiratory volume in the first second ( $FEV_1$ ) changes from week 0 to week 8 of a competitive indoor swim season in participants with exercise-induced bronchoconstriction.



**Figure 19**. Forced expiratory volume in the first second (FEV<sub>1</sub>) percent predicted at weeks 0, 3, 6, and 8 of a competitive indoor swim season in participants with exercise-induced bronchoconstriction.

FVC % predicted (24), PEF, and FEF<sub>25-75%</sub> as absolute values at weeks 3, 6, and 8 were similar to that at study entry (**Figure 20-22**).



**Figure 20**. Forced expiratory volume in the first second divided by forced vital capacity (FEV<sub>1</sub>/FVC) at weeks 0, 3, 6, and 8 of a competitive indoor swim season in participants with exercise-induced bronchoconstriction.

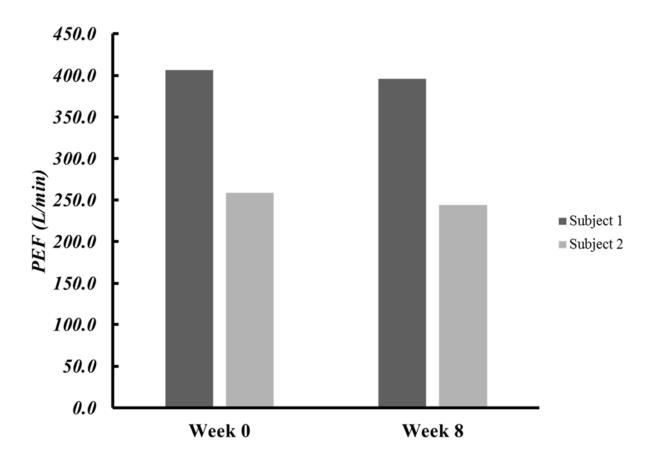


Figure 21. Peak expiratory flow (PEF) for participants with EIB at weeks 0 and 8.

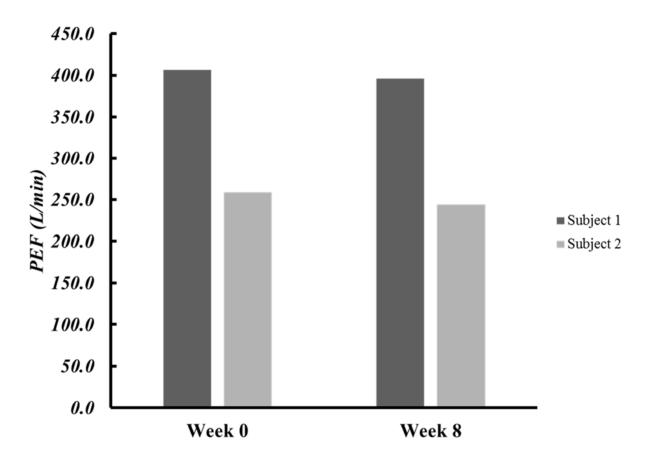


Figure 22. Forced expiratory flow 25–75% (FEF<sub>25-75%</sub>) for participants with EIB at weeks 0 and 8.

#### **Urinary Analysis Measures in EIB Participants**

No relative differences or changes were found in leukotriene concentrations in the urine when normalized for creatinine (Figure 23).

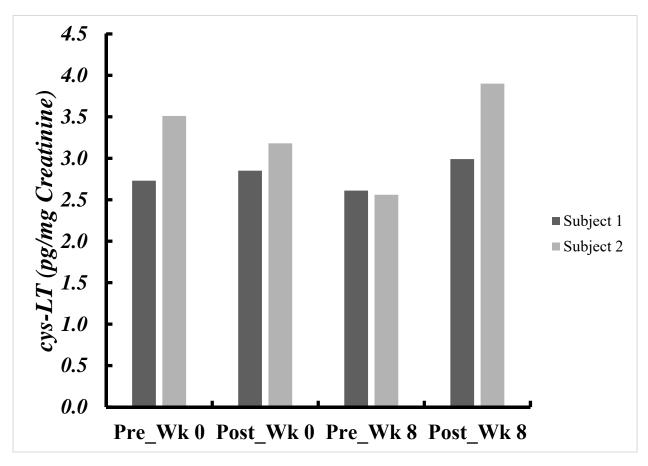


Figure 23. Leukotriene/Creatinine concentrations from week 0 to week 8.

#### **Diet and Bronchodilator Usage**

There were no abnormal amounts or change in amounts of omega-3 consumed from week 0 to week 8 by EIB participants as shown by the self-reported FFQ (**Figure 24 and 25**). Data is expressed as 0 being no consumption (no consumption), 1 being low consumption (once a month), 2 being moderate consumption (once a week), and 3 being high consumption (more than twice a week). Medication logs were issued at each visit. No participants reported being prescribed an inhaler from the beginning to the end of the study.

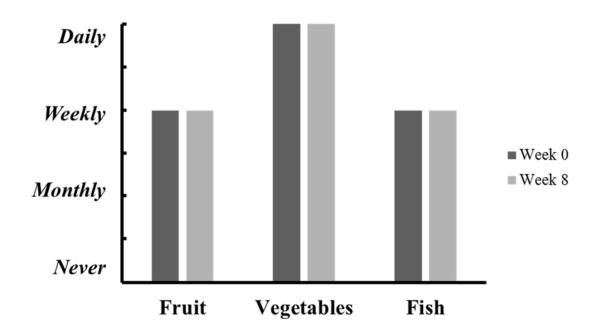


Figure 24. Food Frequency from week 0 to week 8 as reported by Subject 1.

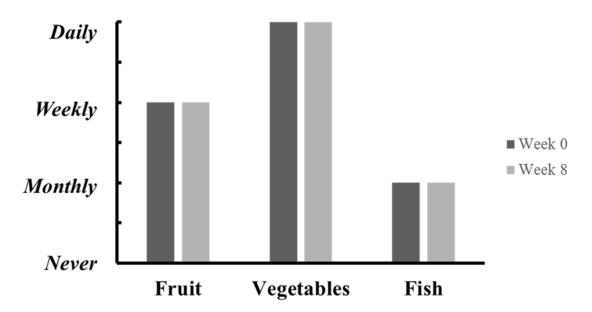


Figure 25. Food Frequency from week 0 to week 8 as reported by Subject 2.

#### Average Yardage

Participants completed 7.1±1.7 swim practices per week during the study. Average pool exposure hours per week was 14.25±1 hours. Average yardage per practice was 5400±250 yards per day. Teams had two pool practices a day on Monday and Wednesdays resulting in a total of 4 hours of pool exposure of those days.

#### Average Pool Chemical Content per University

Both pools used stabilized chlorine disinfectants. Pools were chlorinated using calcium hypochlorite ±ts and manual chorine addition (15-gallon buckets). Pools were in line with recommended levels made by swimming pool and spa management guidelines for temperature and chlorine levels. Both pools maintained a temperature of 26.6 °C over the course of 8 weeks. One location maintained a chlorine level of 1.4ppm and the other was at 2.0ppm. The pH of both pools was 8 during all 8 weeks. The pH level for pools over the course of the 8-week study was higher than recommended.

#### Chapter 5

#### DISCUSSION

The purpose of this study was twofold: 1) to comprehensively examine whether or not spirometric indicators of pulmonary function change over an 8-week portion of the indoor swim season in competitive collegiate swimmers and 2) to perform a pilot investigation on the efficacy of fish-oil supplementation in swimmers with EIB over the course of 8 weeks. Contrary to our hypothesis, no significant changes were observed in FVC or FEV<sub>1</sub> over the course of an 8-week indoor swim season. Further, additional spirometric indicators (PEF, FEF) did not change over the course of the 8 weeks.

Participants in this study were Division I and II college swimmers. The study consistent of 12 females and 1 male 20±2 years of age. Participants were specifically recruited due to their exposure to chloramines on the surface of the pool water during practice. All participants were classified as competitive swimmers (47). The 8 weeks of data collection started at the beginning of the college indoor swim season. Researchers chose this time of year to collect data because participants were just coming back from summer break and it was the time that interfered the least amount with championship season. The beginning of the swim season allowed researchers to look at the change in airway function from the participants' entrance into the pool environment after summer break.

It is well known that swimmers have larger lung volumes when compared to other athletes and general population (12, 15, 57). Pulmonary function measures of all participants were above the lower limits of normal at study entry with nearly all mean values reaching above 90% of predicted (24). Vaccaro et al. found measures of TLC,

FVC, and FEV<sub>1</sub> in child and preadolescent swimmers were approximately 10-16% above normal after 3 years of training (41, 57). When compared to land based activities (runners, basketball, canoeing, and rowing) and sedentary controls, swimmers had superior FEV<sub>1</sub> independent of stature and age (12, 15).

Nordberg et al. (46) reported a significant relationship between the number of hours spent in the swimming pool environment and the amount of acute symptoms associated with asthma and EIB (i.e. dyspnea, cough, nose irritation, throat irritation, eye irritation). Due to the volume of hours swimmers in this current study spent in this pool, it was expected to see a significant decrease in FEV<sub>1</sub> over the course of a swim season. In the Nordberg et al. study, researchers reported a significant decrease in pool attendees (swimmers, lifeguards, etc.) FEV<sub>1</sub> after two hours of exposure to the pool environment (46). Jacobs et al. reported considerable lung function changes and patterns were found in swimming pool instructors (29).

The current investigation took place over 8 weeks of indoor swimming training. However, 8 weeks may not have been sufficient to observe significant changes in pulmonary function over time.

Two different university teams were used as participant pools for this study. Each team practiced at their specific institution throughout the eight weeks of the study. Each team was followed their own training regimen during the course of the study. Yardage and pool time were also collected from coaches of both teams in this study, confirming training load. Participants spent 14.25±1 hours per week in the swimming pool practices. The exposure to the swimming pool environment was greater than 2 hours on days participants doubled up on pool practices (Monday and Wednesday). Swimmers

completed an average of 5400±250 yards of swimming per practice thus indicating a lack of exercise stimulus was not likely a factor in the lack of change in lung function of the participants. The difference in training factors may be in part to why researchers saw no change in measures of pulmonary function over the course of 8 weeks.

To investigate the presence and severity of EIB, conditions for provoking a response were provided. The exercise challenge protocol required the prediction of a target workload to achieve a target Ve and VO2 (2, 14). Though the EVH test is regarded as the gold standard, researchers saw subjects elicit symptoms of EIB, indicating this was an appropriate test to use (2). EVH tests show a higher prevalence of EIB in individuals. The sample size used in the EIB pilot study was small. With the use of an EVH test it can be assumed use of this test could have led to an increase in total study participants in the EIB portion of this study.

No relative differences in leukotriene concentration in the urine pre and post exercise challenge test were observed. Changes in leukotriene levels were not similar to other studies. Kumar et al. (32) found production of LTB4 in lung microsomes almost doubled after exhaustive exercise in the form of swimming. Exhaustive swimming exercise in rats resulted in the enhanced production of LTB4 in lung microsomes. This led researchers to believe an increased number of leukotrienes would be found in the urine post exercise challenge test. However, researchers for this study found relatively no increase in the amount of leukotrienes in the urine post exercise challenge test. It is possible participant's baseline values were not normalized when the pre-exercise test sample was taken. Researcher has shown leukotriene concentration values return to baseline 3 to 7 hours after symptom evoking stimulus (i.e. exercise) (38). All participants

were asked to refrain from exercise at least 4 hours prior to giving a sample and participating in the study. The body may not have fully returned to baseline after 4 hours before the collection causing discrepancy in data.

#### Limitations

Sample size of the EIB pilot study did not allow for any statistical analysis. The absence of a metabolic cart hindered the researcher ability to perform an EVH test compared to an exercise challenge test. The inability to collect ventilation and trichloramine data from each location did not allow for a comparison between pool environments to be made.

#### **Future Directions**

It may be imperative to study coaches and/or lifeguards to determine if there detrimental effects to their pulmonary function as well. Futures studies on pool attendee pulmonary function can determine whether the air on the surface of the water or the pool environment causes more cases of acute asthma and EIB symptoms.

#### Conclusion

In conclusion, it is important to understand the physiological impact inhalation of chemical by-products may have on swimmers throughout their lifetime. Though no significant differences I pulmonary function over 8 weeks were found in this study, respiratory limitations in swimmers have been noted in other research. Looking at the impact of the swimming pool environment on swimmers' lung volumes and acute asthma symptoms may help to ultimately improve the swimmers performance over the course of his/her swimming career. A better understanding of treatments for asthma and EIB symptoms is needed to help abate long term respiratory limitations that may occur due to

pool environment exposure. It is also important to further exam pool quality standards to provide a safe and healthy pool environment for these athletes.

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### **Appendix A: Informed Consent**

## Appalachian State University Informed Consent for Participants in Research Projects Involving Human

Title of Project:	Pulmonary Function in Competitive Swimmers over an Indoor Swim Season		
IRB Study #:	17-0274		
<i>Principal</i> Investigator:	Hannah Snyder, M.S.	Email: snyderhe@appstate.edu	
<b>Research Assistants</b>	: Jonathon Stickford, Ph.D.	Email: stickfordjl@appstate.edu	

This is to certify that I, \_\_\_\_\_\_ have been given the following information with respect to my participation as a volunteer in a program of investigation under the supervision of Hannah Snyder, M.S. to which Jonathon Stickford, Ph.D. may be assisting.

### 1. <u>Purpose of the study:</u>

Repeated exposure to disinfectant by-products in swimming pool environments over an indoor swim season may contribute to poorer respiratory health in swimmers. Fish oil supplementation, however, may play a protective role in airway function.

The purpose of this study is threefold: 1) to asses whether or not spirometric indicators of pulmonary function change over a swim season, 2) to assess if a decrease in FEV1 in swimmers with a decrease in FEV1 of greater than 10% worsen over the course of a swim season, and 3) to assess if fish-oil supplementation abates the worsening of FEV1 in swimmers with a decrease greater than 10 percent over the course of a swim season.

## 2. <u>Inclusion Criteria: You may participate in the study if the following apply to you:</u>

- Sex: Male or Female
- Ethnicity: Any
- Age: 18-25 years
- Without injury or illness that prevents them from practicing/competing
- Interest in participating in a research study assessing pulmonary function and the effects of dietary supplementation on respiratory function
- Understand written and oral instructions in English
- Provides informed consent
- Available during times the data collection is offered
- All subjects must be nonsmokers
- Must be a competitive swimmer

## **Exclusion Criteria: You should not participate in this study if any of the following apply to you:**

- Known cardiovascular, metabolic or renal disease, or signs/symptoms suggestive of cardiovascular, metabolic or renal disease will exclude you from participation.
- Current smoker.
- 3. <u>Procedures:</u> Please read the descriptions of each experimental day and write your *initials in the space provided.*

Table 1. Study procedures listed by visit humber.				
Visit 1	Visit 2	Visit 3	Visit 4	
Informed Consent	Pulmonary	Pulmonary	VO <sub>2</sub> submax Testing	
	Function Test	Function Test		
Medical History			Urine Sample	
			Collection	
VO <sub>2</sub> submax Testing				
Urinary Sample				
Collection				

Table 1. Study procedures listed by visit number.

**initial Consent and Questionnaires:** At this screening visit, the study will be explained in-depth to you by the PI or a trained research assistant. You will be provided time to consider your options and get all questions answered - if you agree to participate, you will then provide your written informed consent.

**initial** <u>Pulmonary Function Testing (PFT)</u>: You will be asked to perform tests of breathing function. The protocol will follow that described by the American Thoracic Society. These tests include measurement of the total volume of air your lungs can hold, the volume of air that you can push out with one maximal breath, the volume of air that you can breathe out in one second, and the maximum voluntary volume of air that you can breathe in 12 seconds (MVV). For all these procedures, you will wear nose clips and breathe through a disposable mouthpiece. These procedures will take ~60 minutes total.

**initial** <u>Submaximal Aerobic Capacity Exercise Test ( $\dot{VO}_{2submax}$ )</u>: You will be asked to perform a submaximal exercise test to examine your cardiovascular fitness. This test will measure your submaximal exercise capacity and is often described as a  $\dot{VO}_{2submax}$  test. You should be rested, well nourished, and hydrated for the test and avoid caffeine and tobacco 3 hours before the test. Additionally, avoid alcohol 12 hours before the test. Report any medication that you are using to the testing staff before the test. When you are ready to perform the test, the investigators will help with necessary adjustments to testing equipment to assure your comfort. You will be fitted with a rubber mouthpiece and nose clip. Your breathing pattern, exercise metabolism, exhaled carbon dioxide, and arterial blood oxygen level (via pulse oximetry) will be monitored during the test. This procedure will require ~40 minutes total, with exercise lasting approximately 8 minutes.

## Treadmill Protocol

Exercise protocol for subject (should be 8 minutes of treadmill running): Minute 1: 60% of target VO2 (tVO2) (manipulate speed and grade) Minute 2: 70% of tVO2 (manipulate speed and grade) Minute 3: 90% of tVO2 (manipulate speed and grade) Minutes 4-8: 100% of tVO2 (manipulate speed and grade) \* Target VO2 (tVO2) will be 40-60% of the predicted maximum voluntary ventilation (MVV, estimated as FEV1x35). Target heart rate for stage 4 is 80-90% of the subject's predicted maximum heart rate (Crapo et al., 2000).

**initial** <u>Urine Sample Collection</u>: A urine cup will be used to collect a small urine sample to measure your markers of inflammation approximately 5 minutes after the submaxVO2 test has ended.

Supplement	Manufacturer	Dosage
Omega 3 Fatty Acid	Nordic Naturals	4.0 g/day
Placebo: Olive Oil	Swanson Organic Extra Virgin Olive Oil	4.0 g/day

**initial** Fish Oil and Placebo Supplementation: You will enter the study on your usual diet and receive either a fish oil or placebo (olive oil) supplement for the duration of the study (8 weeks). You will be randomly placed into the supplementation groups. The supplement compositions are displayed in Table 1. You will be asked to consume the supplement protocol daily.

## 4. Discomforts and risks:

There are <u>minimal</u> risks involved with measuring/monitoring/performing: questionnaires, pulmonary function testing, flow-volume loops, and submaxVO2 tests.

<u>Submaximal Aerobic Capacity Exercise Test (VO<sub>2submax</sub>)</u>: VO<sub>2submax</sub> test risks include abnormal heart beats, abnormal blood pressure responses, muscle cramps, muscle strain and/or joint injury, delayed muscle soreness (1 to 2 days afterwards), light headedness, fatigue, and in rare instances, heart attack.

<u>Loss of Confidentiality</u>: Any time information is collected; there is a potential risk for loss of confidentiality. Every effort will be made to keep your information confidential; however, this cannot be guaranteed.

<u>Other Risks</u>: There may possibly be other side effects that are unknown at this time. If you are concerned about other, unknown side effects, please discuss this with the researchers.

How you can help reduce some of the risks: During your participation in this research, the researchers will closely observe your testing to determine whether there are problems that need medical care. It is your responsibility to do the following:

- Ask questions about anything you do not understand.
- Keep appointments.
- Follow the study researchers' instructions.
- Let the researchers know if your telephone number changes.
- Tell the researchers before you take any new medication.
- Tell your regular doctor about your participation in this research.
- Talk to a family member or friend about your participation in this research.
- 5. a. <u>Benefits to me:</u> There exists the potential that respiratory health could be improved as a result of your involvement in the study.
  - **b.** <u>Potential benefits to society:</u> The results could alter interventional approaches for improving respiratory health in competative swimmers.
  - 6. <u>Alternative procedures that could be utilized:</u> Not participating in the study. The procedures used in this study are frequently used in research and are the most appropriate methods to accomplish the goals of this research.
  - 6. Time duration of the procedures and study:

initial Visit 1 (about 2 hours). initial Visit 2 (about 1 hour).

\_\_\_\_\_ initial Visit 3 (about 1 hour).

\_\_\_\_\_ initial Visit 4 (about 2 hours).

## Approximately 6 hours Total

7. <u>Statement of confidentiality:</u> Volunteers are coded by an identification number for statistical analyses. All records are kept in a secure location. All records associated with your participation in the study will be subject to the university confidentiality standards and in the event of any publication resulting from the research no personally identifiable information will be disclosed.

**8.** <u>**Right to ask questions:**</u> Please contact Hannah Snyder, B.S. (919-368-4735), with questions, complaints, or concerns about this research. If you have any questions about your rights as a research subject, please contact the IRB Administrator at the Appalachian State University Institutional Review Board Office at (828) 262-2692, <u>irb@appstate.edu</u>. This study has been approved on August 30, 2017 by the Institutional Review Board (IRB) at Appalachian State University. This approval will expire on August 30, 2018 unless the IRB renews the approval of this research.

**9.** <u>**Compensation:**</u> You will receive no monetary compensation for your participation in this study.

**10.** <u>Injury Clause:</u> In the unlikely event you become injured as a result of your participation in this study, standard emergency procedures will be followed. If you get hurt or sick when you are not at the research site, you should call your doctor or call 911 in an emergency. If your illness or injury could be related to the research, tell the doctors or emergency room staff about the research study, the name of the Principal Investigator, and provide a copy of this consent form if possible. Please call the PI as soon as possible (Hannah Snyder, B.S. 919-368-4735). You will be responsible for any costs for medical care not paid by your insurance company. No other compensation is offered by Appalachian State University. By signing this document, you are not waiving any legal rights that you have against Appalachian State University for injury resulting from negligence of the University or its investigators.

11. <u>Voluntary participation</u>: Your participation in this study is voluntary. You may withdraw from this study at any time by informing the research personnel. You may decline to answer certain questions and may decide not to comply with certain procedures. However, your being in the study may be contingent upon answering these questions or complying with the procedures. The researcher may end your role in the study without your consent if the researcher deems that your health or behavior adversely affects the study or increases risks to you beyond those approved by the Institutional Review Board and agreed upon by you in this document. You have been given an opportunity to ask any questions you may have, and all such questions or inquiries have been answered to your satisfaction.

You must be 18 years of age or older to take part in this research study. If you agree to take part in this research study and have read the information outlined above, please sign your name and indicate the date below. You will be given a copy of this signed and dated consent form for your records.

Volunteer

I, the undersigned, have defined and explained the studies involved to the above volunteer.

Person Obtaining Consent

Date

Date

## **Appendix B: Script for Class Recruitment**

#### **Script for Class Recruitment**

Hello! Researchers in the Department of Health and Exercise Science at Appalachian State University are interested in to assessing pulmonary function and markers of bronchoconstriction over the course of an indoor season in competitive swimmers. We are also interested in examining the potential influence of fish oil supplementation on pulmonary function and markers of bronchoconstriction in competitive swimmers over the course of an indoor season.

We are looking for competitive swimmers aged 18-25 years to participate in a study. The study will require four visits (or about 6 hours in total). At the first visit, you will complete some questionnaires and we will measure your lung function. During the remaining visits, you will perform pulmonary function tests to assess potential changes throughout the season.

To be eligible, you must be healthy, non-smoking, able to participate in practice and meets throughout the swim season. You must not have any cardiovascular, metabolic, or renal disease.

If you are interested, please contact Hannah Snyder at <u>snyderhe@appstate.edu</u> or 919.368.4735.

## **Appendix C: Script for Email Recruitment**

To whomever it may concern.

The researchers at Appalachian State are conducting a study on competitive swimmers. The purpose of this study is threefold: 1) to asses whether or not spirometric indicators of pulmonary function change over a swim season, 2) to assess if a decrease in FEV1 in swimmers with a decrease in FEV1 of greater than 10% worsen over the course of a swim season, and 3) to assess if fish-oil supplementation abates the worsening of FEV1 in swimmers with a decrease greater than 10 percent over the course of a swim season.

The visits and tests are:

Visit 1:

- Consent & HIPAA review and sign
- Review of Medical History
- Lung Function Tests
- Submax Aerobic Capacity Test
- Urine Collection
- Time commitment: 2 hrs

## Visit 2:

- Lung Function Tests
- Time commitment: .5 hr

## Visit 3:

- Lung Function Tests
- Time commitment: .5 hr

## Visit 4

- Lung Function Tests
- Submax Aerobic Capacity Test
- Urine Collection
- Time commitment: 2 hrs

If you are only participating in this study to evaluate the change in pulmonary function over the course of eight weeks and did not have a fall in FEV1 greater than 10%, you will NOT participate in the Submaximal Aerobic Capacity Exercise Test.

For your safety, we will monitor your heart rate response to during all exercise tests. Please let me know if you have any questions or would like additional information. If you are interested in participating tin the study, please contact me to arrange a time to visit the laboratory.

Hannah Snyder, B.S. snyderhe@appstate.edu; 919-368-4735

# Appendix D: Medication Log

Medication Log					
Medication	Medication Log Dosage	Date Taken			

# **Appendix E: Food Frequency Questionnaire**



Athlete: \_\_\_\_\_ Date: \_\_\_\_\_

## FOOD FREQUENCY QUESTIONNAIRE:

FOOD			ON (select one you do not eat	
	Per Day	Per Week	Per Month	Rarely/Never
Fruit:				
Apple				
Banana				3
Melon				
Plums, Apricots				
Orange				
Pear				
Berries				3
Dried Fruit				
Fruit Juice				
Vegetables:	_			
Corn, Squash, Potato				
Lettuce, spinach, cabbage				
Carrots, beets				
Tomato, mushrooms,				
zucchini				
Legumes: lentils, beans etc				
Meat, Fish, Eggs, Nuts:				
Beef, lamb				9
Egg				
Chicken, Turkey				
Fish				
Ground beef				2
Luncheon meat e.g. ham				
Pork, Veal	-			
Nuts, seeds and their butters				
Sausage				
Tofu				
Breads, Cereals, Grains:				
Bread				
Bagel				
Cereal, cold	1			
Cooked cereal				
Crackers, rice cakes				
Rice		-		
Pasta, Noodles				
Pasta, Nooules Pancakes, Waffles				3
rancakes, wantes				-
			-	



Athlete: \_\_\_\_\_ Date: \_\_\_\_\_

Dairy Products:			
Milk (Skim, 1%, Whole)			 ·
Soy milk, Rice milk			
Cheese – hard e.g. swiss		2 9	
Cheese - soft e.g. cottage			
Icecream, Frozen yogurt		1 	
Yogurt		)()	ç
Oils, Butter, Creams:			
Butter, margarine	-		
Oils e.g. olive			9
Salad dressings			
Athlete Foods:			
Sports drink			
Gels			
Bars e.g. powerbars		) ()	0
Snacks:			
Cake			
Cookies, doughnuts			
Chocolate			
Hard Candy		) ()	9
Corn chips, potato chips			
Granola bars			
Pretzels			
Beverages – other:			
Coffee, Tea, Hot			
Chocolate			
Wine, Beer, Vodka etc			
Energy drinks e.g. red bull			
Supplements			
		i i	
Other			

# Appendix F: 24-Hour Health History Form

Study:	Age:		Height:	Weight:	Sex:
Subject Number:				Date:	
Do you have: Head cold Nasal Congestion Headache Sore Throat Digestive Upset Intestinal Disorder General Fatigue Muscle Soreness	Yes	<b>N</b> ₀ □ □ □ □ □ □ □ □ □ □	How do you feel? Good Fair Not so good Bad	# of hours sleep How was your sleep? Normal Wakeful Restless	# of hours since eating: What did you eat? 
Medicine taken in 24 hours:		- - -	Any leg cramps Since last activity? Yes No	Physical activity in last 24 hours:	Any unusual physical activity in last 24 hours?

## 24-HOUR HEALTH HISTORY

\*\* Take weight with each visit.

Last Menstrual Period (LMP): \_\_\_ (1⁵t Day of LMP)

Vita

Hannah Elizabeth Snyder was born in Fort Collins Colorado, to Douglas and Juanita Snyder, She graduated Magna Cum Laude with Honors from Towson University in Maryland in May 2016 to study Exercise Science and was awarded a Bachelor's of Science degree. She was a three time Academic All-American as a Division I swimmer. In the fall of 2016, she entered Appalachian State University began study toward a Master of Sciences degree in Exercise Science. The M.A. was awarded in May of 2018. Hannah remains active in the swimming community and wishes to pursue a career in the field.