THE EFFECT OF OMEGA-3 FATTY ACIDS ON AIRWAY INFLAMMATION, HYPERPNEA-INDUCED BRONCHOCONSTRICTION, AND AIRWAY SMOOTH MUSCLE CONTRACTILITY IN ASTHMA

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ABSTRACT

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THE EFFECT OF OMEGA-3 FATTY ACIDS ON AIRWAY INFLAMMATION, HYPERPNEA-INDUCED BRONCHOCONSTRICTION, AND AIRWAY SMOOTH MUSCLE CONTRACTILITY IN ASTHMA

Asthma, a chronic inflammatory disease of the airways, affects nearly 25 million Americans. The vast majority of these patients suffer from exercise-induced bronchoconstriction (EIB), a complication of asthma. Although traditionally treated pharmacologically, nutritional strategies provide a promising alternative for managing EIB as the prevalence of asthma may be due in part to changes in diet.

Our objective was to determine the effects of novel nutritional strategies on hyperpnea-induced bronchoconstriction (HIB) in asthmatic individuals. HIB uses rapid breathing to identify EIB in a research or clinical setting. Fish oil, a combination of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docsahexaenoic acid (DHA), has been shown to be effective in suppressing EIB. However, its use in combination with other nutritional supplements, the optimal fish oil formula, and its effect on smooth muscle contractility have not been fully explored.

An *in vivo* study (study 1) was conducted in individuals with both asthma and HIB to determine whether a combination of fish oil and vitamin C was more effective than either one alone in alleviating HIB. Pulmonary function was significantly improved with both fish oil and the combination treatment but not with vitamin C alone. In study 2, individuals with both asthma and HIB were supplemented with DHA alone since the optimal formula for fish oil has yet to be ascertained; previous *in vitro* studies have suggested DHA may be the more potent omega-3 fatty acid in fish oil. However, no significant changes in pulmonary function or airway inflammation were seen with DHA supplementation.

For study 3, canine airway smooth muscle tissue was treated with fish oil to determine the *in vitro* effect of fish oil on smooth muscle contractility. Acute treatment with fish oil relaxed smooth muscle strips that had been contracted with acetylcholine or 5-hydroxytryptamine. These minor relaxations in smooth muscle tension with fish oil may represent significant changes at the level of the smaller airways.

These studies have confirmed that fish oil represents a viable treatment modality for asthmatic individuals with EIB and suggest that fish oil may influence airway smooth muscle contractility.

Timothy D. Mickleborough, Ph.D., Chair

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CHAPTER 1

INTRODUCTION

Asthma

Epidemiology of Asthma

Asthma is a chronic inflammatory disease of the airways characterized by recurrent wheezing, breathlessness, chest tightness, and coughing (87). The hallmark features of asthma are airway inflammation, airway hyperresponsiveness, and airway narrowing (90). In 2009, 24.6 million Americans reported having asthma with 60% of those 5-17 years of age missing at least one day of school and 34% of those over 18 years of age missing at least one day of work due to asthma symptoms; this translated to 10.5 million missed school days and 14.2 missed work days (6). Moreover, around 6% in each of the above age groups reported being limited in their activity due to asthma symptoms (6). In 2007, asthma was reportedly responsible for $19.7 billion in direct and indirect healthcare costs annually; this includes $6.2 billion spent on prescription drugs for treating asthma (1). Since asthma is a multifaceted disease, patients often need multiple medications to optimally control their symptoms. Combination therapies targeting the acute and chronic symptoms of asthma are increasingly prescribed since monotherapy is often inadequate (31). Appropriate asthma treatment and management is thus an important issue due to the substantial burden asthma has placed on American society in terms of lost productivity and healthcare costs.

Airway Smooth Muscle Contractility

As airway narrowing and hyperresponsiveness are key features of asthma, airway smooth muscle contraction is an important mechanism. Consequently, medications that relax airway smooth muscle and thus dilate the airways are among the most widely prescribed treatments for asthma. These include long- and short-acting β_2-agonists, such as salmeterol and albuterol, respectively.

Smooth muscle contraction (figure 1-1) involves membrane depolarization with subsequent calcium release. Calcium binds to calmodulin which activates myosin light chain kinase to phosphorylate myosin, the thick filament in muscle. Phosphorylated myosin binds actin, the thin filament in muscle, to produce contraction. Smooth muscle relaxation occurs with the reuptake of calcium and de-phosphorylation of myosin by myosin light chain phosphatase.

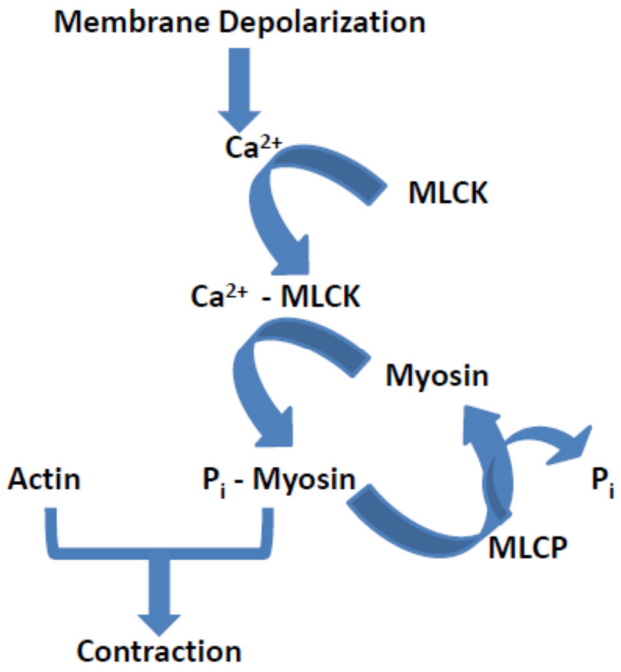

Figure 1-1. ***Mechanism of smooth muscle contraction.*** *Following cell membrane depolarization, the calcium (Ca^{2+}) concentration increases. Myosin light chain kinase (MLCK), whose activation depends on calcium, phosphorylates myosin. This allows myosin to bind with actin to produce smooth muscle contraction. Myosin light chain phosphatase (MLCP) dephosphorylates myosin to cause relaxation.*

Airway smooth muscle contractility has been shown to be dependent on the overlying epithelium. Epithelial functions include creating a barrier between the airways and the external environment as well as secreting many factors (48). Epithelial secretions include arachidonic acid metabolites involved in airway smooth muscle tone, mucus secretion, and inflammation (48). Nitric oxide, growth factors involved in

respiratory tissue repair, and proinflammatory cytokines that recruit inflammatory cells to the airways are also released by the epithelium (48). It has been shown in animal models that *in vitro* airway smooth muscle sensitivity to contractile agonists is increased with the epithelium removed (3, 15). This is important to note as it is known that the epithelium is damaged or denuded in the airways of asthmatics (48). Although it is thus likely that this contributes to airway hyperresponsiveness, it is still not clear whether the epithelial abnormalities are a cause or an effect of asthma (48).

Airway Inflammation

The other key feature of asthma is airway inflammation, which can occur acutely or chronically, and is the target for many other asthma medications. In fact, guidelines for managing asthma tend to concentrate on treating airway inflammation (18). Acute inflammation in asthma includes both an early and a late phase. In the early phase, mast cells and macrophages in the airways are activated and release proinflammatory mediators such as histamine, leukotrienes, prostaglandins, and reactive oxygen species (18). Six to nine hours later, the late phase begins as cytokines released by the mast cells in the early phase recruit eosinophils, basophils, neutrophils, and macrophages to the airways (18). Chronic inflammation in asthma is characterized by activated T-cells, eosinophils, mast cells, macrophages, epithelial cells, fibroblasts, and bronchial smooth muscle cells in the airways (18). The eosinophils in particular secrete proinflammatory mediators, cytotoxic mediators, and cytokines which cause many of the features of asthma, including mucus secretion, smooth muscle contraction, epithelial shedding, and airway hyperresponsiveness (18).

Various treatment strategies are used to treat the inflammation associated with asthma. Since the symptoms of wheezing and shortness of breath result from acute inflammation, β_2-agonists used to dilate the airways can also be used to treat the effects of acute inflammation (18). Medications, such as cromolyn and nedocromil, that

stabilize mast cells to reduce their early phase secretions are also used to treat inflammation. Medications that target the proinflammatory products are routinely prescribed as well. These include enzyme inhibitors, such as zileuton, an inhibitor of 5-lipoxygenase (the enzyme involved in leukotriene production), and leukotriene receptor antagonists, such as montelukast and zafirlukast.

Non-Invasive Markers of Airway Inflammation

Non-invasive methods of assessing the adequacy of disease management can be useful clinically. In addition to changes in pulmonary function and symptoms, the degree of airway inflammation can demonstrate how effective a particular treatment regimen is (58). Exhaled breath condensate and exhaled nitric oxide can each be measured non-invasively to assess airway inflammation in asthma.

Exhaled breath condensate (EBC) pH has been shown to be correlated with airway inflammation (93). Asthmatics tend to have a lower EBC pH (19). The acidic pH likely stems from neutrophil and eosinophil products, such as myeloperoxidase and eosinophil peroxidase, reacting with hydrogen peroxide upon their release to form acids and increase the concentration of hydrogen ions in the airways (19). Markers in EBC can also be measured to evaluate airway inflammation. These markers include inflammatory mediators as well as 8-isoprostane, a marker of oxidative stress (19). 8-Isoprostane is produced by free radical oxidation of arachidonic acid; its concentration is increased in asthmatics reflecting increased levels of oxidative stress (67).

The fraction of exhaled nitric oxide (F_ENO) has been shown to be higher in asthmatics than in healthy individuals (78). This is thought to be due to the elevated activation of inducible nitric oxide synthase (iNOS), whose expression can be increased by proinflammatory cytokines and oxidants (18, 58). It has also been shown that F_ENO levels are increased in subjects with exercise-induced bronchoconstriction as compared to those without it (29).

Exercise-Induced Bronchoconstriction

Exercise-induced bronchoconstriction (EIB) is a complication of asthma that affects 80-90% of people with asthma (87). EIB is characterized by symptoms of wheezing, reduced exercise tolerance, chest pain, cough, stomachache, and sore throat occurring during or after exercise that lasts at least five minutes (87). EIB is clinically diagnosed based on the change in the volume of air exhaled in the first second of a forced exhalation (FEV_1) before and after exercise; it is specifically defined as at least a 10% post-exercise drop in FEV_1 (12). EIB is important to consider as it can deter individuals with asthma from being physically active (43). Moreover, EIB suggests that an individual's asthma is not being adequately managed (49). Consequently, EIB testing can be used to evaluate asthma therapies (49).

Pathophysiology of Exercise-Induced Bronchoconstriction

Currently, there are two major schools of thought on the pathogenesis of EIB, the hyperosmolarity theory and the airway re-warming theory. According to the hyperosmolarity theory, the airway surface liquid becomes hypertonic due to water loss during exercise; the ensuing hyperosmolar environment in the airway cells results in the release of proinflammatory mediators that cause bronchoconstriction (90). Alternatively, the less widely accepted airway re-warming theory suggests that hyperventilation during exercise cools the airway surface cells such that their post-exercise re-warming causes the surrounding bronchiolar vessels to dilate; this leads to hyperemia with fluid exudation and proinflammatory mediator release, which subsequently causes bronchoconstriction (90).

Bronchoprovocation Tests to Diagnose Exercise-Induced Bronchoconstriction

Exercise Testing

Exercise is the actual stimulus for EIB that individuals will encounter outside of the laboratory or doctor's office. However, the standard exercise protocol for diagnosing

EIB requires patients to breathe dry air while exercising for 6-8 minutes at 85-95% of their maximal heart rate (8). Therefore, in addition to the need for large and expensive equipment, not all patients or subjects can complete an exercise protocol (8).

Sport-specific testing is a variation of exercise testing that is important for athletes who regularly perform at the standard exercise protocol level (55). In this case, the testing protocol is based on the physical demands of a particular sport; however, by testing the athlete in his or her workout environment, the ambient conditions cannot be standardized, which may affect the test's ability to reliably elicit bronchoconstriction (55).

Methacholine Challenge

Methacholine is a parasympathomimetic drug that causes bronchoconstriction (76). This widely used method of bronchoprovocation involves the patient inhaling progressively increasing doses of aerosolized methacholine. There are two different protocols wherein the patient is instructed to inhale the methacholine with either normal tidal volume breaths or deep inhalations (76). The patient is deemed to exhibit bronchial hyperresponsiveness if the dose of methacholine that causes a 20% decline in FEV_1 from the pre-challenge value is less than 4.0 mg/ml (76). Importantly, a negative test excludes asthma in a symptomatic patient (76); however, a positive test is not specific for asthma (8). A large number of false positive tests have been reported in athletes (55). Moreover, a negative methacholine challenge test does not exclude EIB (8).

Mannitol Challenge

Mannitol has only recently been approved by the Food and Drug Administration in the United States although it has been used regularly as a bronchoprovocation test in other countries (8). A standardized mannitol test kit provides progressively increasing doses of mannitol in a dry-powder form for patients to inhale (55). The osmotic gradient that subsequently develops across the airways leads to the release of inflammatory mediators that promote bronchoconstriction (8, 55). The test is considered positive for

bronchial hyperresponsiveness if the patient demonstrates a 15% or greater decrease in his or her baseline FEV_1 at a dose less than 635 mg; alternatively, the test is also considered positive if the patient exhibits a 10% or greater decrease in FEV_1 between two consecutive doses of mannitol (10). Unfortunately, the mannitol challenge test is no more sensitive than the methacholine challenge test for identifying EIB (11).

Eucapnic Voluntary Hyperventilation

In a research or clinical setting, EIB can be readily identified with a test involving hyperpnea, or rapid breathing (9). This test, known as eucapnic voluntary hyperventilation (EVH), requires subjects or patients to breathe cold, dry air at a high rate for six minutes (figure1-2) (9). The rate is approximately 85% of the individual's maximal voluntary ventilation and is estimated by multiplying the FEV_1 at rest by 30 (9). EVH is the bronchoprovocation strategy recommended by the International Olympic Committee to identify athletes with EIB (9). It has been shown that changes in FEV_1 following EVH are comparable to those seen following cold air exercise (81).

Figure 1-2. ***Eucapnic voluntary hyperventilation challenge.*** *Eucapnic voluntary hyperventilation (EVH) is a surrogate exercise challenge recommended by the International Olympic Committee for identifying exercise-induced bronchoconstriction. Subjects are asked to breathe at 85% of their maximal voluntary ventilation estimated by multiplying their FEV_1 at rest by 30 (9).*

Pharmacotherapy for Exercise-Induced Bronchoconstriction

Several classes of medications are typically prescribed to prevent EIB. In general, these drugs either target bronchoconstriction or airway inflammation. To alleviate bronchoconstriction acutely or chronically, either short- or long-acting β_2-agonists are typically prescribed, respectively. These agonists act at β_2-adrenergic receptors on the bronchial smooth muscle to promote bronchodilation (43). Short-acting β_2-agonists, especially albuterol, are most often prescribed as “rescue inhalers” for treating acute asthma exacerbations and preventing EIB (85). However, β_2-agonists, such as formoterol, that have both a short response time and longer duration of effectiveness may be more practical. It has been shown that although albuterol and formoterol were both able to prevent EIB within fifteen minutes of administration,

albuterol provided bronchoprotection for four hours while formoterol's bronchoprotection lasted twelve hours (85).

Since β_2-agonists do not affect the inflammation associated with asthma, other types of drugs are often prescribed as well (43). Corticosteroids reduce inflammation over time by inhibiting the production of proinflammatory prostaglandins, leukotrienes, and cytokines as well as by upregulating β-receptor transcription, which enhances responsiveness to β_2-agonists (43). As such, they are typically prescribed in combination with a β_2-agonist rescue inhaler to reduce EIB symptoms since they cannot alleviate an asthma attack themselves. It has been shown that inhaled corticosteroid therapy begins to offer protection against EIB after one week; its effectiveness improves with increased doses and duration of treatment (91).

Drugs that specifically target leukotrienes have repeatedly demonstrated the capability to control EIB (23, 49, 81, 89, 93). Anti-leukotriene medications either inhibit leukotriene synthesis (e.g. zileuton) or bind to leukotriene receptors to reduce the action of leukotrienes (e.g. montelukast) (43). Leff et al. (49) demonstrated that during a 12-week course of treatment with the leukotriene receptor antagonist montelukast, subjects with mild asthma and EIB showed a significant reduction in EIB as compared to placebo. Similarly, Rundell et al. (81) showed that montelukast could diminish EIB after a single dose in most but not in all of the subjects with EIB.

Although pharmacotherapy can thus manage asthma and EIB, patients have heterogeneous responses to these medications (27). This may be due in part to the variable nature of asthma; however, since patients with clinically similar disease can have different responses, it is also likely due to genetic variation affecting the drugs' actions (27). Furthermore, medications typically have side effects. Side effects for asthma medications range from muscle tremors and hoarseness to cardiotoxicity and

neurotoxicity (43). Therefore, asthma patients may try alternative approaches to pharmacological treatment.

Diet and Asthma

Because conventional asthma medications do not always offer optimal protection, there is interest in finding novel therapeutic strategies (23). Dietary strategies are important to consider because changes in nutrition may have contributed to the increase in the prevalence of asthma (30). Anecdotally, the rise in asthma in developed countries has coincided with a shift in diet to less fresh fruit, green vegetables, and fish (34). In general, a low intake of antioxidants has been linked to the increase in asthma in Western societies, and specifically, it has been shown that adults with asthma have lower levels of plasma ascorbic acid (vitamin C) compared to healthy, non-asthmatic adults (66). Sodium intake is also related to increased airway hyperresponsiveness (34); in non-asthmatic subjects with EIB, a high salt diet exacerbated post-exercise changes in pulmonary function whereas a low salt diet improved post-exercise changes in pulmonary function (37). Furthermore, the American diet features a 10:1 ratio of omega-6 to omega-3 fatty acids whereas the World Health Organization recommends a 3:1 or 4:1 ratio (41). In contrast, it has been shown that Eskimos, who consume large amounts of omega-3 fatty acids compared to the typical Western diet, have a lower incidence of inflammatory diseases (40). Thus, a proinflammatory diet may be contributing to the rise in asthma cases (57).

Since multiple medications are often needed to effectively control symptoms (31), the inclusion of nutritional supplements in asthma management may reduce the amount of medication someone with asthma requires. Our laboratory has shown that both fish oil and the leukotriene receptor antagonist montelukast similarly reduce airway inflammation and protect against HIB (93). Thus, nutritional supplements could reduce

reliance on asthma medications, which can have dangerous side effects, diminished efficacy over time, or may be banned for use in athletic competition (36).

Omega-3 Polyunsaturated Fatty Acids

Fish oil, a combination of the omega-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has been used to alleviate the symptoms of asthma. It works through the competition of omega-3 PUFAs with omega-6 PUFAs for enzymes in the production of different sets of leukotrienes and prostaglandins; the omega-3 PUFA products have less proinflammatory activity as compared to the omega-6 PUFA products (figure 1-3) (60).

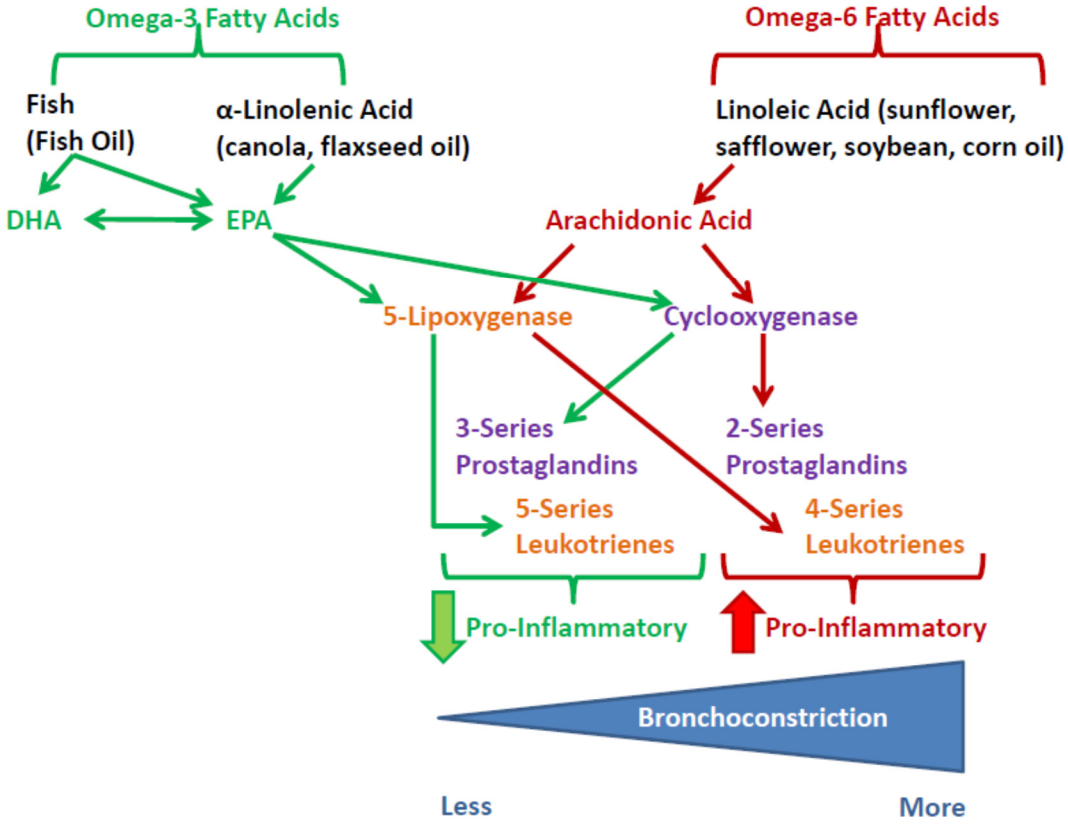

Figure 1-3. ***Competing pathways for omega-3 and -6 polyunsaturated fatty acids.*** *Omega-3 and -6 polyunsaturated fatty acids compete for the 5-lipoxygenase and cyclooxygenase enzymes to produce leukotrienes and prostaglandins with different proinflammatory potentials. The omega-3 products, which are not as proinflammatory as the omega-6 products, trigger less bronchoconstriction than the omega-6 products.*

The digestion and absorption of fish oil is a complex issue. The longer carbon chain length of the omega-3 PUFAs has raised concerns that they may not be as readily hydrolyzed and absorbed as other fatty acids (70). Furthermore, the double bond position in omega-3 PUFAs may affect which digestive enzymes are most important, which may impede the rate of initial lipolysis (70). Nevertheless, this early delay is probably inconsequential given that fat digestion is normally a long process. Once hydrolyzed, omega-3 PUFAs can form chylomicrons and enter the circulation via the lymphatic system similar to other long chain fatty acids (70). Chylomicrons containing omega-3 fatty acids are hydrolyzed as efficiently as other chylomicrons (71); the released fatty acids are subsequently incorporated by nearby tissues (70). The complexity of fish oil digestion arises when the different forms are considered. Commercial fish oil often contains ethyl derivatives of the omega-3 PUFAs in an effort to enhance their concentration (5). The ethyl derivatives are not as well absorbed as their triglyceride counterparts found naturally in fish (28). However, fish oil supplements can also be processed such that the triglyceride structure is retained. Because the manufacture of fish oil has not been standardized, various types are available commercially (5). This may contribute to inconsistent findings between studies.

The literature shows conflicting results concerning the effectiveness of fish oil in treating asthma which may be due to experimental design differences in fish oil dose, treatment period, continued medication use, and bronchoprovocation strategy (13, 17, 57, 59, 61, 68, 96). Nevertheless, our laboratory has consistently shown that fish oil significantly prevents EIB (61, 62, 93). In 2006, Mickleborough et al. (61) investigated the effect of three weeks of fish oil supplementation on EIB in adults with asthma. Compared to taking a placebo, fish oil reduced the change in post-exercise pulmonary function to below the threshold for EIB in these subjects. Likewise, Tecklenburg-Lund et al. (93) showed that three weeks of supplementation with fish oil or the leukotriene

receptor antagonist medication montelukast (Singulair®) decreased the change in pulmonary function following eucapnic voluntary hyperventilation, a surrogate exercise challenge that involves rapid breathing. The post-challenge FEV_1 changes were similar for fish oil and montelukast in these adult subjects with asthma and EIB. Thus, fish oil is an effective means of reducing EIB.

Docosahexaenoic Acid (DHA)

There is no consensus on which component of fish oil, EPA or DHA, is the more potent contributor to the positive effects seen with supplementation (86). Knowing this would allow for the optimization of the fish oil formula for clinical and research purposes. To date, studies comparing EPA and DHA have focused on markers of inflammation and immune function, not airway responsiveness. Results from these comparative studies, which include *in vivo* studies in humans and mice as well as *in vitro* studies on human macrophage cells, do not agree as to which omega-3 PUFA is more potent (47, 64, 86, 98). Kew et al. (47) compared the effect of chronic supplementation with either EPA-rich fish oil, DHA- rich fish oil, or placebo on immune function in healthy, non-asthmatic adults. They determined that DHA suppressed T-cell activation while other immune function markers were not affected by either EPA or DHA.

DHA promotes health in many physiological systems, including the central nervous and cardiovascular systems, in addition to alleviating various types of inflammatory diseases (41). The mechanism of action for DHA relieving inflammation is likely through its metabolite protectin D1 (figure 1-4) (52). Discovered by Serhan et al. (84), protectins are chemical mediators that actively resolve inflammation by reducing proinflammatory signaling. In this study, novel DHA products were isolated from murine exudates in mice injected with DHA during an inflammatory response. Human microglial cells involved in neural tissue host defense and inflammation were incubated with the novel DHA products; as a result, tumor necrosis factor-α (TNF-α)-induced cytokine

production was inhibited indicating that the novel products were anti-inflammatory. Although there have been no human studies on airway responsiveness following supplementation with DHA alone, Levy et al. (52) analyzed protectin D1 levels in asthmatic patients. They found that compared to three healthy volunteers, four patients having an acute asthma exacerbation had significantly lower levels of protectin D1 in their exhaled breath condensate. Levy et al. (52) also examined protectin D1 in a mouse model for airway hyperresponsiveness. Compared to mice injected with saline, mice injected with protectin D1 30 minutes prior to an aerosol challenge had less bronchoalveolar lavage fluid inflammation as measured by reduced eosinophils, airway mucus, and proinflammatory leukotrienes and prostaglandins. Bronchoconstriction following exposure of the mice to increasing concentrations of inhaled methacholine was also decreased. In these experiments, lung tissue was removed from some mice and homogenized following sensitization and aerosol challenge. When DHA was added *ex vivo*, the protectin D1 concentration increased significantly suggesting that DHA can be converted to protectin D1 by respiratory tissues during airway inflammation. Thus, since respiratory DHA levels are reduced in diseases featuring airway inflammation, such as asthma, increasing DHA levels through supplementation should increase the availability of protectin D1 to alleviate airway inflammation and bronchoconstriction (52).

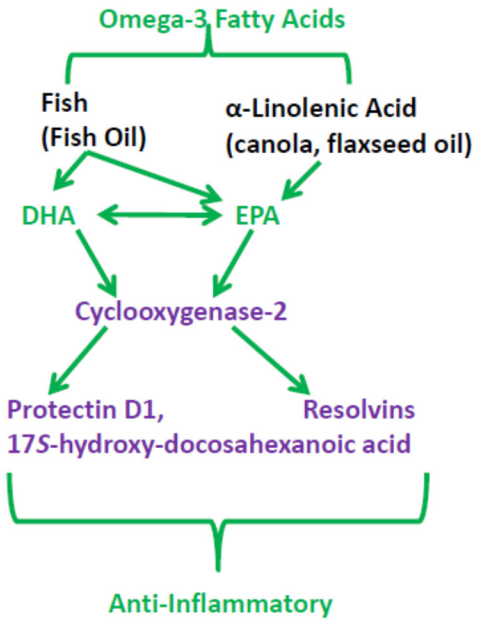

Figure 1-4. ***EPA and DHA produce resolvins and protectins.*** *Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are metabolized by cyclooxygenase-2 to produce resolvins and protectins, rescpectively. These metabolites have anti-inflammatory activity.*

Antioxidants

Reactive oxygen species (ROS) are produced by normal cellular metabolism. They are physiologically important yet toxic to cell structure and function if not properly regulated by antioxidant defenses (35). ROS can cause airway epithelial damage and inflammation; they may have an important role in the pathophysiology of asthma since their production is enhanced in asthmatics (15). Moreover, patients with asthma have been documented to have reduced concentrations of antioxidants such as vitamin C and carotene (66). This imbalance between excess ROS and lack of antioxidants leads to oxidative stress, which occurs with chronic inflammation (35). Oxidative stress in asthma can be resolved by restoring the balance between ROS and antioxidants, either by inhibiting ROS production or by increasing antioxidant availability (80).

Vitamin C is the major antioxidant in the lung's pulmonary protective lining (18). Its antioxidant activity includes direct scavenging of ROS (3). Vitamin C may also affect

arachidonic acid metabolism and the cyclooxygenase pathway. It can change prostaglandin synthesis from the bronchoconstrictor PGF_2 to the bronchodilator PGE_2 (88). Using a 2-week 1500 mg/day protocol, our laboratory showed that vitamin C supplementation reduces exercise-induced airway narrowing and inflammation in asthmatic subjects with EIB (94).

Combination of Nutritional Supplements

Since asthma is known to be a multifaceted disease that often requires multiple medications for optimal management, it is likely that a combination of nutritional supplements will be more effective in alleviating symptoms and protecting against asthma than any one supplement alone (26). For example, ROS are thought to be just one contributor to the development of asthma (80) while it has been shown that the leukotriene pathway only accounts for up to 50-60% of EIB (81). It is thus possible that addressing both of these contributors could lead to better asthma management though nutrition.

Furthermore, there is a possible additive effect with the combination of vitamin C and fish oil as both substances affect arachidonic acid metabolism. ROS cause increased 5-lipoxygenase activity, an enzyme involved in the lipoxygenase pathway of arachidonic acid metabolism; individuals with asthma have enhanced lipid peroxidation in their airways (16). ROS-induced lipid peroxidation of cell membrane phospholipids releases arachidonic acid which subsequently forms proinflammatory prostaglandins and leukotrienes (figure 1-5) (15). The omega-3 PUFAs in fish oil compete with the more proinflammatory omega-6 PUFAs, including arachidonic acid, in the cyclooxygenase and lipoxygenase pathways resulting in the increased production of less proinflammatory prostaglandins and leukotrienes (figure 1-6) (63). Fish oil treatment has been shown to alter cell membrane phospholipid content such that the concentration of arachidonic acid is reduced (61). The effect of the combination of fish oil and antioxidant supplements on

HIB and airway inflammation in adults with asthma has not been studied. Biltagi et al. (17) found that a combination of fish oil, vitamin C, and zinc, which is a cofactor in prostaglandin synthesis, was more effective than any one supplement alone in treating children with moderately persistent asthma. It is important to now study the effect of combining fish oil with another nutritional supplement in adults with asthma as the disease process of childhood asthma is different from that in adults (45).

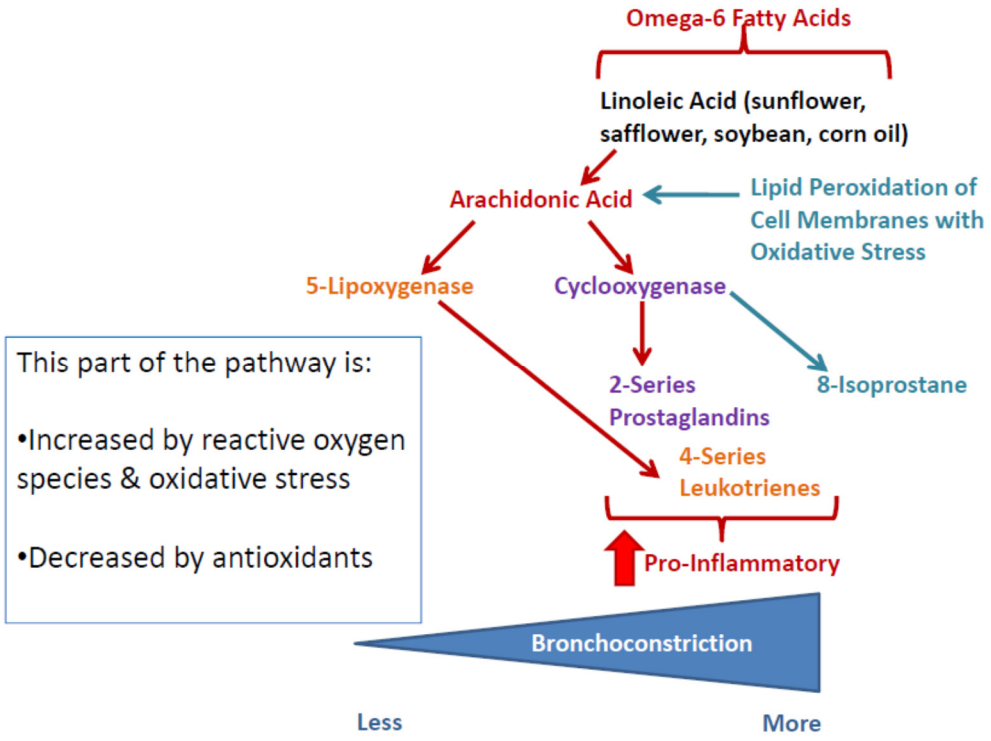

Figure 1-5. ***Site of action for antioxidant supplementation.*** *Reactive oxygen species and oxidative stress increase the lipid peroxidation of cell membranes which results in an increased concentration of arachidonic acid, a precursor for proinflammatory leukotrienes and prostaglandins. Antioxidants, which combat reactive oxygen species and reduce oxidative stress, may decrease this part of the pathway in asthmatics leading to less bronchoconstriction.*

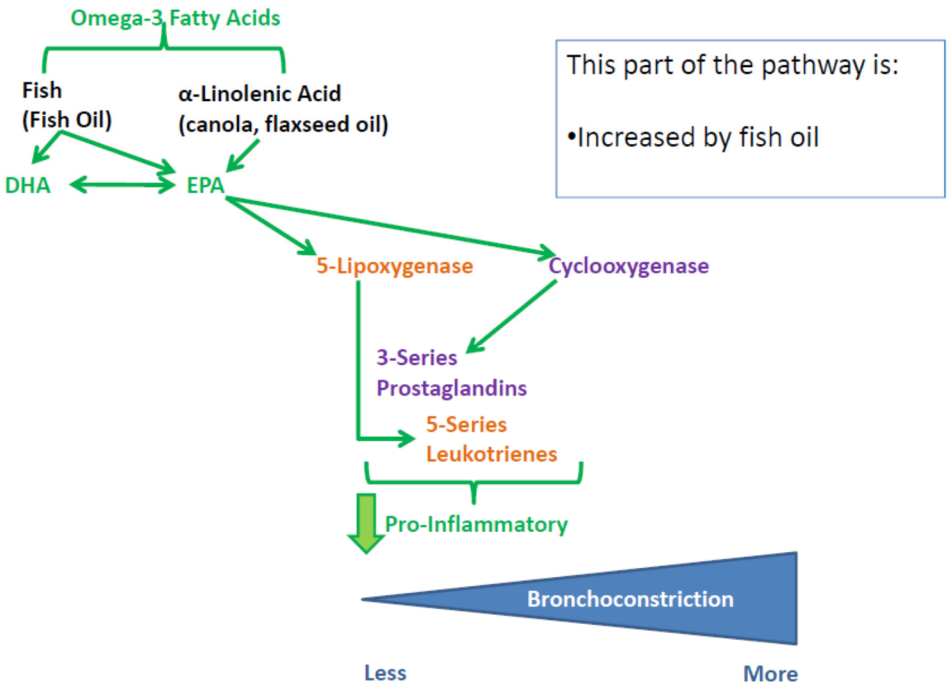

Figure 1-6. ***Site of action for fish oil supplementation.*** *The omega-3 polyunsaturated fatty acids in fish oil produce prostaglandins and leukotrienes that are less proinflammatory than their omega-6 fatty acid counterparts. Fish oil supplementation thus increases this pathway to reduce bronchoconstriction.*

Omega-3 Fatty Acids and Smooth Muscle Contractility

Conflict exists in the current literature regarding the association between the exposure to fish oil or one of its components and smooth muscle contractility. Both vascular and airway smooth muscle have been studied with vascular smooth muscle having received more attention.

Vascular Smooth Muscle

More extensive research has been dedicated to fish oil's effect on vascular smooth muscle due its use in reducing cardiovascular disease. Since vascular and airway smooth muscle tissue differ physiologically, results from these studies cannot be assumed to hold true for airway smooth muscle tissue. Nevertheless, Yanagisawa et al. (101) showed that EPA acutely relaxes pre-contracted rabbit and cat aortic rings in an endothelium-dependent manner. Similarly, Engler et al. (32) showed that EPA acutely relaxes pre-contracted rat aortic rings in a concentration-dependent manner that is

abolished with a cyclooxygenase inhibitor and/or an ATP-sensitive K^+ channel inhibitor. This result suggests that EPA exerts its relaxing effect through the production of K^+ channel-activating prostaglandins. However, these results conflict with their earlier research (30) that showed EPA- and DHA-induced relaxations of pre-contracted rat aortic rings were not affected by cyclooxygenase or lipoxygenase inhibitors. Thus, they had suggested that their action on the vessel wall may be more important than prostaglandin production. Engler et al. (33) also suggested this in an earlier study where DHA relaxed rat aortic rings at baseline tension and after pre-contraction. Because washouts failed to diminish the relaxation response, Engler et al. (33) proposed that DHA may have been incorporated which would increase cell membrane fluidity and change enzyme and receptor activities at the membrane; however, this was not measured.

Airway Smooth Muscle

It has been shown that fish oil diminishes airway inflammation in asthma (64, 98, 105), which can in turn reduce bronchoconstriction; however, its impact on airway smooth muscle is not as well-defined. Although airway inflammation is significant in asthma, airway narrowing is of the utmost concern clinically (44). Thus, determining fish oil's impact on airway smooth muscle contractility is important. Few studies have addressed this issue. Hichami et al. (39) determined that adding free (non-conjugated) DHA to a tissue bath relaxes guinea pig bronchial smooth muscle basal tone whereas adding other diacylglycerols causes contraction. They concluded that the fatty acid structure affects its modulation of airway smooth muscle tone through its activation of protein kinase C and smooth muscle contraction. Interestingly, DHA failed to cause a relaxation in tissue pre-contracted with carbamylcholine, which differs from the Engler et al. (33) study where DHA was able to relax pre-contracted rat aortic rings. Although not a study on fish oil, Abeywardena et al. (4) tested the contractility of airway smooth

muscle obtained from guinea pigs chronically fed a diet rich in olive, canola, or safflower oil. Although lipid analysis of the tissue showed an overall increase in omega-3 PUFAs with the canola oil diet as compared to the other two diets, there was no significant change in airway contractility. However, this does not rule out a possible association between fish oil incorporation and reduced smooth muscle contractility because there was not a significant change in EPA or DHA composition with any of the diets as has been shown in the lung tissue of mice chronically fed fish oil (102). Furthermore, the results of these studies must be interpreted in the context that guinea pig airway smooth muscle basal tone is modulated by local prostaglandin production (74), which itself is known to be affected by fish oil exposure. In a study on human tissue, Morin et al. (20) showed that the EPA metabolite 17(18)-epoxyeicosatetraenoic acid relaxes non-stimulated bronchial smooth muscle tissue that has an initial load applied and following contraction with methacholine; K^+ channels may be involved as this was inhibited with K^+ channel blockers. The results were similar following 48-hour incubation in TNF-α to induce airway hyper-responsiveness. Despite some experiments on tissues incubated with fish oil, Morin et al. (69) did not evaluate omega-3 PUFA incorporation.

The existence of an acute or chronic effect of fish oil or one of its components on airway smooth muscle contractility is thus unclear. Should an association exist, the reason for reduced airway smooth muscle contractility with fish oil may be a decrease in the formation of proinflammatory omega-6 PUFA products as a result of less arachidonic acid content in smooth muscle cell membranes, an increase in omega-3 PUFA content in smooth muscle cell membranes to compete for common enzymes, or an alteration in cell membrane properties, such as fluidity and enzyme function, from increased omega-3 PUFA content.

Summary and Proposed Experimental Aims

Asthma is a chronic disease that may require multiple medications for adequate management (26), and oftentimes, a considerable burden of disease will nevertheless remain unaddressed (27). Importantly, prescription medications account for over a third of the healthcare costs attributed to asthma (1). Consequently, asthmatics have sought out alternative non-pharmacological treatments to replace or supplement their current treatment regimen (95). Nutritional supplements have been investigated as an alternative strategy since changes in diet may be partially responsible for the prevalence of asthma (34).

The vast majority of asthmatics exhibit EIB, with estimates as high as 90% (87). For the most part, EIB has been treated pharmacologically (60). However, several nutritional strategies, such as fish oil or vitamin C supplementation and salt-reduction, have recently been shown to be effective in preventing EIB (61, 62, 93, 94). This shows promise for reducing reliance on asthma medications that may have side effects, show decreased effectiveness with chronic use, or not be allowed for athletic competitions (60).

Despite the encouraging results to date with fish oil, several important questions surrounding fish oil remain unanswered. First, the ability of fish oil to work in conjunction with traditional medications or other nutritional supplements has just begun to be explored (93). Although it has been shown that the combination of fish oil, zinc, and vitamin C is more effective in improving moderate asthma in children than taking fish oil alone (17), the effect of taking fish oil with another nutritional supplement in adults with asthma is unknown. This is important to study because improvement in pulmonary function in asthmatics with EIB beyond that attained with fish oil is physiologically possible. Mickleborough et al. (61) have demonstrated that fish oil supplementation reduced the percent change in FEV_1 to below the diagnostic threshold for EIB, but

further improvement is possible as the normal response to exercise is dilation of the airways such that the post-exercise percent change in FEV_1 is zero or positive. Additionally, it is estimated that blocking the leukotriene pathway, as with fish oil, offers a 50-60% reduction in bronchoconstriction in EIB suggesting that one or more pathways are responsible for the remaining portion (81). Furthermore, since the diverse nature of asthma is better managed with several pharmacologic agents, it is possible that more than one nutritional supplement may be necessary for optimal treatment.

Second, the optimal formula for fish oil has yet to be ascertained. It is not known which omega-3 fatty acid in fish oil is more potent (86). Although Levy et al. (52) have recently demonstrated that the DHA metabolite protectin D1 decreases airway inflammation and bronchoconstriction in a mouse model, treating EIB with DHA supplements has not been attempted in human subjects. Finally, several studies have indicated that fish oil treatment is associated with a reduction in inflammation (64, 98, 105); however, there is not a clear indication in the literature whether fish oil treatment is similarly associated with reduced airway smooth muscle contractility (39, 69), which, along with airway inflammation, is largely responsible for the symptoms of asthma. Further research on fish oil and airway smooth muscle contractility is thus necessary.

Our objective is to determine the effects of novel nutritional strategies on hyperpnea-induced bronchoconstriction (HIB) and airway inflammation in asthmatic individuals. HIB uses rapid breathing to identify EIB in a research or clinical setting (9). We will also explore airway smooth muscle as a target of fish oil's action to explain its effectiveness as a therapeutic agent. The *central hypothesis* is that nutritional supplementation with omega-3 polyunsaturated fatty acids effectively controls HIB and airway inflammation. The *secondary hypothesis* is that omega-3 fatty acid treatment involves reduced arachidonic acid content and increased EPA and DHA content in smooth muscle cell membranes with an associated decrease in the airway smooth

muscle responsiveness to a contractile agonist. These hypotheses will be tested in studies investigating the following specific aims (figure 1-7):

1. **Determine the effect of fish oil and antioxidant supplementation and their combination on hyperpnea-induced bronchoconstriction (HIB) and airway inflammation in asthmatic individuals.** Previous work has shown that fish oil and the antioxidant vitamin C individually reduce EIB (59, 61, 94). It is *hypothesized* that fish oil and vitamin C supplementation taken in isolation will be effective in attenuating HIB and airway inflammation and that the two treatments combined will confer even greater protection than either intervention alone.
2. **Determine the effects of docosahexaenoic acid (DHA), a component of fish oil, on hyperpnea-induced bronchoconstriction (HIB) and airway inflammation in asthmatic individuals.** The DHA component of fish oil may effectively manage asthma and prevent HIB as suggested by an *in vitro* study where pure DHA was more potent than eicosapentaenoic acid (EPA) in relieving inflammation (98). It is *hypothesized* that DHA supplementation will diminish HIB and airway inflammation as compared to placebo.
3. **Determine whether fish oil is associated with a reduction in the contractility of canine tracheal smooth muscle.** Asthma is characterized by airway narrowing due to airway smooth muscle contraction. Research on fish oil and airway smooth muscle tissue contractility is limited. It is *hypothesized* that fish oil treatment of tracheal smooth muscle tissue will be associated with a decrease in its contractility.

The proposed research builds upon our previous findings. Our laboratory has shown that fish oil and vitamin C supplementation each effectively alleviate EIB. Despite improvements in pulmonary function with these supplements, there is still unaddressed disease that may be minimized with a combination of the supplements. Furthermore,

since it has been shown that fish oil is an effective treatment for EIB, we will begin to determine the optimal formula for fish oil by supplementing with pure DHA only. Lastly, exploring the effect of fish oil incubation on airway smooth muscle contractility will be a step toward reducing the current confusion on this matter in the literature. This project is a key step in the practice of evidence-based medicine since it will provide scientific support for using fish oil to treat asthma and will attempt to provide a rationale for its effectiveness. The combination treatment study will afford answers about complementary mechanisms involved in the development of HIB. Overall, a major benefit of this research will be that it will continue the effort to offer alternative treatment options to traditional asthma medications.

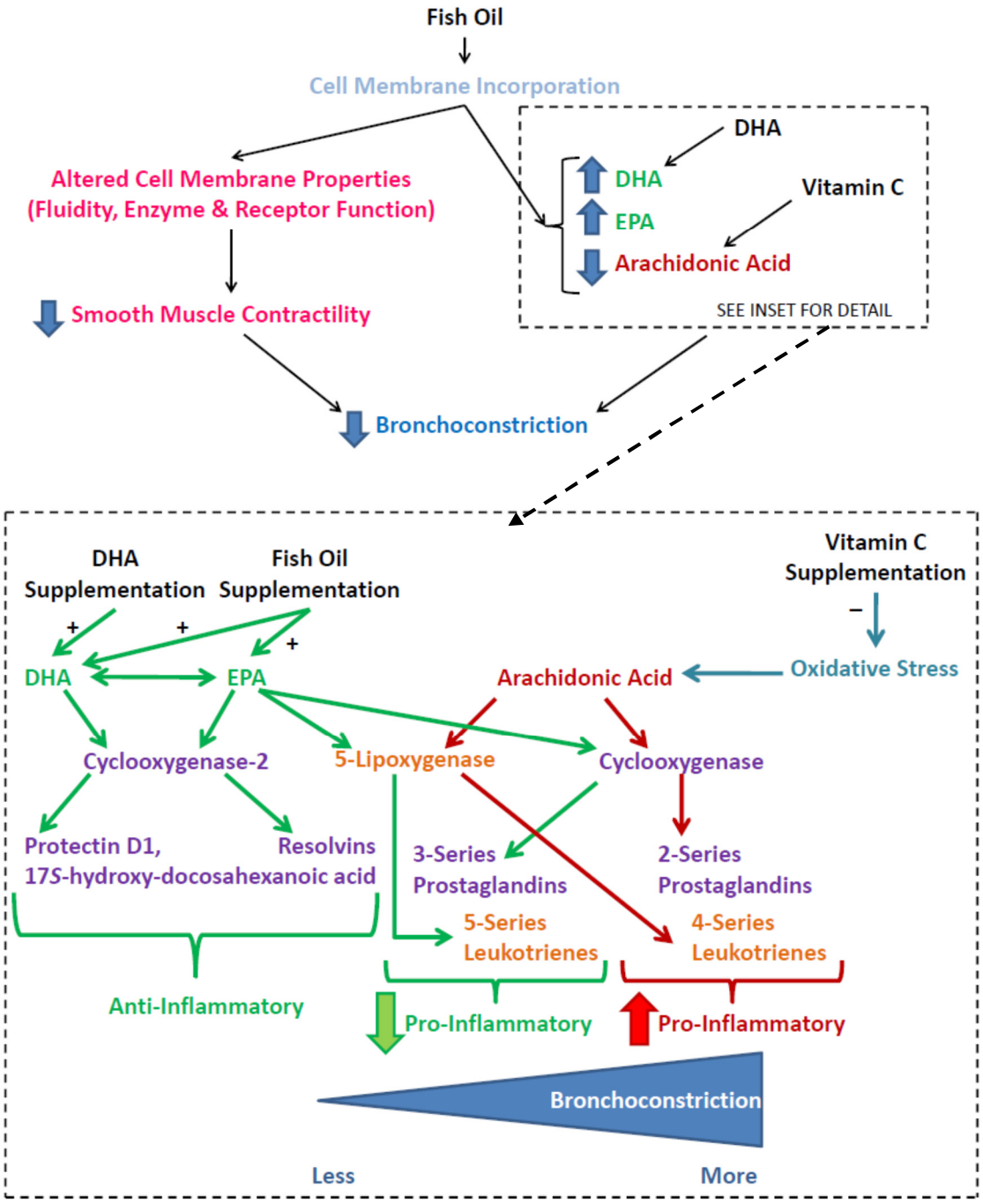

Figure 1-7. ***Proposed mechanism of how omega-3 fatty acids reduce airway inflammation and constriction in hyperpnea-induced bronchoconstriction.*** *We propose that omega-3 fatty acids in the form of fish oil or pure DHA reduce bronchoconstriction through associated decreases in inflammation and smooth muscle contractility. The proposed mechanism also indicates where vitamin C may act to have an additive effect with fish oil.*

CHAPTER 2

THE EFFECT OF FISH OIL, VITAMIN C, AND THEIR COMBINATION ON HYPERPNEA-INDUCED BRONCHOCONSTRICTION IN ADULTS WITH ASTHMA

Abstract

Background: Asthma is a multi-faceted disease that often includes exercise-induced bronchoconstriction (EIB). Hyperpnea-induced bronchoconstriction (HIB) has been shown to reliably detect EIB. Previous research has demonstrated that individual nutritional supplements, such as fish oil and vitamin C, alleviate EIB.

Purpose: Determine whether the combination of fish oil and vitamin C supplementation offers increased protection against HIB over either one alone.

Methods: Fourteen subjects (18 to 29 years) with asthma and HIB participated in a randomized, double-blind, parallel group trial consisting of two treatment periods in which subjects first received either active fish oil (n = 7) or vitamin C supplements (n = 7) for 3 weeks. After a 2-week washout period, all subjects received both active fish oil and vitamin C for 3 weeks. Subjects visited the laboratory following an initial 2-week run-in phase and after each supplementation phase for a total of three visits. Bronchoprovocation was elicited with eucapnic voluntary hyperventilation (EVH), a surrogate exercise challenge involving rapid breathing (hyperpnea). Pulmonary function, fraction of exhaled nitric oxide (F_ENO), and exhaled breath condensate pH were measured pre- and post-EVH. Subjects recorded daily symptoms, peak expiratory flow, and bronchodilator use throughout the study.

Results: Post-EVH pulmonary function measures significantly improved with fish oil and the combination treatment of fish oil and vitamin C while airway inflammation was affected by vitamin C supplementation as well as by the combination treatment of fish oil and vitamin C.

Conclusions: Although previous research has shown that either fish oil or vitamin C can reduce bronchoconstriction and airway inflammation on their own, the variable responses obtained in this study suggest that for subjects not reaping the full benefits from either supplement alone, a combination of fish oil and vitamin C may be more effective.

Introduction

Nearly 25 million Americans have asthma, a chronic inflammatory disease featuring airway inflammation, hyperresponsiveness, and narrowing (6, 90). A complication of this disease that affects up to 90% of asthmatics is exercise-induced bronchoconstriction (EIB) (87). EIB is specifically defined as a 10% or greater decrease in a person's forced expiratory volume in one second (FEV_1) following a bout of exercise lasting at least five minutes (12, 87).

Because EIB suggests that an individual's asthma is not being adequately managed, it is important to test for clinically to evaluate the effectiveness of asthma therapies (49). Rapid breathing, or hyperpnea, can be used in place of exercise to provoke bronchoconstriction; the eucapnic voluntary hyperventilation (EVH) challenge is currently recommended by the International Olympic Committee to identify athletes with EIB (9). Moreover, since it has been previously shown that the changes in FEV_1 following the EVH challenge are comparable to those seen following cold air exercise, hyperpnea-induced bronchoconstriction (HIB) is a reliable indicator of EIB (81).

Traditional pharmacologic approaches to managing asthma do not always provide optimal protection (27). This may be due to patients' variable responses to medications or side effects of the drugs, including reduced efficacy with chronic use. Thus, patients may seek out alternatives to asthma medications. Since it has been suggested that the prevalence of asthma is related to dietary factors such as a high ratio

of omega-6:omega-3 polyunsaturated fatty acids (PUFAs) or a low antioxidant intake, numerous nutritional strategies have been tried (41, 66).

Of these nutritional strategies, fish oil supplementation is a promising approach. Fish oil is composed of the omega-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It is thought that fish oil reduces bronchoconstriction by competing with the proinflammatory omega-6 PUFA pathway (60). Both omega-6 and omega-3 PUFAs produce leukotrienes and prostaglandins via the 5-lipoxygenase and cyclooxygenase enzymes, respectively; however, the omega-3 products are less proinflammatory than the omega-6 products and thus cause less bronchoconstriction (60). Fish oil supplementation has been shown to effectively reduce EIB in elite athletes without concurrent asthma (62) as well as in adults with asthma (61). It has also been determined that fish oil and the leukotriene receptor antagonist montelukast (Singulair®) each prevent HIB to a similar extent (93).

As it has been shown that adults with asthma have lower levels of plasma ascorbic acid (vitamin C) compared to healthy, non-asthmatic adults (66), vitamin C supplementation has been tried as another nutritional approach to managing asthma. The mechanism supporting vitamin C supplementation is related to its antioxidant role. Asthmatics have enhanced production of reactive oxidant species (ROS), which can damage airway epithelium and cause airway inflammation (14, 35). To reduce this oxidative stress, increased concentrations of antioxidants are needed to regulate the ROS (38). In a recent meta-analysis investigating vitamin C as a treatment for asthma, Kaur et al. (46) determined that vitamin C has demonstrated only limited effectiveness although this may be due to the incongruent protocols for supplementation and bronchoprovocation among the current literature. Nevertheless, Tecklenburg et al. (94) showed that a two-week, 1500 mg/day regimen of pharmaceutical grade vitamin C

significantly reduced exercise-induced narrowing and inflammation in adults with asthma and EIB.

Given that asthma is known to be a multi-faceted disease and that pharmacological treatment often includes multiple medications, it is likely that a nutritional approach incorporating multiple nutrients will be more effective than any one nutritional supplement alone (26). In addition to simultaneously addressing different aspects of the disease, combining nutritional supplements may have an additive effect as well. Specifically, fish oil and vitamin C both affect arachidonic acid metabolism, which is a component of the omega-6 PUFA pathway. As previously mentioned, the omega-3 PUFAs EPA and DHA in fish oil compete with the omega-6 PUFA arachidonic acid for common enzymes to produce mediators with less proinflammatory activity (63). Furthermore, it had been demonstrated that fish oil supplementation increases the concentration of EPA and DHA in the cell membrane phospholipid bi-layer while reducing that of arachidonic acid (61). This is important as it has been shown that asthmatics have increased ROS-induced lipid peroxidation of cell membranes in their airways (16). Thus, by reducing ROS activity via an increase in the antioxidant concentration with vitamin C supplementation while decreasing the availability of arachidonic acid-derived mediators through an alteration of the cell membrane content and through competition for enzymes with fish oil supplementation, vitamin C and fish oil may work together to alleviate asthma.

The purpose of this study is to determine whether the combination of fish oil and vitamin C supplementation offers increased protection against HIB over either one alone. We will test the hypothesis that fish oil and vitamin C supplementation taken in isolation will be effective in the attenuation of HIB and airway inflammation and that the two treatments combined will confer even greater protection.

Methods

Subjects. Fourteen subjects (8 male, 6 female) between the ages of 18-29 years with both physician-diagnosed asthma and hyperpnea-induced bronchoconstriction (HIB) were recruited from a university and community setting. Subjects were evaluated for HIB at the first laboratory test in order to only include mild to moderate asthmatics. This was determined by a resting forced expiratory volume in one second (FEV_1) greater than 60% of the predicted value based on age, height, weight, and sex (76) as well as at least a 10% decrease in the FEV_1 following a surrogate exercise challenge used to diagnose EIB (9). Subjects were not allowed to take asthma maintenance medications during the study other than their prescribed short-acting β_2-agonists (e.g. albuterol) to be used *ad libitum* except for six hours prior to reporting to the laboratory for testing. One subject stopped taking ADVAIR DISKUS® (fluticasone propionate and salmeterol) with his doctor's written permission for four weeks before starting the study (61); no other subjects were taking asthma maintenance medications at the time of enrollment. Furthermore, subjects could not be taking nutritional supplements containing vitamin C or fish oil at the time of enrollment or during the study. Subjects were instructed to limit their fish consumption to one meal per week and to avoid vitamin C rich foods for the duration of the study. Exclusion criteria for the study included pregnancy or a history of diabetes, hypertension, hyperlipidemia, bleeding disorders, delayed clotting time, or seizures.

This investigation was approved by the Indiana University Institutional Review Board (protocol # 0910000751) and was registered as a clinical trial with clinicaltrials.gov (study # NT01057615). Informed consent was obtained from all subjects prior to their enrollment. A control group of healthy, non-asthmatic subjects was not included in this study design as it has previously been reported that fish oil supplementation does not

significantly affect pulmonary function or inflammatory mediators in individuals without asthma or EIB (62).

Study Design. This study (figure 2-1) was conducted as a randomized, double-blind, parallel group trial consisting of two treatment periods in which subjects first received either active fish oil or ascorbic acid for 3 weeks. After a 2-week washout period, all subjects received both active fish oil and ascorbic acid for 3 weeks. Subjects came to the laboratory for testing following a 2-week run-in phase at the beginning of the study and after each supplementation phase for a total of three visits.

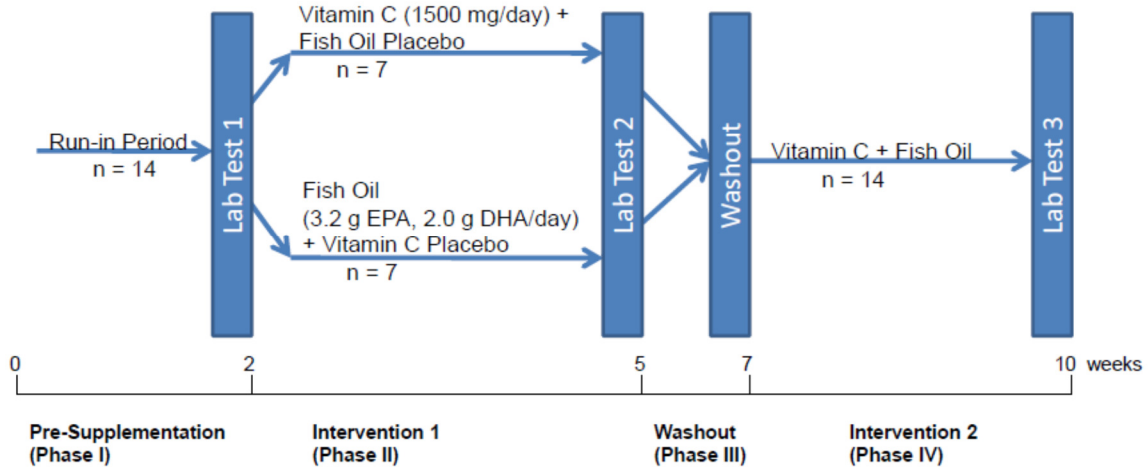

Figure 2-1. ***Schematic of study design.*** *Subjects entered the study on their normal diet. Following a 2-week run-in period, subjects completed a placebo-controlled parallel group trial consisting of two 3-week supplementation periods separated by a 2-week washout phase. Subjects were first supplemented with either 1500 mg of vitamin C or fish oil composed of 3.2 g eicosapentaenoic acid (EPA) and 2.0 g docosahexaenoic acid (DHA). All subjects received both vitamin C and fish oil for the second supplementation period.*

Subjects entered the study on their normal diet and were randomly divided, but matched by sex, into one of two groups. Following their first laboratory test, subjects received either [1] vitamin C capsules containing a total of 1500 mg of pharmaceutical grade ascorbic acid (NOW Foods, Bloomingdale, IL) per day and placebo fish oil capsules containing soybean oil (n = 7) or [2] placebo vitamin C capsules containing

sucrose and active fish oil capsules (Nordic Naturals, Watsonville, CA) containing a total of 3.2 g EPA and 2.0 g DHA per day (n = 7). Subjects were supplemented for 3 weeks (61, 62) and then returned to the laboratory for a second test. Subjects then underwent a 2-week washout period where they did not take any capsules (61, 62). After the washout, all subjects (n = 14) took both active vitamin C and fish oil for 3 weeks. At the end of this treatment phase, all subjects returned to the laboratory for testing.

Subjects were not aware of when they received placebo treatments. An independent investigator having no contact with subjects and no involvement in data collection or analysis used a computerized random number generator (http://www.randomizer.org/form.htm) to create the randomization sequence which was stratified by sex with a 1:1 allocation using a fixed random blocks size of two. The active fish oil and ascorbic acid capsules were identical in appearance to their respective placebo counterpart. Identical packages of capsules were numbered by the independent investigator. After the principal investigator obtained the subject's consent, the independent investigator was asked to provide the allocation assignment for the subject. A list of subject number and randomized allocation number was kept confidential by the independent investigator and only revealed to the principal investigator after data collection and initial data analysis were completed.
Subjects reported to the laboratory having abstained from exercising for 24 hours, having caffeine for 8 hours, and using their short-acting β_2-agonist for 6 hours (93). They performed the same tests during each visit. Bronchoprovocation was elicited with eucapnic voluntary hyperventilation (EVH), a surrogate exercise challenge. Prior to and following EVH, markers of inflammation and pulmonary function were measured. In between laboratory visits, each subject was instructed to record his or her daily peak expiratory flow, short-acting β_2-agonist (albuterol) usage, and symptoms in log books submitted at each visit. Two 24-hour dietary recalls were conducted during each phase

of the study to gauge any changes in nutrient intake over the course of the study. Compliance with supplementation was assessed by counting the pills returned by the subjects at their second and third laboratory tests.

Eucapnic Voluntary Hyperventilation. Bronchoprovocation was provided by the eucapnic voluntary hyperventilation (EVH) challenge, which requires subjects to breathe cold, dry air at a rapid rate. While wearing nose clips, subjects were asked to breathe through a non-rebreathing two-way valve (Hans Rudolph, Inc., Kansas City, MO) connected to a reservoir bag continually filled with 21% oxygen, 5% carbon dioxide, and balance nitrogen from a compressed gas tank containing less than 3 mg $H_2O.L^{-1}$ air (93). Subjects were instructed to breathe for 6 minutes at 85% of their maximal voluntary ventilation as estimated by thirty times their resting FEV_1 (9). In order to verify the ventilatory rate, a flow sensor measured ventilation (Vmax 22 Metabolic Measurement Cart, SensorMedics, Yorba Linda, CA) (81).

Pulmonary Function Tests. Pulmonary function was measured pre-EVH and post-EVH at 5, 10, 15, and 20 minutes using a calibrated computerized pneumotachograph spirometer (Vmax 22 Metabolic Measurement Cart, SensorMedics, Yorba Linda, CA) (93). In accordance with American Thoracic Society (ATS) recommendations, each subject performed three acceptable spirograms, of which the largest and second largest forced vital capacity (FVC) and FEV_1 values did not vary by more than 0.15 L (65). The largest value of each was recorded. Values for forced expiratory flow at 25-75% of the FVC ($FEF_{25-75\%}$) were recorded from the trial with the greatest sum of FVC and FEV_1 (65). The bronchoconstrictor response to EVH was also determined as the area under the curve of the percentage fall in post-exercise FEV_1 plotted against time for 20 minutes, using trapezoidal integration (93).

Fraction of Exhaled Nitric Oxide. Before and 30 minutes following EVH challenge, online measurement of the fraction of exhaled nitric oxide (F_ENO) was

recorded using a restricted exhaled breath protocol (NOA 280i Nitric Oxide Analyzer, Accurate NO Breath Kit, Thermal Mass Flowmeter, NO Analysis Software Version 3.21, Sievers Instruments, Boulder, CO) (93). American Thoracic Society guidelines were followed (2). Accordingly, subjects were instructed to inhale maximally to their total lung capacity and immediately exhale against expiratory resistance at a rate of 50 ± 10 mL/s for at least 6 seconds to produce a nitric oxide plateau lasting at least 3 seconds; real time feedback was provided visually on a computer screen. Subjects performed this maneuver while wearing nose clips with at least 30 seconds of rest between each trial (93). The F_ENO was recorded as the mean of three exhalations with the individual F_ENO values within 10% of the mean F_ENO (2).

Exhaled Breath Condensate. Exhaled breath condensate (EBC) was collected pre-EVH and post-EVH at 0-10 minutes (93) according to American Thoracic Society and European Respiratory Society recommendations (42). Subjects were instructed to breathe normally into a non-rebreathing valve attached to a condensing chamber (ECoScreen, Viasys Healthcare-Jaeger, Germany) for 10 minutes while wearing nose clips (93). This condensing chamber maintains an internal temperature of -20 ˚C to immediately freeze the exhaled breath sample during collection (93). The pH of the non-deaerated EBC was measured within 5 minutes of collection (Orion 2 Star pH benchtop meter, ROSS™ Glass Combination Micro pH electrode, Thermo Fisher Scientific, Inc., Beverly, MA).

Symptoms and Short-Acting β_2-Agonist Usage. Subjects were instructed to rate their symptoms by filling out a symptom diary every day throughout the study. This diary adapted from Santanello et al. (83) contained four questions about daytime symptoms with a seven point scale and one question about nighttime symptoms with a four point scale. Symptom scores were calculated for each subject by averaging the mean score from each day (83).

Subjects recorded their short-acting β_2-agonist use in log books provided to them. Subjects were instructed to mark down the number of puffs taken per day throughout the study.

Peak Flow Measurements. Electronic peak flow meters (PiKo-1, Ferraris Medical, Louisville, CO) were given to subjects to measure their morning and evening peak expiratory flow throughout the study. Subjects were instructed to perform the maneuver by inhaling fully to their total lung capacity and then exhaling forcefully through the flow meter according to manufacturer instructions. Subjects were instructed to record the best of three trials upon waking and before going to bed.

Nutrient Intake. To evaluate nutrient intake during the study, 24-hour dietary recalls were conducted for each subject using the nutrition data system for research (NDSR) dietary assessment computer program from the University of Minnesota. Subjects were called twice during each phase of the study to be interviewed about what food and beverages they consumed in the previous 24-hour period. These calls were made unannounced so that subjects could not change their eating habits in anticipation of the interview.

Data Analysis. A power analysis of data collected by our laboratory was used to determine the number of subjects. In separate studies, our laboratory tested pulmonary function in asthmatics receiving fish oil or ascorbic acid supplements (93, 94). Based on the maximum percent drop in FEV_1 following an exercise challenge in these subjects, it was determined with the aid of the G*Power3.0.5 program (Universität Kiel, Germany) that a minimum of seven subjects per group would be needed to show a significant difference between baseline and post-supplementation pulmonary function with a power of 0.80. Since it was hypothesized that the combination would be more effective than either alone, it was expected that fewer subjects would be needed to show a significant

difference. Thus, this study should be sufficiently powered with seven subjects per group.

Data was analyzed with SPSS version 18.0 statistical software (SPSS Inc., Chicago, IL). Pairwise comparisons were made using dependent t-tests to assess changes between baseline, each supplement, or the combination of supplements for each group of subjects in the parallel study design. A Bonferroni adjustment was made to the p-value to account for the multiple t-tests. Thus, for this set of analyses, significance was held at $p < 0.016$. This strategy for statistical analysis was undertaken in order to avoid a large type II error wherein the null hypothesis would be accepted when it is actually false; a repeated measures ANOVA with Tukey's post-hoc would be too conservative for the small sample size ($n = 6$ or 7) in this study. An independent t-test was used to determine whether the groups of subjects were significantly different from each other at the pre-supplementation test. Where the groups were not significantly different from each other, the subjects were pooled in order to examine the overall effect of the combination of treatments compared to pre-supplementation values with dependent t-tests. Significance was held at $p < 0.05$ for these analyses. Repeated measures analysis of variance (ANOVA) was used to assess nutrient intake and at-home measurements during the four phases of the study (run-in phase, one treatment phase, washout phase, and combination of treatments phase). Mauchley's test was conducted to determine if sphericity was violated; if it was, a Greenhouse-Geisser adjustment was used. Where a significant F-ratio was found ($p < 0.05$), Tukey's post-hoc test was used to isolate differences in group means. Significance was held at $p < 0.05$. Repeated measures ANOVA with a Tukey's post-hoc was chosen here because the additional measurement under analysis for this set of measurements would increase the number of dependent t-tests to six and thus lower the p-value for significance to

0.008 with a Bonferroni adjustment; this would increase the risk of committing type I error. The data is reported as mean ± standard error of the mean.

Results

Subjects. One subject reported gastrointestinal symptoms with supplementation; these symptoms subsided with increased water consumption. The subjects' measurements at the pre-supplementation laboratory visit were considered their baseline values (table 2-1). There were no significant differences within ($p > 0.016$) or between ($p > 0.05$) the two groups of subjects in terms of resting pulmonary function among the three laboratory tests. However, one subject's post-eucapnic voluntary hyperventilation (EVH) pulmonary function progressively worsened from the pre-supplementation test to the one treatment test to the combination treatment test. Since this was in contrast to the response of the rest of the subjects, this subject was deemed a "non-responder" and the subject's data was removed from analysis. Descriptive statistics of the baseline characteristics were thus recalculated for the remaining 13 subjects who responded positively to treatment (table 2-2). The mean percent predicted values for resting pulmonary function for each group were not significantly different ($p > 0.016$) among the three laboratory tests (tables 2-3, 2-4). A summary of the results for the main dependent variables with means for each group as well as for all 13 subjects encapsulates the treatment effects (tables 2-5, 2-6, 2-7).

Males	8
Females	6
Age, yr (range)	22.1 ± 0.8 (18-29)
Height, m	1.74 ± 0.47
Weight, kg	77.1 ± 20.6
BMI, kg/m^2	24.9 ± 6.7
Morning Peak Flow, L/min	420.0 ± 38.1
Evening Peak Flow, L/min	426.1 ± 37.8
FEV_1/FVC	77.68 ± 2.25
Percent Predicted FVC, %	102.1 ± 3.8
Percent Predicted FEV_1, %	96.6 ± 3.6

Table 2-1. ***Baseline characteristics of the subjects at their first (pre-supplementation) laboratory visit.*** *The values are averages of all the subjects who completed the protocol and are reported as mean ± standard error of the mean. BMI, body mass index; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity*

Males	8
Females	5
Age, yr (range)	22.2 ± 0.9 (18-29)
Height, m	1.75 ± 0.49
Weight, kg	79.3 ± 22.0
BMI, kg/m2	25.4 ± 7.1
Morning Peak Flow, L/min	430.2 ± 39.6
Evening Peak Flow, L/min	435.6 ± 39.5
FEV1/FVC	77.78 ± 2.43
Percent Predicted FVC, %	103.4 ± 3.8
Percent Predicted FEV1, %	97.9 ± 3.6

Table 2-2. ***Baseline characteristics of the "responders" at their first (pre-supplementation) laboratory visit.*** *The values are averages of the 13 subjects who responded positively to treatment and upon whom data analysis was performed. The values are reported as mean ± standard error of the mean. BMI, body mass index; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity*

	Pre-Supplementation	Fish Oil	Fish Oil + Vitamin C
FVC	105.46 ± 7.69	102.70 ± 7.16	104.64 ± 8.21
FEV_1	99.20 ± 6.80	94.93 ± 5.73	99.13 ± 6.82
$FEF_{25-75\%}$	81.93 ± 9.54	76.17 ± 9.37	79.95 ± 10.03

Table 2-3. ***Resting pulmonary function of the subjects in the Fish Oil Group.*** *These subjects (n=6) received fish oil supplements for the first treatment phase. There were no significant differences in the resting pulmonary function values among pre-supplementation, the fish oil treatment, and the combination treatment prior to the eucapnic voluntary hyperventilation challenge. The pulmonary function values are expressed as percentages of the subjects' predicted values based on age, height, weight, and sex. They are reported as mean ± standard error of the mean. FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; $FEF_{25=75\%}$, forced expiratory flow at 25-75% of the FVC*

	Pre-Supplementation	Vitamin C	Fish Oil + Vitamin C
FVC	101.65 ± 3.19	102.96 ± 4.36	101.76 ± 3.79
FEV_1	96.84 ± 3.97	101.82 ± 3.05	100.36 ± 3.03
$FEF_{25-75\%}$	81.99 ± 10.06	91.03 ± 8.72	92.81 ± 8.71

Table 2-4. ***Resting pulmonary function of the subjects in the Vitamin C Group.*** *These subjects (n=7) received vitamin C supplements for the first treatment phase. There were no significant differences in the resting pulmonary function values among pre-supplementation, the vitamin C treatment, and the combination treatment prior to the eucapnic voluntary hyperventilation challenge. The pulmonary function values are expressed as percentages of the subjects' predicted values based on age, height, weight, and sex. They are reported as mean ± standard error of the mean. FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; $FEF_{25=75\%}$, forced expiratory flow at 25-75% of the FVC*

Variable	Pre-Supplementation	Fish Oil	Fish Oil + Vitamin C
Post-EVH max drop FVC (L)	0.53 ± 0.10	0.24 ± 0.05 *	0.25 ± 0.06
Post-EVH max drop FEV_1 (L)	0.72 ± 0.11	0.39 ± 0.08 *	0.43 ± 0.13
Post-EVH max drop $FEF_{25-75\%}$ (L/s)	1.07 ± 0.21	0.58 ± 0.13	0.63 ± 0.13
Post-EVH max % drop FVC	9.79 ± 2.13	4.49 ± 1.03 *	4.43 ± 0.97
Post-EVH max % drop FEV_1	17.29 ± 1.98	9.94 ± 1.90 *	10.19 ± 3.35
Post-EVH max % drop $FEF_{25-75\%}$	28.33 ± 3.33	18.43 ± 4.52	17.75 ± 3.26
AUC FEV_1	202.72 ± 12.18	112.36 ± 30.26	91.21 ± 49.03
Pre-EVH Exhaled Breath Condensate pH	6.92 ± 0.13	7.18 ± 0.11	6.85 ± 0.12
Post-EVH Exhaled Breath Condensate pH	6.85 ± 0.08	7.22 ± 0.08	6.68 ± 0.18
Pre-EVH F_ENO	33.5 ± 7.0	41.4 ± 9.5	31.0 ± 4.7
Post-EVH F_ENO	32.8 ± 8.4	36.1 ± 7.8	27.8 ± 4.9

Table 2-5. ***Summary of the treatment effects in the Fish Oil Group at each laboratory visit.*** *These subjects (n = 6) received fish oil for the first treatment phase and both fish oil and vitamin C for the second treatment phase. Values are reported as mean ± standard error of the mean. FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; $FEF_{25=75\%}$, forced expiratory flow at 25-75% of the FVC; AUC FEV_1, area under the curve of the percent change in FEV_1, FENO, fraction of exhaled nitric oxide; *, significantly different from pre-supplementation*

Variable	Pre-Supplementation	Vitamin C	Fish Oil + Vitamin C
Post-EVH max drop FVC (L)	0.63 ± 0.20	0.56 ± 0.27	0.18 ± 0.08
Post-EVH max drop FEV_1 (L)	0.88 ± 0.19	0.76 ± 0.32	0.34 ± 0.09 *
Post-EVH max drop $FEF_{25-75\%}$ (L/s)	1.29 ± 0.20	1.23 ± 0.39	0.71 ± 0.17 *
Post-EVH max % drop FVC	12.07 ± 2.94	9.98 ± 4.39	3.29 ± 1.50 *
Post-EVH max % drop FEV_1	23.48 ± 4.50	18.03 ± 7.05	8.64 ± 2.14
Post-EVH max % drop $FEF_{25-75\%}$	39.28 ± 6.08	32.73 ± 10.45	18.45 ± 3.73 *
AUC FEV_1	346.99 ± 81.61	232.63 ± 106.22	103.41 ± 36.11
Pre-EVH Exhaled Breath Condensate pH	7.05 ± 0.11	6.94 ± 0.09	6.91 ± 0.08
Post-EVH Exhaled Breath Condensate pH	7.10 ± 0.11	7.06 ± 0.09	7.20 ± 0.10 †
Pre-EVH F_ENO	89.7 ± 44.9	82.7 ± 38.9	66.1 ± 19.6
Post-EVH F_ENO	65.8 ± 26.9	72.6 ± 39.3 †	54.8 ± 18.0 †

Table 2-6. ***Summary of the treatment effects in the Vitamin C Group at each laboratory visit.*** *These subjects (n = 7) received vitamin C for the first treatment phase and both fish oil and vitamin C for the second treatment phase. Values are reported as mean ± standard error of the mean. FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; $FEF_{25=75\%}$, forced expiratory flow at 25-75% of the FVC; AUC FEV_1, area under the curve of the percent change in FEV_1, FENO, fraction of exhaled nitric oxide; *, significantly different from pre-supplementation; †, post-EVH value significantly different from the pre-EVH value at the same laboratory visit*

Variable	Pre-Supplementation	Fish Oil + Vitamin C
Post-EVH max drop FVC (L)	0.58 ± 0.11	0.21 ± 0.05 *
Post-EVH max drop FEV_1 (L)	0.81 ± 0.11	0.38 ± 0.07 *
Post-EVH max drop $FEF_{25-75\%}$ (L/s)	1.19 ± 0.14	0.67 ± 0.10 *
Post-EVH max % drop FVC	11.02 ± 1.82	3.82 ± 0.90 *
Post-EVH max % drop FEV_1	20.62 ± 2.65	9.36 ± 6.68 *
AUC FEV_1	280.39 ± 47.46	97.78 ± 28.57 *
Pre-EVH Exhaled Breath Condensate pH	6.98 ± 0.08	6.88 ± 0.07
Pre-EVH F_ENO	63.7 ± 24.8	49.9 ± 11.5
Post-EVH F_ENO	50.6 ± 15.2	42.3 ± 10.4 †

Table 2-7. ***Summary of the treatment effects for all subjects at the pre-supplement and combination treatment tests.*** *Data from the Fish Oil and Vitamin C groups were pooled for variables in which the subjects were not significantly different from each other (n = 13). Values are reported as mean ± standard error of the mean. FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; $FEF_{25=75\%}$, forced expiratory flow at 25-75% of the FVC; AUC FEV_1, area under the curve of the percent change in FEV_1, FENO, fraction of exhaled nitric oxide; *, significantly different from each other*

Pulmonary Function. At the initial laboratory test, subjects who would receive fish oil during the first supplementation period (Fish Oil Group) showed a mean maximum drop in post-EVH FEV_1 of 0.72 ± 0.11 L or 17.29 ± 1.98% of their resting FEV_1. Subjects who would receive vitamin C during the first supplementation period (Vitamin C Group) showed a mean maximum drop in post-EVH FEV_1 of 0.88 ± 0.19 L or 23.48 ± 4.50% of their resting FEV_1. Since there was no significant difference in the pre-supplementation FEV_1 values between the Fish Oil Group and Vitamin C Group ($p > 0.05$), the subjects were pooled to determine the overall effect of the combination treatment. Thus, the maximum volume and percent changes in FEV_1 were significantly

lower ($p < 0.05$) with the combination treatment (0.42 ± 0.08 L, 11.36 ± 2.63%) as compared to pre-supplementation (0.77 ± 0.11 L, 19.92 ± 2.55%) (figures 2-2, 2-3).

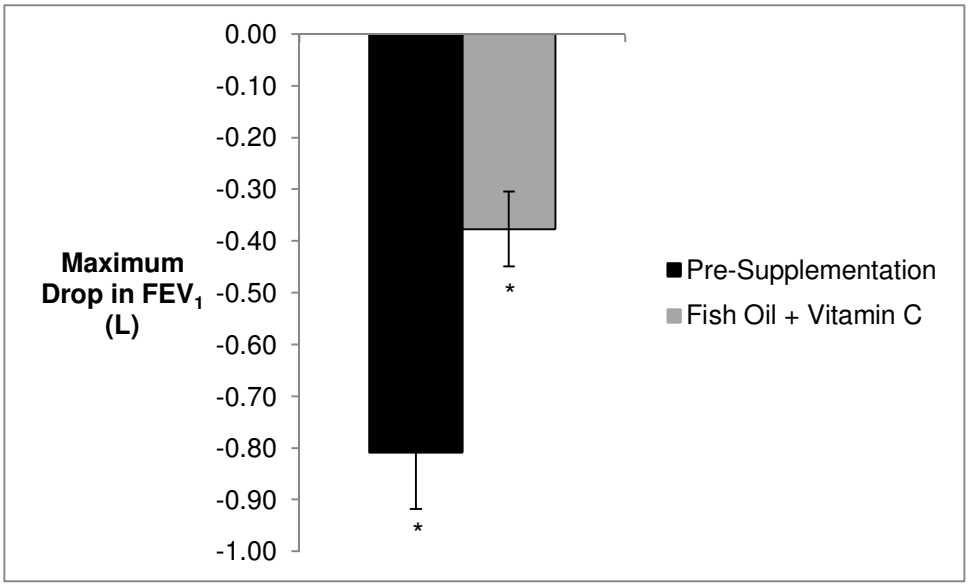

Figure 2-2. ***The maximum drop in FEV_1 volume for all subjects at the pre-supplementation and combination treatment tests following the eucapnic voluntary hyperventilation challenge.*** *The maximum post-challenge change in the volume of the FEV_1 was significantly reduced with the combination treatment. Error bars represent standard error of the mean. FEV_1, forced expiratory volume in one second; *, significantly different from each other*

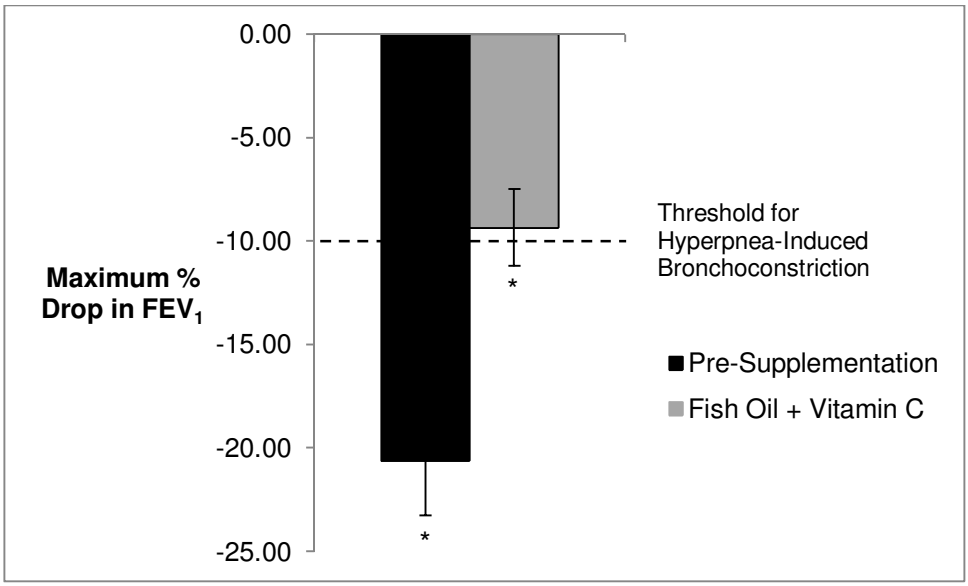

Figure 2-3. ***The maximum percent drop in FEV_1 for all subjects at the pre-supplementation and combination treatment tests following the eucapnic voluntary hyperventilation challenge.*** *The maximum post-challenge percent change in FEV_1 was significantly reduced with the combination treatment. Error bars represent standard error of the mean. FEV_1, forced expiratory volume in one second; *, significantly different from each other*

When examined in isolation, subjects in the Fish Oil Group demonstrated significant differences ($p < 0.016$) in their FEV_1 values between pre-supplementation and fish oil treatment. The maximum volume and percent changes in FEV_1 (figures 2-4, 2-5) were significantly reduced ($p < 0.016$) with fish oil (0.39 ± 0.07 L, 10.80 ± 1.82%) as compared to pre-supplementation (0.66 ± 0.11 L, 16.36 ±1.92%). However, there were no significant differences ($p > 0.016$) between the combination treatment (0.50 ± 0.13 L, 14.07 ± 4.80%) and pre-supplementation or between the combination treatment and the fish oil treatment.

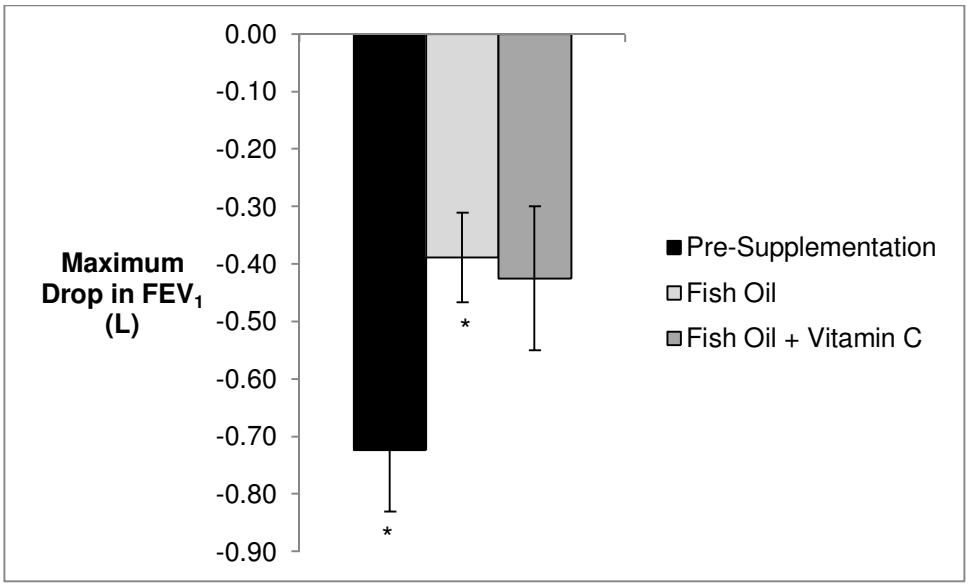

Figure 2-4. ***The maximum drop in FEV_1 volume for the Fish Oil Group at each laboratory test.*** *The maximum drop in the post-eucapnic voluntary hyperventilation challenge was significantly reduced with fish oil supplementation compared to pre-supplementation for these subjects (n = 6). There were no significant changes between pre-supplementation and the combination treatment or between the fish oil treatment and the combination treatment. Error bars represent standard error of the mean. FEV_1, forced expiratory volume in one second; *, significantly different from each other*

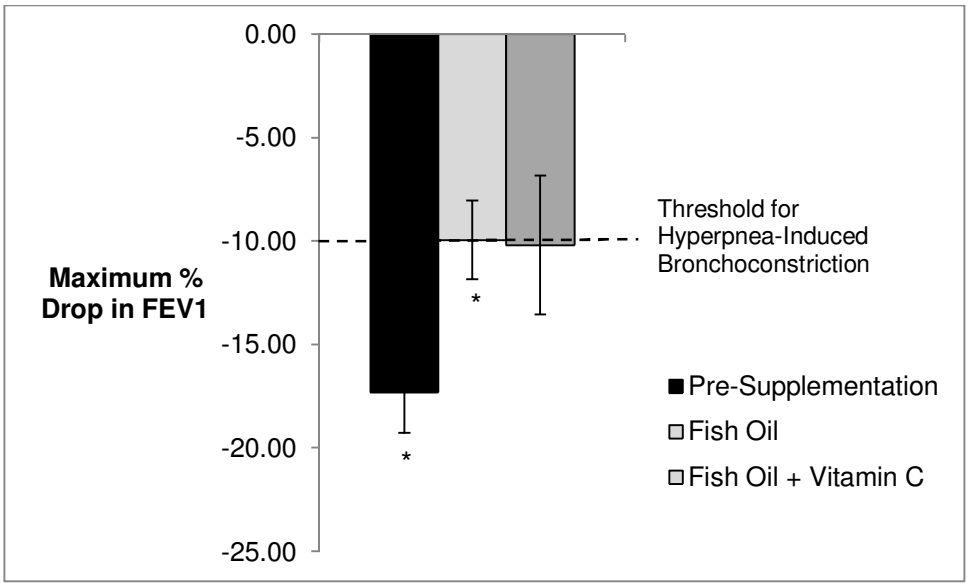

Figure 2-5. ***The maximum percent drop in FEV_1 for the Fish Oil Group at each laboratory test.*** *There was a significant decrease in the maximum percent drop in the post-eucapnic voluntary hyperventilation FEV_1 with the fish oil treatment compared to pre-supplementation (n = 6). There was not a significant change between pre-supplementation and the combination treatment or between the fish oil treatment and the combination treatment. Error bars represent standard error of the mean. FEV_1, forced expiratory volume in one second; *, significantly different from each other*

In contrast, the subjects in the Vitamin C Group only showed a significant change in FEV_1 on the combination treatment as compared to pre-supplementation. The mean maximum drop in post-EVH FEV_1 significantly decreased ($p < 0.016$) with the combination treatment (0.34 ± 0.09 L) compared to pre-supplementation (0.88 ± 0.19 L) (figure 2-6). There was not a significant change ($p > 0.016$) in the maximum percent drop in post-EVH FEV_1 between the combination treatment (8.64 ± 2.14%) and pre-supplementation (23.48 ± 4.50%) (figure 2-7). Also, as a group, there were no significant differences ($p > 0.016$) between the combination treatment and the vitamin C treatment (0.76 ± 0.32 L, 18.03 ± 7.05%) or between the vitamin C treatment and pre-supplementation.

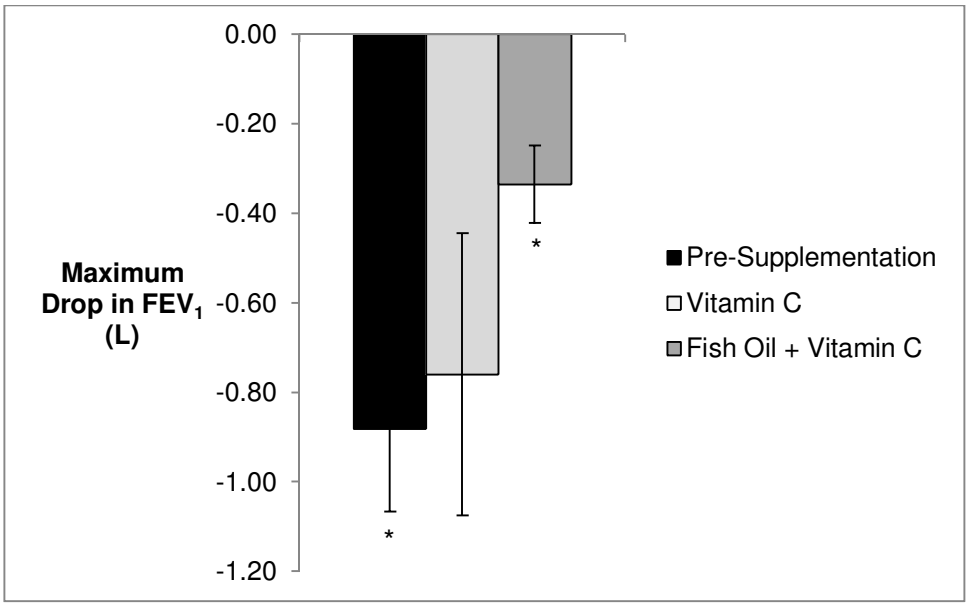

Figure 2-6. ***The maximum drop in FEV_1 volume for the Vitamin C Group at each laboratory test.*** *The maximum drop in the post-eucapnic voluntary hyperventilation challenge was significantly reduced with the combination treatment of fish oil and vitamin C compared to pre-supplementation for these subjects (n = 7). There were no significant changes between pre-supplementation and the vitamin C treatment or between the vitamin C treatment and the combination treatment. Error bars represent standard error of the mean. FEV_1, forced expiratory volume in one second; *, significantly different from each other*

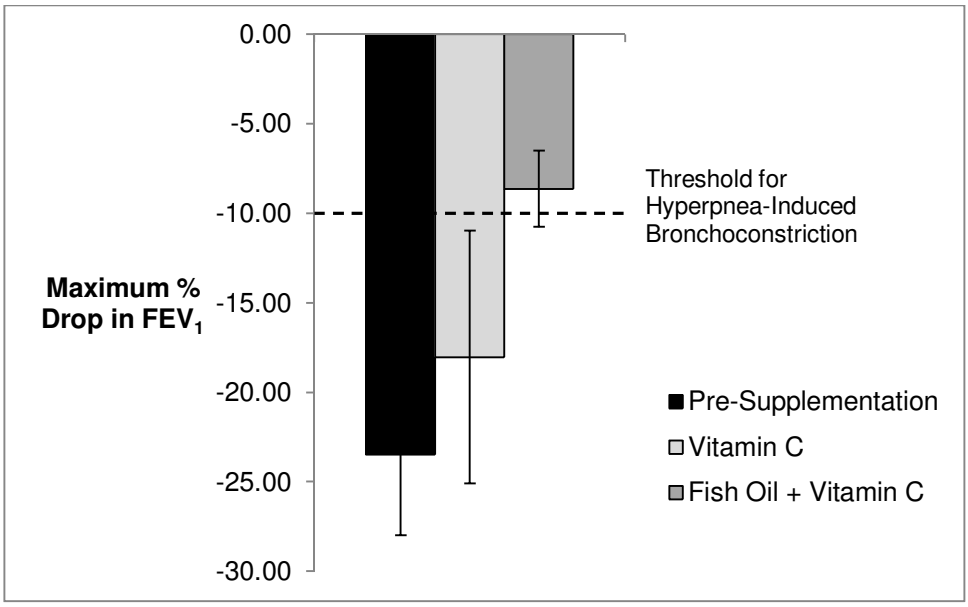

Figure 2-7. ***The maximum drop in FEV_1 volume for the Vitamin C Group at each laboratory test.*** *There was not a significant change in the maximum percent drop in the post-eucapnic voluntary hyperventilation FEV_1 among the pre-supplementation, vitamin C, and the combination treatment (n = 7). Error bars represent standard error of the mean. FEV_1, forced expiratory volume in one second*

Since exercise-induced bronchoconstriction is defined as a post-exercise drop in FEV_1 of at least 10% and most often occurs between 5 to 15 minutes following exercise, it is important to examine the effects of treatment on this response in hyperpnea-induced bronchoconstriction. The Fish Oil Group and Vitamin C Group were not significantly different ($p > 0.05$) from each other in terms of the percent change in FEV_1 at any of the time points; thus, the subjects were pooled to evaluate the overall effect of the combination treatment compared to pre-supplementation. The percent drop in FEV_1 was significantly reduced ($p < 0.05$) with the combination treatment at 5, 10, 15, and 20 minutes post-EVH (figure 2-8). Additionally, the area under the FEV_1 curve for the 20 minutes following EVH ($AUC_{0\text{-}20}$) was significantly reduced ($p < 0.05$) with the combination treatment (97.78 ± 28.57) as compared to pre-supplementation (280.40 ± 47.46) (figure 2-9).

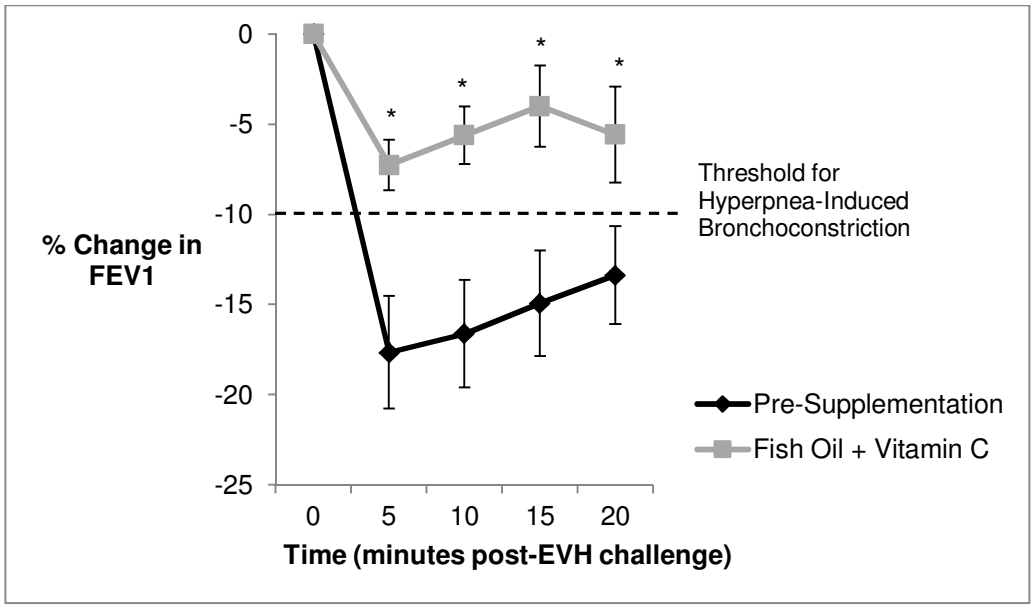

Figure 2-8. ***The mean percent change in FEV_1 volume for all subjects for 20 minutes following the eucapnic voluntary hyperventilation (EVH) challenge.*** *At each of the post-EVH time points tested, the percent change in FEV_1 was significantly lower with the combination treatment compared to pre-supplementation (n = 13). Error bars represent standard error of the mean. FEV_1, forced expiratory volume in one second; *, significantly different from pre-supplementation*

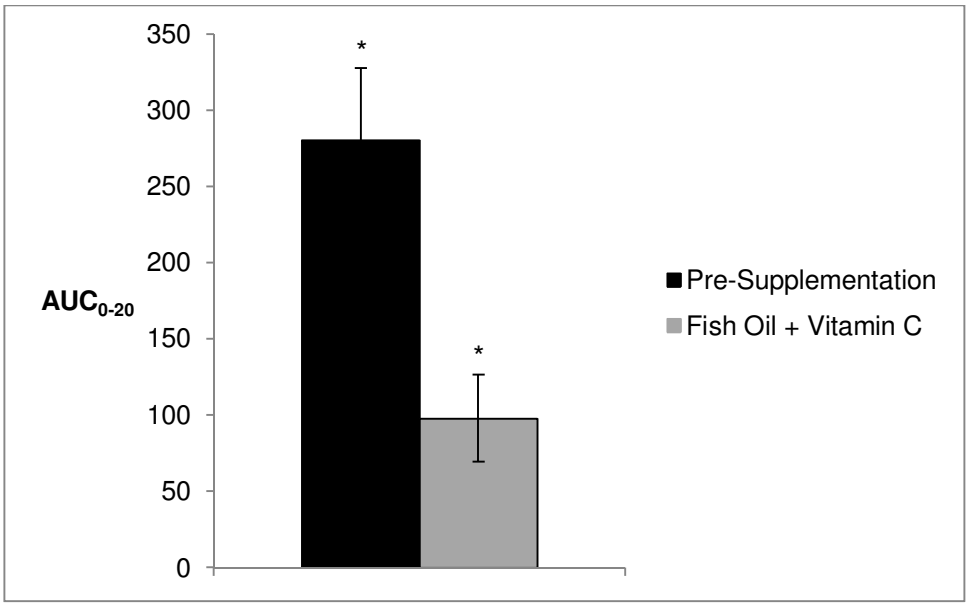

Figure 2-9. ***The area under the FEV_1 curve for the 20 minutes following EVH (AUC_{0-20}) for all subjects.*** *The bronchoconstrictor response, as measured by the AUC_{0-20}, was significantly reduced with the combination treatment compared to pre-supplementation (n = 13). Error bars represent standard error of the mean. FEV_1, forced expiratory volume in one second; *, significantly different from each other*

For the Fish Oil Group, the percent drop in FEV_1 was significantly reduced ($p < 0.016$) at 5 minutes post-EVH with the combination treatment compared to pre-supplementation (figure 2-10). There were no significant differences ($p > 0.016$) in AUC_{0-20} among the pre-supplementation (202.72 ± 12.18), fish oil treatment (112.36 ± 30.26), and combination treatment (91.21 ± 49.03) tests (figure 2-11).

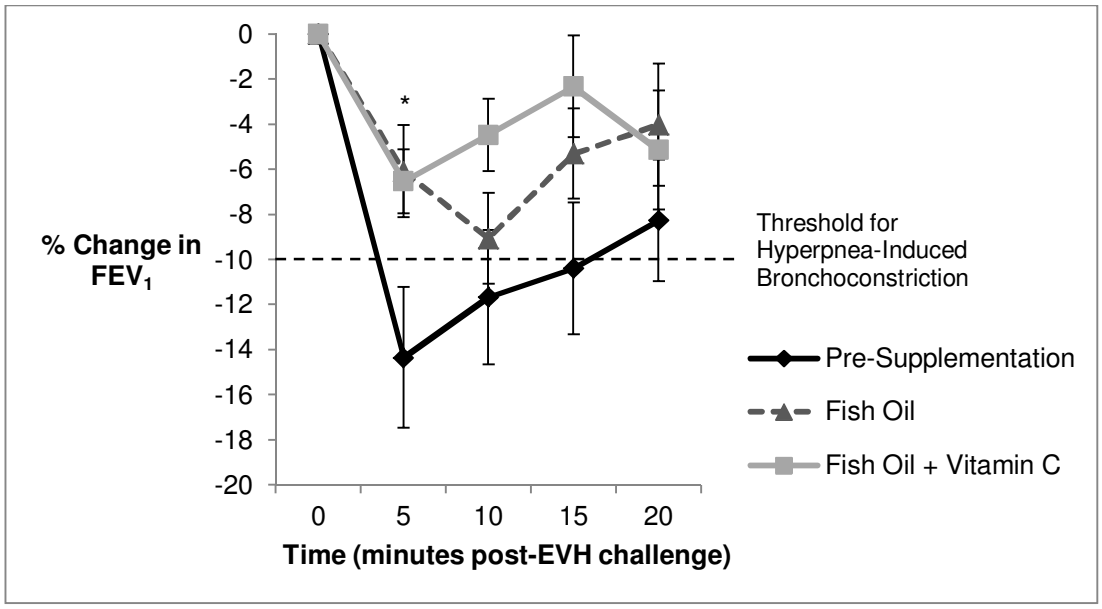

Figure 2-10. ***The mean percent change in FEV_1 volume for the Fish Oil Group for 20 minutes following the eucapnic voluntary hyperventilation (EVH) challenge.*** *At five minutes post-EVH, the percent change in FEV_1 was significantly lower with the combination treatment compared to pre-supplementation (n = 6). There were no other significant differences at any other time points. Error bars represent standard error of the mean. FEV_1, forced expiratory volume in one second; *, significantly different pre-supplementation*

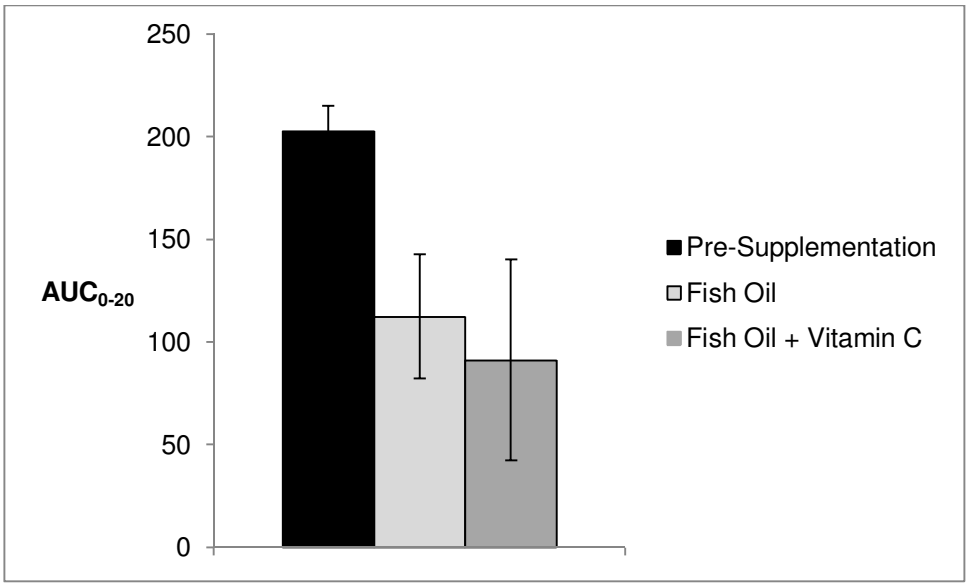

Figure 2-11. ***The area under the FEV_1 curve for the 20 minutes following EVH (AUC_{0-20}) in the Fish Oil Group.*** *There were no significant differences in the AUC_{0-20} among the pre-supplementation, fish oil treatment, and combination treatment tests (n = 6). Error bars express standard error of the mean. FEV_1, forced expiratory volume in one second*

The Vitamin C Group did not demonstrate any significant changes ($p > 0.016$) in the percent drop in FEV_1 at any of the post-EVH time points among the three laboratory tests (figure 2-12). Furthermore, there were no significant changes ($p > 0.016$) in the AUC_{0-20} among the pre-supplementation (346.98 ± 81.61), vitamin C treatment (232.63 ± 106.22), and combination treatment (103.41 ± 36.11) laboratory tests (figure 2-13).

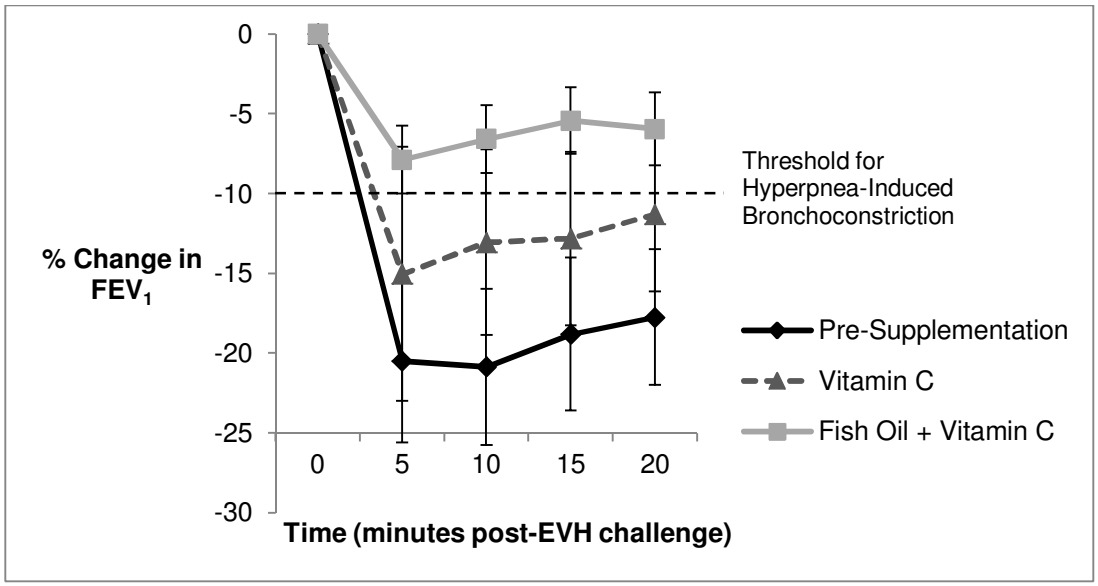

Figure 2-12. ***The mean percent change in FEV_1 volume for the Vitamin C Group for 20 minutes following the eucapnic voluntary hyperventilation (EVH) challenge.*** *There were no significant changes in the post-EVH percent change in FEV_1 at any of the times points tested among the three laboratory visits for these subjects (n = 7). Error bars express standard error of the mean. FEV_1, forced expiratory volume in one second*

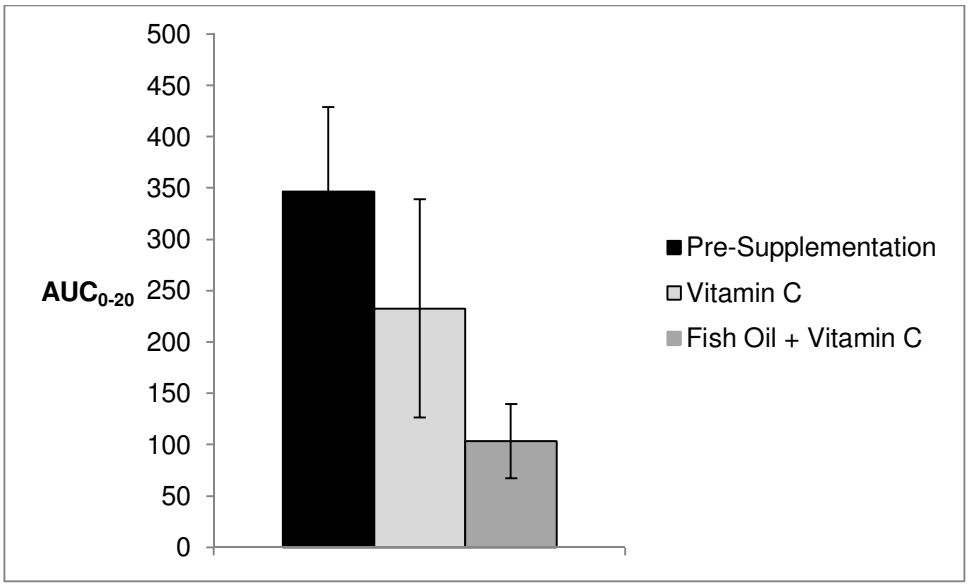

Figure 2-13. ***The area under the FEV_1 curve for the 20 minutes following EVH (AUC_{0-20}) in the Vitamin C Group.*** *There were no significant differences in the AUC_{0-20} among the pre-supplementation, vitamin C, and combination treatment laboratory tests (n = 7). Error bars express standard error of the mean. FEV_1, forced expiratory volume in one second*

At the pre-supplementation lab test, the Fish Oil Group had a mean maximum drop in post-EVH FVC of 0.53 ± 0.10 L or 9.79 ± 2.13% of their resting FVC while the Vitamin C Group had a mean maximum drop in post-EVH FVC of 0.63 ± 0.20 or 12.07 ± 2.94% of their resting FVC. The groups were not significantly different ($p > 0.05$). The groups were thus pooled to determine the effect of the combination treatment on FVC. The maximum volume and percent changes in FVC were significantly lower ($p < 0.05$) with the combination treatment (0.22 ± 0.05 L, 4.11 ± 0.89%) as compared to pre-supplementation (0.55 ± 0.11 L, 10.35 ± 1.81%) (figures 2-14, 2-15).

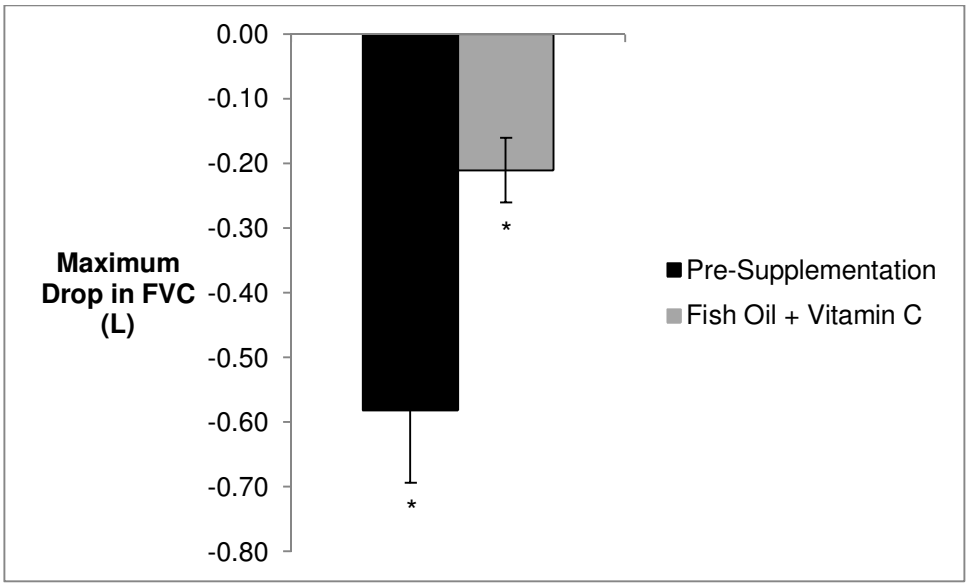

Figure 2-14. ***Maximum drop in FVC following the eucapnic voluntary hyperventilation challenge for all subjects.*** *The maximum post-challenge drop in FVC was significantly reduced with the combination treatment compared to pre-supplementation (n = 13). Error bars express standard error of the mean. FVC, forced vital capacity; *, significantly different from each other*

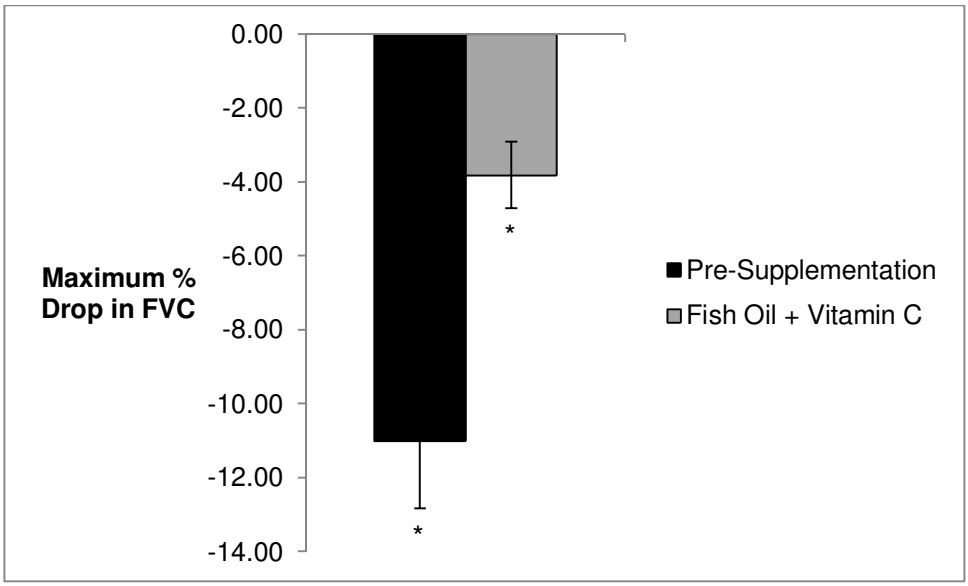

Figure 2-15. ***Maximum percent drop in FVC following the eucapnic voluntary hyperventilation challenge for all subjects.*** *The combination treatment significantly decreased the maximum percent drop in FVC as compared to pre-supplementation (n = 13). Error bars express standard error of the mean. FVC, forced vital capacity; *, significantly different from each other*

The fish oil treatment alone significantly improved the FVC in the Fish Oil Group. After taking fish oil, these subjects demonstrated significant reductions ($p < 0.016$) in both the maximum volume (0.23 ± 0.05 L) and percent changes (4.56 ± 0.88%) in their post-EVH FVC values compared to pre-supplementation (0.46 ± 0.11 L, 8.64 ± 2.14%) (figures 2-16, 2-17). However, there were no significant differences ($p > 0.016$) between the combination treatment (0.25 ±0.05 L, 4.94 ± 0.96%) and pre-supplementation or between the combination treatment and the fish oil treatment.

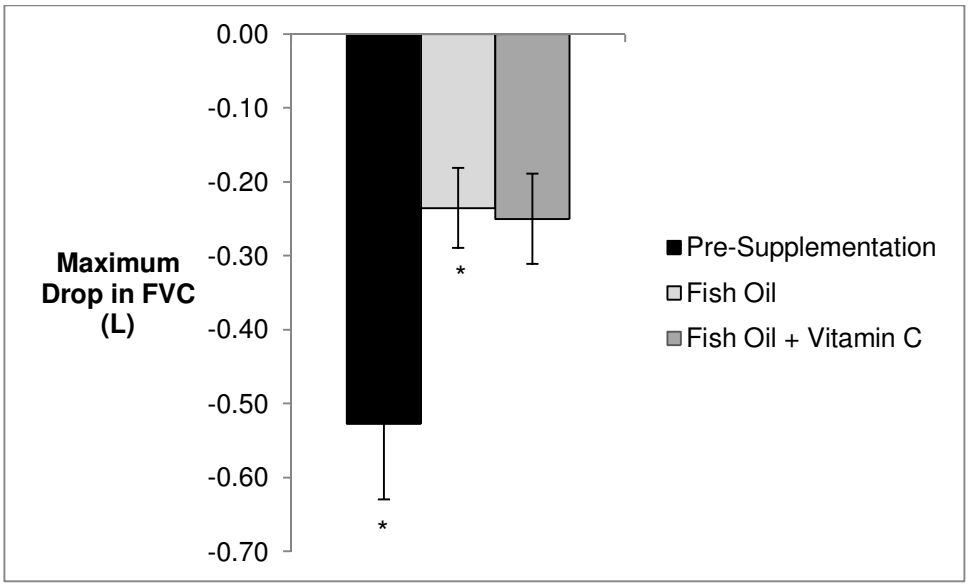

Figure 2-16. ***Maximum drop in FVC following the eucapnic voluntary hyperventilation challenge for the Fish Oil Group.*** *The maximum drop in FVC was significantly reduced with the fish oil treatment compared to pre-supplementation (n = 6). There were no significant differences between pre-supplementation and the combination treatment or between the fish oil treatment and the combination treatment. Error bars express standard error of the mean. FVC, forced vital capacity; *, significantly different from each other*

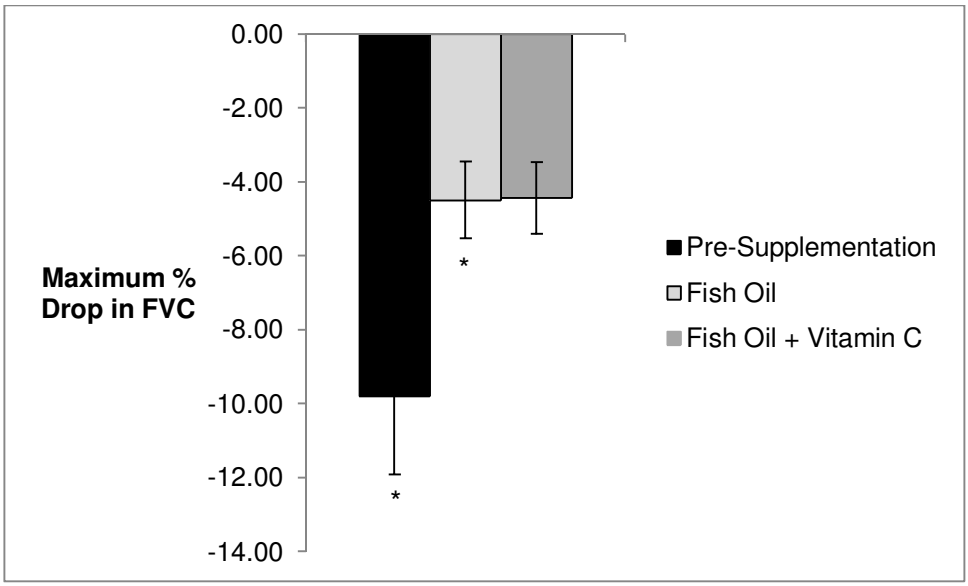

Figure 2-17. ***Maximum percent drop in FVC following the eucapnic voluntary hyperventilation challenge for the Fish Oil Group.*** *The fish oil treatment significantly decreased the maximum drop in FVC as compared to pre-supplementation (n = 6). There were no significant differences between pre-supplementation and the combination treatment or between the fish oil treatment and the combination treatment.Error bars express standard error of the mean. FVC, forced vital capacity; *, significantly different from each other*

Although the vitamin C treatment alone did not significantly alter the FVC of the Vitamin C Group compared to pre-supplementation, the combination treatment was effective. There was not a significant change ($p > 0.016$) in the maximum post-EVH FVC volume with the combination treatment (0.18 ± 0.08 L) compared to pre-supplementation (0.63 ± 0.20 L) or to the vitamin C treatment (0.56 ± 0.27 L) (figure 2-18). However, the mean maximum percent drop in post-EVH FVC was significantly lower ($p < 0.016$) with the combination treatment (3.29 ± 1.50%) than at pre-supplementation (12.07 ± 2.94%) (figure 2-19).

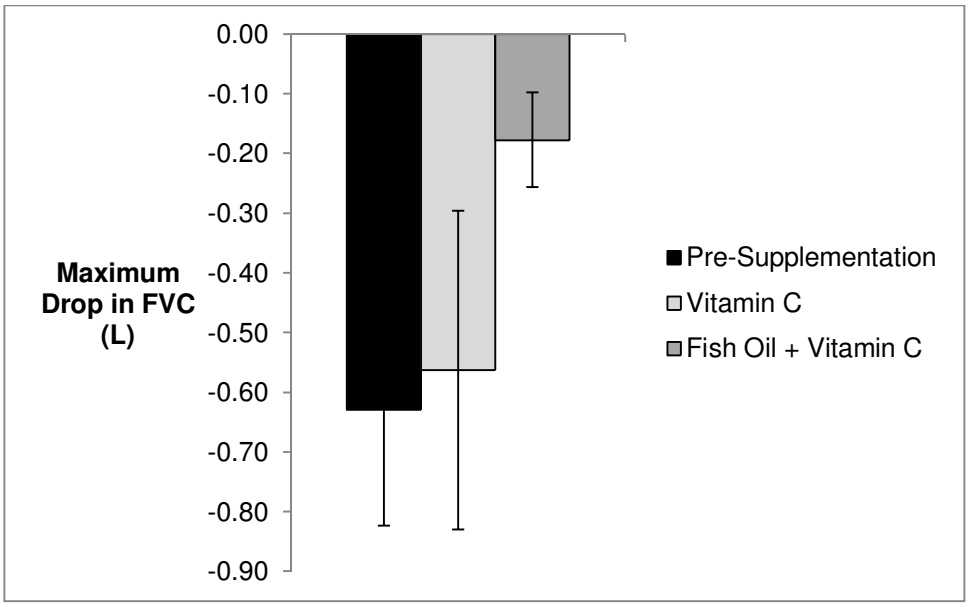

Figure 2-18. ***Maximum drop in FVC following the eucapnic voluntary hyperventilation challenge for the Vitamin C Group.*** *There were no significant changes in the maximum drop in FVC among the pre-supplementation, vitamin C treatment, or combination treatment laboratory tests (n = 7). Error bars express standard error of the mean. FVC, forced vital capacity*

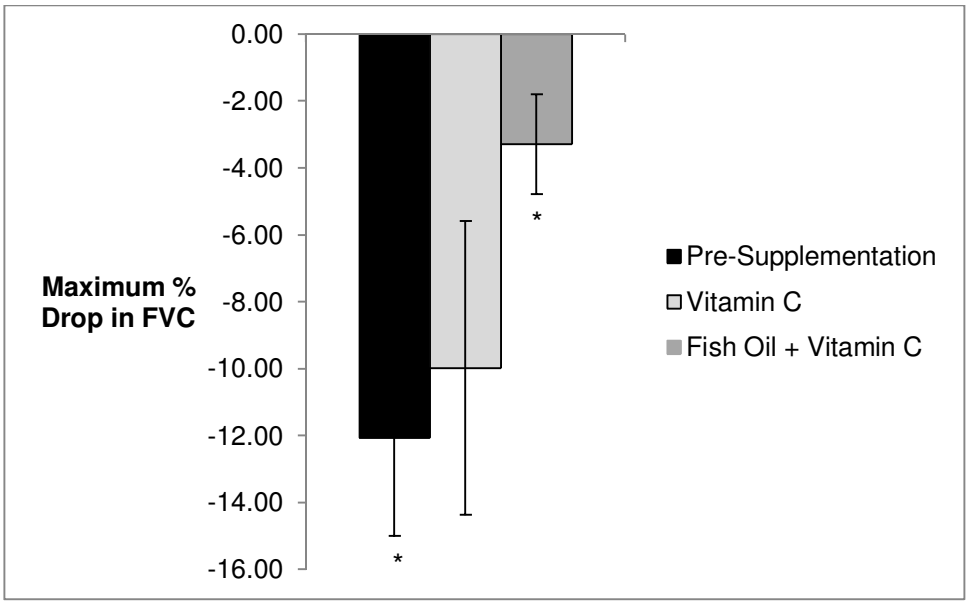

Figure 2-19. ***Maximum percent drop in FVC following the eucapnic voluntary hyperventilation challenge for the Vitamin C Group.*** *The maximum percent drop in FVC was significantly decreased with the combination treatment compared to pre-supplementation (n = 7). There were no significant differences between pre-supplementation and the vitamin C treatment or between the vitamin C treatment and the combination treatment. Error bars express standard error of the mean. FVC, forced vital capacity; *, significantly different from each other*

The Fish Oil Group had a mean maximum drop in post-EVH $FEF_{25-75\%}$ of 1.07 ± 0.21 L/s or 28.33 ± 3.33% of their resting $FEF_{25-75\%}$ at the first laboratory test. The Vitamin C Group had a mean maximum drop in post-EVH $FEF_{25-75\%}$ of 1.29 ± 0.20 L/s or 39.28 ± 6.08% of their resting $FEF_{25-75\%}$ at the first laboratory test. Although the groups did not significantly differ in the change in flow rate ($p > 0.05$), the groups were significantly different at some time points when this value was expressed in terms of a percentage of the resting $FEF_{25-75\%}$ ($p < 0.05$). Therefore, only the data on the change in flow rate was pooled for the subjects to determine the effect of the combination treatment on $FEF_{25-75\%}$. The maximum flow rate change in $FEF_{25-75\%}$ was significantly lower ($p < 0.05$) with the combination treatment (0.72 ± 0.11 L/s) as compared to pre-supplementation (1.15 ± 0.14 L/s) (figure 2-20).

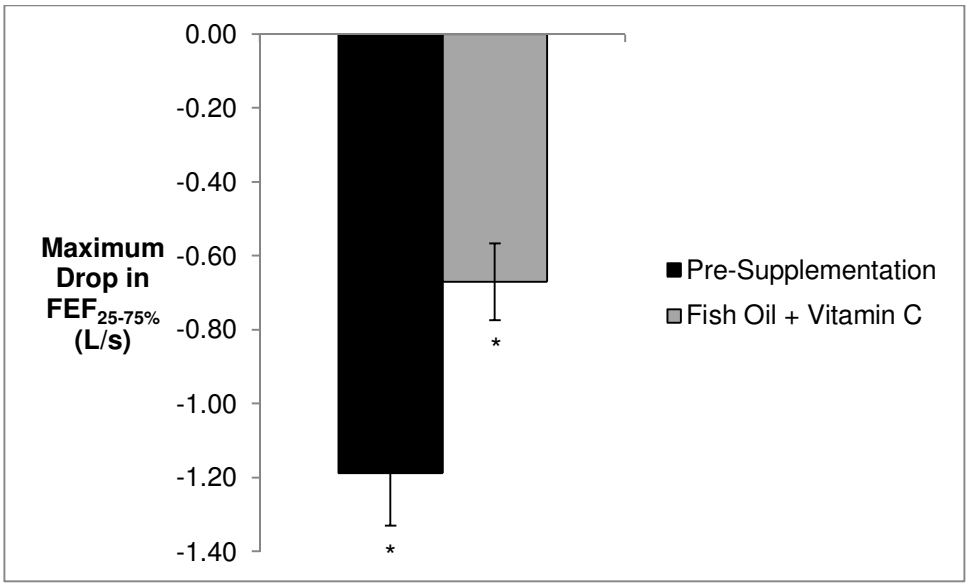

Figure 2-20. ***Maximum percent drop in $FEF_{25-75\%}$ following the eucapnic voluntary hyperventilation challenge for all subjects.*** *The combination treatment significantly reduced the maximum drop in $FEF_{25-75\%}$ as compared to pre-supplementation (n = 13). Error bars express standard error of the mean. $FEF_{25-75\%}$, forced expiratory flow at 25-75% of the forced vital capacity; *, significantly different from each other*

The Fish Oil Group did not demonstrate any significant differences ($p > 0.016$) in the maximum drop in $FEF_{25-75\%}$ in terms of flow rate or percent change among the pre-supplementation (1.00 ± 0.19 L/s, 28.08 ± 2.82%), fish oil treatment (0.56 ± 0.11 L/s, 19.16 ± 3.89), and combination treatment (0.73 ± 0.15 L/s, 24.49 ± 7.27%) laboratory visits (figures 2-21, 2-22). However, while on the combination treatment, the Vitamin C Group had a mean maximum drop in post-EVH $FEF_{25-75\%}$ that was significantly reduced ($p < 0.016$) in terms of the change in flow rate (0.71 ± 0.17 L/s) and the change in the percentage of the resting value (18.45 ± 3.73%) in comparison to pre-supplementation (1.29 ± 0.20 L/s, 39.28 ± 6.08%) (figures 2-23, 2-24). There were no significant differences ($p > 0.016$) in the post-EVH $FEF_{25-75\%}$ between the combination treatment and the vitamin C treatment (1.23 ± 0.39 L/s, 32.73 ± 10.45%) or between the vitamin C treatment and pre-supplementation.

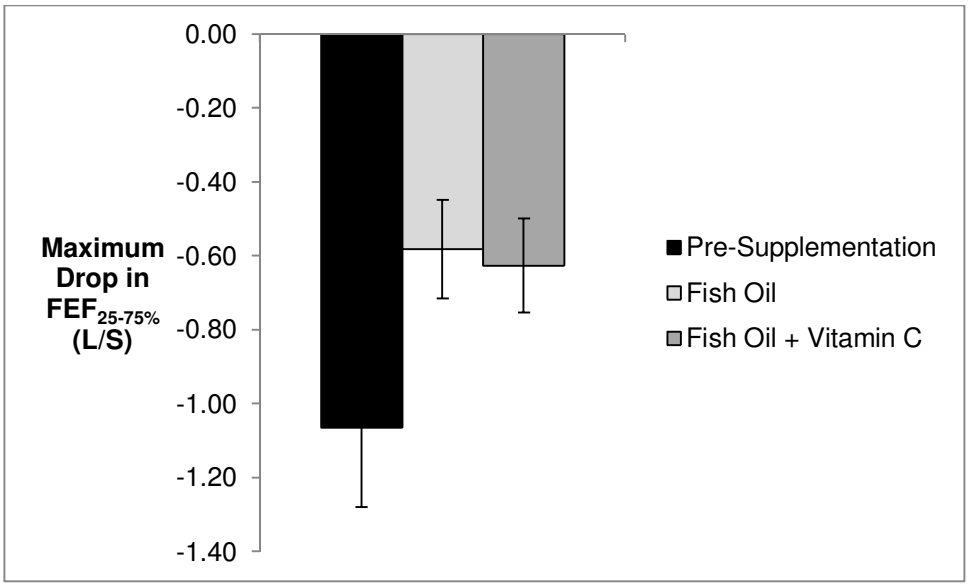

Figure 2-21. ***Maximum drop in $FEF_{25-75\%}$ following the eucapnic voluntary hyperventilation challenge for the Fish Oil Group.*** *There were no significant changes in the maximum drop in $FEF_{25-75\%}$ among the pre-supplementation, fish oil treatment, and combination treatment laboratory tests (n = 6). Error bars express standard error of the mean. $FEF_{25-75\%}$, forced expiratory flow at 25-75% of the forced vital capacity*

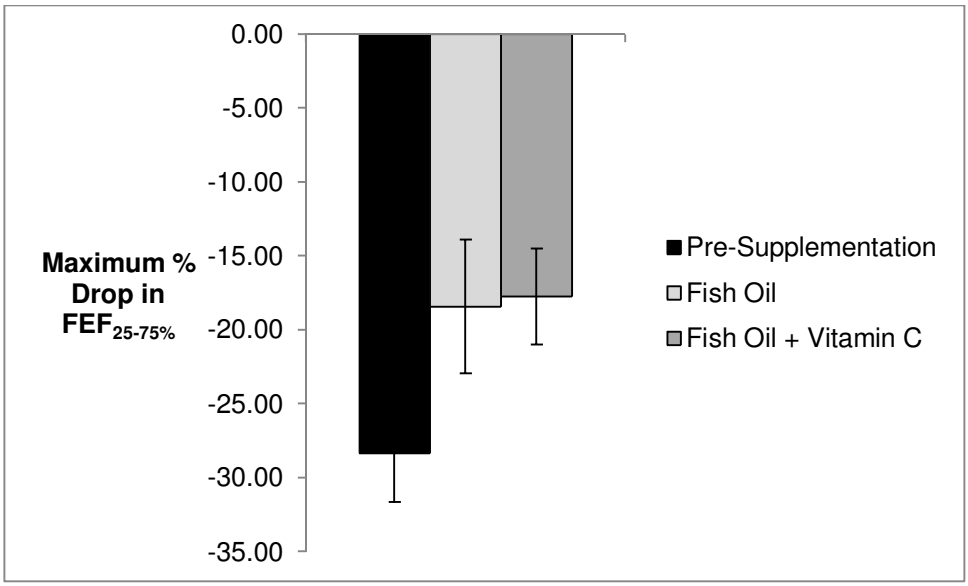

Figure 2-22. ***Maximum percent drop in $FEF_{25-75\%}$ following the eucapnic voluntary hyperventilation challenge for the Fish Oil Group.*** *There were no significant differences in the maximum percent drop in $FEF_{25-75\%}$ among the three laboratory visits (n = 6). Error bars express standard error of the mean. $FEF_{25-75\%}$, forced expiratory flow at 25-75% of the forced vital capacity*

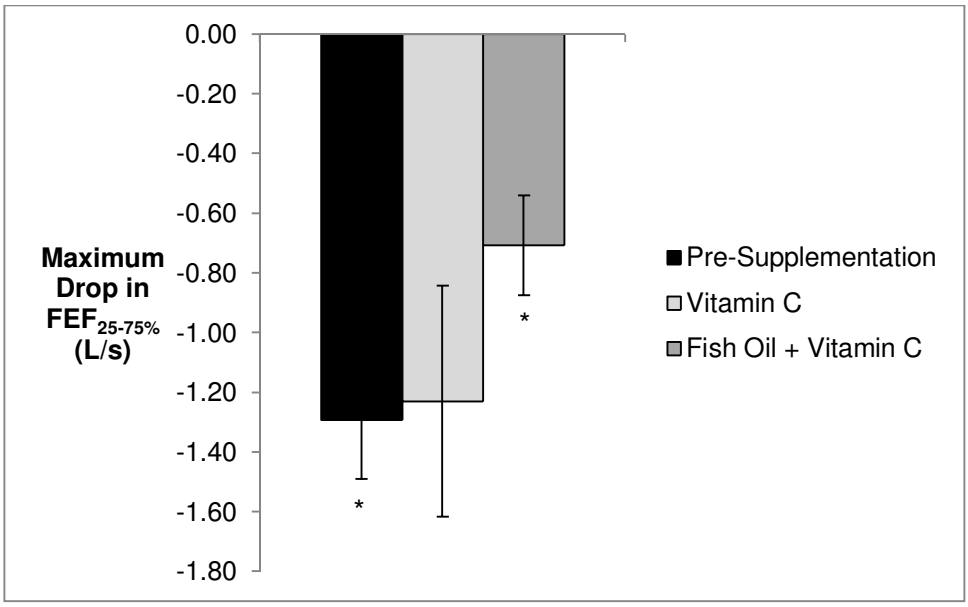

Figure 2-23. ***Maximum drop in $FEF_{25-75\%}$ following the eucapnic voluntary hyperventilation challenge for the Vitamin C Group.*** *The combination treatment significantly reduced the maximum drop in $FEF_{25-75\%}$ as compared to pre-supplementation (n = 7). There were no significant differences between pre-supplementation and the vitamin C treatment or between the vitamin C treatment and the combination treatment. Error bars express standard error of the mean. $FEF_{25-75\%}$, forced expiratory flow at 25-75% of the forced vital capacity; *, significantly different from each other*

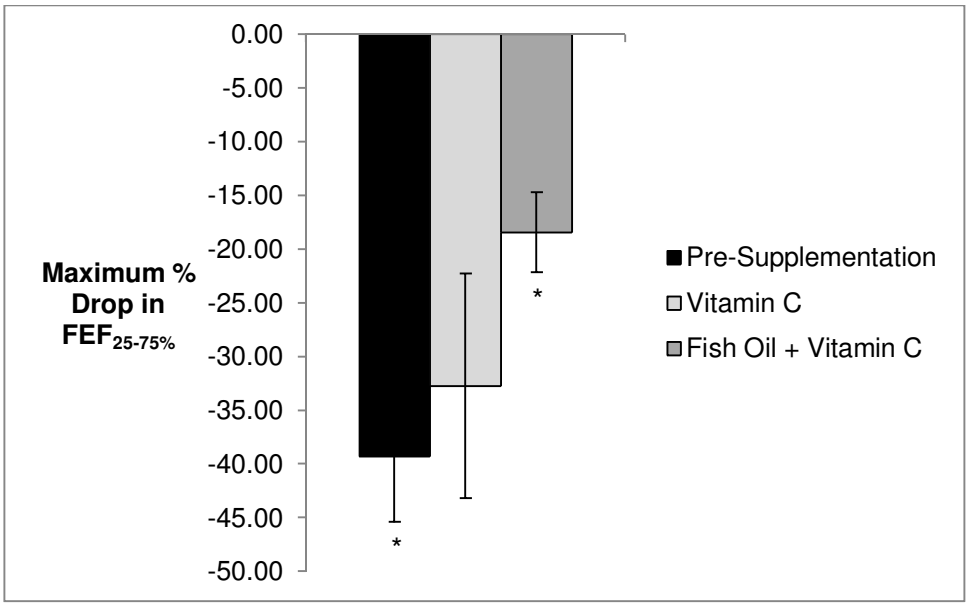

Figure 2-24. ***Maximum percent drop in $FEF_{25-75\%}$ following the eucapnic voluntary hyperventilation challenge for the Vitamin C Group.*** *The maximum percent drop in $FEF_{25-75\%}$ was significantly decreased with the combination treatment compared to pre-supplementation (n = 7). There were no significant changes between pre-supplementation and the vitamin C treatment or between the vitamin C treatment and the combination treatment. Error bars express standard error of the mean. $FEF_{25-75\%}$, forced expiratory flow at 25-75% of the forced vital capacity; *, significantly different from each other*

Fraction of Exhaled Nitric Oxide. There were no significant differences ($p > 0.05$) between the Fish Oil Group and the Vitamin C Group for either the pre-or post-EVH fraction of exhaled nitric oxide (F_ENO); therefore, the groups were again pooled to determine overall differences between pre-supplementation and the combination treatment. There were no significant differences ($p > 0.05$) between the pre-EVH F_ENO values at the combination treatment test (58.2 ± 13.5 ppb) compared to the pre-supplementation test (67.0 ± 23.2 ppb) or between the post-EVH F_ENO values at the combination treatment test (48.2 ± 11.2 ppb) compared to the pre-supplementation test (53.3 ± 14.3 ppb). Moreover, there was not a significant difference ($p > 0.05$) between the pre- and post-EVH values at the pre-supplementation test; the mean change score

between the values was -13.1 ± 10.2 ppb. However, the post-EVH F_ENO was significantly lower ($p < 0.05$) than the pre-EVH F_ENO with the combination treatment; the mean change score between the values was -7.6 ± 2.1 ppb (figure 2-25).

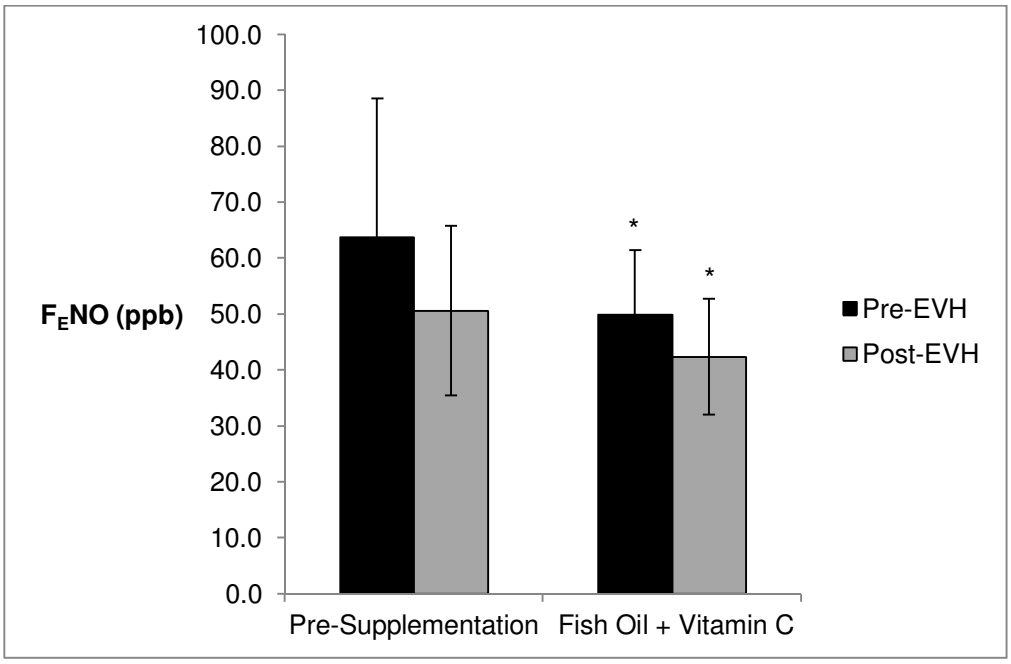

Figure 2-25. ***The fraction of exhaled nitric oxide (F_ENO) pre- and post-eucapnic voluntary hyperventilation (EVH) challenge for all subjects.*** *The post-EVH F_ENO was significantly decreased from the pre-EVH F_ENO at the combination treatment test but not at the pre-supplementation test (n = 13). There were no significant differences in the pre-EVH F_ENO or in the post-EVH F_ENO between the pre-supplementation and combination treatment laboratory tests. Error bars express standard error of the mean. *, significantly different from each other*

The Fish Oil Group did not show any significant changes ($p > 0.016$) in pre-F_ENO with either the fish oil treatment (41.4 ± 9.5 ppb) or the combination treatment (31.0 ± 4.7 ppb) as compared to each other or to the pre-supplementation value (33.5 ± 7.0) (figure 2-26). Also, the Fish Oil Group did not have any significant changes ($p > 0.016$) in post-F_ENO among pre-supplementation (32.8 ± 8.4 ppb), fish oil treatment (36.1 ± 7.8 ppb), and combination treatment (27.8 ± 4.9 ppb). The mean change scores between pre- and post-EVH F_ENO were -0.7 ± 2.4 ppb at pre-supplementation, -5.3 ± 4.2 ppb at the fish oil treatment test, and -3.2 ± 1.5 ppb at the combination treatment test. In contrast, the Vitamin C Group demonstrated a significant decrease ($p < 0.016$) in the post-EVH F_ENO as compared to the pre-EVH F_ENO with both the vitamin C treatment and the combination treatment (figure 2-27). The mean change scores between pre- and post-EVH F_ENO were -23.8 ± 18.5 ppb at pre-supplementation, -10.1 ± 2.5 ppb at the vitamin C treatment test, and -11.3 ± 3.1 ppb at the combination treatment test. However, there were no significant changes ($p > 0.016$) between pre-EVH F_ENO values at the vitamin C treatment test (82.7 ± 38.9 ppb) compared to the pre-supplementation test (89.7 ± 44.9 ppb) or at the combination treatment test (66.1 ± 19.6 ppb) compared to the pre-supplementation test. There were also no significant changes ($p > 0.016$) in the post-EVH F_ENO values among the pre-supplementation test (65.8 ± 26.9 ppb), vitamin C treatment test (72.6 ± 39.3 ppb), and combination treatment test (54.8 ± 18.0 ppb).

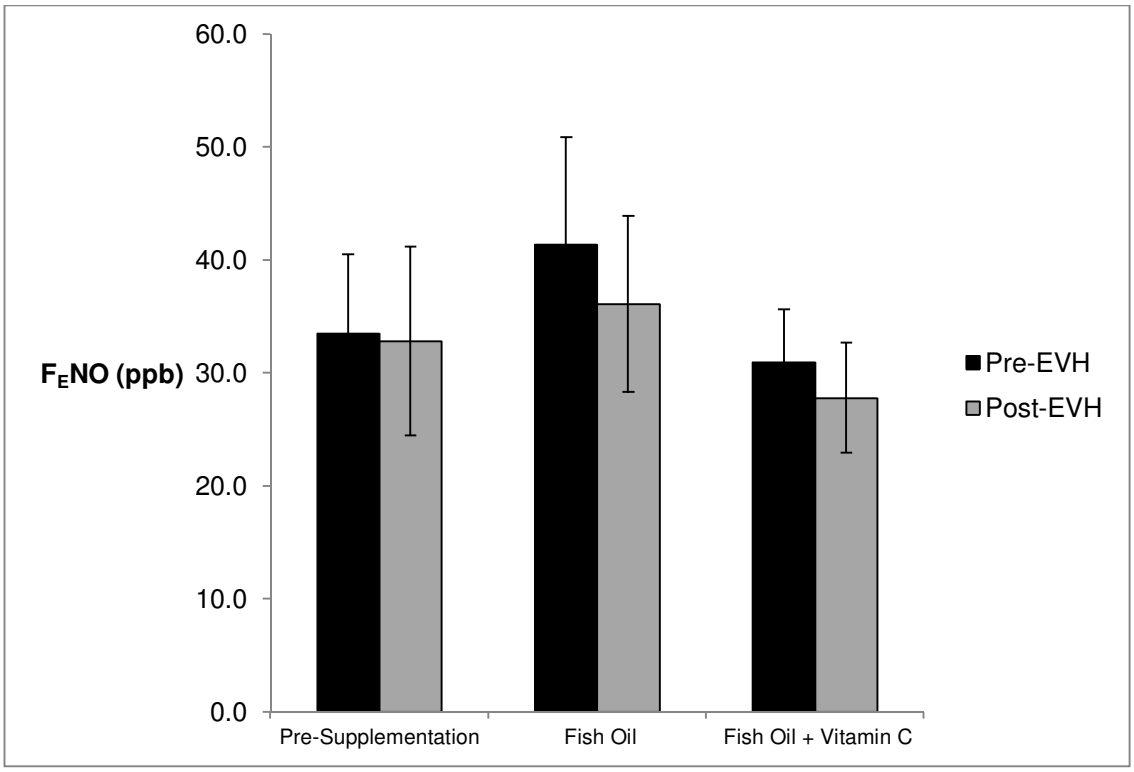

Figure 2-26. ***The fraction of exhaled nitric oxide (F_ENO) pre- and post-eucapnic voluntary hyperventilation (EVH) challenge for the Fish Oil Group.*** *There were no significant changes in F_ENO for the Fish Oil Group (n = 6). Error bars express standard error of the mean.*

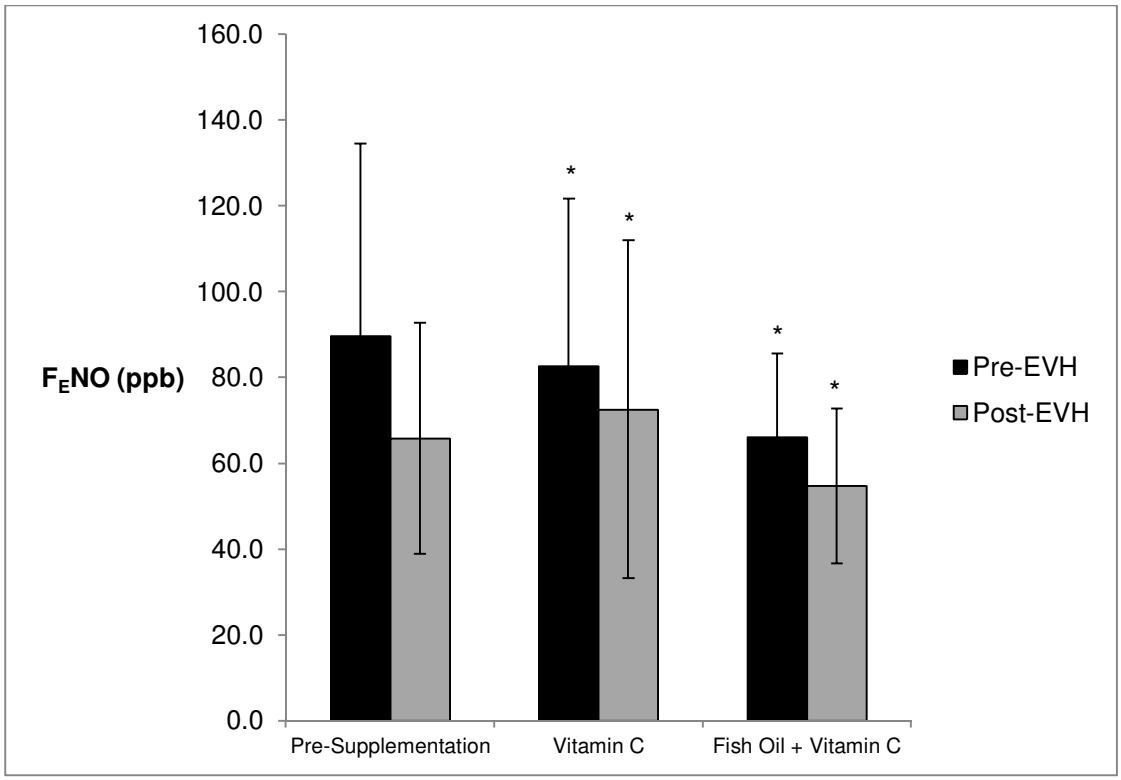

Figure 2-27. ***The fraction of exhaled nitric oxide (F_ENO) pre- and post-eucapnic voluntary hyperventilation (EVH) challenge for the Vitamin C Group.*** *The post-EVH F_ENO was significantly lower than the pre-EVH F_ENO at the vitamin C treatment and combination treatment tests but not at the pre-supplementation test (n = 7). There were no significant differences in the pre-EVH or post-EVH F_ENO values among the three laboratory tests. Error bars express standard error of the mean. *, significantly different from the pre-EVH value at the same laboratory test*

Exhaled Condensate Breath pH. Although the Fish Oil Group and the Vitamin C Group were not significantly different ($p > 0.05$) in the pre- or post-EVH exhaled breath condensate (EBC) pH at the pre-supplementation test, the groups were significantly different ($p < 0.05$) in the post-EVH EBC pH at the combination treatment test. Thus, the groups were not pooled to examine the overall effect of the combination treatment on the EBC pH.

The Fish Oil Group's pre-EVH EBC pH did not significantly change ($p > 0.016$) among the pre-supplementation (6.92 ± 0.32), fish oil treatment (7.18 ± 0.10), and combination treatment tests (6.85 ± 0.11). However, their post-EVH EBC pH was significantly increased ($p < 0.016$) with the fish oil treatment (7.22 ± 0.08) as compared to the combination treatment (6.68 ± 0.17) (figure 2-28). There were no significant differences ($p > 0.016$) between the pre-supplement value (6.85 ± 0.07) and the fish oil treatment value or the combination treatment value.

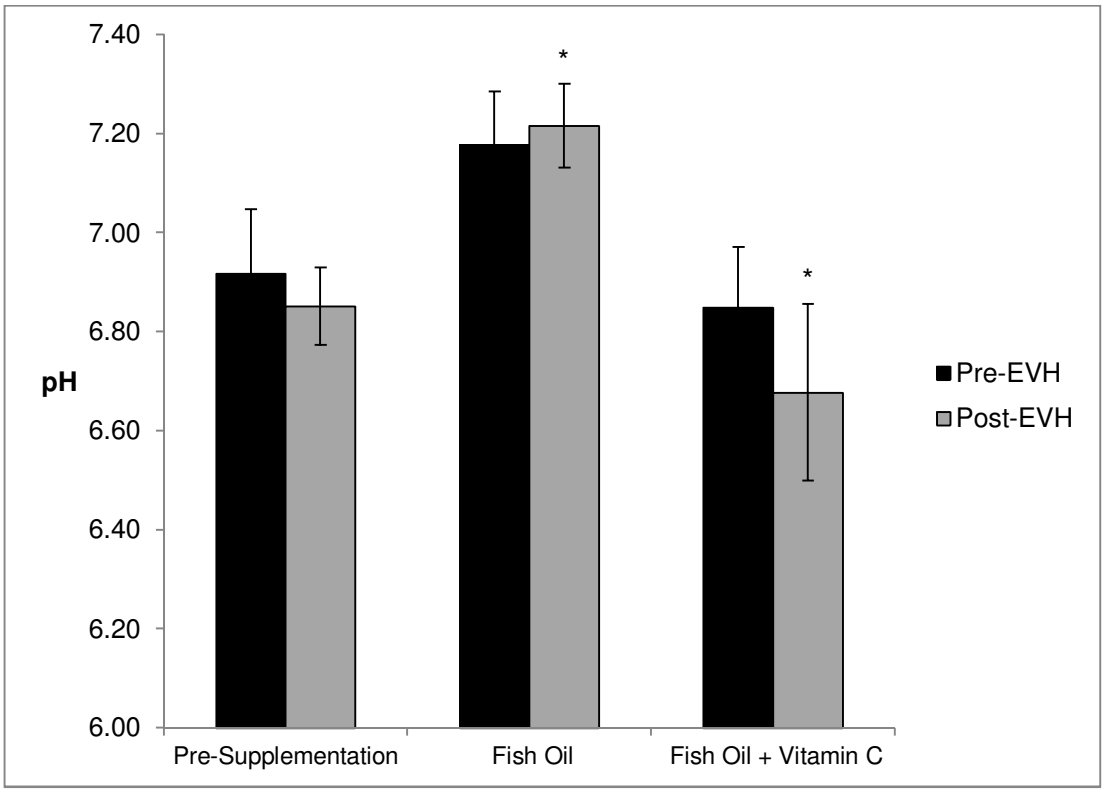

Figure 2-28. ***Exhaled breath condensate (EBC) pH pre- and post-eucapnic voluntary hyperventilation (EVH) challenge for the Fish Oil Group.*** *There were no significant changes in the pre-EVH EBC pH values among the laboratory tests (n = 6). The post-EVH EBC pH values were significantly different from each other at the fish oil treatment and combination treatment laboratory tests. Error bars express standard error of the mean. *, significantly different from each other*

Six out of the seven subjects in the Vitamin C Group provided adequate EBC samples to test the pH at all three lab tests. This group did not show any significant changes ($p > 0.016$) in the pre-EVH EBC pH among the pre-supplementation (7.05 ± 0.11), vitamin C treatment (6.94 ± 0.09), and combination treatment (6.91 ± 0.21) tests. There were also no significant changes ($p > 0.016$) in the post-EVH EBC pH among the pre-supplementation (7.10 ± 0.11), vitamin C treatment (7.06 ± 0.09), and combination treatment (7.20 ± 0.10) tests for the Vitamin C Group (figure 2-29).

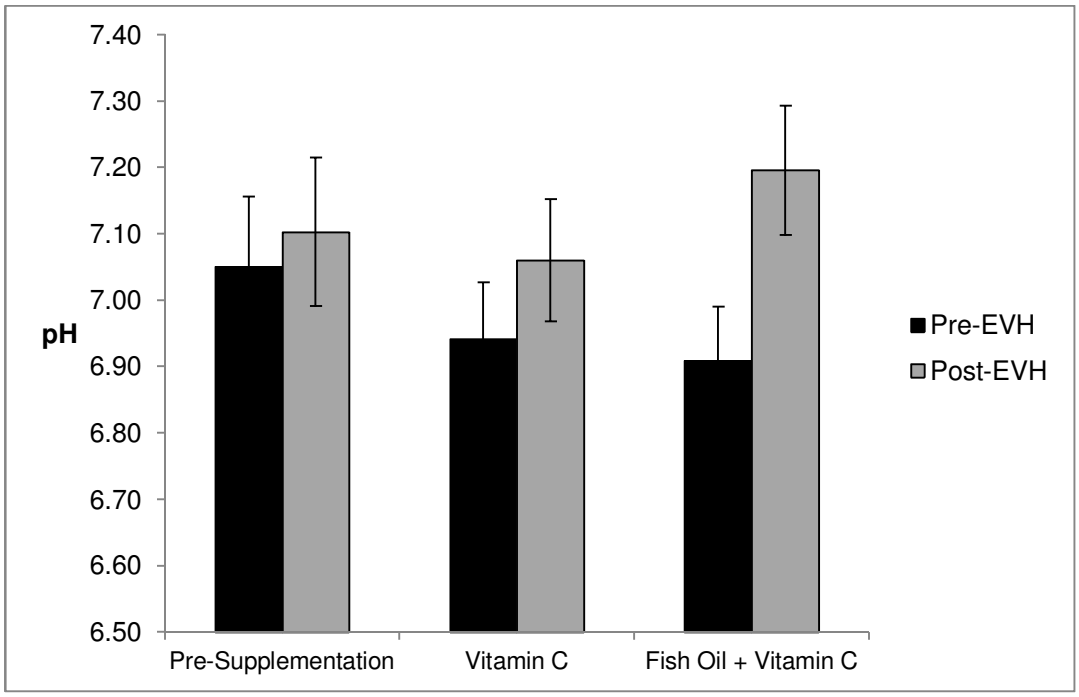

Figure 2-29. ***Exhaled breath condensate pH pre- and post-eucapnic voluntary hyperventilation (EVH) challenge.*** *There were no significant differences in the pre-EVH or post-EVH EBC pH values among the pre-supplementation, vitamin C treatment, and combination treatment laboratory tests (n = 6). Error bars express standard error of the mean.*

Symptoms and Short-Acting β-Agonist Usage. During the run-in phase, first treatment phase, washout phase, and combination treatment phase, subjects recorded their daytime symptoms and nighttime symptoms using a symptom diary (83). They also reported their short-acting β-agonist usage by recording the number of puffs taken each day. The Fish Oil Group's daytime symptom score decreased throughout the study phases from 1.13 ± 0.18 during the run-in phase, to 0.90 ± 0.18 during the fish oil treatment phase, to 0.79 ± 0.17 during the washout phase, and to 0.76 ± 0.15 during the combination treatment phase; the washout and combination treatment phases were both significantly lower ($p < 0.05$) than the run-in phase (figure 2-30). The Vitamin C Group did not exhibit any significant changes ($p > 0.05$) in the daytime symptom score between any of the study's phases (run-in phase: 1.63 ± 0.25, vitamin C treatment phase: 1.21 ± 0.27, washout phase: 1.50 ± 0.25, combination treatment phase: 1.52 ± 0.27). Neither the Fish Oil Group nor the Vitamin C Group demonstrated significant changes ($p > 0.05$) in the nighttime symptom score among the run-in phase (Fish Oil Group: 0.10 ± 0.09, Vitamin C Group: 0.21 ± 0.08), one treatment phase (Fish Oil Group: 0.04 ± 0.04, Vitamin C Group: 0.05 ± 0.05), washout phase (Fish Oil Group: 0.00 ± 0.00, Vitamin C Group: 0.15 ± 0.08), and combination treatment phase (Fish Oil Group: 0.00 ± 0.00, Vitamin C Group: 0.14 ± 0.08). Furthermore, neither group had a significant difference ($p > 0.05$) in their short-acting β-agonist usage from the run-in phase (Fish Oil Group: 0.38 ± 0.30 puffs per day, Vitamin C Group: 0.84 ± 0.65 puffs per day), to the one treatment phase (Fish Oil Group: 0.04 ± 0.03 puffs per day, Vitamin C Group: 0.58 ± 0.48 puffs per day), to the washout phase (Fish Oil Group: 0.08 ± 0.04 puffs per day, Vitamin C Group: 0.38 ± 0.32 puffs per day), and to the combination treatment phase (Fish Oil Group: 0.08 ± 0.04 puffs per day, Vitamin C Group: 0.37 ± 0.24 puffs per day) (figures 2-31, 2-32).

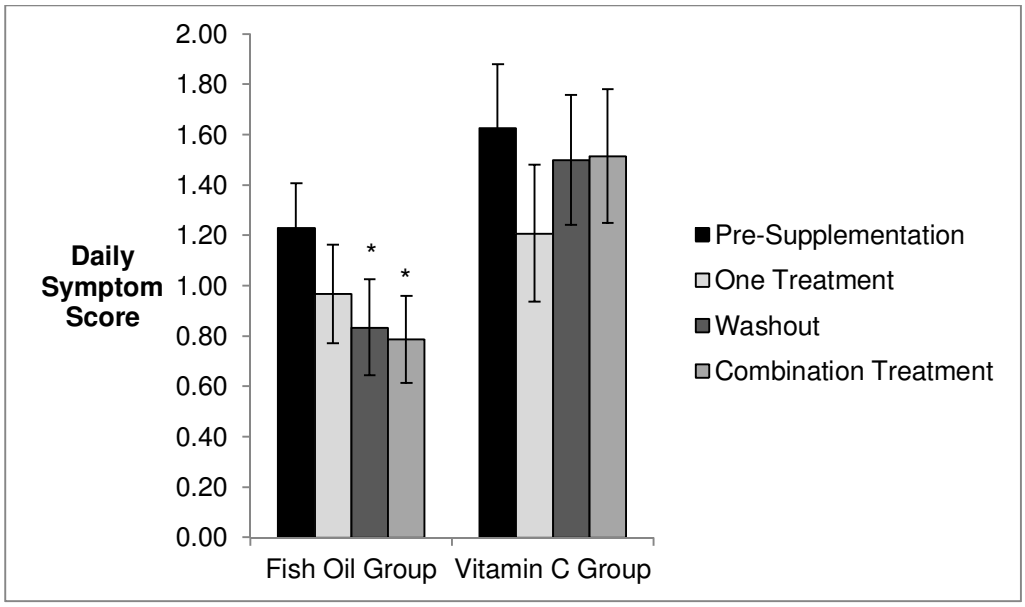

Figure 2-30. ***Daily symptom scores for the Fish Oil Group and Vitamin C Group during each of the study's phases.*** *The Fish Oil Group's daytime symptom score was significantly lower during the washout and combination treatment phases than during the pre-supplementation phase (n = 6). There were no significant differences in the daily symptom scores among the pre-supplementation, one treatment, washout, and combination treatment study phases for the Vitamin C Group (n = 7). Error bars express standard error of the mean. *, significantly different from pre-supplementation*

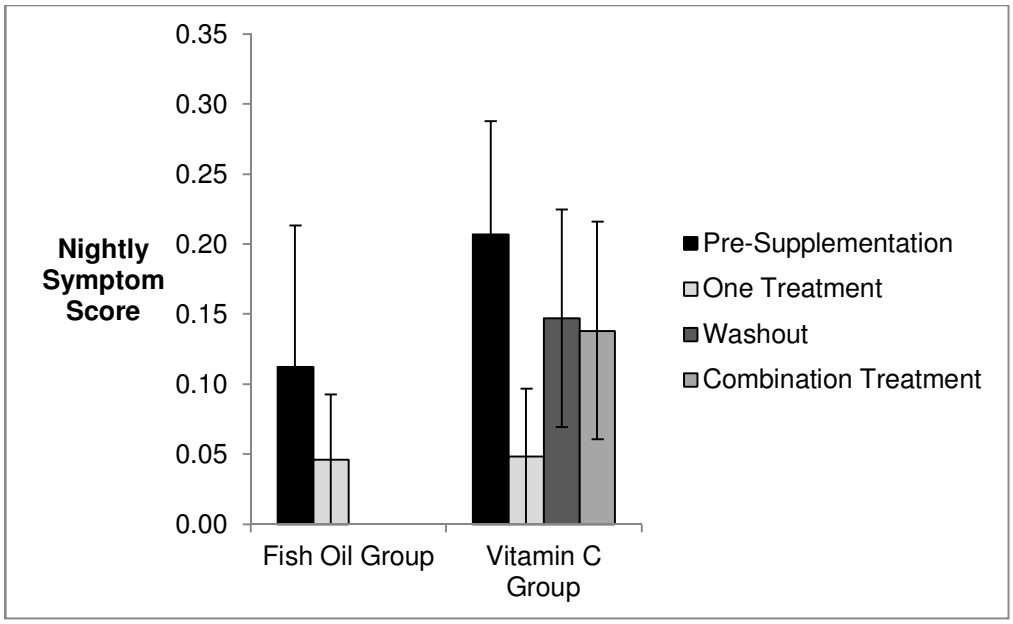

Figure 2-31. ***Nightly symptom scores for the Fish Oil Group and Vitamin C Group during each of the study's phases.*** *There were no significant changes in the nightly symptom scores among the four study phases for either the Fish Oil Group (n = 6) or the Vitamin C Group (n = 7). Error bars express standard error of the mean.*

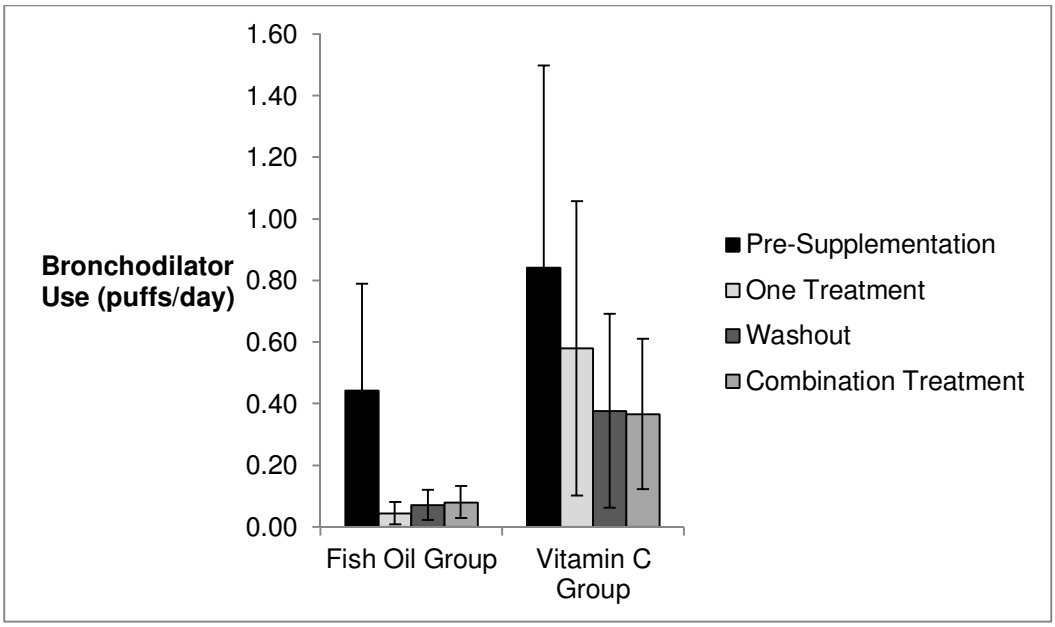

Figure 2-32. ***Average daily bronchodilator use for the Fish Oil Group and Vitamin C Group during each of the study's phases.*** *There were no significant differences in the subjects' bronchodilator use among the pre-supplementation, one treatment, washout, and combination treatment study phases for either the Fish Oil Group (n = 6) or the Vitamin C Group (n = 7). Error bars express standard error of the mean.*

Peak Flow Measurements. Each subject used an electronic meter to measure his or her peak expiratory flow every morning and evening during the run-in phase, first treatment phase, washout phase, and combination treatment phase. Although there were no significant differences ($p > 0.05$) in their morning peak flow measurements during the course of the study (figure 2-33), the Fish Oil Group exhibited a significant increase ($p < 0.05$) in their mean evening peak flow during the combination treatment phase (491.61 ± 62.44 L/min) as compared to the pre-supplementation phase (448.29 ± 63.27 L/min) (figure 2-34). The fish oil treatment phase (473.94 ± 67.80 L/min) and washout phase (480.28 ± 63.69 L/min) did not significantly differ ($p > 0.05$) from any of the other phases. The Vitamin C Group did not demonstrate any significant changes ($p > 0.05$) in their morning or evening peak flow throughout the study.

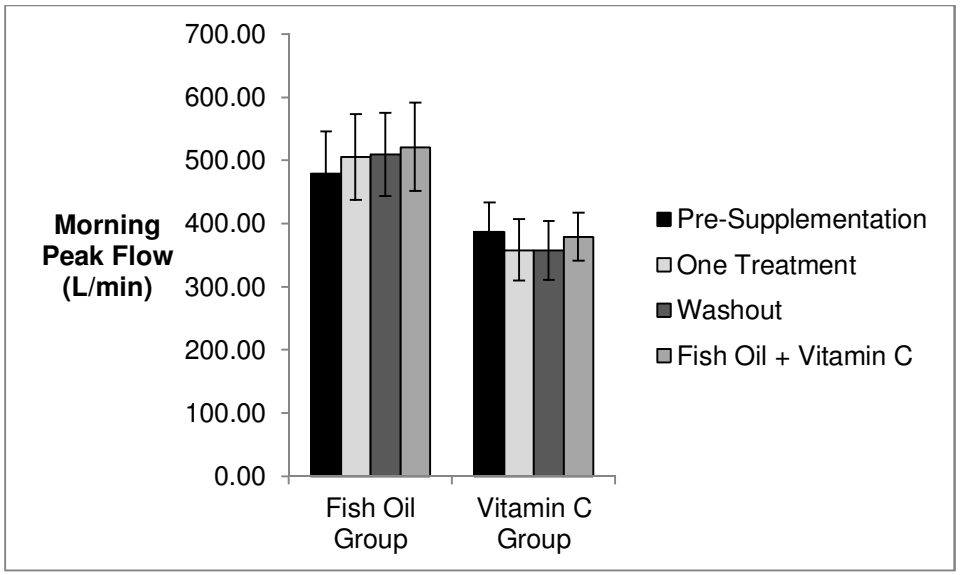

Figure 2-33. ***Morning peak expiratory flow values for the Fish Oil Group and Vitamin C Group during each of the study's phases.*** *There were no significant differences in the morning peak flow values among the pre-supplementation, one treatment, washout, and combination treatment study phases for either the Fish Oil Group (n = 6) or the Vitamin C Group (n = 7). Error bars express standard error of the mean.*

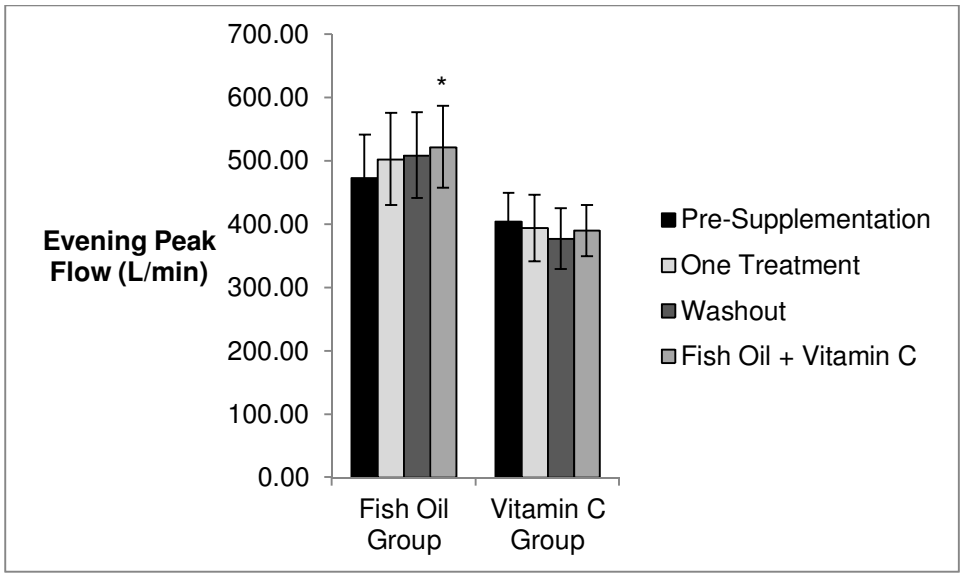

Figure 2-34. ***Evening peak expiratory flow values for the Fish Oil Group and Vitamin C Group during each of the study's phases.*** *The Fish Oil Group demonstrated a significant increase in its mean evening peak flow with the combination treatment compared to pre-supplementation (n = 6). There were no significant changes in the mean evening peak flow for the Vitamin C Group among the four study phases (n = 7). Error bars express standard error of the mean. *, significantly different from pre-supplementation*

Nutrient Intake. Twenty-four hour dietary recalls were used to gauge each subject's nutrient intake during the run-in phase, first treatment phase, washout phase, and combination treatment phase. Neither the Fish Oil Group nor the Vitamin C Group significantly changed ($p > 0.05$) their nutrient intake among any of the study phases (table 2-8).

		Energy (kcal)	Total Fat (g)	Total Carbohydrate (g)	Total Protein (g)	Total Dietary Fiber (g)	Vitamin C (mg)	Arachidonic Acid (g)	EPA (g)	DHA (g)
Pre-Supplementation										
Fish Oil Group	**Mean**	3032.23	109.37	364.62	126.20	26.43	93.55	0.20	0.03	0.08
	SEM	395.85	17.91	67.48	24.21	6.77	23.38	0.04	0.01	0.03
Vitamin C Group	**Mean**	2042.51	77.83	238.45	85.94	18.60	88.73	0.20	0.09	0.29
	SEM	308.96	12.10	41.78	16.67	3.80	24.48	0.05	0.04	0.15
One Treatment										
Fish Oil Group	**Mean**	2713.83	88.40	369.45	113.95	26.05	198.97	0.10	0.03	0.05
	SEM	369.40	14.53	54.66	21.15	8.24	134.66	0.02	0.02	0.02
Vitamin C Group	**Mean**	2584.30	103.10	299.20	109.62	20.45	70.38	0.18	0.02	0.07
	SEM	477.30	20.15	58.65	17.23	3.99	19.72	0.05	0.01	0.02
Washout										
Fish Oil Group	**Mean**	2842.75	96.15	361.30	111.20	28.29	148.33	0.12	0.06	0.12
	SEM	560.92	30.62	74.20	18.84	8.12	60.79	0.03	0.03	0.06
Vitamin C Group	**Mean**	1845.08	61.59	245.38	75.92	16.50	86.72	0.14	0.01	0.04
	SEM	347.90	14.14	44.93	16.55	3.74	21.99	0.05	0.00	0.02
Combination Treatment										
Fish Oil Group	**Mean**	3264.28	123.09	414.21	122.95	25.76	114.57	0.11	0.04	0.13
	SEM	674.89	36.78	77.10	21.88	5.59	36.53	0.02	0.03	0.10
Vitamin C Group	**Mean**	2042.51	77.83	238.45	85.94	18.60	88.73	0.20	0.09	0.29
	SEM	308.96	12.10	41.78	16.67	3.80	24.48	0.05	0.04	0.15

Table 2-8. ***Average intake amounts of selected nutrients for the Fish Oil Group and the Vitamin C Group.*** *There were no significant changes in diet for the Fish Oil Group (n = 6) or the Vitamin C Group (n = 7) among the four study phases as assessed by nutrient intake. The average values for the intake of only selected nutrients are presented here. SEM, standard error of the mean; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid*

Discussion

This study was undertaken to determine whether a combination of nutritional supplements would improve pulmonary function and airway inflammation to a greater extent than an individual nutritional supplement in adults with asthma and hyperpnea-induced bronchoconstriction (HIB). Based on previous research demonstrating the effectiveness of fish oil and vitamin C supplementation in attenuating exercise-induced bronchoconstriction (EIB), the combination of these supplements was tested (61, 62, 93, 94). We have demonstrated that pulmonary function was improved with both fish oil and the combination of fish oil and vitamin C but not with vitamin C alone. Moreover, airway inflammation seems to have been affected by vitamin C supplementation as well as by the combination treatment of fish oil and vitamin C.

Pulmonary function was evaluated with spirometry before and after bronchoprovocation. The forced expiratory volume in one second (FEV_1) is the primary measure used in diagnosing EIB or HIB because it is known to be highly reproducible. The area under the curve of the percent change in FEV_1 for a given time period (AUC_{20}) represents the bronchoconstrictor response; it thus indicates the degree of airway narrowing and recovery following bronchoprovocation. The forced vital capacity (FVC) represents the usable volume of air in the lungs. The forced expiratory flow at 25-75% of the FVC ($FEF_{25-75\%}$) is a measure of constriction in the peripheral airways; it is these small, distal airways that are typically obstructed in asthma. It was expected that these pulmonary function measures would improve with each supplement as based on previous research and to a greater degree with the combination of fish oil and vitamin C based on the proposed mechanism for an additive effect. Significant improvements were seen with either fish oil alone or the combination of supplements but not with vitamin C alone.

These results for fish oil supplementation support previous research from our laboratory showing that fish oil alleviates airway narrowing in EIB and HIB (61, 62, 93). However, the lack of significant improvement in any of the pulmonary function measures with vitamin C supplementation is in disagreement with Tecklenburg et al. (94). The discrepancy may be due to experimental differences between the studies. Tecklenburg et al. (94) allowed subjects to continue freely using their prescribed asthma medications throughout the study except for in the hours to days before laboratory tests where use was restricted. Furthermore, the method of bronchoprovocation differed from the current study as Tecklenburg et al. (94) used a sub-maximal treadmill exercise test instead of eucapnic voluntary hyperventilation (EVH). Lastly, although the vitamin C supplement composition and daily dose were the same between the studies, the current study treated subjects for an additional week in order to match the treatment period required for fish oil. As it known that some antioxidants, such as vitamin C, can become pro-oxidant in large amounts, this difference in treatment periods may be important (75). Nevertheless, this study's vitamin C results support the finding by Cohen et al. (22) that not all asthmatics with exercise-induced bronchoconstriction respond positively to vitamin C supplementation. Cohen et al. (22) found that following a single 2000 mg dose of ascorbic acid, only nine of twenty asthmatic subjects had improved post-exercise pulmonary function compared to placebo. In the current study, four of the seven subjects in the Vitamin C Group had reduced decreases in their FEV_1 values after the EVH challenge at the laboratory test following vitamin C supplementation. In future nutritional studies, subjects should first be screened for vitamin C responsiveness to allow for a more homogeneous study population.

The combination treatment significantly improved pulmonary function in comparison to pre-supplementation values for the Vitamin C Group as well as for all subjects overall. However, a limitation of this study's research design is that it cannot

determine whether fish oil is solely responsible for the overall significant improvements in pulmonary function between pre-supplementation and post-combination treatment values. Because subjects in the Vitamin C Group demonstrated significant changes from pre-supplementation to the combination treatment but not between pre-supplementation and the vitamin C treatment, it is not clear whether the improvement is due to the combination of fish oil and vitamin C or just due to fish oil. Since the Fish Oil Group demonstrated significant improvement with fish oil alone, the post-combination treatment improvements in the Vitamin C Group may be due to these subjects being supplemented with active fish oil. Nevertheless, although the lack of significant changes between all treatments makes it difficult to ascertain the effect of the combination treatment, improvements observed in individual subjects in both groups throughout the study suggest that the combination of fish oil and vitamin C may be effective in a subset of the asthma population.

Changes in airway inflammation were evaluated by measuring the fraction of exhaled nitric oxide (F_ENO) and exhaled breath condensate (EBC) pH. These measures are recognized as indirect markers of airway inflammation. It was anticipated that F_ENO would be decreased both pre-EVH and post-EVH with supplementation. However, no significant changes were demonstrated in the pre-EVH or post-EVH F_ENO values with any of the treatments compared to pre-supplementation. The lack of significant changes in F_ENO with vitamin C supplementation agrees with the results from Baumann et al. (16). These researchers tracked changes in pre-EVH F_ENO in subjects with EIB who were supplemented with a placebo or undenatured whey protein, an antioxidant supplement, and determined that there were no significant changes in F_ENO. However, fish oil supplementation not affecting the pre-EVH F_ENO in this study differs from Tecklenburg-Lund et al. (93) who showed a significant reduction in pre-EVH F_ENO with fish oil treatment.

The lack of changes in post-EVH F_ENO is supported by similar findings by Tecklenburg-Lund (92). It was suggested that there were no significant changes in the post-EVH F_ENO among treatment with fish oil, the leukotriene-receptor antagonist montelukast (Singulair®), or the combination of the two as compared to pre-supplementation because bronchoconstriction may limit the amount of nitric oxide that leaves the lungs during an exhalation (24). Therefore, lower F_ENO values may be due to either reduced airway inflammation or nitric oxide being trapped in the inflamed airways of the lungs, which thus confounds the ability of the test to detect changes in airway inflammation as intended.

Additionally, the pre- and post-EVH F_ENO values were compared for each laboratory test. Overall, in all subjects, there was no significant change ($p > 0.05$) between the pre- and post-EVH F_ENO at the pre-supplementation test whereas the post-EVH F_ENO was significantly lower ($p < 0.05$) than the pre-EVH F_ENO with the combination treatment, which could suggest either less airway inflammation or increased bronchoconstriction as previously mentioned. In looking at the graph of this data (figure 2-25), it appears that the large variability in the pre-supplementation measures are masking any significant changes. ElHalawani et al. (29) demonstrated that a wide range of F_ENO values exist in subjects with EIB (median ± standard deviation, 23 ± 42.2 ppb) and without EIB (19.95 ± 18.47 ppb). Moreover, examination of the change scores between pre- and post-EVH F_ENO at the pre-supplementation and combination treatment laboratory tests, indicates that there is a smaller decrease in F_ENO with the combination treatment (-7.6 ± 2.1 ppb) than at pre-supplementation (-13.1 ± 10.2 ppb), which suggests reduced airway inflammation with supplementation. Thus, in this case, statistical significance or insignificance does not seem to appropriately reflect physiological significance.

Similarly, in the Vitamin C Group, there was no significant difference ($p > 0.016$) between pre- and post-EVH F_ENO at the pre-supplementation test. However, the post-EVH F_ENO was significantly lower ($p < 0.016$) than the pre-EVH at the vitamin C treatment test and combination treatment test despite smaller change scores (-10.1 ± 2.5 ppb and -11.3 ± 3.1 ppb, respectively) compared to the pre-supplementation test (-23.8 ± 18.5 ppb). In addition to the variability in the subjects' pre-supplementation responses, the drastic improvement of one particular subject's pre- and post-EVH F_ENO from 353.3 ppb and 221.8 ppb, respectively, at pre-supplementation, 311.3 ppb and 305.7 ppb with the vitamin C treatment, and 169.0 ppb and 152.0 ppb with the combination treatment may have also impacted the detection of statistical significance.

Since it has been shown that people with asthma have a lower EBC pH than healthy subjects, it was expected that nutritional supplementation would increase the EBC pH. Because the Fish Oil Group and Vitamin C Group were significantly different from each other in terms of the EBC pH, the groups were not pooled to examine the overall effect of the combination of fish oil and vitamin C. There were no significant differences in the pre-EVH EBC pH for either group among pre-supplementation, one supplement treatment (i.e. fish oil or vitamin C), and the combination treatment. These results are in contrast to those obtained by Tecklenburg-Lund et al. (93) who showed that fish oil supplementation significantly improved the mean pre-EVH EBC pH. However, the post-EVH EBC pH was significantly greater with fish oil supplementation compared to the combination treatment in the Fish Oil Group. This suggests that these subjects had better buffering capabilities with just fish oil than with vitamin C and fish oil. Since vitamin C did not improve EBC pH either singly or in combination with fish oil, it suggests that vitamin C supplementation does not improve the proinflammatory acidic environment of the airways in asthma and may impede fish oil's positive effect on airway inflammation.

Subjects were asked to provide records of their morning and evening peak expiratory flow (PEF) measurements, short-acting β-agonist usage, and symptoms throughout the four phases of the study (run-in, one treatment, washout, and combination treatment phases). It was expected that their PEF would increase while their short-acting β-agonist usage and symptoms would decrease throughout the study to illustrate improved asthma management with the nutritional supplements. The Fish Oil Group demonstrated a significant increase in the evening PEF and a significant decrease in their symptoms with the combination treatment. There were no significant changes seen in their short-acting β-agonist usage. The Vitamin C Group did not demonstrate any significant changes in their PEF, symptoms, or short-acting β-agonist usage.

It has been suggested that self-reported symptoms cannot reliably diagnose EIB (82). However, Santanello et al. (83) have demonstrated that a symptom diary containing daytime and nighttime questions with scaled responses was reliable, valid, and responsive to changes in clinical trials where subjects also recorded their PEF and short-acting β-agonist usage, which is what subjects were asked to do in the current study. This concordance between subjective symptoms and objective measurements was seen in this study as the decrease in the daytime symptom score for the Fish Oil Group on the combination treatment was accompanied by an increase in the evening PEF. Although there was not a concurrent decrease in short-acting β-agonist usage, this should not discount the significance of the other results because the subjects in the current study reported low baseline short-acting β-agonist usage; overall, 5 of the 13 analyzed subjects averaged 0 puffs per day while only 2 subjects averaged greater than 1 puff per day during the pre-supplementation phase. Because the significant decrease in the daytime symptom score for the Fish Oil Group during the washout phase was not accompanied by changes in either objective measure, this change may not be as

reliable. Therefore, treatment phases of the study for which there was not a significant change in symptom score and PEF did not significantly improve the day-to-day control of asthma in these subjects.

In conclusion, this study has confirmed the effectiveness of fish oil in preventing HIB with a 3-week regimen of 3.2 g of eicosapentaenoic acid (EPA) and 2.0 g of docosahexaenoic acid (DHA) per day. Future studies should determine the optimal duration and formula for treatment with fish oil in order to best inform clinical recommendations to patients. Additionally, this study has demonstrated that vitamin C supplementation as a therapy for HIB is not universally effective. However, it can improve pulmonary function in a subset of patients. Thus, in the future, studies assessing vitamin C supplementation should include an additional screening period to first identify asthmatics who respond to vitamin C supplementation. This would help reduce variability in the subject population. Due to the multi-faceted nature of asthma, studying the most homogenous population of subjects may be a necessary, though difficult, task to best assess treatment efficacy. A vitamin C loading test could also be administered at the start of the study to determine the appropriate dose since individuals differ in the amount of vitamin C they absorb (97). Furthermore, there was not an additive effect with fish oil and vitamin C as expected. It is possible that subjects in both the Vitamin C and Fish Oil Groups who did not respond to vitamin C may have masked the potential additive effect. Examination of individual subjects' data reveals cases where the subject improved with one treatment and then improved further with the combination treatment. These results encourage future research on combining nutritional supplements to treat HIB.

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CHAPTER 3

THE EFFECT OF THE OMEGA-3 POLYUNSATURATED FATTY ACID DOCOSAHEXAENOIC ACID (DHA) ON HYPERPNEA-INDUCED BRONCHOCONSTRICTION IN ADULTS WITH ASTHMA

Abstract

Background: Hyperpnea, or rapid breathing, can be used to identify exercise-induced bronchoconstriction, which is a complication of asthma that has been shown to be attenuated by supplementation with fish oil. An optimal formula for fish oil has not been determined although previous *in vitro* studies have suggested that docosahexaenoic acid (DHA) may be the more potent omega-3 fatty acid in fish oil in terms of reducing inflammation.

Purpose: Determine whether supplementation with DHA can attenuate bronchoconstriction and airway inflammation in adults with asthma as compared to placebo.

Methods: Nine subjects (18-30 years) with asthma and hyperpnea-induced bronchoconstriction (HIB) participated in a randomized, double-blind, placebo-controlled crossover trial where they received either DHA or placebo capsules for 3 weeks. Following a 2-week washout phase, subjects then received the opposite supplement for 3 weeks. Subjects were tested in a laboratory following an initial 2-week run-in phase and after each supplementation phase. At each laboratory test, bronchoprovocation was elicited with eucapnic voluntary hyperventilation (EVH), a surrogate exercise challenge involving rapid breathing (hyperpnea). Prior to and following the EVH challenge, pulmonary function, fraction of exhaled nitric oxide (F_ENO), exhaled breath condensate pH, and the concentrations of 8-isoprostane and the DHA metabolites 17*S*-hydroxy-docosahexaenoic acid and protectin D1 in exhaled breath condensate were measured.

Subjects submitted records of their daily symptoms, peak expiratory flow measures, and bronchodilator use at each laboratory visit.

Results: There were no significant changes in pulmonary function, F_ENO, exhaled breath condensate pH, the concentration of 8-isoprostane, or the concentrations of the DHA metabolites 17*S*-hydroxy-docosahexaenoic acid and protectin D1 with DHA supplementation.

Conclusion: The data indicate that supplementation with 4.0 g DHA for 3 weeks did not significantly attenuate HIB or airway inflammation in asthmatic subjects as compared to baseline or placebo. Supplementation with eicosapentaenoic acid (EPA) should be similarly tested to determine if it is the more effective component of fish oil.

Introduction

The Center for Disease Control recently reported that 8.2% of the United States population has asthma (6). Exercise-induced bronchoconstriction (EIB) is an important complication of this chronic inflammatory disease of the airways as patients with asthma often report limitations in their physical activity (43). Moreover, EIB is an indication that a patient's current asthma treatment is inadequate (49). Because asthma is a multi-faceted disease, multiple medications targeting the acute and chronic symptoms are often prescribed (26). Nearly a third of the estimated $19.7 billion in direct and indirect healthcare costs for asthma in 2007 stemmed from prescription medications (1). Therefore, there is a growing interest in non-pharmacological alternatives.

A nutritional approach is an appealing alternative as the prevalence of asthma has been linked to societal changes in diet, such as increased sodium intake along with decreased antioxidant and omega-3 polyunsaturated fatty acid (PUFA) intake (60). Moreover, dietary supplement use is already popular in both the general and asthmatic populations with approximately 50% in each group reporting use in the last month in a recent National Health and Nutrition Examination Survey (54). It is thus important to

study these nutritional supplements and their effect on asthma in order to have a scientific basis upon which clinicians can recommend their proper and safe use.

Omega-3 PUFA supplementation is reportedly used by 6.7% of asthmatics (54). The omega-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) are the primary components of fish oil. Previous research has shown that supplementation with fish oil (3.2 g EPA and 2.0-2.2 g DHA per day for 3 weeks) reduces airway inflammation and EIB in elite athletes without asthma (62) and in adults with asthma (61). The mechanism of action of the omega-3 PUFAs is thought to involve decreasing the availability of the more proinflammatory omega-6 PUFA products through competition for common enzymes (60). The omega-3 PUFA products cause less bronchoconstriction than the omega-6 PUFA products (60).

The optimal fish oil formula, dose, and length of treatment period used for alleviating EIB has yet to be determined; this is partly due to the fact that there is no consensus as to which component of fish oil, EPA or DHA, is the more potent contributor to the positive effects seen with supplementation in asthma or EIB (86). Although there have been several *in vivo* studies in humans and mice (47, 86) as well as *in vitro* studies on human macrophage cells (64, 98) comparing the anti-inflammatory effects of EPA and DHA, the existing research primarily focuses on markers of inflammation and immune function, not airway responsiveness. This is a notable shortcoming of the available literature comparing EPA and DHA since airway responsiveness is clinically important for patients with asthma.

Studies by Serhan et al. (84) and Levy et al. (52) provide the principal support for DHA as the more potent ingredient in fish oil. Serhan et al. (84) have demonstrated that a metabolite of DHA, now called protectin D1, can actively resolve inflammation by reducing proinflammatory signaling. Applying this mechanism to *in vivo* murine studies, Levy et al. (52) have shown that injecting mice with protectin D1 decreased their

subsequent bronchoconstriction during a methacholine challenge. Furthermore, Levy et al. (52) have demonstrated that adding DHA to the homogenized murine lung tissue *ex vivo* yielded a significant increase in the protectin D1 concentration, which suggests that DHA can be converted to its anti-inflammatory metabolite by respiratory tissues (52). In addition, it was shown that during an asthma attack, patients had significantly lower levels of protectin D1 in their exhaled breath condensate as compared to healthy individuals (52). Therefore, the next step is to determine whether asthmatics taking DHA supplements will increase their exhaled breath condensate concentration of protectin D1 and thus experience less bronchoconstriction upon provocation.

The purpose of this study was to investigate whether DHA can attenuate hyperpnea-induced bronchoconstriction and airway inflammation in adults with asthma as compared to placebo. It is hypothesized that DHA supplementation will attenuate hyperpnea-induced bronchoconstriction (HIB) and airway inflammation in asthmatic individuals as compared to placebo.

Methods

Subjects. Nine subjects (6 male, 3 female) between the ages of 18 to 30 years with physician-diagnosed asthma and exercise-induced bronchoconstriction (EIB) were recruited from a university setting. Mild to moderate asthmatics were included based on their pulmonary function evaluated during their first laboratory test. Each subject's forced expiratory volume in one second (FEV_1) was measured at rest and following eucapnic voluntary hyperventilation, a surrogate exercise challenge used to diagnose EIB (9). Subjects who demonstrated a 10 to 50% change in their FEV_1 from pre- to post-challenge were classified as mildly to moderately asthmatic and permitted to continue in the study. All subjects were allowed to continue to use their prescribed short-acting β_2-agonist (albuterol) throughout the study except for in the six hours before they reported to the laboratory for testing. No other prescribed maintenance medications for asthma

were allowed during the study. This required one subject to stop taking ADVAIR DISKUS® (fluticasone propionate and salmeterol) with the written permission of his physician for four weeks prior to beginning the study (61). Exclusion criteria included current fish oil supplementation, pregnancy, or a history of seizures, diabetes, hypertension, hyperlipidemia, bleeding disorders, or delayed clotting time. Subjects were asked to limit their fish consumption to one meal per week throughout the course of the study.

This study was approved by the Indiana University Institutional Review Board (protocol # 1005001346) and was registered as a clinical trial with clinicaltrials.gov (study # NCT01200446). All subjects gave their informed consent before enrolling in the study. Healthy, non-asthmatic subjects were not recruited to this study to act as a control group because it has been demonstrated that fish oil supplementation does not significantly change pulmonary function or inflammatory mediators in individuals without asthma or EIB (62).

Study Design. This study (figure 3-1) was conducted as a randomized, double-blind, placebo-controlled crossover trial where subjects received either active capsules containing 4.0 g of DHA (Martek Biosciences Corporation, Columbia, MD) (n = 5) or placebo capsules containing a corn and soy oil blend (n = 5) for 3 weeks. Following a 2-week washout period, subjects who were given placebo capsules received active DHA capsules and subjects who were given active DHA capsules received placebo capsules for 3 weeks.

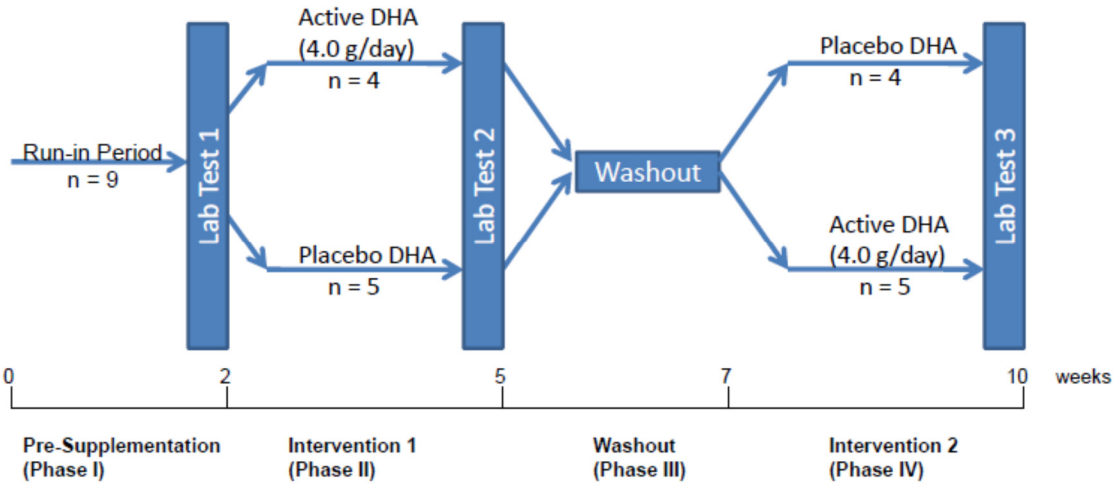

Figure 3-1. ***Schematic of study design.*** *Subjects entered the study on their normal diet. Following a 2-week run-in period, subjects completed a placebo-controlled crossover trial of two 3-week supplementation periods separated by a 2-week washout phase. Subjects were supplemented with either 4.0 g of docosahexaenoic acid or placebo containing a mixture of corn and soy oils.*

Subjects were enrolled while on their normal diet. The order of supplementation was randomly assigned with the use of a computerized random number generator (http://www.randomizer.org/form.htm). The randomization sequence was created using a fixed random block size of two to correspond to the two treatments (i.e. active and placebo). The company supplying the active and placebo capsules delivered sealed pill bottles labeled with one of two material numbers. The principle investigator was not informed which material number corresponded to each treatment until after data collection and initial data analysis was completed. The active and placebo capsules were identical in appearance so that subjects were not aware of which treatment they received.

At each laboratory visit, subjects completed the same series of tests. They reported to the laboratory having abstained from exercising for 24 hours, having caffeine for 8 hours, and using their short-acting β_2-agonist for 6 hours (93). Eucapnic voluntary hyperventilation (EVH) was the bronchoprovocation test used at each visit to the

laboratory. Before and after EVH, inflammatory markers and pulmonary function were evaluated. Food frequency questionnaires were employed to assess changes in diet between phases of the study. Subjects were instructed to track their peak expiratory flow values, short-acting β_2-agonist (albuterol) usage, and symptoms every day at home and to submit these records at each laboratory visit. Compliance with supplementation was determined through pill counts of the bottles returned by each subject at the second and third laboratory visits.

Eucapnic Voluntary Hyperventilation. Bronchoprovocation was provided by the eucapnic voluntary hyperventilation (EVH) challenge, which requires subjects to breathe cold, dry air at a rapid rate. While wearing nose clips, subjects were asked to breathe through a non-rebreathing two-way valve (Hans Rudolph, Inc., Kansas City, MO) connected to a reservoir bag continually filled with 21% oxygen, 5% carbon dioxide, and balance nitrogen from a compressed gas tank containing less than 3 mg H_2O*L^{-1} air (93). Subjects were instructed to breathe for 6 minutes at 85% of their maximal voluntary ventilation as estimated by thirty times their resting FEV_1 (9). In order to verify the ventilatory rate, a flow sensor measured ventilation (Vmax 22 Metabolic Measurement Cart, SensorMedics, Yorba Linda, CA) (81).

Pulmonary Function Tests. Pulmonary function was measured pre-EVH and post-EVH at 5, 10, 15, and 20 minutes using a calibrated computerized pneumotachograph spirometer (Vmax 22 Metabolic Measurement Cart, SensorMedics, Yorba Linda, CA) (93). In accordance with American Thoracic Society (ATS) recommendations, each subject performed three acceptable spirograms, of which the largest and second largest forced vital capacity (FVC) and FEV_1 values did not vary by more than 0.15 L (65). The largest value of each was recorded. Values for the forced expiratory flow at 25-75% of the FVC ($FEF_{25-75\%}$) were recorded from the trial with the greatest sum of FVC and FEV_1 (65). The bronchoconstrictor response to EVH was also

determined as the area under the curve of the percentage fall in post-exercise FEV_1 plotted against time for 20 min, using trapezoidal integration (93).

Fraction of Exhaled Nitric Oxide. Before and 30 minutes following EVH challenge, online measurement of the fraction of exhaled nitric oxide (F_ENO) was recorded using a restricted exhaled breath protocol (NOA 280i Nitric Oxide Analyzer, Accurate NO Breath Kit, Thermal Mass Flowmeter, NO Analysis Software Version 3.21, Sievers Instruments, Boulder, CO) (93). American Thoracic Society guidelines were followed (2). Accordingly, subjects were instructed to inhale maximally to their total lung capacity and immediately exhale against expiratory resistance at a rate of 50 ± 10 mL/s for at least 6 seconds to produce a nitric oxide plateau lasting at least 3 seconds; real time feedback was provided visually on a computer screen. Subjects performed this maneuver while wearing nose clips with at least 30 seconds of rest between each trial (93). The F_ENO was recorded as the mean of three exhalations with the individual F_ENO values within 10% of the mean F_ENO (2).

Exhaled Breath Condensate. Exhaled breath condensate (EBC) was collected from seven subjects (6 male, 1 female) pre-EVH and post-EVH at 0-10 minutes (93) according to American Thoracic Society and European Respiratory Society recommendations (42). Subjects were instructed to breathe normally into a non-rebreathing valve attached to a condensing chamber (ECoScreen, Viasys Healthcare-Jaeger, Germany) for 10 minutes while wearing nose clips (93). This condensing chamber maintained an internal temperature of -20 ˚C to immediately freeze the exhaled breath sample during collection (93). The pH of the non-deaerated EBC was measured within 5 minutes of collection (Orion 2 Star pH benchtop meter, ROSS™ Glass Combination Micro pH electrode, Thermo Fisher Scientific, Inc., Beverly, MA).

The EBC samples were then stored at -80 ˚C until liquid chromatography analysis was performed. Quantification of the DHA metabolites 17*S*-hydroxy-

docosahexaenoic acid and protectin D1 as well as the oxidative stress marker 8-isoprostane was performed using the QTRAP 4000 instrument (ABI Sciex, Foster City, CA). A Dionex UltiMate 3000 LC system (Dionex Corporation, Sunnyvale, CA) consisting of a binary pump, a temperature-controlled autosampler maintained at 5 ˚C, and a column oven compartment maintained at 25 ˚C, was interfaced to an ESI Turbo V ion source of the triple quadrupole 4000 QTRAP instrument. The samples (~200 µL) were lyophilized following the addition of 2 µL of 10 pg/µL of the internal standard (IS) docohexanoic acid d5 dissolved in 50:50 (v:v) methanol:H_2O. The samples were then re-suspended in 25 µL of 50:50 (v:v) methanol:H_2O, and 20 µL were injected into the mass spectrometer. Calibration curve solutions of all three metabolites at concentrations of 1, 5, 10, and 20 pg/µL containing the IS at a concentration of 0.8 pg/µL in each of the solutions were prepared starting from a working solution mixture containing all three metabolites at a concentration of 1 ng/µL in 50:50 (v:v) methanol:H_2O. The internal standard working solution had a concentration of 10 pg/µL. The analysis was performed in negative ion mode using a reversed-phase Acclaim® RSLC 120 C18 column (100 mm × 2.1 mm, 2.2 µm particle size, 120 A˚ diameter) from Dionex. The flow rate was 150 µL/minute and mobile phase A consisted of methanol:water:acetic acid (65:35:0.01, v:v:v); mobile phase B was methanol. The gradient conditions were from 0% B to 100% B from 8-30 minutes followed by 3 minutes at 100% B. The total run time was 41 minutes. The mobile phase and gradient conditions were similar to those used by Lu et al. (53)

The mass spectrometer was operated in negative ion multiple reaction monitoring (MRM) mode where the radiofrequency (rf) and direct current (dc) in both Q1 and Q3 were jumped to transmit different precursor/product ion pairs. The $[M-H]^-$ precursor ions were used for all species monitored in this experiment. The MRM transitions employed were 332/288 for docohexanoic acid-d5, 343/281 for 17*S*-hydroxy-

docosahexaenoic acid, 375/140.9 for protectin D1, and 353/193 for 8-isoprostane. The Turbo V ion source parameters were common to all analytes in the MRM method. The capillary was operated at 4500 V, and the source temperature was set to 250 ˚C. The curtain gas (N_2) and collision gas (N_2) settings were 10 psi; the nebulization gas setting was 40 psi and the vaporization gas setting was 50 psi. A dwell time of 20 msec, a declustering potential (DP) of -78 V, and a collision energy (CE) value of -25 V were used.

Symptoms and Short-Acting β_2-Agonist Usage. Subjects were instructed to rate their symptoms by filling out a symptom diary everyday throughout the study. This diary adapted from Santanello et al. (83) contained four questions about daytime symptoms with a seven point scale and one question about nighttime symptoms with a four point scale. Daily symptom scores were calculated for each subject by averaging the mean score from each day (83).

Subjects recorded their short-acting β_2-agonist use in log books provided to them. Subjects were instructed to mark down the number of puffs taken per day throughout the study.

Peak Flow Measurements. Electronic peak flow meters (PiKo-1, Ferraris Medical, Louisville, CO) were given to subjects to measure their morning and evening peak expiratory flow throughout the study. Subjects were instructed to perform the maneuver by inhaling fully to their total lung capacity and then exhaling forcefully through the flow meter according to manufacturer instructions. Subjects were instructed to record the best of three trials upon waking and before going to bed.

Nutrient Intake. The GSEL version of food frequency questionnaires developed by the Nutrient Assessment Shared Resource (NASR) of the Fred Hutchinson Cancer Research Center (FHCRC) was used to evaluate subjects' nutrient intake during the study. Subjects were asked to complete a questionnaire at the end of each phase of the

study. They were instructed to refer to their diet during the course of that particular phase of the study to answer the questions. It has been previously demonstrated that food frequency questionnaires are a valid and reliable method of collecting dietary data (99).

Data Analysis. A power analysis was conducted using data from our laboratory to determine the number of subjects needed for the present study (61). Based on the maximal post-exercise drop in FEV_1 (L) in asthmatics supplemented with fish oil, it was determined with the aid of the G*Power3.0.5 program that at least three subjects would be needed to achieve a power of 0.80. Because the current study used DHA, which is a different fish oil formula, we recruited additional subjects with asthma.

Data was analyzed with SPSS version 18.0 statistical software (SPSS Inc., Chicago, IL). Repeated measures ANOVA assessed differences among pre-supplementation, placebo, and DHA supplementation values at the laboratory tests as well as among the pre-supplementation, placebo, DHA supplementation, and washout phases for nutrient intake and the at-home measures. Mauchley's test was conducted to determine if sphericity was violated; if it was, a Greenhouse-Geisser adjustment was applied. When a significant F-ratio was present ($p < 0.05$), Tukey's post-hoc test was used to isolate differences in group means. To determine the presence of a carry-over effect between the two treatment periods, a 2 x 2 cross-over trial split-plot ANOVA was conducted. Significance was held at $p < 0.05$ for all statistical tests. The data is presented as mean ± standard error of the mean.

Results

Subjects. There were no reported adverse effects with supplementation. According to the 2 x 2 cross-over design split-plot ANOVA, there was not a significant carry-over effect ($p > 0.05$) between the two treatment periods (figure 3-2). The subjects' measurements at the pre-supplementation laboratory visit were considered

their baseline values (table 3-1). The subjects' resting pulmonary function was not significantly different ($p > 0.05$) among the three laboratory visits (table 3-2). A summary of the treatment effects for the main dependent variables demonstrates that there were no significant differences ($p > 0.05$) between DHA supplementation and placebo (table 3-3).

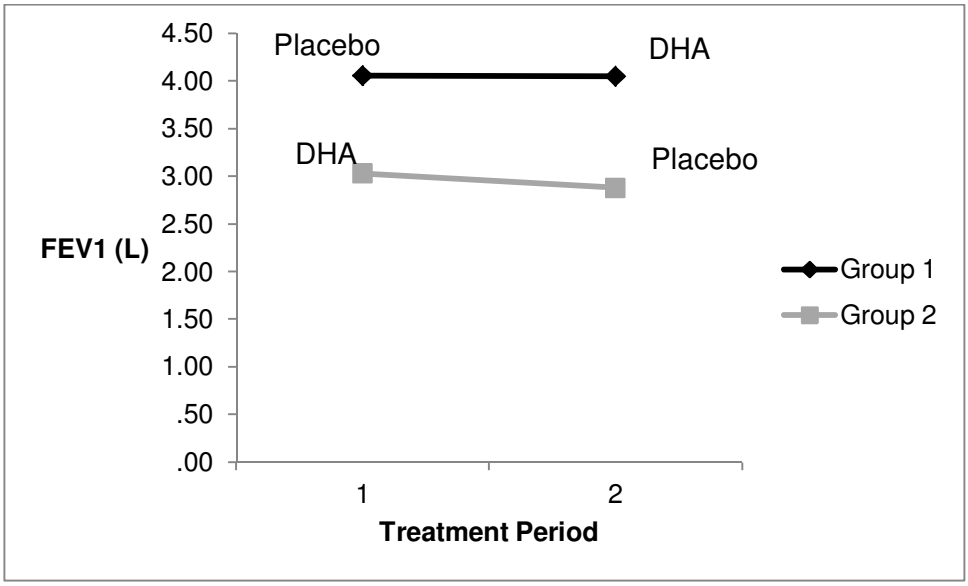

Figure 3-2. ***Analysis of the treatment periods for a carry-over effect.*** *The mean forced expiratory volume in one second (FEV_1) at 5 minutes post-eucapnic voluntary hyperventilation challenge for each group at each treatment period is shown. There was not a significant ($p > 0.05$) carry-over effect between the treatment periods for the two groups. Group 1 contains the subjects who received the placebo during the first treatment period and docosahexaenoic acid (DHA) supplements during the second treatment period ($n = 5$). Group 2 contains the subjects who received DHA supplements during the first treatment period and the placebo during the second treatment period ($n = 4$).*

Males	6
Females	3
Age, yr (range)	22.36 ± 1.31 (18-30)
Height, m	1.77 ± 0.02
Weight, kg	75.77 ± 2.43
BMI, kg/m^2	24.17 ± 0.86
Morning Peak Flow, L/min	467.68 ± 43.61
Evening Peak Flow, L/min	483.17 ± 46.33
FEV_1/FVC	79.11 ± 3.44
Percent Predicted FVC, %	110.89 ± 4.39
Percent Predicted FEV_1, %	103.65 ± 3.69

Table 3-1. ***Baseline characteristics of the subjects at their first (pre-supplementation) laboratory visit.*** *Values are reported as mean ± standard error of the mean. BMI, body mass index; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity*

	Pre-Supplementation (%)	**Placebo** (%)	**DHA** (%)
FVC	110.89 ± 4.39	108.59 ± 5.20	108.04 ± 4.08
FEV_1	103.65 ± 3.69	101.00 ± 4.14	103.01 ± 4.04
$FEF_{25-75\%}$	90.67 ± 11.70	88.95 ± 9.56	91.16 ± 9.65

Table 3-2. ***Resting pulmonary function.*** *There were no significant differences in the resting pulmonary function of the subjects among the pre-supplementation, placebo, and docosahexaenoic acid (DHA) supplementation laboratory tests prior to the eucapnic voluntary hyperventilation challenge. The pulmonary function values are expressed as percentages of the subjects' predicted values based on age, height, weight, and sex. They are reported as mean ± standard error of the mean. FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; $FEF_{25-75\%}$, forced expiratory flow at 25-75% of the FVC*

Variable	Pre-Supplementation	Placebo	DHA
Post-EVH maximum drop FVC (L)	0.66 ± 0.06	0.55 ± 0.10	0.49 ± 0.12
Post-EVH maximum drop FEV_1 (L)	0.87 ± 0.11	0.69 ± 0.13	0.79 ± 0.18
Post-EVH maximum drop $FEF_{25-75\%}$ (L/s)	1.17 ± 0.30	0.91 ± 0.23	0.97 ± 0.30
Post-EVH maximum % drop FVC	12.23 ± 1.43	10.49 ± 1.89	10.06 ± 2.77
Post-EVH maximum % drop FEV_1	21.07 ± 2.65	17.20 ± 3.27	17.27 ± 3.73
Post-EVH maximum % drop $FEF_{25-75\%}$	31.56 ± 6.37	26.86 ± 5.93	26.85 ± 6.78
AUC FEV_1	296.69 ± 46.09	232.31 ± 56.23	251.69 ± 61.84
Pre-EVH Exhaled Breath Condensate pH	6.86 ± 0.12	7.15 ± 0.04	6.86 ± 0.06
Post-EVH Exhaled Breath Condensate pH	6.86 ± 0.09	6.96 ± 0.13	7.01 ± 0.19
Pre-EVH F_ENO	73.02 ± 20.96	43.26 ± 9.42	68.96 ± 21.64
Post-EVH F_ENO	63.50 ± 16.67	36.39 ± 8.36 *	59.87 ± 18.20
Pre-EVH 8-Isoprostane Concentration (pg/µL)	3.08 ± 1.50	6.16 ± 2.12	4.48 ± 1.20
Post-EVH 8-Isoprostane Concentration (pg/µL)	2.21 ± 1.67	3.47 ± 1.82	6.59 ± 3.71
Pre-EVH Protectin D1 Concentration (pg/µL)	< 0	< 0	< 0
Post-EVH Protectin D1 Concentration (pg/µL)	< 0	< 0	< 0
Pre-EVH 17*S*-hydroxy-docosahexaenoic acid (pg/µL)	< 0	< 0	< 0
Post-EVH 17*S*-hydroxy-docosahexaenoic acid (pg/µL)	< 0	< 0	< 0

Table 3-3. ***Summary of the treatment effects.*** *Values are reported as mean ± standard error of the mean. DHA, docosahexaenoic acid; EVH, eucapnic voluntary hyperventilation; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; $FEF_{25-75\%}$, forced expiratory flow at 25-75% of the FVC; AUC FEV_1, area under the curve of the percent change in FEV_1; FENO, fraction of exhaled nitric oxide; *, significantly different from pre-supplementation*

Pulmonary Function. At the pre-supplementation laboratory test, the mean maximum drop in FEV_1 following the eucapnic voluntary hyperventilation (EVH) challenge was 21.07 ± 2.65%. At the subsequent laboratory tests, the mean maximum drop in FEV_1 remained greater than the diagnostic threshold of a 10% post-challenge decrease in FEV_1; the mean maximum drop in FEV_1 was not significantly different ($p > 0.05$) among the pre-supplementation, placebo (17.20 ± 3.27%), and DHA supplementation (17.27 ± 3.73%) values (figure 3-3). When examined as a change in volume, there were still no significant differences ($p > 0.05$) in the values for the maximum drop in FEV_1 among the pre-supplementation (0.87 ± 0.11 L), placebo (0.69 ± 0.13 L), and DHA supplementation (0.79 ± 0.18 L) tests (figure 3-4).

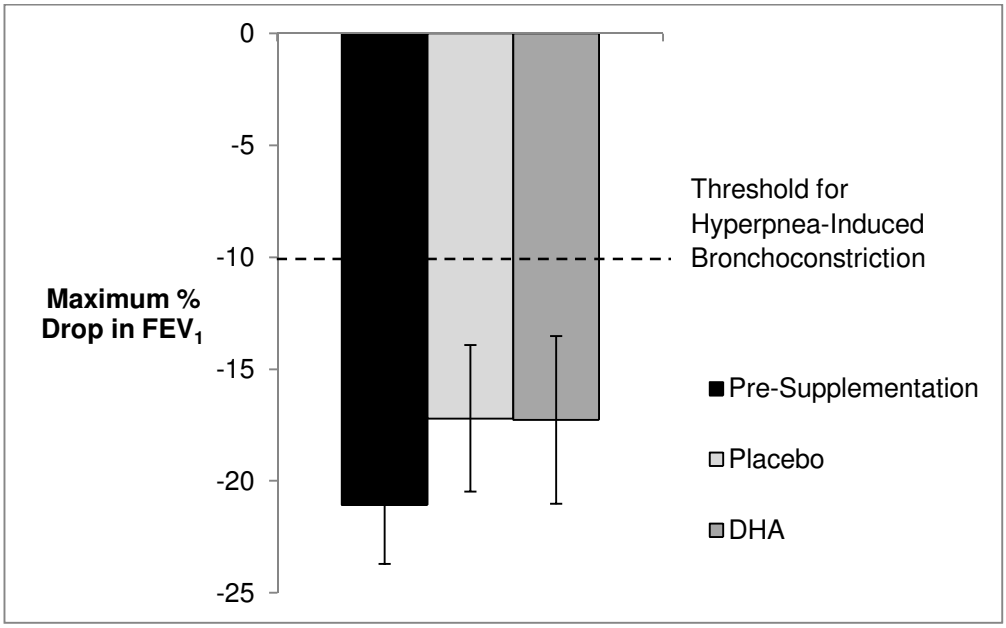

Figure 3-3. ***Maximum percent drop in FEV_1 following the eucapnic voluntary hyperventilation challenge.*** *The mean maximum percent drop in FEV_1 exceeded the diagnostic threshold for hyperpnea-induced bronchoconstriction (HIB) at all three laboratory visits. There were no significant differences ($p > 0.05$) among pre-supplementation, placebo, and DHA supplementation. Error bars express standard error of the mean. DHA, docosahexaenoic acid; FEV_1, forced expiratory volume in one second*

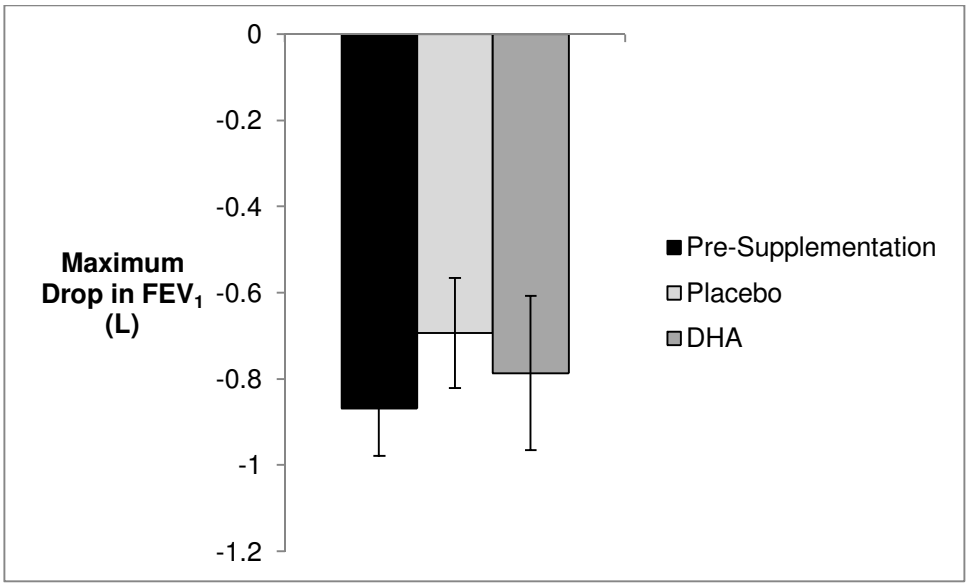

Figure 3-4. ***Maximum drop in FEV_1 following the eucapnic voluntary hyperventilation challenge.*** *There were no significant differences ($p > 0.05$) in the post-challenge FEV_1 volumes at the three laboratory visits. Error bars express standard error of the mean. DHA, docosahexaenoic acid; FEV_1, forced expiratory volume in one second*

Furthermore, there were no significant differences ($p > 0.05$) between maximal changes in FVC expressed as a percentage (figures 3-5) or as a volume (figures 3-6) for pre-supplementation (12.23 ± 1.43%, 0.66 ± 0.6 L), placebo (10.49 ± 1.89%, 0.55 ± 0.10 L), and DHA supplementation (10.16 ± 2.77%, 0.49 ± 0.12 L).

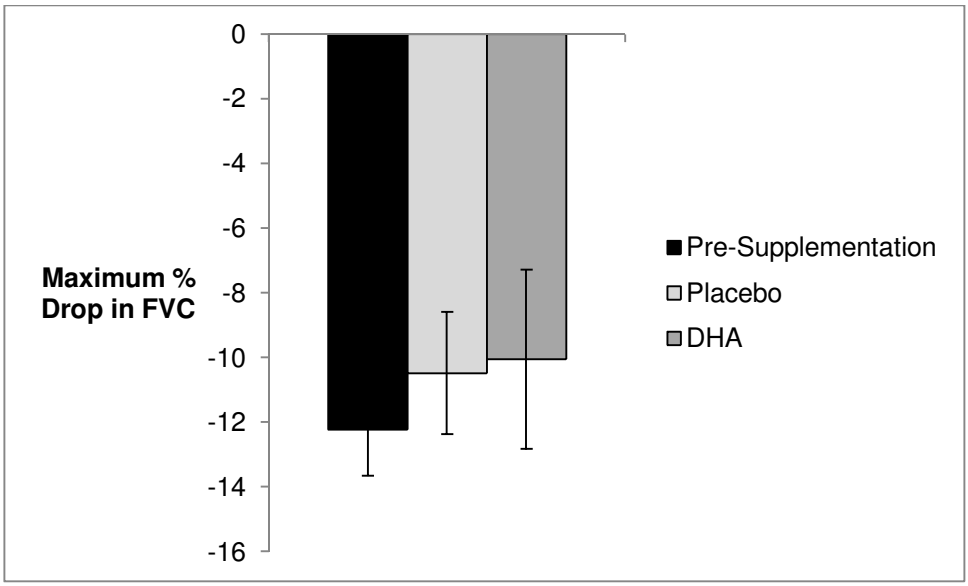

Figure 3-5. ***Maximum percent drop in FVC following the eucapnic voluntary hyperventilation challenge.*** *There were no significant differences (p > 0.05) between pre-supplementation, placebo, and docosahexaenoic acid (DHA) supplementation. Error bars express standard error of the mean. FVC, forced vital capacity*

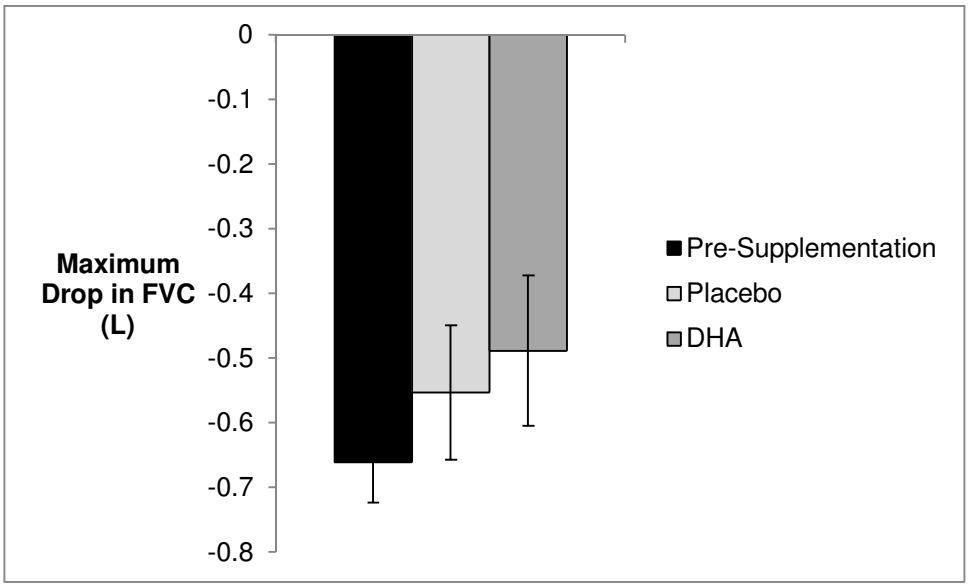

Figure 3-6. ***Maximum drop in FVC following the eucapnic voluntary hyperventilation challenge.*** *There were no significant differences (p > 0.05) in the post-challenge FVC volumes at the three laboratory tests. Error bars express standard error of the mean. DHA, docosahexaenoic acid; FVC, forced vital capacity*

There were also no significant differences ($p > 0.05$) in $FEF_{25-75\%}$ in terms of percentages (figure 3-7) or flow rates (figure 3-8) among the pre-supplementation (31.57 ± 6.37%, 1.17 ± 0.30 L/s), placebo (26.86 ± 5.93%, 0.91 ± 0.23 L/s), and DHA supplementation (26.85 ± 6.78%, 0.97 ±0.30 L/s) laboratory tests.

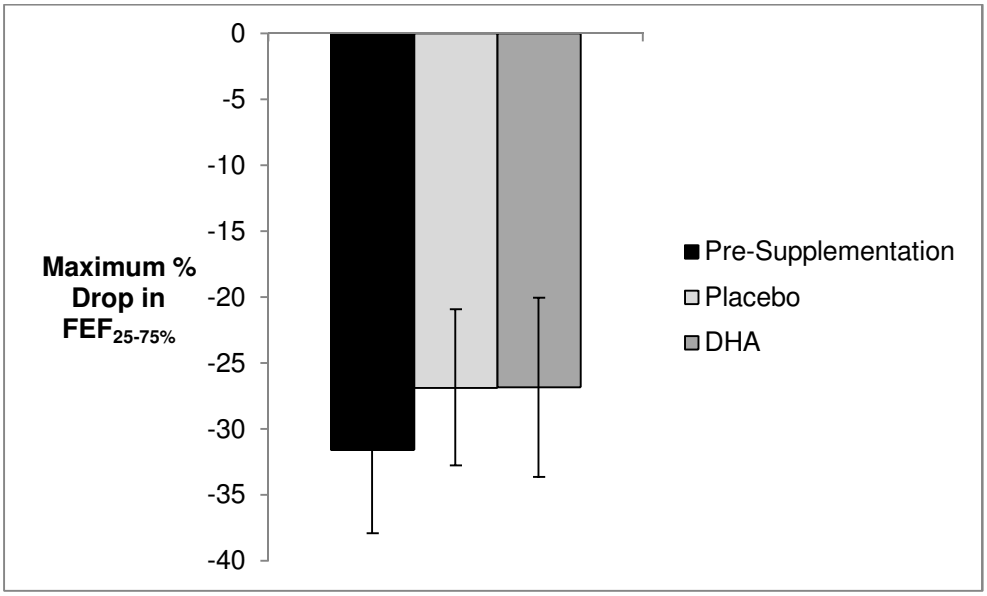

Figure 3-7. ***Maximum percent drop in $FEF_{25-75\%}$ following the eucapnic voluntary hyperventilation challenge.*** *There were no significant differences ($p > 0.05$) between pre-supplementation, placebo, and docosahexaenoic acid (DHA) supplementation. Error bars express standard error of the mean. $FEF_{25-75\%}$, forced expiratory flow at 25-75% of the forced vital capacity*

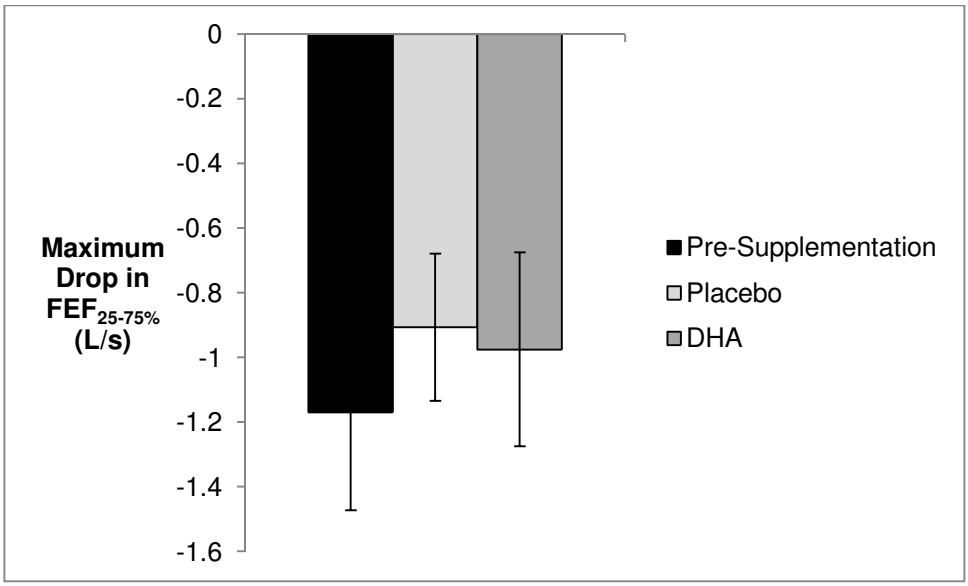

Figure 3-8. ***Maximum drop in $FEF_{25-75\%}$ following the eucapnic voluntary hyperventilation challenge.*** *There were no significant differences ($p > 0.05$) in the post-challenge $FEF_{25-75\%}$ flow rates at the three laboratory visits. Error bars express standard error of the mean. DHA, docosahexaenoic acid; $FEF_{25-75\%}$, forced expiratory flow at 25-75% of the FVC*

Additionally, no significant differences ($p > 0.05$) were found between pre-supplementation, placebo, and DHA supplementation in terms of the post-eucapnic voluntary hyperventilation (EVH) percent changes in FEV_1, FVC, or $FEF_{25-75\%}$ at any of the time points tested (5, 10, 15, and 20 minutes post-EVH) (figures 3-9, 3-10, 3-11).

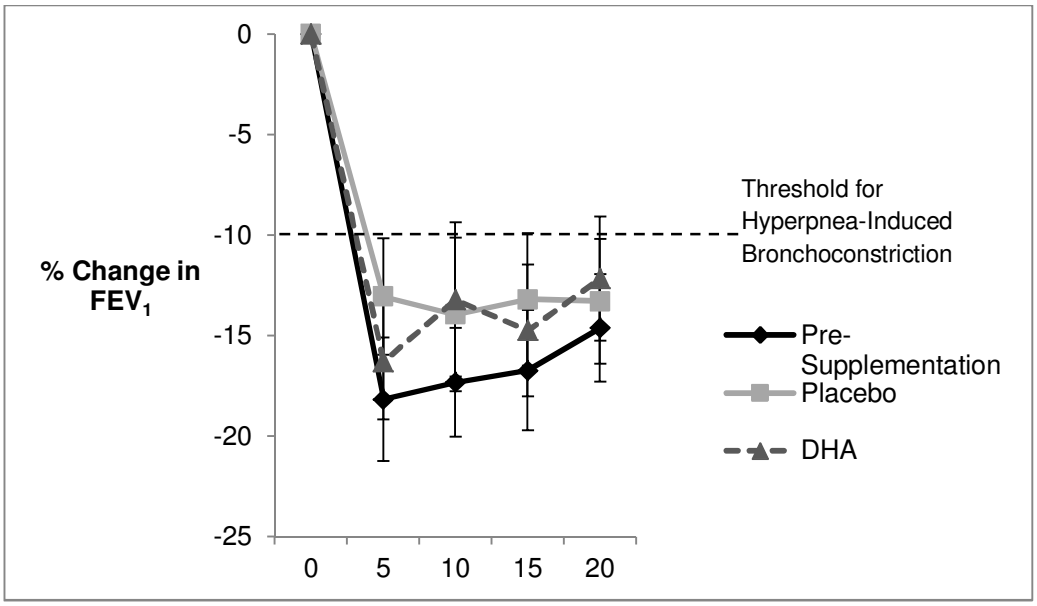

Figure 3-9. ***The percent change in FEV_1 at 5, 10, 15, and 20 minutes after the eucapnic voluntary hyperventilation challenge.*** *There were no significant differences ($p > 0.05$) between pre-supplementation, placebo, and docosahexaenoic acid (DHA) supplementation at any of the time points. Error bars express standard error of the mean. FEV_1, forced expiratory volume in one second*

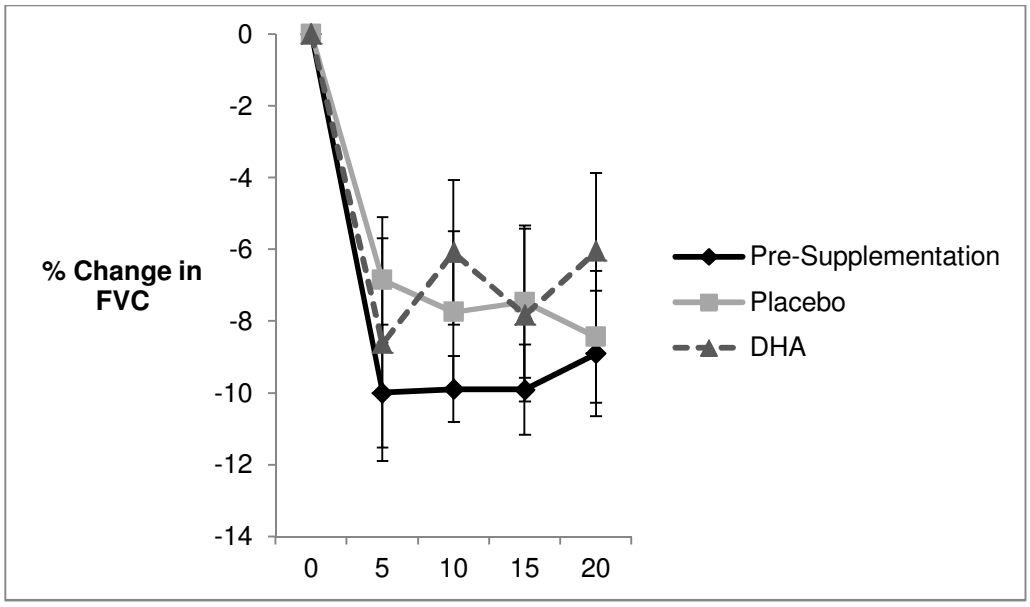

Figure 3-10. ***The percent change in FVC at 5, 10, 15, and 20 minutes after the eucapnic voluntary hyperventilation challenge.*** *There were no significant differences ($p > 0.05$) between pre-supplementation, placebo, and docosahexaenoic acid (DHA) supplementation at any of the time points. Error bars express standard error of the mean. FVC, forced vital capacity*

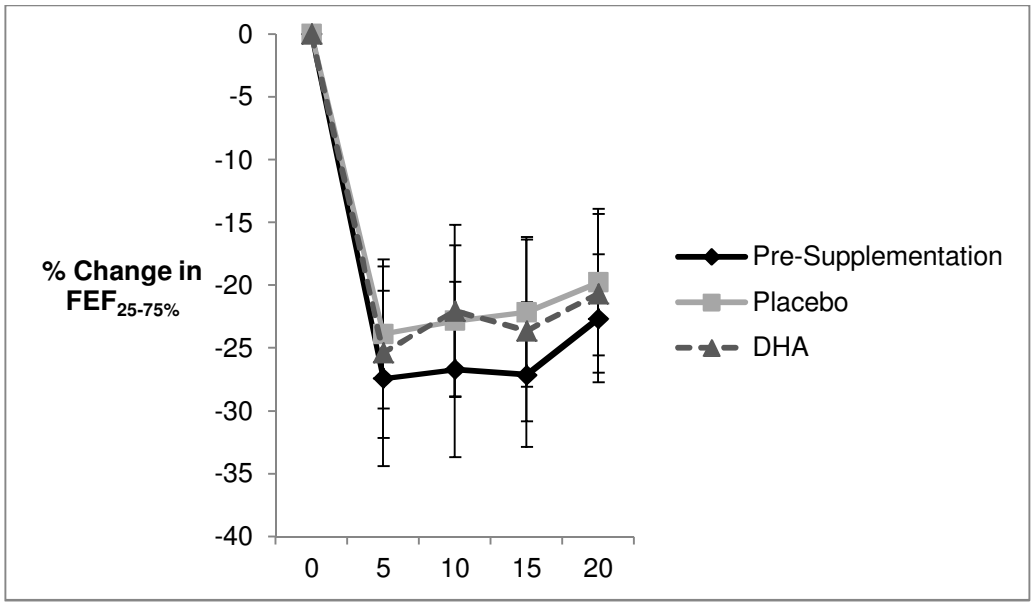

Figure 3-11. ***The percent change in $FEF_{25-75\%}$ at 5, 10, 15, and 20 minutes after the eucapnic voluntary hyperventilation challenge.*** *There were no significant differences ($p > 0.05$) between pre-supplementation, placebo, and docosahexaenoic acid (DHA) supplementation at any of the time points. Error bars express standard error of the mean. $FEF_{25-75\%}$, forced expiratory flow at 25-75% of the FVC*

Lastly, there were no significant differences ($p > 0.05$) between the values for the area under the curve of the percent change in FEV_1 for the 20 minutes following EVH (AUC_{0-20}) among the pre-supplementation (296.69 ± 46.09), placebo (232.31 ± 56.23), and DHA supplementation (251.69 ± 61.84) tests (figure 3-12).

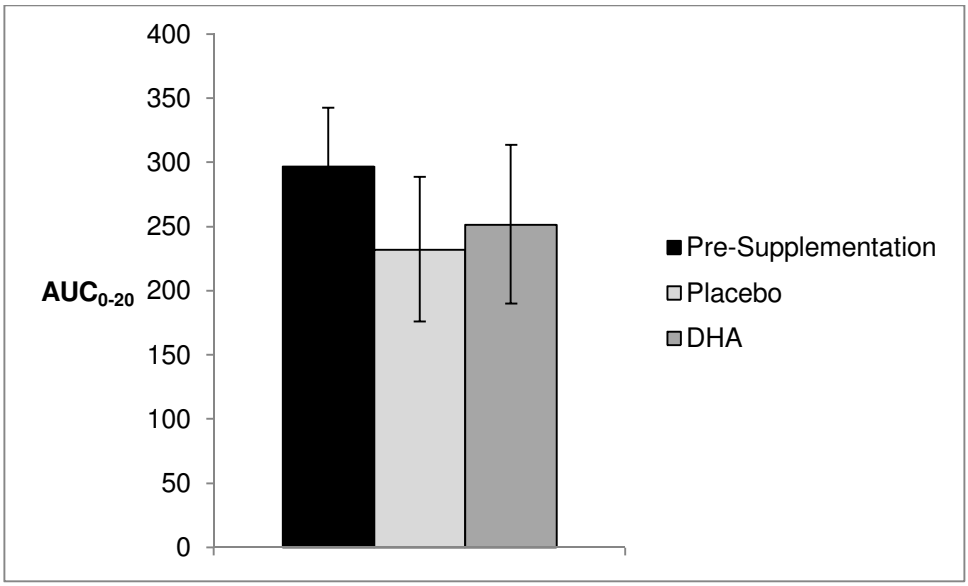

Figure 3-12. ***The area under the curve of the percent change in FEV_1 for 20 minutes (AUC_{0-20}).*** *This measure represents the bronchoconstrictor response to the eucapnic voluntary hyperventilation challenge. There were no significant differences ($p > 0.05$) in the AUC_{0-20} at the three laboratory visits. Error bars express standard error of the mean. DHA, docosahexaenoic acid; FEV_1, forced expiratory volume in one second*

Fraction of Exhaled Nitric Oxide. The pre-EVH fraction of exhaled nitric oxide (F_ENO) was not significantly different ($p > 0.05$) between pre-supplementation (73.02 ± 20.96 ppb), placebo (43.26 ± 9.42 ppb), and DHA supplementation (68.96 ± 21.64 ppb) (figure 3-13). However, the placebo value for post-EVH F_ENO (36.39 ± 8.36 ppb) was significantly lower ($p < 0.05$) than the pre-supplementation value for post-EVH F_ENO (63.50 ± 16.67 ppb). The post-EVH F_ENO following DHA supplementation (59.87 ± 18.20 ppb) was not significantly different ($p > 0.05$) from either the pre-supplementation or placebo values.

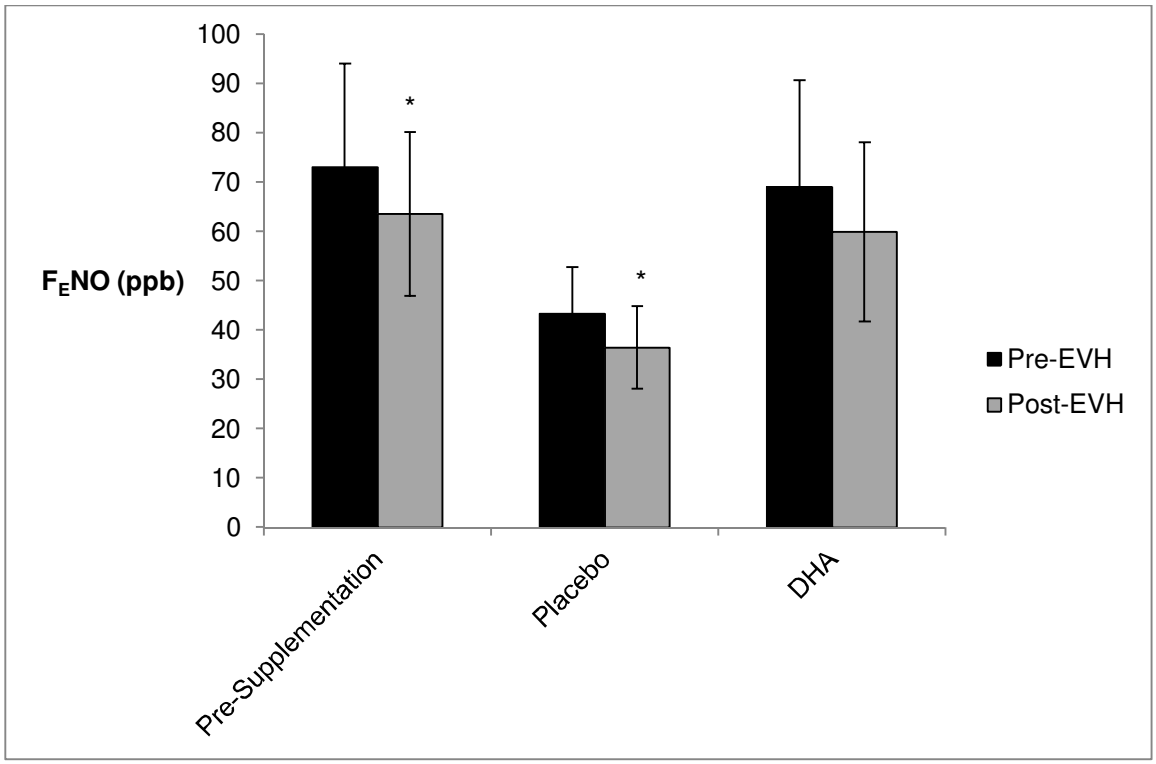

Figure 3-13. ***The fraction of exhaled nitric oxide (F_ENO) pre- and post-eucapnic voluntary hyperventilation (EVH) challenge.*** *There were no significant differences ($p > 0.05$) between the pre-EVH values from any of the laboratory visits. The post-EVH F_ENO was significantly lower with the placebo treatment compared to the pre-supplementation value. The post-EVH F_ENO with docosahexaenoic acid (DHA) supplementation was not significantly different from pre-supplementation or placebo values. Error bars express standard error of the mean. *, significantly different from each other*

Exhaled Breath Condensate. Seven subjects provided exhaled breath condensate samples at each of the laboratory tests. Neither the pre- nor post-EVH exhaled breath condensate (EBC) pH values significantly changed ($p > 0.05$) among the pre-supplementation (pre-EVH: 6.88 ± 0.09, post-EVH: 6.82 ± 0.09), placebo (pre-EVH: 7.09 ± 0.07, post-EVH: 6.93 ± 0.12), and DHA supplementation (pre-EVH: 6.90 ± 0.07, post-EVH: 7.02 ± 0.16) laboratory tests (figure 3-14).

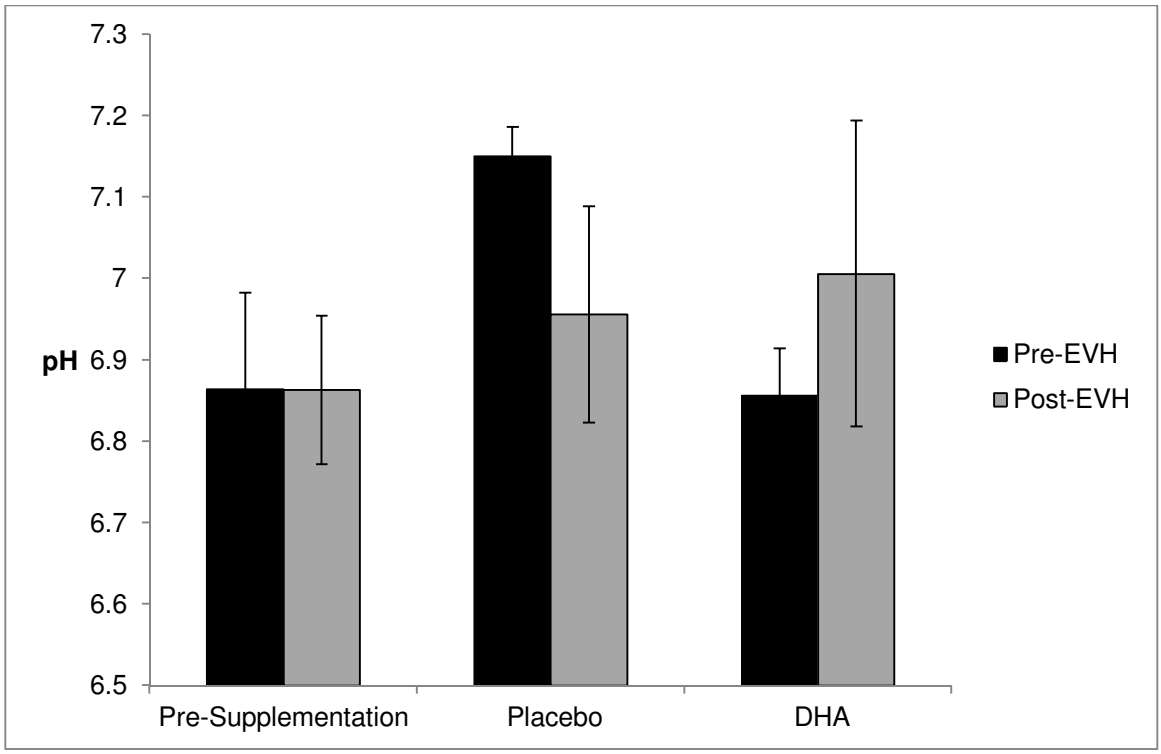

Figure 3-14. ***Exhaled breath condensate pH pre- and post-eucapnic voluntary hyperventilation (EVH) challenge.*** *There were no significant differences ($p > 0.05$) between the pre-EVH values or between the post-EVH values at any of the laboratory visits ($n = 7$). Error bars express standard error of the mean.*

For these seven subjects, EBC was also analyzed by liquid chromatography for the oxidative stress marker 8-isoprostane and the DHA metabolites protectin D1 and 17*S*-hydroxy-docosahexaenoic acid at each laboratory test before and after the EVH challenge. The concentration of 8-isoprostane did not significantly change among the three laboratory tests (figure 3-15). The mean pre-EVH concentration of 8-isoprostane was 3.08 ± 1.50 pg/μL at the pre-supplementation test, 6.16 ± 2.12 pg/μL following placebo supplementation, and 4.48 ± 1.20 pg/μL following DHA supplementation. The post-EVH concentration of 8-isoprostane was 2.21 ± 1.67 pg/μL at the pre-supplementation test, 3.47 ± 1.82 pg/μL following placebo supplementation, and 6.59 ± 3.71 pg/μL following DHA supplementation.

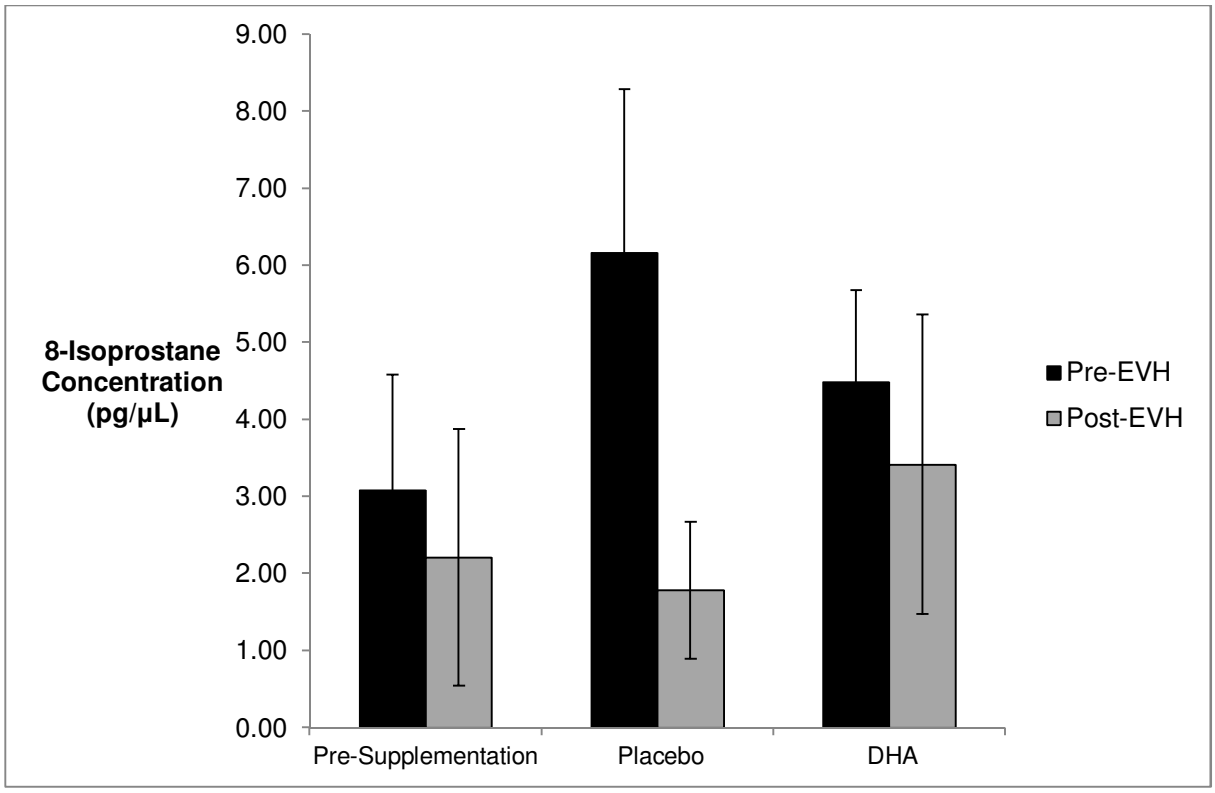

Figure 3-15. ***Exhaled breath condensate 8-isoprostane concentration pre- and post-eucapnic voluntary hyperventilation (EVH) challenge.*** *8-isoprostane is a marker of oxidative stress. There were no significant differences ($p > 0.05$) between the pre-EVH values or between the post-EVH values at any of the laboratory visits. Error bars express standard error of the mean.*

The levels of protectin D1 and 17*S*-hydroxy-docosahexaenoic acid were too low for detection both pre- and post-EVH at each of the three laboratory tests. Thus, the pre- and post-EVH concentration was < 0 pg/µL for each metabolite at all the laboratory tests.

Symptoms and Short-Acting β_2-Agonist Usage. Eight subjects returned their symptom diaries and logs of their bronchodilator usage for each phase of the study. There were no significant changes ($p > 0.05$) in the subjects' daily symptom scores among the pre-supplementation (1.12 ± 0.24), placebo (0.66 ± 0.15), washout (0.74 ± 0.13), and DHA supplementation (0.70 ± 0.14) phases (figure 3-16). There were also no

significant changes ($p > 0.05$) in the subjects' nightly symptom scores among the pre-supplementation (0.17 ± 0.10), placebo (0.00 ± 0.00), washout (0.01 ± 0.01), and DHA supplementation (0.02 ± 0.02) phases (figure 3-17). Moreover, the subjects did not significantly change ($p > 0.05$) their short-acting β_2-agonist use during the course of the study (figure 3-18); the mean bronchodilator usage was 0.17 ± 0.05 puffs per day during pre-supplementation, 0.04 ± 0.02 puffs per day during the placebo phase, 0.03 ± 0.03 puffs per day during the washout phase, and 0.10 ± 0.05 puffs per day during the DHA supplementation phase.

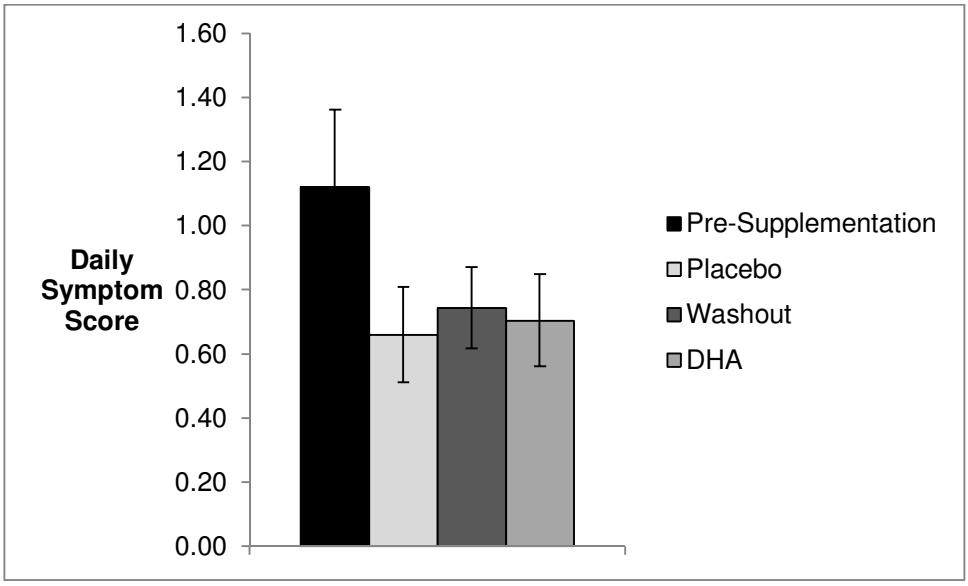

Figure 3-16. ***Daily symptom scores for each phase of the study.*** *There were no significant differences ($p > 0.05$) in the daily symptom scores between any of the four study phases. Error bars express standard error of the mean.*

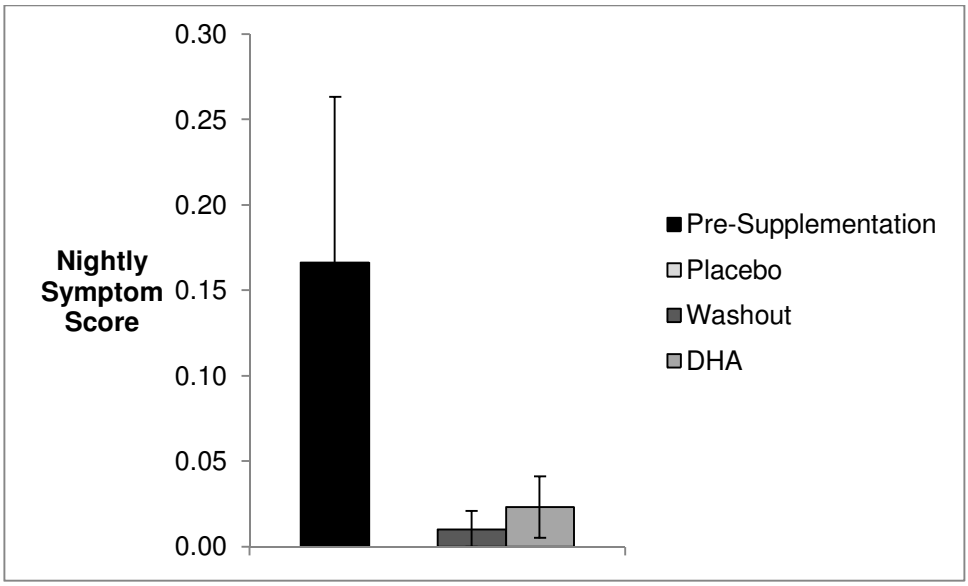

Figure 3-17. ***Nightly symptom scores for each phase of the study.*** *There were no significant differences ($p > 0.05$) in the nightly symptom scores between any of the four study phases. Error bars express standard error of the mean.*

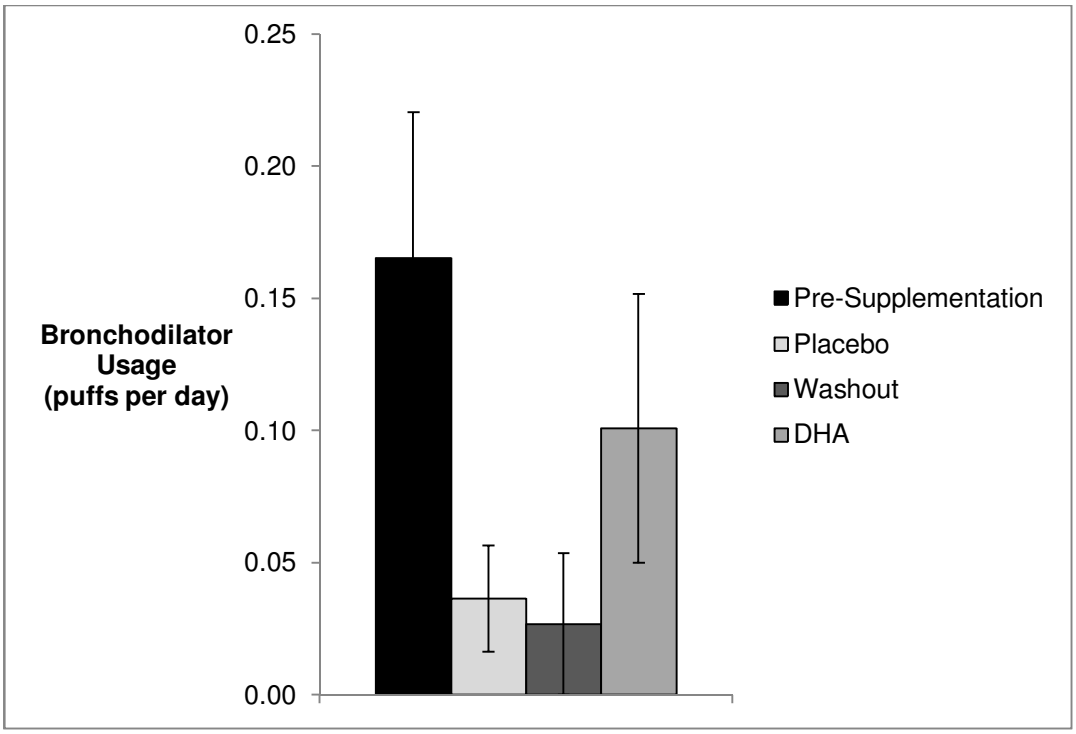

Figure 3-18. ***Bronchodilator usage during each phase of the study.*** *There were no significant differences (p > 0.05) in the subjects' bronchodilator usage between any of the four study phases. Error bars express standard error of the mean.*

Peak Flow Measurements. Eight subjects returned logs of their peak flow measurement data for all four phases of the study. The at-home measurements of the morning peak expiratory flows were not significantly different ($p > 0.05$) among the pre-supplementation (446.63 ± 43.31 L/min), placebo (464.12 ± 42.07 L/min), washout (463.19 ± 40.96 L/min), and DHA supplementation (468.92 ± 42.51 L/min) phases (figure 3-19). Also, the evening peak expiratory flow measurements were not significantly different ($p > 0.05$) among the pre-supplementation (456.37 ± 42.85 L/min), placebo (470.99 ± 42.80 L/min), washout (462.37 ± 46.72 L/min), and DHA supplementation (474.12 ± 47.91 L/min) phases (figure 3-20).

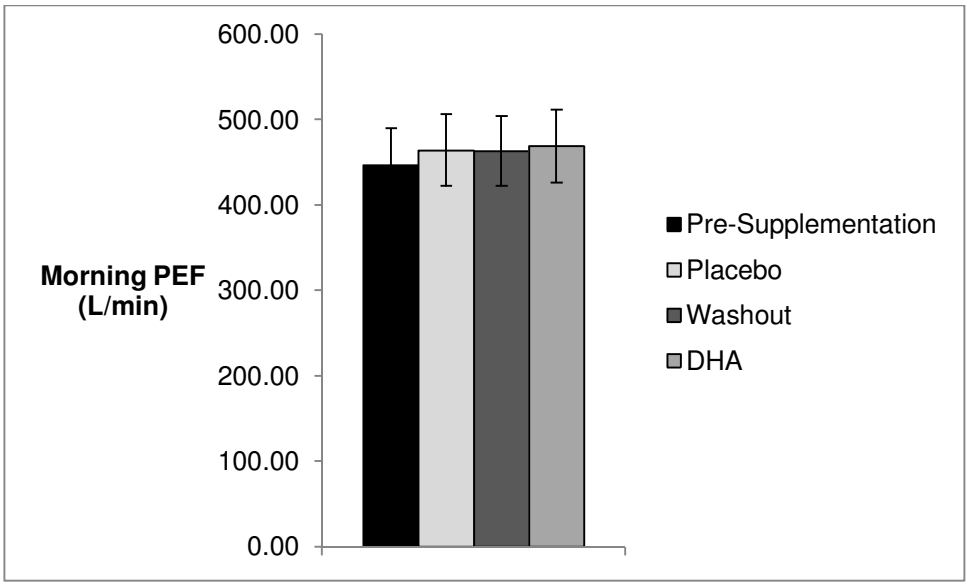

Figure 3-19. ***Morning peak expiratory flow (PEF) during each phase of the study.*** *There were no significant differences ($p > 0.05$) in the subjects' morning PEF measurements among the four study phases. Error bars express standard error of the mean.*

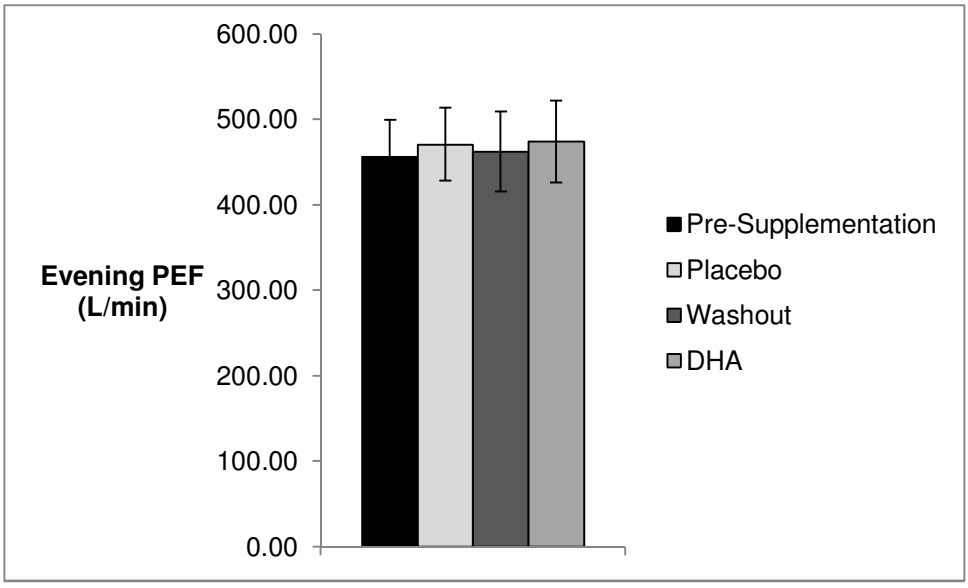

Figure 3-20. ***Evening peak expiratory flow (PEF) during each phase of the study.*** *There were no significant differences in the subjects' evening PEF measurements among the four study phases. Error bars express standard error of the mean.*

Nutrient Intake. Eight subjects completed a food frequency questionnaire for each phase of the study. There were no significant changes ($p > 0.05$) in nutrient intake among the pre-supplementation, DHA, placebo, and washout phases (table 3-4).

Study Phase	Total Grams	Energy (kcal)	Total Fat (g)	Total Carbohydrate (g)	Total Protein (g)	Total Dietary Fiber (g)	Arachidonic Acid (g)	EPA (g)	DHA (g)
Pre-Supplementation									
Mean	3184.26	2602.13	98.00	316.77	110.87	25.52	0.19	0.02	0.05
SEM	603.89	499.10	20.20	66.83	20.77	5.39	0.04	0.01	0.01
DHA Supplementation									
Mean	2704.66	1894.00	65.31	236.91	81.56	20.19	0.17	0.02	0.06
SEM	522.92	257.95	10.08	37.31	13.76	4.99	0.04	0.01	0.02
Placebo Supplementation									
Mean	2891.81	2142.87	80.09	257.03	96.33	21.57	0.18	0.02	0.05
SEM	568.02	401.92	16.67	46.44	20.26	4.10	0.05	0.01	0.01
Washout									
Mean	2274.70	1655.35	61.54	188.71	69.62	14.44	0.16	0.02	0.07
SEM	287.99	207.22	11.14	25.41	10.97	2.06	0.04	0.01	0.04

Table 3-4. ***Average intake amounts of selected nutrients.*** *There were no significant changes in diet for the subjects (n = 8) among the four study phases as assessed by nutrient intake. The average values for the intake of selected nutrients are presented here. SEM, standard error of the mean; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid*

Discussion

Since it has been shown that fish oil effectively attenuates bronchoconstriction and airway inflammation (61, 62, 93), the main purpose of this study was to determine whether supplementation with docosahexaenoic acid (DHA), an omega-3 fatty acid found in fish oil, could reduce hyperpnea-induced bronchoconstriction (HIB) and airway inflammation in adults with asthma. Using a placebo-controlled crossover design, the present study has shown that supplementation with 4.0 g of DHA per day for 3 weeks does not significantly alter pulmonary function, markers of airway inflammation, or DHA metabolite concentrations in comparison to placebo or baseline values in a group of nine adults with asthma.

There are several reasons why DHA supplementation may not have had a significant impact on the pulmonary function measures, inflammatory markers, or symptoms as anticipated. First, it is possible that DHA supplementation simply cannot improve HIB on its own. This may be because eicosapentaenoic acid (EPA) is the more important component of fish oil in terms of attenuating hyperpnea-induced bronchoconstriction and airway inflammation. Although there is not a consensus in the literature whether EPA or DHA is the more potent component of fish oil, there is substantial evidence to support DHA's effectiveness in reducing inflammation (41, 52, 98, 103). DHA administration has also been shown to reduce bronchial hyperresponsiveness in mice (52, 103). Nevertheless, a recent study from Mickleborough et al. (64) showed that EPA was more effective than DHA in reducing inflammatory responses in lipopolysaccharide-stimulated macrophages. This was in contrast to an earlier *in vitro* study from Weldon et al. (98) demonstrating that DHA decreased proinflammatory cytokine production to a greater extent than EPA in macrophages. The discrepancy in the findings between these two studies may be explained by the different cell lines used for the experiments. Weldon et al. (98) studied

THP-1 macrophages, a monocytic leukemia cell line, whereas Mickleborough et al. (64) studied human asthmatic alveolar macrophages, which suggests that their data may be more relevant to patients with asthma.

Second, it is possible that our study design used an inappropriate dose or time course. Because this was a novel application of DHA supplementation, we used the 3-week time course that has been shown to be effective for fish oil supplementation and a 4.0 g dose of DHA. The duration of the washout phase was sufficient as there was not a significant carry-over effect between the treatment periods. Since the exhaled breath condensate (EBC) concentration of the DHA metabolites protectin D1 and 17*S*-hydroxy-docosahexaenoic acid did not increase with supplementation, it suggests that a higher dose of DHA would be necessary to have an effect. Levy et al. (52) reported that there were only "trace amounts" of protectin D1 in the EBC of four adults during an acute exacerbation of their asthma. In the current study, the concentrations of protectin D1 and 17*S*-hydroxy-docosahexaenoic acid were not detectable at baseline, following placebo supplementation, or following DHA supplementation. In addition to perhaps requiring a larger dose of DHA to produce significant changes in the DHA metabolites as hypothesized, a different method of supplementation may be necessary. DHA supplementation was accomplished via oral intake of gel capsules as in previous fish oil studies. However, murine studies demonstrating the effectiveness of DHA administered the omega-3 fatty acid via aerosol (103) or intravenously in its metabolite form (52). Thus, the efficacy of DHA may have been affected by the means of administration.

Third, the variability in our subject population was greater than expected. There was no suitable data available on DHA supplementation in adults with asthma to use to determine an appropriate sample size. Therefore, an *a priori* power analysis was conducted using data from Mickleborough et al.'s (61) study on fish oil supplementation in adults with asthma because it used a similar placebo-controlled crossover design.

Due to the large effect size in the Mickleborough et al. (61) study, it was determined that three subjects would be needed to show a significant reduction in the maximum drop in FEV_1 volume with DHA supplementation compared to placebo. However, we were unable to demonstrate a significant difference in this or any other pulmonary function measure in the nine subjects in the present study. The coefficients of variation for the maximum drop in FEV_1 volume for our subjects were 0.38 at pre-supplementation, 0.55 with placebo, and 0.68 with DHA supplementation; in contrast, the coefficients of variation for the subjects in the Mickleborough et al. (61) study were 0.30 at pre-supplementation, 0.23 with placebo, and 0.26 with fish oil supplementation. Therefore, the subjects in the current study showed greater variability in their pulmonary function responses, and this may have impaired our ability to detect statistically significant differences between treatments.

However, before concluding that pure DHA is not effective in alleviating HIB in asthmatic individuals, variations of the current study should be undertaken. First, time course trials using different doses should be completed. Additionally, a different route of administrating DHA should be attempted. Although intravenous administration of DHA may be problematic in humans, aerosolized DHA may be a viable option (103). Furthermore, it would also be worthwhile to conduct a similar study using EPA instead of DHA. Significant *in vivo* improvements in pulmonary function and airway inflammation with EPA supplementation in adults with asthma would indicate that EPA is the more potent component in fish oil and the optimization of a formula for omega-3 fatty acid supplementation in asthma could proceed.

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CHAPTER 4

THE ASSOCIATION BETWEEN FISH OIL TREATMENT OF ISOLATED CANINE TRACHEAL SMOOTH MUSCLE TISSUE AND THEIR CONTRACTILITY

Abstract

Background: Fish oil supplementation has been shown to reduce exercise-induced bronchoconstriction. However, the association between airway smooth muscle contractility and its exposure to fish oil or one of its components has received only limited attention in the literature.

Purpose: Determine whether fish oil exposure is associated with a reduction in the contractility of canine tracheal smooth muscle tissue.

Methods: Canine tracheal smooth muscle strips were exposed to fish oil, soybean oil (placebo), or control with vehicle media either chronically or acutely. Contractility was measured *in vitro* before and after exposure. Lipid analysis via gas chromatography was performed on select tissue samples to determine the incorporation of the omega-3 polyunsaturated fatty acids in fish oil.

Results: Significant incorporation ($p < 0.05$) of the fish oil omega-3 fatty acids was evident following 24 hours of incubation in fish oil but not after 15 hours ($p > 0.016$). Following 4 hours of incubation in fish oil, there was a statistically significant ($p < 0.016$), though likely not physiologically significant, increase in DHA incorporation. Smooth muscle contractility in response to acetylcholine was significantly elevated ($p < 0.05$) for tissues incubated for 15 hours in fish oil medium compared to those incubated in control with vehicle medium. There were no significant changes ($p > 0.05$) in contractility or the effective dose (ED) 50 following incubation periods of 2, 4, and 24 hours. Acute administration of fish oil significantly relaxed ($p < 0.05$) tissues contracted with either 10^{-6} M acetylcholine or 10^{-6} M 5-hydroxytryptamine.

Conclusions: These experiments suggest that fish oil exposure can be associated with changes in airway smooth muscle contractility. Although there does not appear to be a relationship between airway smooth muscle contractility and the lipid profile of the tissue according to the current data, this may be due to differences in the experimental protocols at the time points tested; additional experiments to reconcile these differences may be necessary in the future.

Introduction

Airway inflammation, narrowing, and hyperresponsiveness are the hallmark features of asthma (90). Asthma pharmacotherapy typically targets either airway inflammation or airway narrowing. Clinically, airway narrowing is the greater concern (44). Therefore, addressing the impact of asthma treatment strategies on airway smooth muscle contractility is important.

Fish oil is a non-pharmacological alternative for treating asthma. Recent studies have demonstrated that fish oil supplementation suppresses exercise-induced bronchoconstriction, which can be a complication of asthma (61, 62, 93). The mechanism for fish oil's effectiveness in this regard has not been fully elucidated. It is generally thought that fish oil works through a reduction in airway inflammation via the competition of its omega-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), for enzymes also used by omega-6 polyunsaturated fatty acids. Because the omega-3 fatty acids produce leukotrienes and prostaglandins that are less proinflammatory than those produced by the omega-6 fatty acids, less bronchoconstriction is consequently expected with fish oil supplementation (60). However, the impact of fish oil on the airway smooth muscle itself has only received modest attention.

Few studies have focused on the association between airway smooth muscle contractility and its exposure to fish oil or one of its components. In an *in vitro* study

involving guinea pig bronchial smooth muscle, Hichami et al. (39) determined that acute administration of DHA reduced basal tone but not contractions with carbamylcholine. Moreover, Morin et al. (69) demonstrated that treatment with an EPA metabolite resulted in the relaxation of human bronchial smooth muscle tissue that had not been stimulated as well as following contraction of the tissue with methacholine. By studying the effects of ion channel blockers on the changes in smooth muscle contractility elicited with omega-3 fatty acid exposure, these studies suggested that ion channels were involved (39, 69). However, they did not address the possibility of the tissue's responsiveness having been affected by omega-3 polyunsaturated fatty acid incorporation in the phospholipid bi-layer.

Although the existing literature contains additional studies on vascular smooth muscle treated with fish oil or one of its components, these findings cannot be assumed to hold true for airway smooth muscle due to the physiological differences between these tissue types. Nevertheless, they are worth noting in that these studies have suggested that lipid incorporation of omega-3 fatty acids may affect smooth muscle contractility via an alteration in cell membrane properties, such as fluidity and enzyme function (33, 101). While these studies did not measure lipid incorporation, *in vivo* human and mice studies involving fish oil supplementation have shown increased omega-3 fatty acid content in plasma phoshoplipids (47), neutrophils (47, 61), and lung tissue (102).

The purpose of this study is to determine whether fish oil exposure is associated with a reduction in the contractility of canine tracheal smooth muscle tissue. The hypothesis is that the treatment of canine tracheal smooth muscle with fish oil will reduce arachidonic acid content and increase EPA and DHA content in smooth muscle cell membranes and will be associated with a decrease in the airway smooth muscle responsiveness to a contractile agonist.

Methods

Study Design. Canine tracheal smooth muscle strips were exposed to fish oil, soybean oil (placebo), or control with vehicle media either chronically or acutely. Chronic exposure to the treatments ranged from 2 to 24 hours for the contractility experiments and from 4 hours to 6 days for the lipid analysis experiments while acute exposure to the treatments lasted 30 to 40 minutes. Contractility was measured *in vitro* before and after exposure to the treatments.

Smooth Muscle Tissue Preparation. All procedures involving animal tissues were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Indiana University School of Medicine. Tracheal smooth muscle tissue was obtained as previously described (104). Briefly, mongrel dogs were sacrificed by exsanguination following injection with sodium pentobarbital (30 mg/kg). A section of the trachea was removed immediately and immersed in physiological saline solution (PSS) at 22 ℃ containing 110 mM NaCl, 3.4 mM KCl, 2.4 mM $CaCl_2$, 0.8 mM $MgSO_4$, 25.8 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 5.6 mM glucose. The canine tracheal smooth muscle tissue was excised from the trachea with the connective tissue removed and then cut into strips. The tissue was subsequently placed in PSS at 37 ℃ in 25 ml organ baths aerated with 95% O_2 and 5% CO_2 to maintain a pH of 7.4.

Treatments. Fish oil and soybean oil (Nordic Naturals, Watsonville, CA) were dissolved in a 0.5% by volume mixture of 1:1 ethanol and dimethyl sulfoxide (DMSO). Controls with vehicle were prepared with equivalent volumes of PSS (acute) or incubation media (chronic) dissolved in ethanol and DMSO. Controls without vehicle were also used in each protocol.

Lipid Analysis. To determine the extent of canine tracheal smooth muscle incorporation of fish oil during incubation periods of various lengths, cell membrane fatty acid content was analyzed via gas chromatography. Following incubation at 37 ℃ and

5% CO_2 in Dulbecco's modified eagle medium (DMEM) with antibiotics in fish oil or control without vehicle for 1, 2, 4, and 6 days in a high dose (8 mM) of fish oil, the muscle strips were rinsed with PSS containing 1% bovine serum albumin to remove unincorporated lipids and then frozen with liquid nitrogen for storage. Tissue samples from 4 hour and 15 hour incubations (see below for details) were also frozen with liquid nitrogen after contraction experiments were completed. The samples were then stored at -80 ℃ before analysis. A modified protocol of Lepage and Roy's (50, 51) one-step transesterification method developed by Xu et al. (100) for the optimal determination of fatty acid concentrations was employed. Briefly, the internal standard tricosanoate-methyl ester and methanol-benzene were added to the dried tissue samples; this mixture was vortexed, purged with nitrogen for 10 seconds, capped, and bathed in dry ice for 10 minutes. Acetyl chloride was added, and then, the samples were transferred to room temperature until the reaction mixture melted. The sample tubes were then placed on a 100 ℃ heating plate for 15 minutes, sonicated for 10 seconds, put back on the heating plate for 45 minutes, and cooled in an ice bucket. To end the transesterification reaction and to allow the mixture to neutralize, a 6% K_2CO_3 solution was added slowly to the samples. After the samples were centrifuged at 200 rpm for 10 minutes at 25 ℃, the benzene layer was removed and the remaining contents analyzed by split injection gas chromatography at 250 ℃ using the Shimadzu GC2010 system and RT-2560 column. The equation $C_{(GC)} = A_{(FA)} / A_{(IS)} \times C_{(IS)}$, where $C_{(GC)}$ is the concentration of the particular fatty acid, $A_{(FA)}$ is the peak area of the particular fatty acid, $A_{(IS)}$ is the peak area of the internal standard, and $C_{(IS)}$ is the concentration of the internal standard, was used to calculate the concentration of each fatty acid (100). The percent contribution of arachidonic acid, EPA, and DHA to the smooth muscle sample's total fatty acid composition was determined.

Chronic Exposure to Fish Oil. Contractility was measured as previously described (104). Each strip was attached to a force transducer (Grass Technologies, West Warwick, RI) to measure force with the aid of the PolyVIEW program (Grass Technologies, West Warwick, RI). The optimal length for muscle contraction was determined by progressively increasing the muscle's length until the active isometric force elicited by 10^{-5} M acetylcholine (ACh) reached a maximum. The tissue samples were maintained at the optimal length for 30 to 60 minutes without stimulation. Using half-logarithmic doses from 10^{-9} M to 10^{-4} M, the active isometric force in response to ACh at the optimal muscle length was measured.

After the ACh dose-response contractions, the muscle strips were attached to metal mounts at their optimal lengths. They were incubated at 37 ℃ and 5% CO_2 in DMEM with antibiotics in fish oil, soybean oil, control with vehicle, or control without vehicle overnight (15 hours) or for 24 hours. Following incubation, the muscle strips were rinsed in PSS containing 1% bovine serum albumin to remove the incubation media and then studied immediately. The strips were placed at their optimal lengths in the same tissue baths as before to repeat the half-logarithmic dose response to ACh.

Shorter incubation periods of 2 and 4 hours were also evaluated. For these time periods, the tissues were maintained in the tissue baths attached to the transducers and exposed to the treatments for the desired time period; a dose response curve to ACh was then repeated.

Acute Exposure to Fish Oil. Each strip was attached to a force transducer (Grass Technologies, West Warwick, RI) to measure force with the aid of the PolyVIEW program (Grass Technologies, West Warwick, RI). The smooth muscle strips were contracted with 10^{-6} M acetylcholine (ACh) to achieve peak force. After the tension reached a plateau, fish oil, soybean oil, control with vehicle, or control (sham) treatments were added to the tissue bath. Tissues were evaluated for 30 to 40 minutes after the

treatments to determine whether there was a change in tension. The percent relaxation was calculated for each smooth muscle strip.

To assess the effect of a weaker contractile stimulus, the experiment was repeated with 10^{-7} M ACh. In this experiment, the smooth muscle strips were first conditioned with 10^{-5} M ACh to determine the optimal length for each strip. After this was determined, the agonist was washed out of the tissue baths and the tissue was contracted with 10^{-7} M ACh. Once the tension reached a plateau, fish oil, soybean oil, or control with vehicle treatments were added to the tissue baths. Tissues were evaluated for 30 to 40 minutes after the treatments, and the percent relaxation was calculated for each smooth muscle strip.

Contractions with Other Agonists. To determine whether the results would be affected by using a weaker contractile agonist, either prostaglandin F_2 (PGF_2) or 5-hydroxytryptamine (5-HT) was administered to untreated canine tracheal smooth muscle strips attached to force transducers. Since only 5-HT elicited contractions in the muscle strips, select experiments were repeated with 5-HT as the contractile agonist. These experiments included the chronic exposure to fish oil for 4 and 24 hours as well as the acute exposure to fish oil. Contractility was measured in the manner already described with two exceptions: half-logarithmic doses from 10^{-9} M to 10^{-4} M 5-HT were used in the chronic exposure experiment and 10^{-6} M 5-HT and 10^{-7} M 5-HT were used in the acute exposure experiment after peak force with 10^{-5} M ACh had been achieved.

Data Analysis. Statistical analysis was performed with SPSS version 18.0 statistical software (SPSS Inc., Chicago, IL).

For the lipid analysis, the composition of EPA, DHA, and arachidonic acid were each calculated as a percent of the total fatty acid composition of the tissues. The percentages reflect the mean of the smooth muscle strips that received the same treatment for a particular length of time. Independent t-tests were used to determine

differences between tissues incubated in fish oil or control media at each time point (1, 2, 4, and 6 days). Significance was set at $p < 0.05$. Independent t-tests were also used to determine differences between tissues incubated in fish oil medium and soybean oil, control with vehicle, or control media for 4 hours or 15 hours. A Bonferroni adjustment of the p-value was made to correct for the three sets of independent t-tests; thus, for this analysis, significance was set at $p < 0.016$.

To analyze the effects of chronic exposure to fish oil, the post-incubation contractility of the tissue samples throughout the dose response curves was calculated as a percent of the maximum pre-incubation force for each smooth muscle strip. A two-way mixed analysis of variance (ANOVA) was then performed to examine the dose response to the contractile agonists following incubation; the contractile agonist dose was the within-subjects factor while the treatment was the between-subjects factor. Mauchley's test was conducted to determine if sphericity was violated; if it was, a Greenhouse-Geisser adjustment was used. Where a significant interaction was observed, a multivariate analysis of variance (MANOVA) was performed to examine the simple effect of treatment at each level of the contractile agonist dose. Separate analyses were performed to compare fish oil to control with vehicle, fish oil to soybean oil, and control with vehicle to soybean oil. Significance was set at $p < 0.05$.

The maximum force generated for each smooth muscle strip was established as the highest post-incubation force that was previously calculated as a percent of that smooth muscle strip's maximum pre-incubation force. One-way independent measures ANOVAs were then used to determine differences in the maximum force among the fish oil, soybean oil, and control with vehicle incubation treatments at each of the time points studied (24 hours, 15 hours, 4 hours, and 2 hours). Where a significant difference was detected, a Fisher's least significant difference (LSD) post-hoc test was used to determine where the differences lay. Significance was set at $p < 0.05$.

SigmaPlot 12 (Systat Software Inc., San Jose, CA) was used to determine the effective dose (ED) 50 for each smooth muscle strip. To determine differences in the ED 50 among the fish oil, soybean oil, and control with vehicle incubation treatments, one-way independent ANOVAs were performed for each of the time points studied (24 hours, 15 hours, 4 hours, and 2 hours). If a significant difference was detected, Fisher's LSD post-hoc was employed. Significance was set at $p < 0.05$.

The effect of acute exposure to fish oil was studied by determining the degree of relaxation for each tissue in response to fish oil, soybean oil, vehicle, or control (sham) treatments. This was calculated by determining the peak force generated by the tissue in response to a single dose of the contractile agonist. The minimum force produced by the tissue following treatment was also determined. The percent relaxation was then calculated as 100 – [(minimum force post-treatment / peak force pre-treatment) * 100]. One-way independent ANOVAs were performed for each dose and type of contractile agonist to determine differences among the treatments. When significant differences were detected, Fisher's LSD was used to isolate the differences between treatments. Significance was set at $p < 0.05$.

Results

Lipid Analysis. The 1 to 6 day time course revealed that the percent contribution of EPA and DHA to the total fatty acid composition continually increased with incubation in the fish oil medium. The difference between incubation in fish oil medium and control medium for both the percent composition of EPA and the percent composition of DHA was significant ($p < 0.05$) at 1 and 6 days (figures 4-1, 4-2). There was not a significant difference ($p > 0.05$) between incubation in fish oil medium and control medium for the percent composition of arachidonic acid at any of the time points measured (figure 4-3).

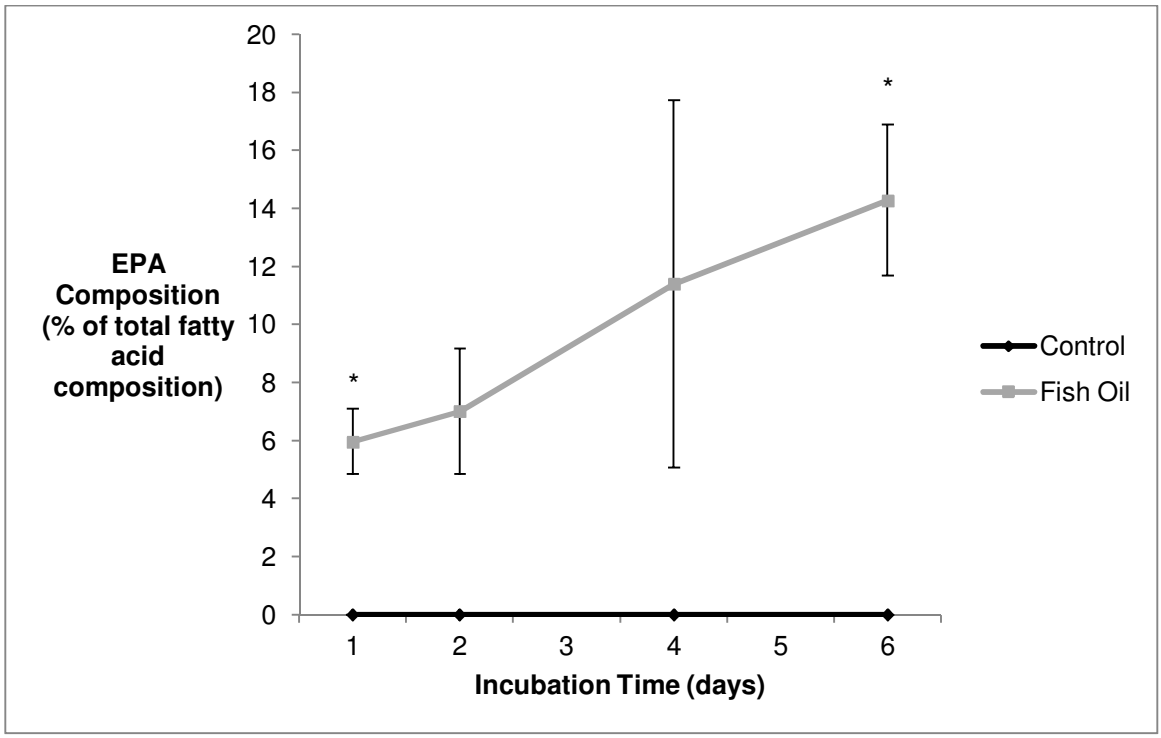

Figure 4-1. ***Eicosapentaenoic acid (EPA) composition of canine tracheal smooth muscle tissue incubated in either control or fish oil media for 1 to 6 days.*** *The percent of the total fatty acid composition containing EPA continually increased with incubation in the fish oil medium for 1 to 6 days. The EPA composition following incubation in the fish oil medium was significantly greater ($p < 0.05$) than that of tissues incubated in the control medium at 1 day and 6 days. Data points reflect the mean values of the incubated tissue strips (n = 2 for control; n = 2 for fish oil at 1, 2, and 4 days; n = 3 for fish oil at 6 days). Error bars represent standard error of the mean. *, significantly different from control*

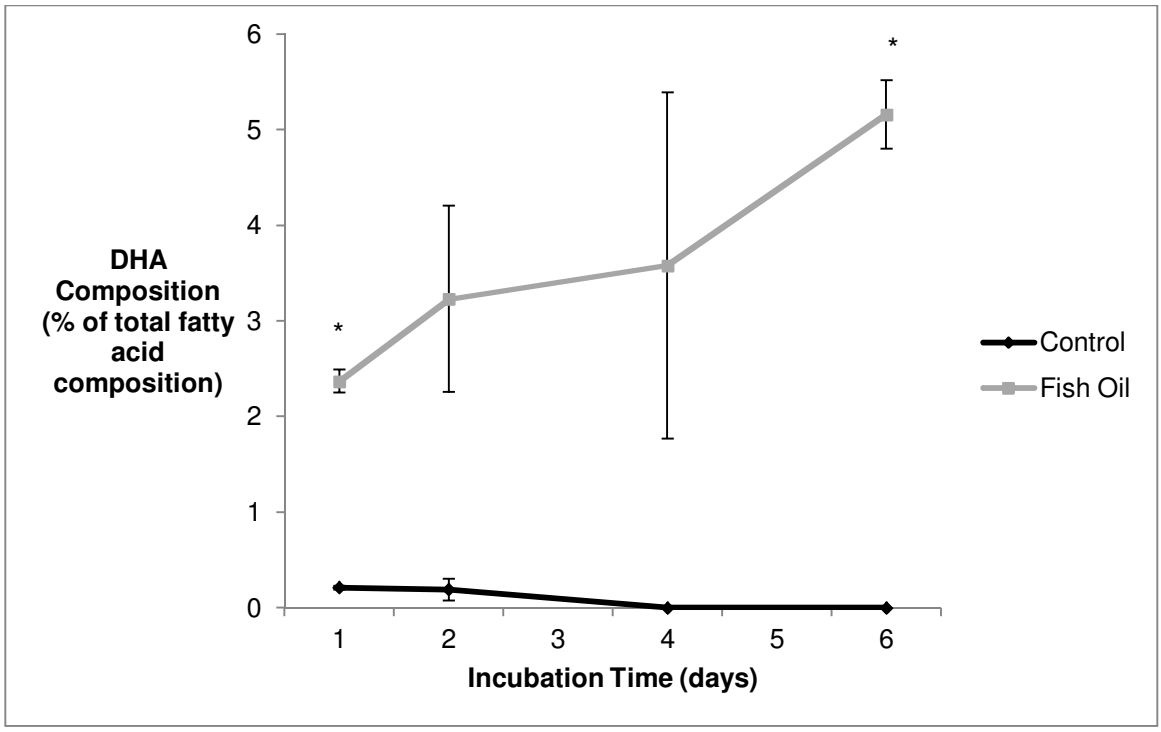

Figure 4-2. ***Docosahexaenoic acid (DHA) composition of canine tracheal smooth muscle tissue incubated in either control or fish oil media for 1 to 6 days.*** *The percent of the total fatty acid composition containing DHA continually increased with incubation in the fish oil medium for 1 to 6 days. The DHA composition following incubation in the fish oil medium was significantly greater ($p < 0.05$) than that of tissues incubated in the control medium at 1 day and 6 days. Data points reflect the mean values of the incubated tissue strips (n = 2 for control; n = 2 for fish oil at 1, 2, and 4 days; n = 3 for fish oil at 6 days). Error bars represent standard error of the mean. *, significantly different from control*

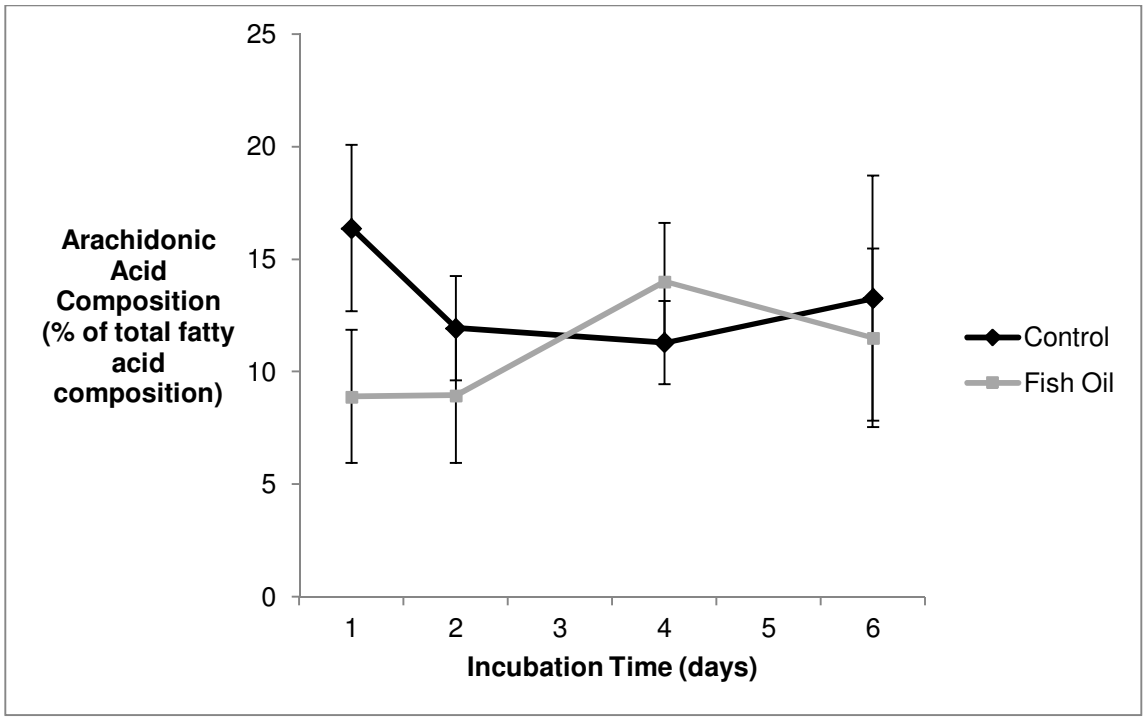

Figure 4-3. ***Arachidonic acid composition of canine tracheal smooth muscle tissue incubated in either control or fish oil media for 1 to 6 days.*** *The percent of the total fatty acid composition containing arachidonic acid following incubation in the fish oil medium was not significantly different from that of tissues incubated in the control medium at 1 day and 6 days. Data points reflect the mean values of the incubated tissue strips (n = 2 for control; n = 2 for fish oil at 1, 2, and 4 days; n = 3 for fish oil at 6 days). Error bars represent standard error of the mean.*

Lipid analysis was also performed on tissues that had been incubated in fish oil, soybean oil, control with vehicle, and control media for 4 hours in the tissue baths or for 15 hours in an incubator. These tissues had been studied for their contractility pre- and post-incubation. There was not a significant difference ($p > 0.016$) in the percent contribution of EPA or arachidonic acid to the total fatty acid composition between incubation in fish oil and incubation in any of the other three treatments for tissues incubated for 4 hours; however, there was a significant increase ($p < 0.016$) in the percent contribution of DHA to the total fatty acid composition between incubation in fish oil and incubation in either control or control with vehicle media (table 4-1). There was not a significant difference ($p > 0.016$) in the percent contribution of EPA, DHA, or

arachidonic acid to the total fatty acid composition between incubation in fish oil and incubation in any of the other three treatments for tissues incubated for 15 hours (table 4-2).

	Control (%)	Vehicle (%)	Soybean Oil (%)	Fish Oil (%)
Arachidonic Acid	2.38 ± 0.69	10.04 ± 4.58	3.90 ± 1.09	1.32 ± 0.06
EPA	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
DHA	0.00 ± 0.00	0.00 ± 0.00	0.39 ± 0.09	0.14 ± 0.02*

Table 4-1. ***Percent composition of arachidonic acid, eicosapentaentoic acid (EPA), and docosahexaenoic acid (DHA) in tissues incubated in control, vehicle, soybean oil, or fish oil media for 4 hours.*** *There were not any significant differences (p > 0.016) in arachidonic acid or EPA composition between fish oil and the other three treatments following the 4-hour incubation in control (n = 3), vehicle (n = 3), soybean oil (n = 3), or fish oil media (n = 3). There was a significant difference (p < 0.016) in DHA composition between fish oil and the control or vehicle treatments. The data are presented as mean percent ± standard error of the mean. *, significantly different from control and vehicle*

	Control (%)	Vehicle (%)	Soybean Oil (%)	Fish Oil (%)
Arachidonic Acid	2.48 ± 0.19	2.25 ± 0.21	3.45 ± 0.81	2.06 ± 0.32
EPA	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
DHA	0.38 ±0.06	0.3 ± 0.06	0.41 ± 0.12	0.19 ± 0.02

Table 4-2. ***Percent composition of arachidonic acid, eicosapentaentoic acid (EPA), and docosahexaenoic acid (DHA) in tissues incubated in control, vehicle, soybean oil, or fish oil media for 15 hours.*** *There were not any significant differences (p > 0.016) in arachidonic acid, EPA, or DHA composition between fish oil and the other three treatments following the 15-hour incubation in control (n = 3), vehicle (n = 3), soybean oil (n = 3), or fish oil media (n = 3). The data are presented as mean percent ± standard error of the mean.*

Chronic Exposure to Fish Oil. Following a 24-hour incubation, there was not a significant interaction ($p > 0.05$) between treatment and the ACh dose or a significant difference ($p > 0.05$) in the post-incubation contractility of the tissues incubated in fish oil medium compared to the tissues incubated in control with vehicle medium when treated with half-logarithmic doses of ACh from 10^{-9} to 10^{-4} M (figure 4-4). Moreover, there was not a significant difference ($p > 0.05$) in the maximum force produced by the tissues

incubated in the fish oil medium as compared to that of the tissues incubated in the control with vehicle medium (figure 4-4). There was also no significant difference ($p > 0.05$) in the effective dose (ED) 50 between the tissues incubated in the fish oil medium and the tissues incubated in the control with vehicle medium (figure 4-5).

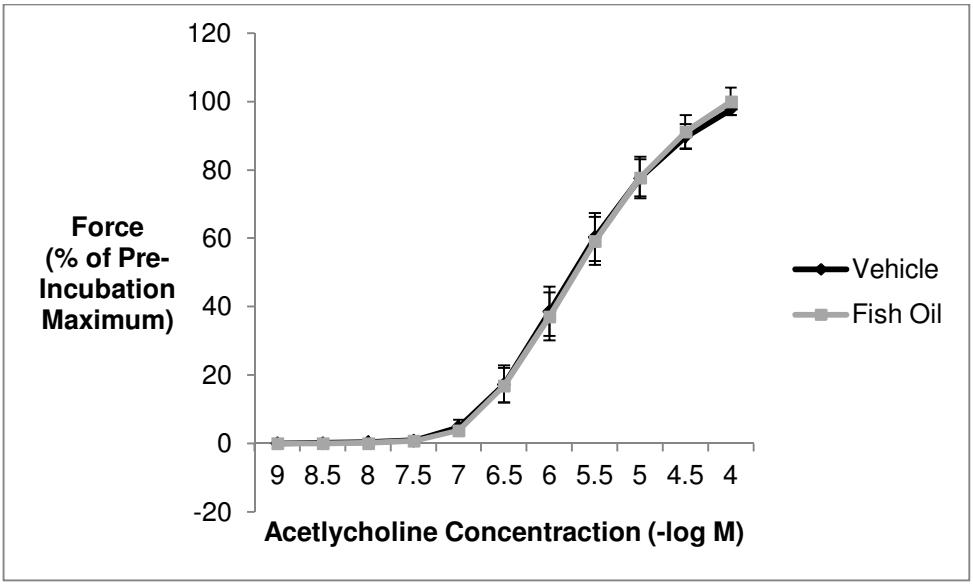

Figure 4-4. ***The force produced by canine tracheal smooth muscle strips in response to half-logarithmic doses of acetylcholine following 24 hours of incubation in control with vehicle or fish oil media.*** *There was not a significant difference ($p > 0.05$) between the control with vehicle ($n = 7$) and fish oil ($n = 7$) treatments in terms of the post-incubation contractility or maximum force generated. Force was calculated as a percent of the pre-incubation maximum force produced by each smooth muscle strip. Error bars represent standard error of the mean.*

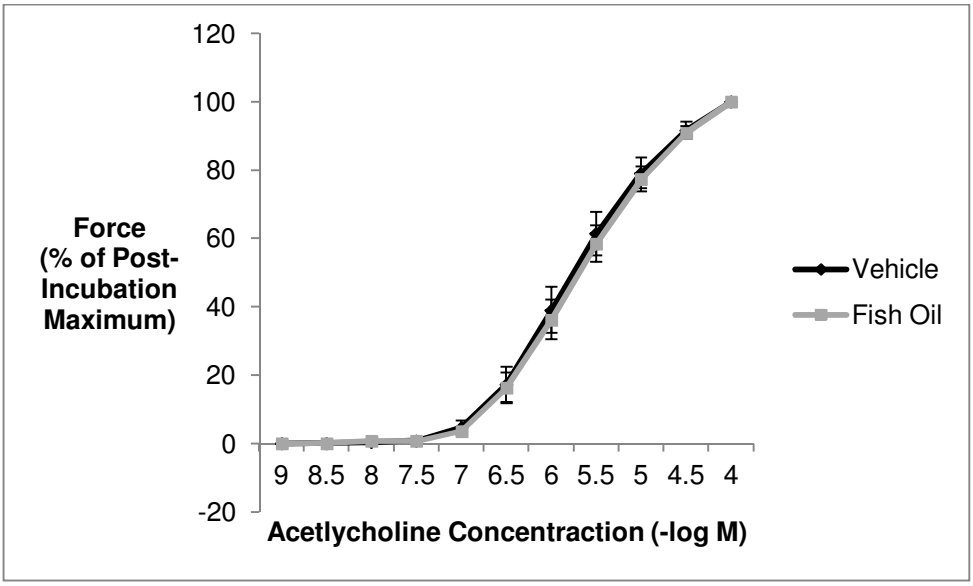

Figure 4-5. ***The effective dose (ED) 50 was not significantly altered by treatment.*** *The force produced in response to half-logarithmic doses of acetylcholine following incubation was normalized with respect to the maximum post-incubation force produced by each canine tracheal smooth muscle strip to illustrate the ED 50 of each treatment. There were no significant differences ($p > 0.05$) in the ED 50 between the 24-hour incubation in control with vehicle medium ($n = 7$) and incubation in fish oil medium ($n = 7$). Error bars represent standard error of the mean.*

For tissue strips incubated overnight (15 hours) in fish oil medium and control with vehicle medium, the two-way mixed ANOVA demonstrated a significant interaction ($p < 0.05$) between the treatment and ACh dose. Consequently, the contractility of the tissue incubated in fish oil medium was determined with a MANOVA to be significantly greater ($p < 0.05$) than that of the tissue incubated in control with vehicle medium at the six highest doses of ACh: 3.16×10^{-7} M, 10^{-6} M, 3.16×10^{-6} M, 10^{-5} M, 3.16×10^{-5} M, and 10^{-4} M (figure 4-6).

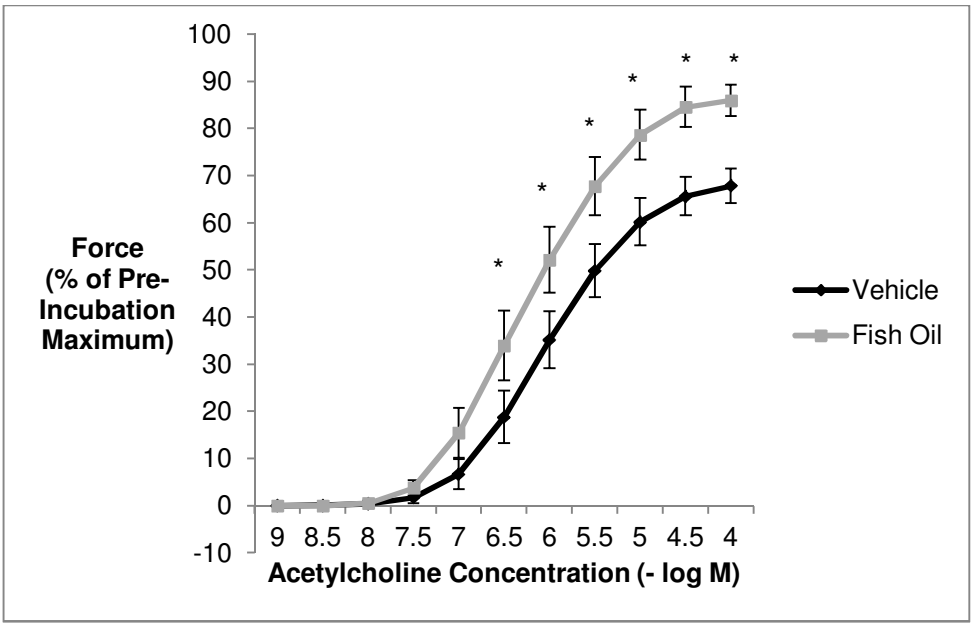

Figure 4-6. ***The force produced by canine tracheal smooth muscle strips in response to half-logarithmic doses of acetylcholine following 15 hours of incubation in control with vehicle or fish oil media.*** *There was a significant difference ($p < 0.05$) between the control with vehicle ($n = 7$) and fish oil ($n = 7$) treatments at the six highest doses of acetylcholine. Force was calculated as a percent of the pre-incubation maximum force produced by each smooth muscle strip. Error bars represent standard error of the mean. *, significantly different from control with vehicle*

There was not a significant interaction ($p > 0.05$) between treatment and ACh dose for tissues incubated overnight in the fish oil medium compared to the soybean oil medium. Moreover, the post-incubation contractility of the tissue samples incubated in the fish oil medium was not significantly different ($p < 0.05$) from the samples incubated in the soybean oil medium (figure 4-7). Additionally, there was neither a significant interaction ($p < 0.05$) between the treatment and ACh dose nor a significant difference ($p < 0.05$) in contractility between the tissues incubated in the control with vehicle medium compared to those incubated in the soybean oil medium (figure 4-8).

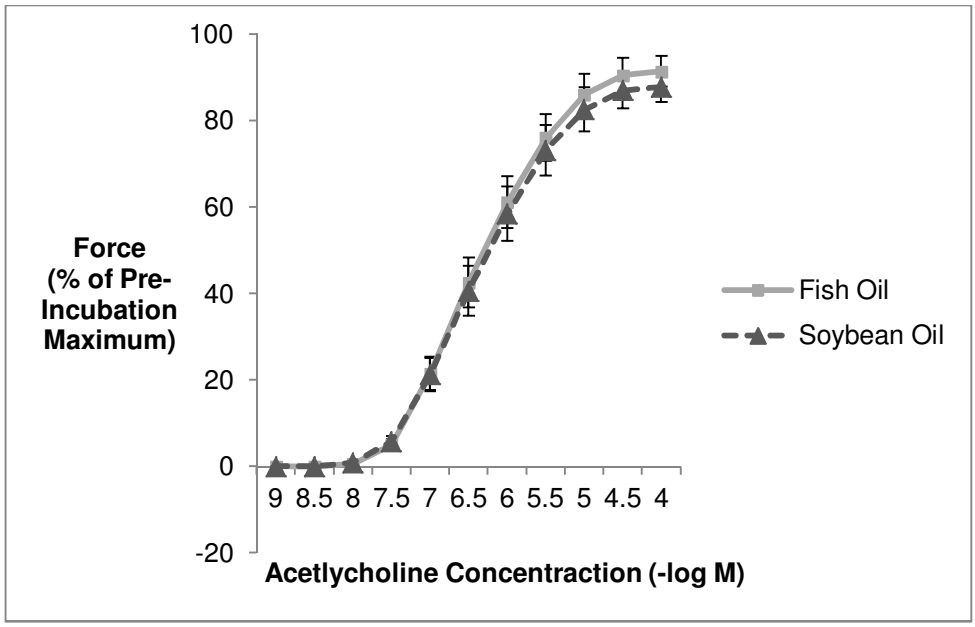

Figure 4-7. ***The force produced by canine tracheal smooth muscle strips in response to half-logarithmic doses of acetylcholine following 15 hours of incubation in fish oil or soybean oil media.*** *There was not a significant difference ($p > 0.05$) between the fish oil ($n = 9$) and soybean oil ($n = 10$) treatments. Force was calculated as a percent of the pre-incubation maximum force produced by each smooth muscle strip. Error bars represent standard error of the mean.*

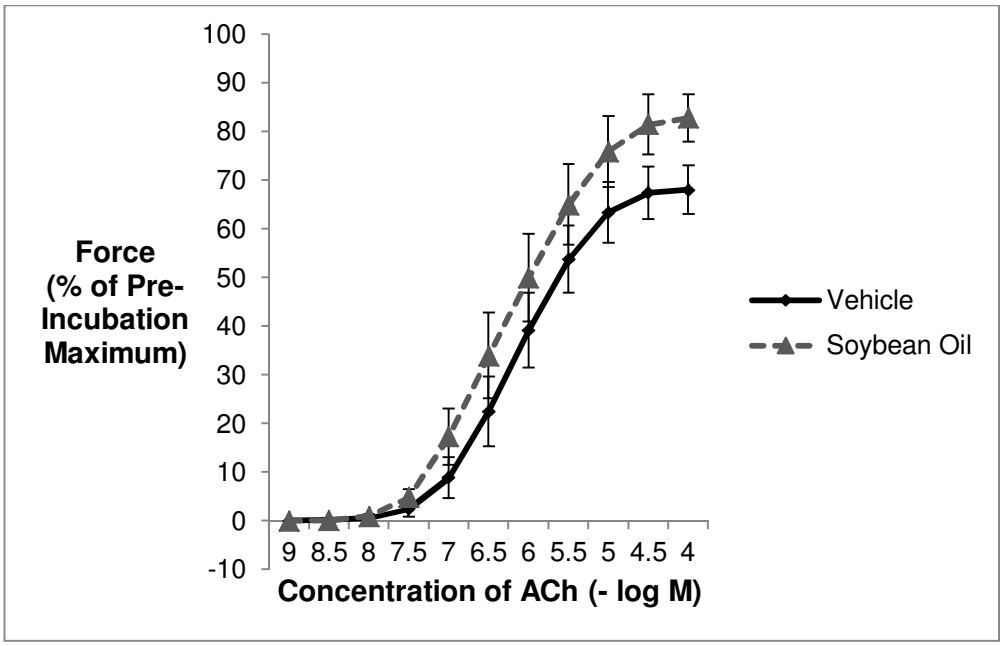

Figure 4-8. ***The force produced by canine tracheal smooth muscle strips in response to half-logarithmic doses of acetylcholine following 15 hours of incubation in control with vehicle or soybean oil media.*** *There was not a significant difference ($p > 0.05$) between the control with vehicle ($n = 5$) and soybean oil ($n = 6$) treatments. Force was calculated as a percent of the pre-incubation maximum force produced by each smooth muscle strip. Error bars represent standard error of the mean.*

A one-way independent measures ANOVA revealed significant differences ($p < 0.05$) among the maximum force generated by tissues following incubation in fish oil, soybean oil, or control with vehicle media (figure 4-9). Fisher's LSD post-hoc indicated that the maximum contraction of the fish oil-treated tissues and soybean oil-treated tissues were each significantly greater ($p < 0.05$) than that of the control with vehicle-treated tissues while there was no significant difference ($p > 0.05$) between the maximum contraction of the fish oil-treated and soybean oil-treated tissues. The ED 50 was compared among the tissues incubated in fish oil medium, soybean oil medium, or control with vehicle medium with a one-way repeated measures ANOVA; there were no significant differences ($p > 0.05$) among the incubation treatments (figure 4-10).

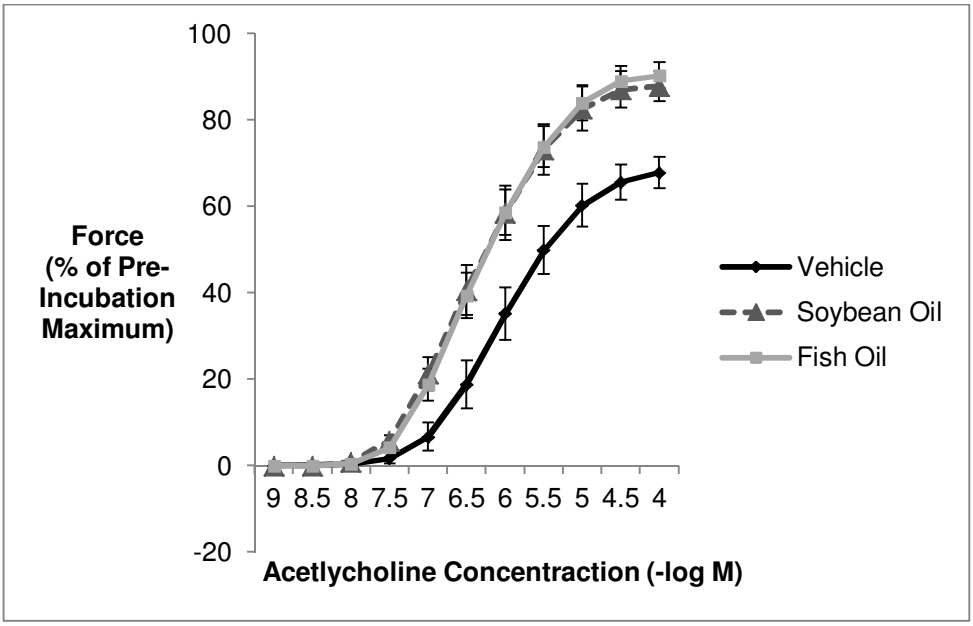

Figure 4-9. ***The maximum force generated by the canine tracheal smooth muscle strips was significantly altered by treatment.*** *The force produced in response to half-logarithmic doses of acetylcholine following incubation was normalized with respect to the maximum pre-incubation force produced by each canine tracheal smooth muscle strip. The tissue incubated in either fish oil (n = 11) or soybean oil (n = 10) media demonstrated significantly greater ($p < 0.05$) maximum force generation than the tissue incubated in control with vehicle (n = 7). There was no significant difference ($p > 0.05$) between the fish oil and soybean oil treatments. Error bars represent standard error of the mean.*

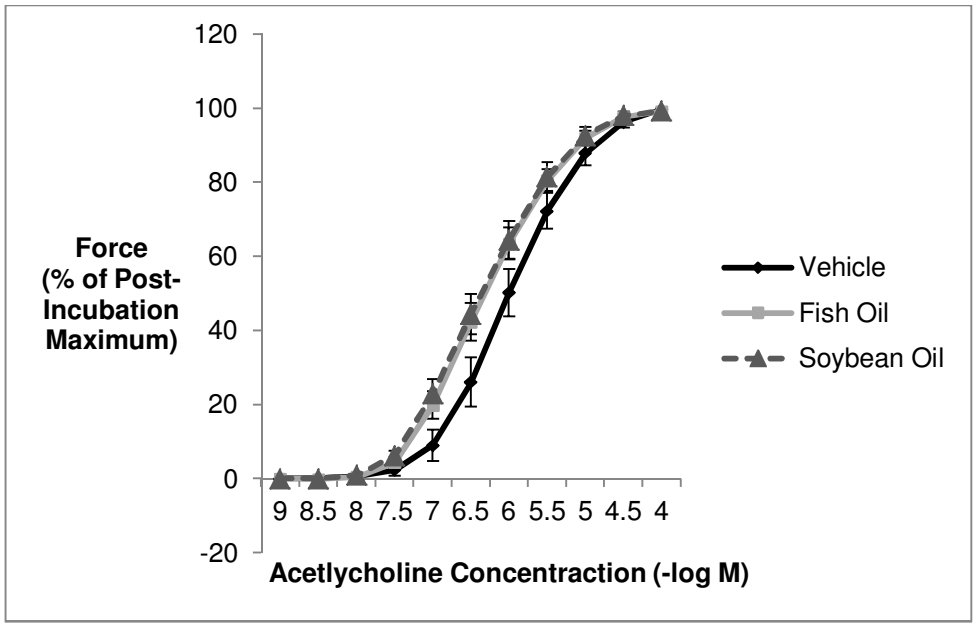

Figure 4-10. ***The effective dose (ED) 50 was not significantly altered by treatment.*** *The force produced in response to half-logarithmic doses of acetylcholine following incubation was normalized with respect to the maximum post-incubation force produced by each canine tracheal smooth muscle strip to illustrate the ED 50 of each treatment. There were no significant differences ($p > 0.05$) in the ED 50 among the 15-hour incubation in control with vehicle medium ($n = 7$), incubation in fish oil medium ($n = 11$), and incubation in soybean oil medium ($n = 10$). Error bars represent standard error of the mean.*

Tissues incubated for 4 hours in their baths in either fish oil or control with vehicle treatment did not demonstrate a significant interaction ($p > 0.05$) between treatment and ACh dose. There was not a significant difference ($p > 0.05$) in the post-incubation contractility between the fish oil treatment and control with vehicle treatment (figure 4-11).

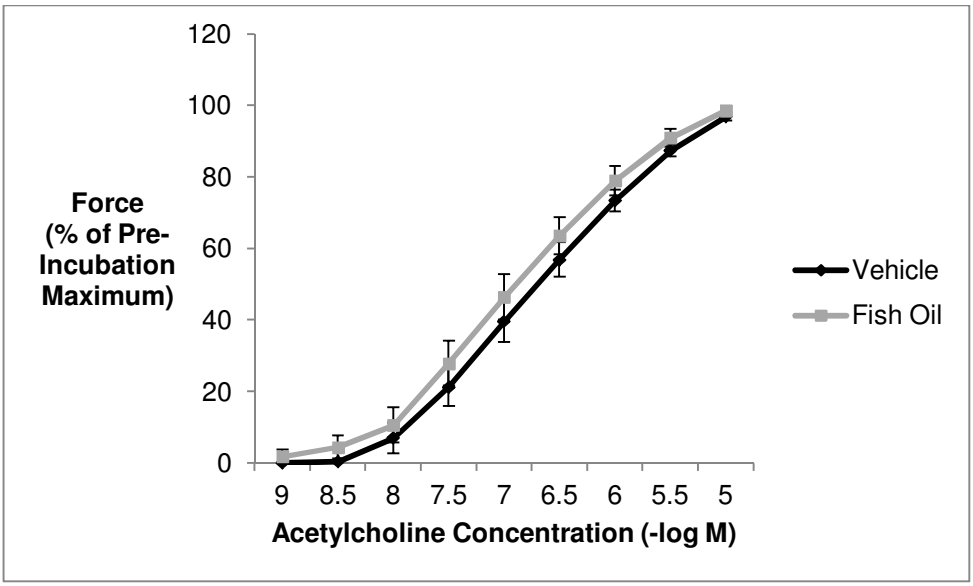

Figure 4-11. ***The force produced by canine tracheal smooth muscle strips in response to half-logarithmic doses of acetylcholine following 4 hours of incubation in control with vehicle or fish oil media.*** *There was not a significant difference ($p > 0.05$) between the control with vehicle ($n = 5$) and fish oil ($n = 5$) treatments. Force was calculated as a percent of the pre-incubation maximum force produced by each smooth muscle strip. Error bars represent standard error of the mean.*

Similarly, there was not a significant interaction ($p > 0.05$) between treatment and ACh dose for tissue strips incubated in either fish oil or soybean oil media. Also, there was not a significant difference ($p > 0.05$) between the fish oil and soybean oil treatments in the post-incubation contractility (figure 4-12). Lastly, there was not a significant interaction ($p > 0.05$) between the treatment and ACh dose or a significant difference ($p > 0.05$) in the post-incubation contractility between the treatments for tissues incubated in control with vehicle medium and those incubated in soybean oil medium (figure 4-13).

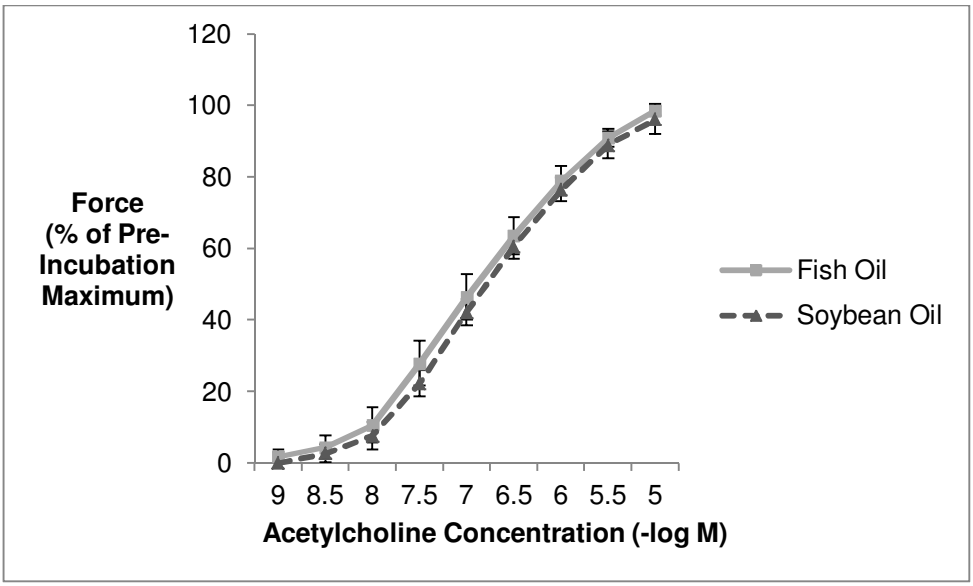

Figure 4-12. ***The force produced by canine tracheal smooth muscle strips in response to half-logarithmic doses of acetylcholine following 4 hours of incubation in fish oil or soybean oil media.*** *There was not a significant difference ($p > 0.05$) between the fish oil ($n = 5$) and soybean oil ($n = 6$) treatments. Force was calculated as a percent of the pre-incubation maximum force produced by each smooth muscle strip. Error bars represent standard error of the mean.*

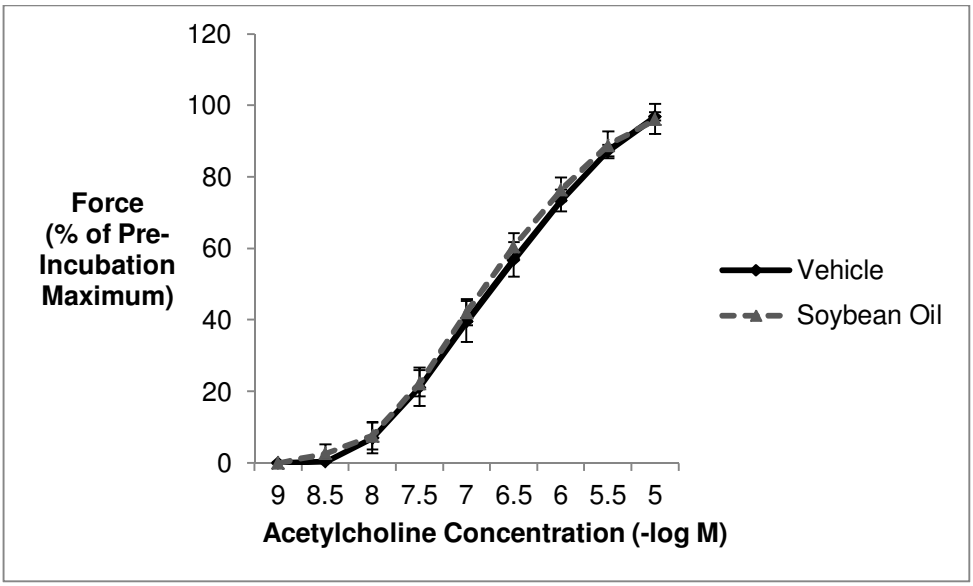

Figure 4-13. ***The force produced by canine tracheal smooth muscle strips in response to half-logarithmic doses of acetylcholine following 4 hours of incubation in control with vehicle or soybean oil media.*** *There was not a significant difference ($p > 0.05$) between the control with vehicle ($n = 5$) and soybean oil ($n = 6$) treatments. Force was calculated as a percent of the pre-incubation maximum force produced by each smooth muscle strip. Error bars represent standard error of the mean.*

There were no significant differences in either the post-incubation maximum force generated by the tissues ($p < 0.05$) or the ED 50 for the tissues ($p > 0.05$) among the fish oil, soybean oil, and control with vehicle incubation treatments (figures 4-14, 4-15).

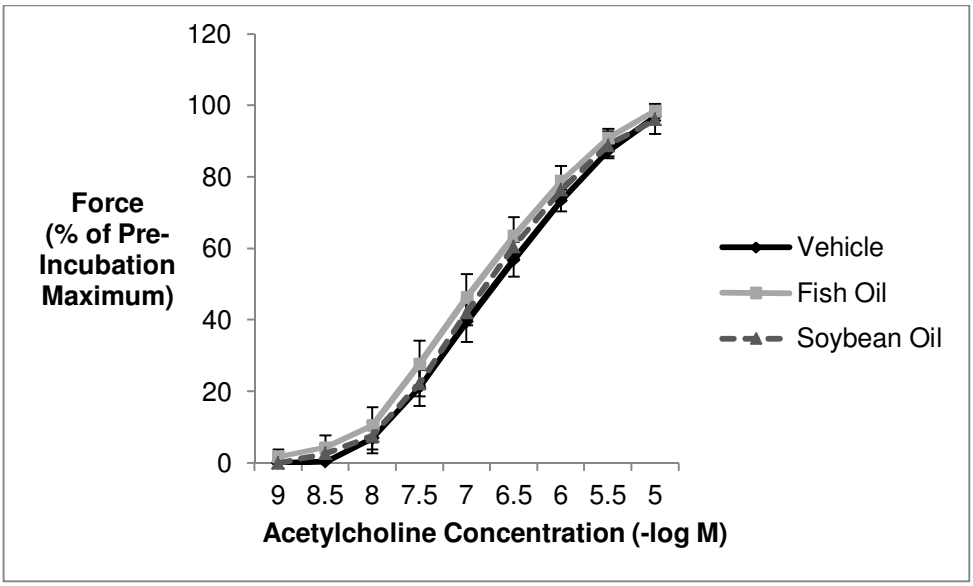

Figure 4-14. ***The maximum force generated by the canine tracheal smooth muscle strips was not significantly altered by treatment.*** *The force produced in response to half-logarithmic doses of acetylcholine following incubation was normalized with respect to the maximum pre-incubation force produced by each canine tracheal smooth muscle strip. There were no significant differences ($p > 0.05$) in the maximum force generated among the control with vehicle ($n = 5$), fish oil ($n = 5$), and soybean oil ($n = 6$) treatments. Error bars represent standard error of the mean.*

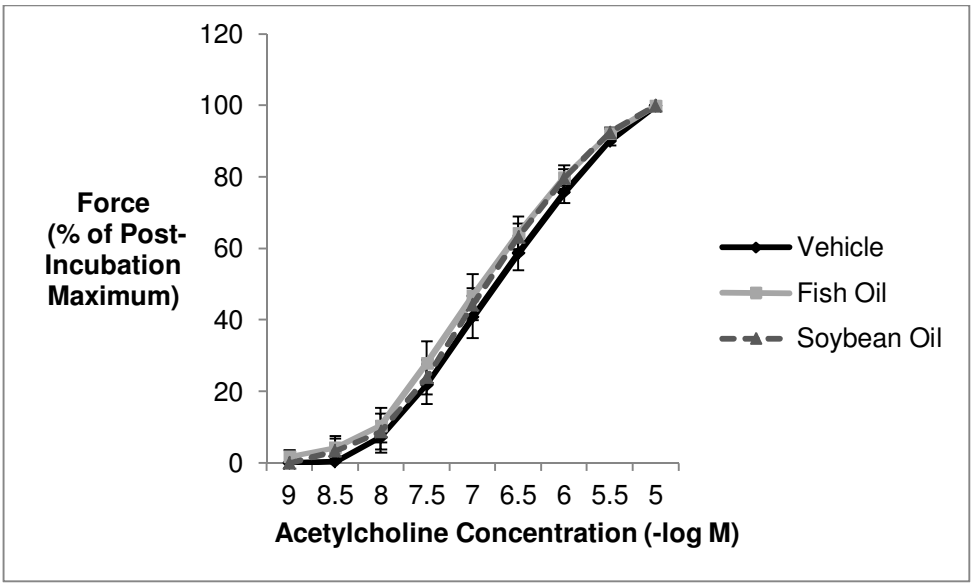

Figure 4-15. ***The effective dose (ED) 50 was not significantly altered by treatment.*** *The force produced in response to half-logarithmic doses of acetylcholine following incubation was normalized with respect to the maximum post-incubation force produced by each canine tracheal smooth muscle strip to illustrate the ED 50 of each treatment. There were no significant differences ($p > 0.05$) in the ED 50 among the 4-hour incubation in control with vehicle medium ($n = 5$), incubation in fish oil medium ($n = 5$), and incubation in soybean oil medium ($n = 6$). Error bars represent standard error of the mean.*

A 2-hour incubation in the tissue baths was also completed. There was not a significant interaction ($p > 0.05$) between the treatment and ACh dose or a significant difference ($p > 0.05$) between the treatments for tissues incubated in fish oil or in control with vehicle (figure 4-16).

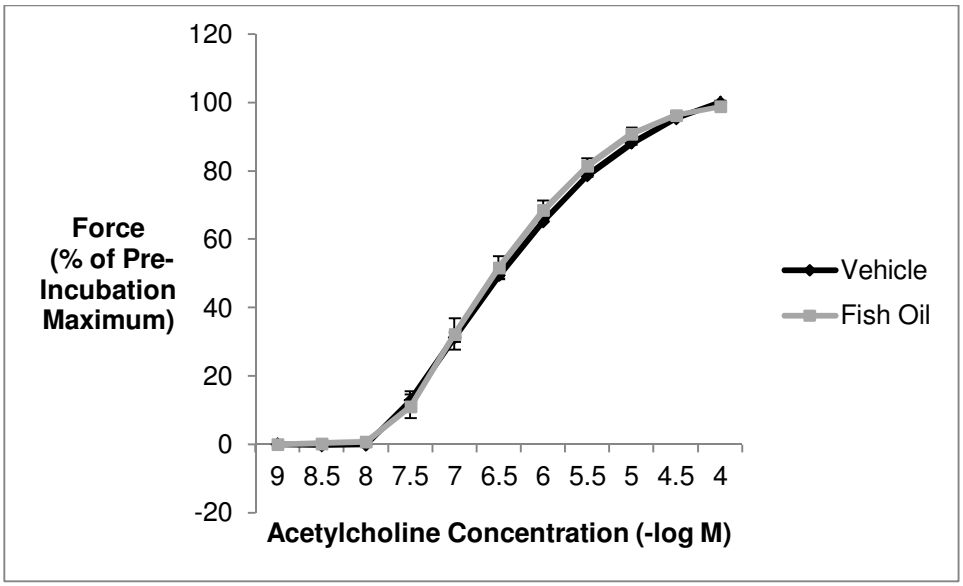

Figure 4-16. ***The force produced by canine tracheal smooth muscle strips in response to half-logarithmic doses of acetylcholine following 2 hours of incubation in control with vehicle or fish oil media.*** *There was not a significant difference ($p > 0.05$) between the control with vehicle ($n = 2$) and fish oil ($n = 3$) treatments. Force was calculated as a percent of the pre-incubation maximum force produced by each smooth muscle strip. Error bars represent standard error of the mean.*

Similarly, there was not a significant interaction ($p > 0.05$) between the treatment and ACh dose or a significant difference ($p > 0.05$) in the post-incubation contractility between the treatments for tissues incubated in fish oil compared to soybean oil (figure 4-17) or for tissues incubated in control with vehicle compared to soybean oil (figure 4-18).

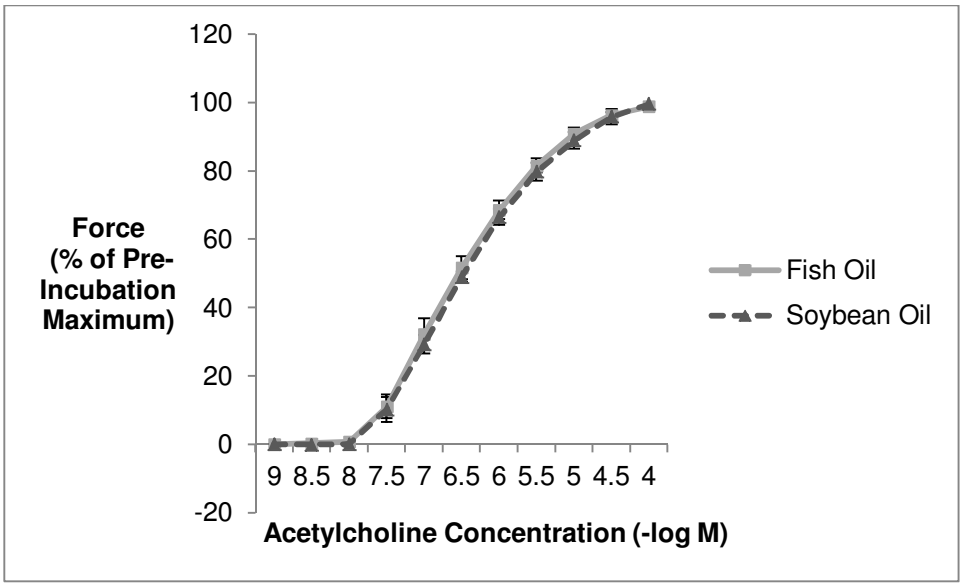

Figure 4-17. ***The force produced by canine tracheal smooth muscle strips in response to half-logarithmic doses of acetylcholine following 4 hours of incubation in fish oil or soybean oil media.*** *There was not a significant difference ($p > 0.05$) between the fish oil ($n = 3$) and soybean oil ($n = 3$) treatments. Force was calculated as a percent of the pre-incubation maximum force produced by each smooth muscle strip. Error bars represent standard error of the mean.*

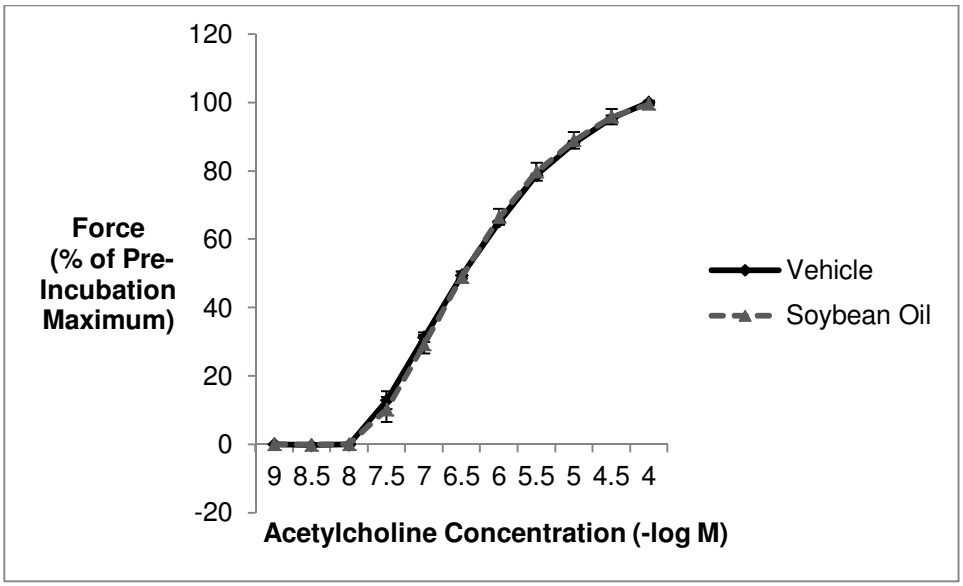

Figure 4-18. ***The force produced by canine tracheal smooth muscle strips in response to half-logarithmic doses of acetylcholine following 2 hours of incubation in control with vehicle or soybean oil media.*** *There was not a significant difference ($p > 0.05$) between the control with vehicle ($n = 2$) and soybean oil ($n = 3$) treatments. Force was calculated as a percent of the pre-incubation maximum force produced by each smooth muscle strip. Error bars represent standard error of the mean.*

Finally, there were no significant differences in either the post-incubation maximum force generated by the tissues ($p < 0.05$) or the ED 50 for the tissues ($p > 0.05$) among the fish oil, soybean oil, and control with vehicle incubation treatments (figures 4-19, 4-20).

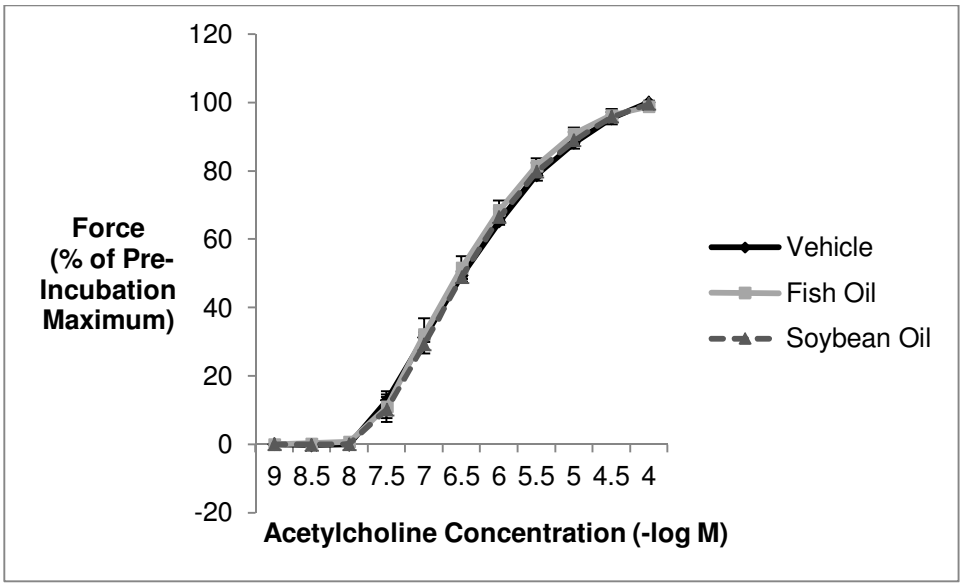

Figure 4-19. ***The maximum force generated by the canine tracheal smooth muscle strips was not significantly altered by treatment.*** *The force produced in response to half-logarithmic doses of acetylcholine following incubation was normalized with respect to the maximum pre-incubation force produced by each canine tracheal smooth muscle strip. There were no significant differences ($p > 0.05$) in the maximum force generated among the control with vehicle ($n = 2$), fish oil ($n = 3$), and soybean oil ($n = 3$) treatments. Error bars represent standard error of the mean.*

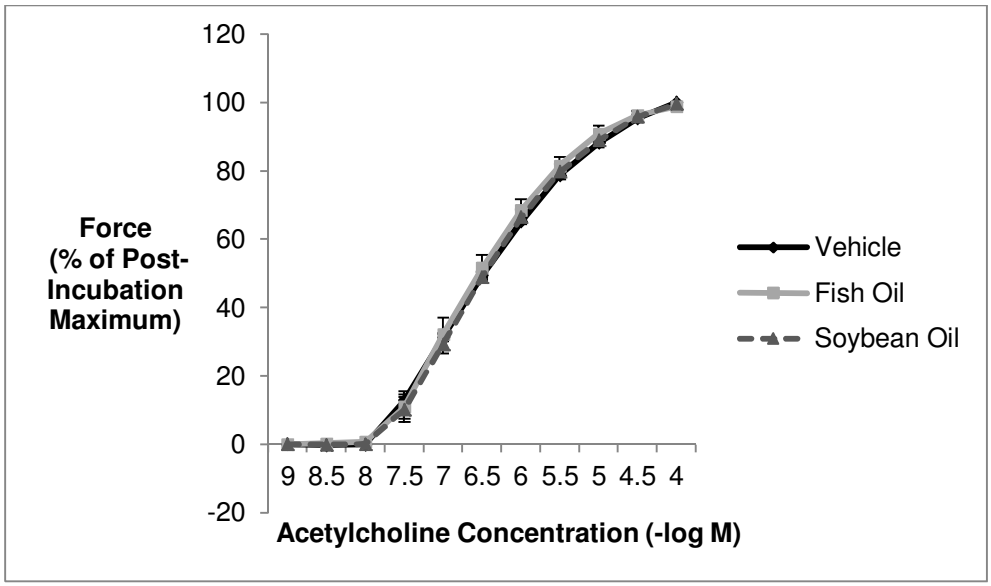

Figure 4-20. ***The effective dose (ED) 50 was not significantly altered by treatment.*** *The force produced in response to half-logarithmic doses of acetylcholine following incubation was normalized with respect to the maximum post-incubation force produced by each canine tracheal smooth muscle strip to illustrate the ED 50 of each treatment. There were no significant differences ($p > 0.05$) in the ED 50 among the 2-hour incubation in control with vehicle medium ($n = 2$), incubation in fish oil medium ($n = 3$), and incubation in soybean oil medium ($n = 3$). Error bars represent standard error of the mean.*

Acute Exposure to Fish Oil. Once a plateau in the force generated by each smooth muscle strip treated with a single dose of ACh was established, treatments were added to the tissue baths. A significant relaxation in the force elicited by 10^{-6} M ACh was observed among the fish oil, soybean oil, vehicle, and control (sham) treatments (figure 4-21). Fisher's LSD post-hoc determined that the percent relaxation with the fish oil treatment was significantly greater ($p < 0.05$) than both the vehicle and control treatments; there was no significant difference between the fish oil and soybean oil treatments. When the smooth muscle strips were contracted with 10^{-7} M ACh, there was not a significant difference ($p > 0.05$) in the percent relaxation occurring with fish oil, soybean oil, and vehicle treatments (figure 4-22).

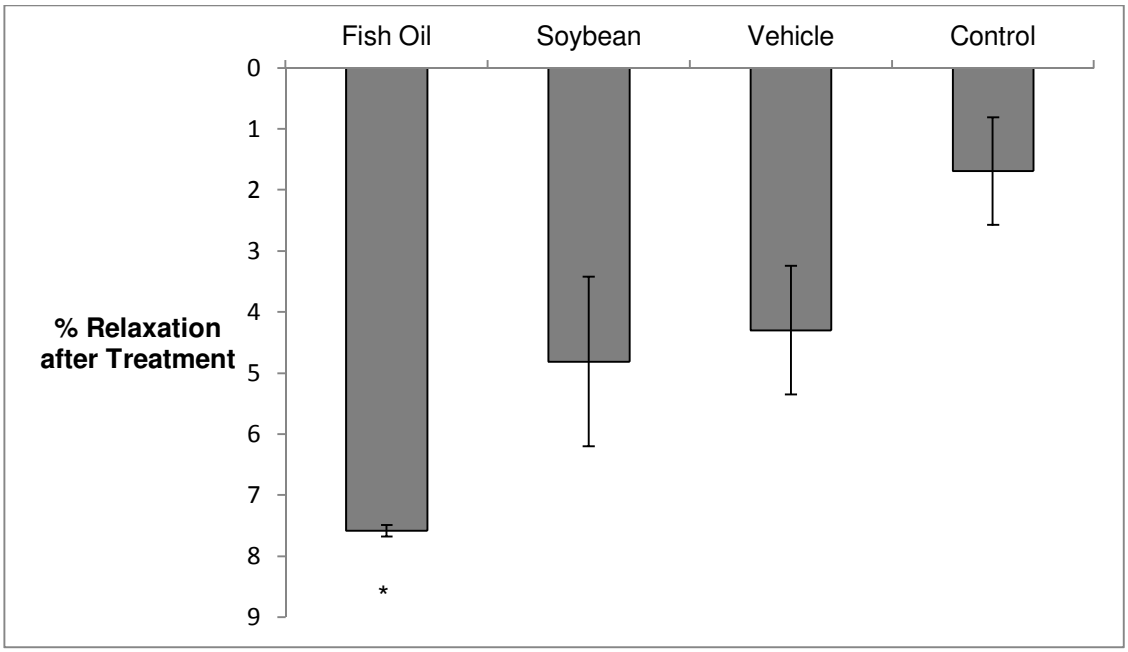

Figure 4-21. ***Canine tracheal smooth muscle strips relaxed in response to the acute exposure of fish oil.*** *Following contraction of the tissue with 10^{-6} M acetylcholine, fish oil (n = 3), soybean oil (n = 3), vehicle (n = 3), or control (sham) (n = 2) treatments were administered to the tissue baths. The percent relaxation associated with the fish oil treatment was significantly greater ($p < 0.05$) than that of the vehicle and control treatments. There were no significant differences ($p > 0.05$) between the fish oil and soybean oil treatments. Error bars represent standard error of the mean. *, significantly different from vehicle and control*

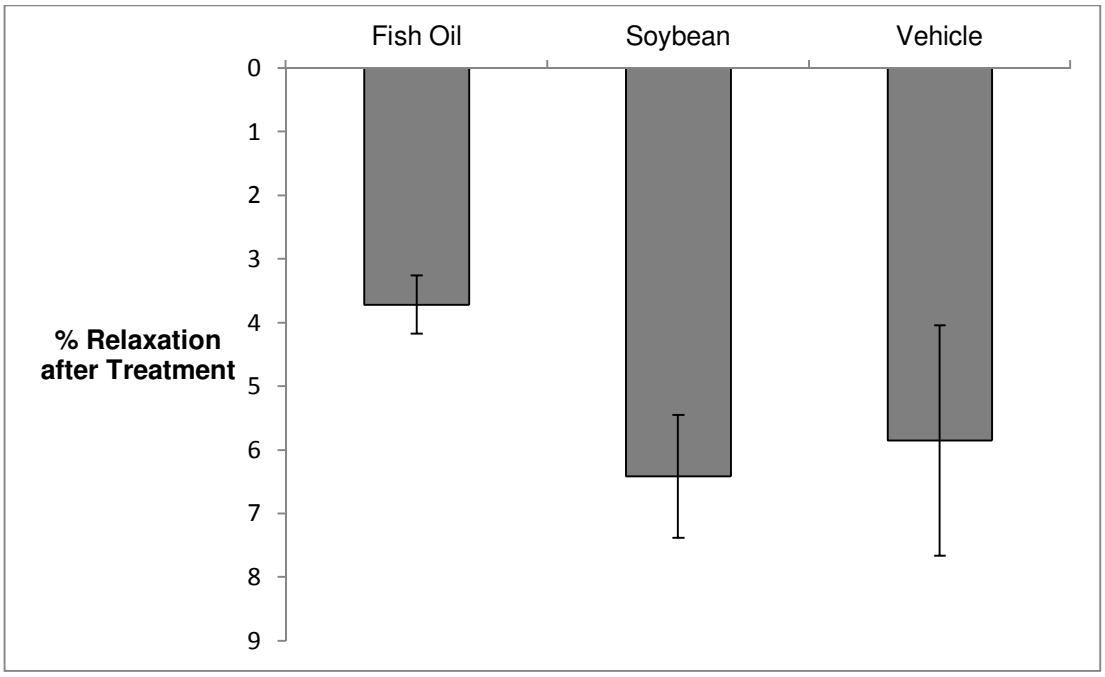

Figure 4-22. ***Acute exposure to fish oil did not significantly relax the canine tracheal smooth muscle response to 10^{-7} M acetylcholine.*** *Following contraction of the tissue with 10^{-7} acetylcholine, fish oil (n = 4), soybean oil (n = 4), or control with vehicle (n = 4) treatments were administered to the tissue baths. There were no significant differences ($p > 0.05$) in the percent relaxation among the fish oil, soybean oil, and vehicle treatments. Error bars represent standard error of the mean.*

Contractions with Other Agonists. PGF_2 failed to contract smooth muscle strips at any half-logarithmic dose between 10^{-9} to 10^{-4} M. The same doses of ACh were tested in tandem as a positive control. Additional studies using PGF_2 as the contractile agonist were not conducted.

A dose response curve was obtained with half-logarithmic doses of 5-HT between 10^{-9} to 10^{-4} M. To demonstrate the repeatability of this response, the dose response curve was repeated 4 hours later. Again, the same doses of ACh were tested in tandem as a positive control. The tissues demonstrated good retention of their responses to both contractile agonists with the repeated dose response curve (figures 4-23, 4-24). Thus, additional studies using 5-HT as the contractile agonist were undertaken.

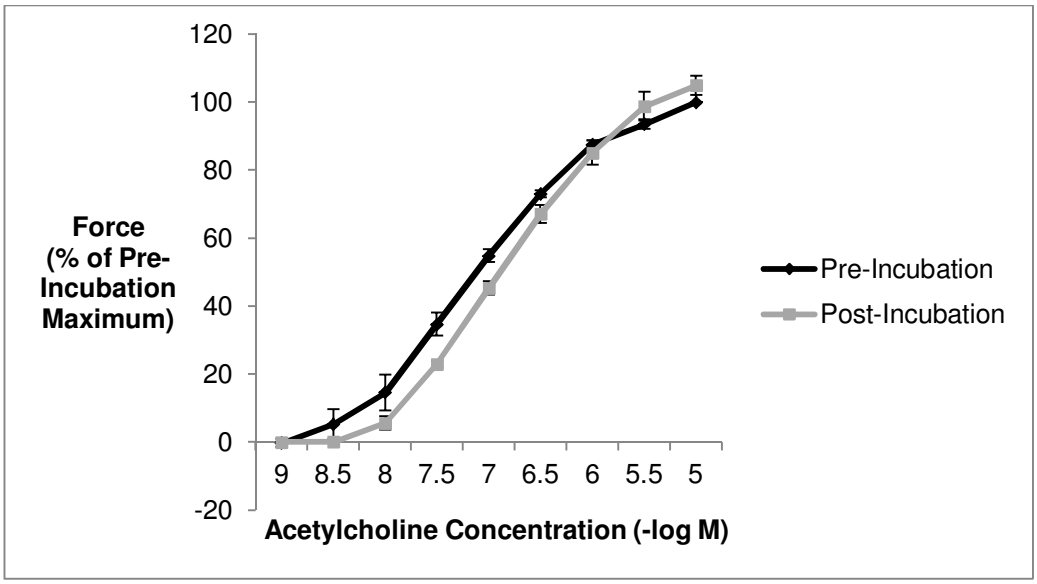

Figure 4-23. ***The force produced by canine tracheal smooth muscle strips in response to half-logarithmic doses of acetylcholine prior to and following a 4-hour incubation in physiologic saline solution.*** *The reproducibility of smooth muscle strips' (n = 4) response to acetylcholine after 4 hours of incubation in tissue baths filled with physiologic saline solution was tested. Force was calculated as a percent of the pre-incubation maximum force produced by each smooth muscle strip. Error bars represent standard error of the mean.*

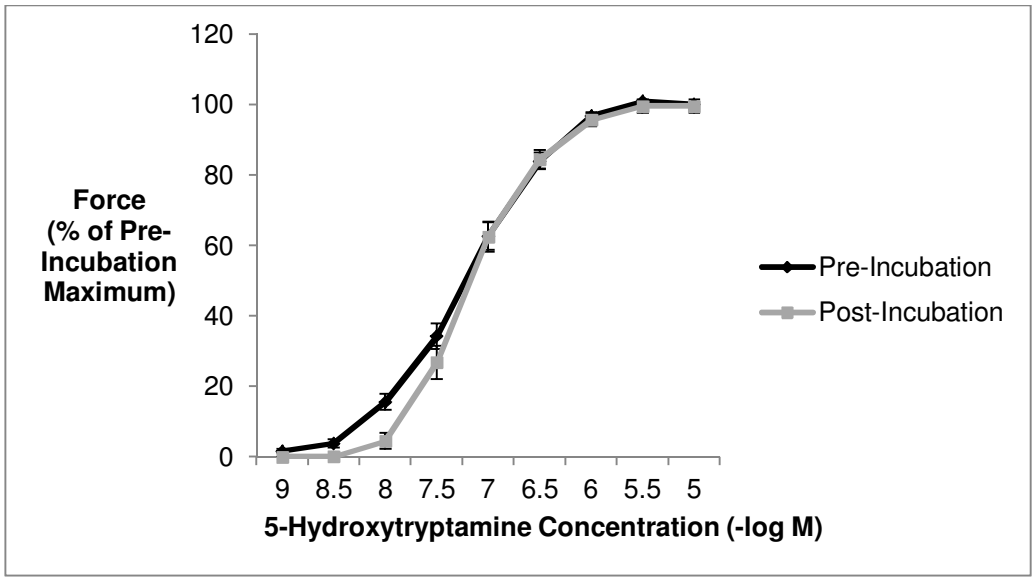

Figure 4-24. ***The force produced by canine tracheal smooth muscle strips in response to half-logarithmic doses of 5-hydroxytryptamine prior to and following a 4-hour incubation in physiologic saline solution.*** *The reproducibility of smooth muscle strips' (n = 4) response to 5-hydroxytryptamine after 4 hours of incubation in tissue baths filled with physiologic saline solution was tested. Force was calculated as a percent of the pre-incubation maximum force produced by each smooth muscle strip. Error bars represent standard error of the mean.*

Several experiments examining the effect of the chronic exposure of fish oil were repeated using half-logarithmic doses of 5-HT as the contractile agonist in place of ACh. Following 24 hours of incubation in fish oil or control with vehicle media, there was no significant interaction between treatment and 5-HT dose. Moreover, there was not a significant difference in the post-incubation contractility between the fish oil and control with vehicle treatments (figure 4-25). Neither the maximum force produced by the tissues nor the ED 50 of the tissues (figure 4-26) were significantly different ($p > 0.05$) between the fish oil and control with vehicle treatments.

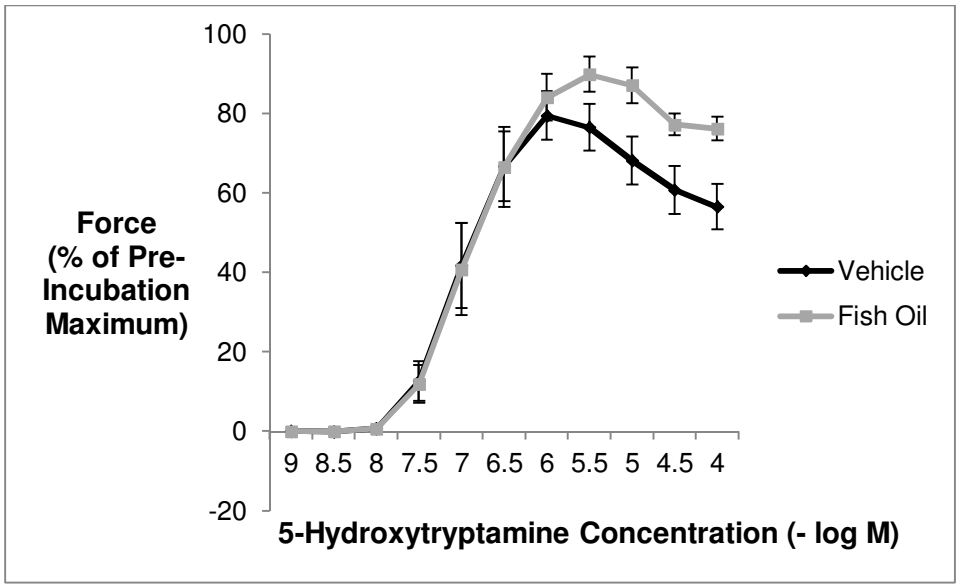

Figure 4-25. ***The force produced by canine tracheal smooth muscle strips in response to half-logarithmic doses of 5-hydroxytryptamine following 24 hours of incubation in control with vehicle or fish oil media.*** *There was not a significant difference ($p > 0.05$) between the control with vehicle ($n = 6$) and fish oil ($n = 5$) treatments in terms of the post-incubation contractility or maximum force generated. Force was calculated as a percent of the pre-incubation maximum force produced by each smooth muscle strip. Error bars represent standard error of the mean.*

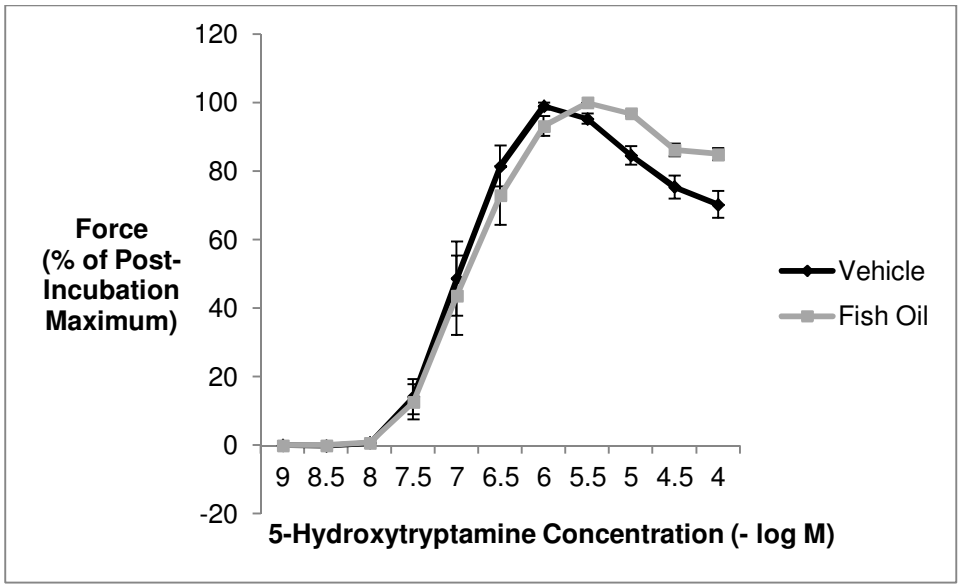

Figure 4-26. ***The effective dose (ED) 50 was not significantly altered by treatment.*** *The force produced in response to half-logarithmic doses of 5-hydroxytryptamine following incubation was normalized with respect to the maximum post-incubation force produced by each canine tracheal smooth muscle strip to illustrate the ED 50 of each treatment. There was no significant difference ($p > 0.05$) in the ED 50 between the 24-hour incubation in control with vehicle medium ($n = 6$) and incubation in fish oil medium ($n = 5$). Error bars represent standard error of the mean.*

The 4-hour incubation in the tissue baths was also conducted with 5-HT as the contractile agonist. There was no significant interaction ($p > 0.05$) between treatment and 5-HT dose at this time point for tissues incubated in fish oil or control with vehicle media. There was also no significant difference ($p > 0.05$) in the post-incubation contractility between the fish oil and control with vehicle treatments (figure 4-27).

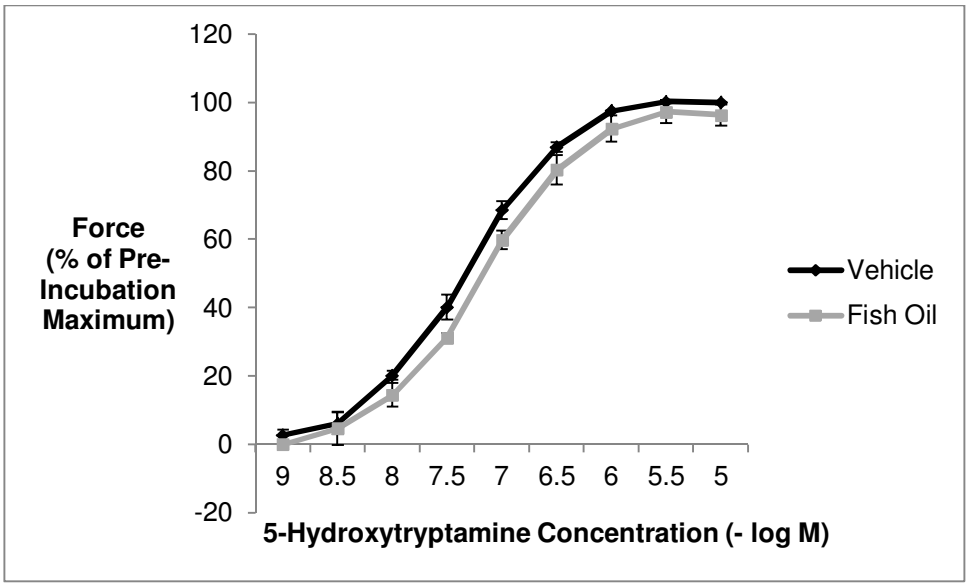

Figure 4-27. ***The force produced by canine tracheal smooth muscle strips in response to half-logarithmic doses of 5-hydroxytryptamine following 4 hours of incubation in control with vehicle or fish oil media.*** *There was not a significant difference ($p > 0.05$) between the control with vehicle ($n = 2$) and fish oil ($n = 2$) treatments. Force was calculated as a percent of the pre-incubation maximum force produced by each smooth muscle strip. Error bars represent standard error of the mean.*

Similarly, there was not a significant interaction ($p > 0.05$) between the treatment and 5-HT dose or a significant difference ($p > 0.05$) in the post-incubation contractility of tissues incubated in fish oil or soybean oil media (figure 4-28). Tissues incubated in control with vehicle medium or soybean oil medium did not express a significant interaction ($p > 0.05$) between the treatment and 5-HT dose; there was also no significant difference ($p > 0.05$) in the post-incubation contractility of these treatments (figure 4-29).

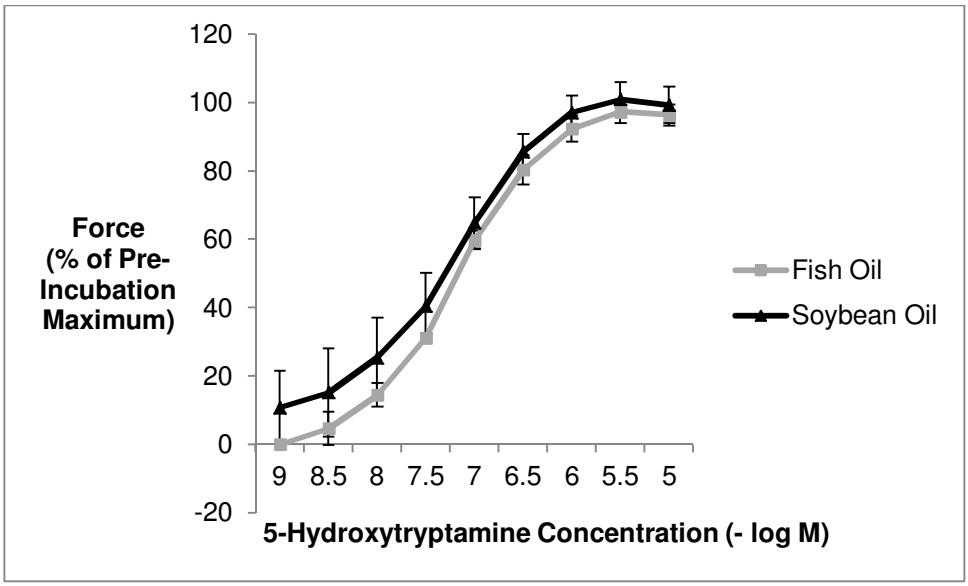

Figure 4-28. ***The force produced by canine tracheal smooth muscle strips in response to half-logarithmic doses of 5-hydroxytryptamine following 4 hours of incubation in fish oil or soybean oil media.*** *There was not a significant difference ($p > 0.05$) between the fish oil ($n = 2$) and soybean oil ($n = 3$) treatments. Force was calculated as a percent of the pre-incubation maximum force produced by each smooth muscle strip. Error bars represent standard error of the mean.*

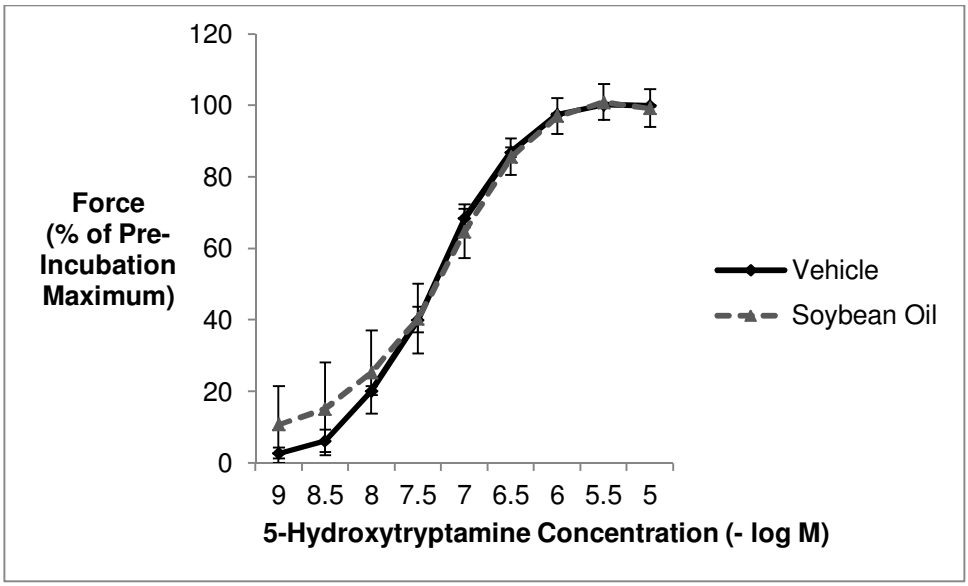

Figure 4-29. ***The force produced by canine tracheal smooth muscle strips in response to half-logarithmic doses of 5-hydroxytryptamine following 4 hours of incubation in control with vehicle or soybean oil media.*** *There was not a significant difference ($p > 0.05$) between the control with vehicle ($n = 2$) and soybean oil ($n = 3$) treatments. Force was calculated as a percent of the pre-incubation maximum force produced by each smooth muscle strip. Error bars represent standard error of the mean.*

The maximum force generated by the tissues was not significantly different among the fish oil, soybean oil, and control with vehicle treatments (figure 4-30). Furthermore, there was not a significant difference in the ED 50 for the tissues treated with fish oil, soybean oil, or control with vehicle treatments (figure 4-31).

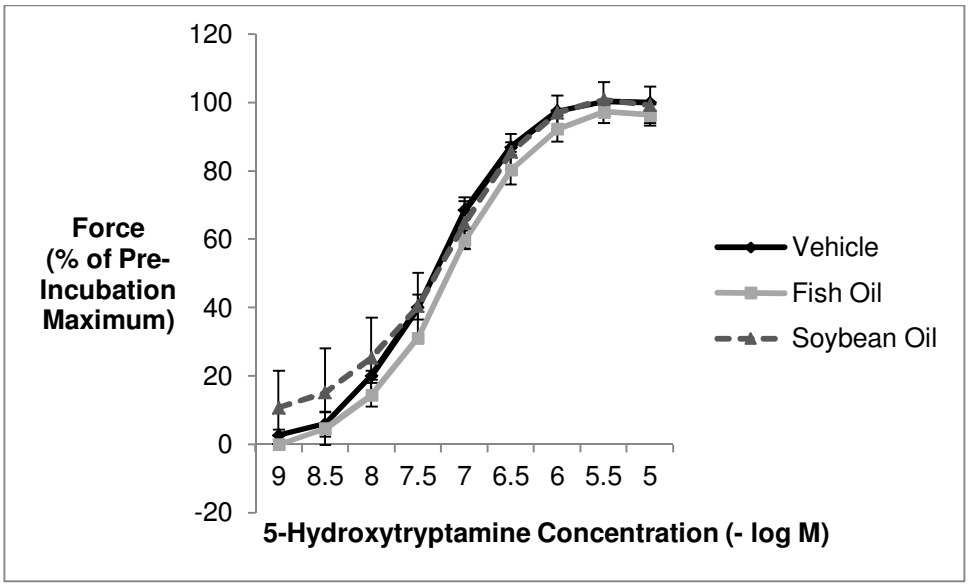

Figure 4-30. ***The maximum force generated by the canine tracheal smooth muscle strips was not significantly altered by treatment.*** *The force produced in response to half-logarithmic doses of 5-hydroxytryptamine following a 4-hour incubation was normalized with respect to the maximum pre-incubation force produced by each canine tracheal smooth muscle strip. There were no significant differences ($p > 0.05$) in the maximum force generated among the control with vehicle ($n = 2$), fish oil ($n = 2$), and soybean oil ($n = 3$) treatments. Error bars represent standard error of the mean.*

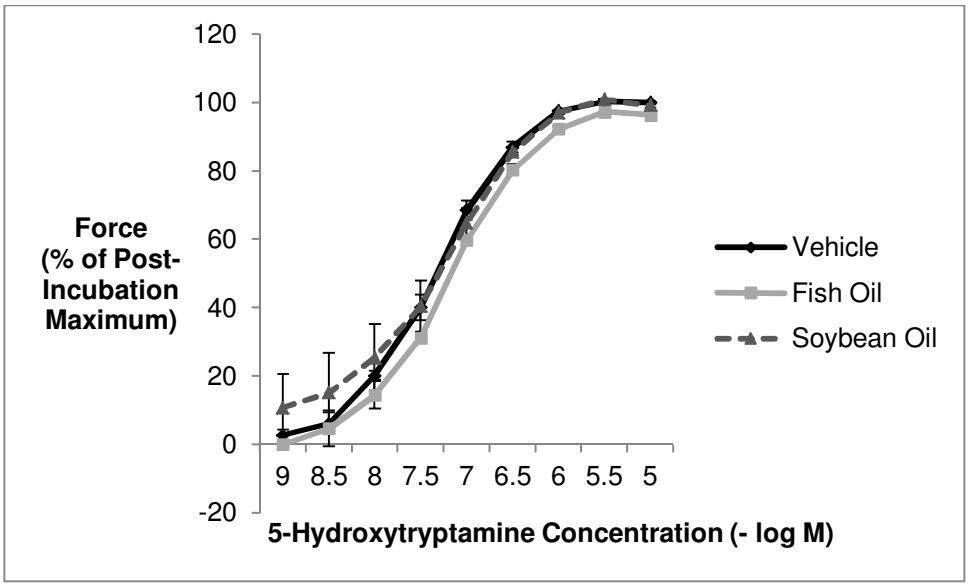

Figure 4-31. ***The effective dose (ED) 50 was not significantly altered by treatment.*** *The force produced in response to half-logarithmic doses of 5-hydroxytryptamine following a 4-hour incubation was normalized with respect to the maximum post-incubation force produced by each canine tracheal smooth muscle strip to illustrate the ED 50 of each treatment. There were no significant differences ($p > 0.05$) in the ED 50 among the 4-hour incubation in control with vehicle medium ($n = 2$), incubation in fish oil medium ($n = 2$), and incubation in soybean oil ($n = 3$). Error bars represent standard error of the mean.*

The acute exposure of fish oil was also assessed using 5-HT as the contractile agonist. After a plateau in the force generated by each smooth muscle strip treated with a single dose of 5-HT was established, treatments were added to the tissue baths. A significant relaxation ($p < 0.05$) in the force generated by 10^{-6} 5-HT was demonstrated between fish oil and soybean oil, vehicle, or control (sham) (figure 4-32); there were no significant differences ($p > 0.05$) among the percent relaxation associated with soybean oil, vehicle, or control treatment administration.

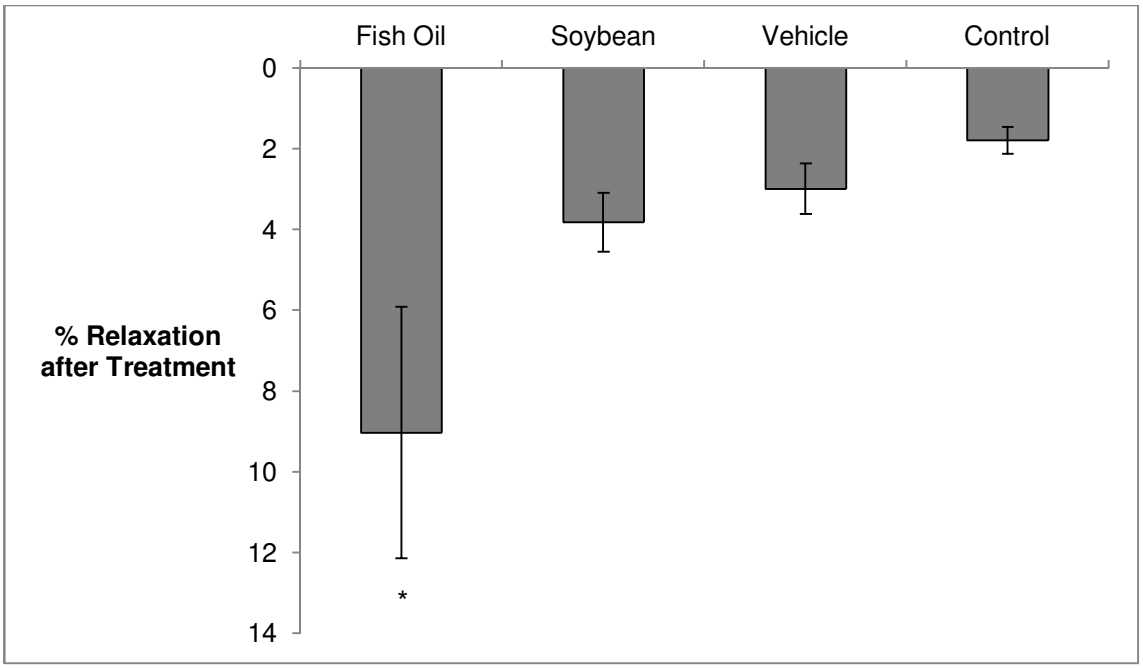

Figure 4-32. ***Canine tracheal smooth muscle strips relaxed in response to the acute exposure of fish oil.*** *Following contraction of the tissue with 10^{-6} M 5-hydroxytryptamine, fish oil (n = 9), soybean oil (n = 6), vehicle (n = 6), or control (sham) (n = 5) treatments were administered to the tissue baths. The percent relaxation associated with the fish oil treatment was significantly greater ($p < 0.05$) than that of the three other treatments. There were no significant differences ($p > 0.05$) among the soybean oil, vehicle, and control (sham) treatments. Error bars represent standard error of the mean. *, significantly different*

Using 10^{-7} 5-HT to contract the smooth muscle strips, this acute experiment protocol was repeated. There were no significant differences among the percent relaxations provoked by the addition of fish oil, soybean oil, and control with vehicle treatments (figure 4-33).

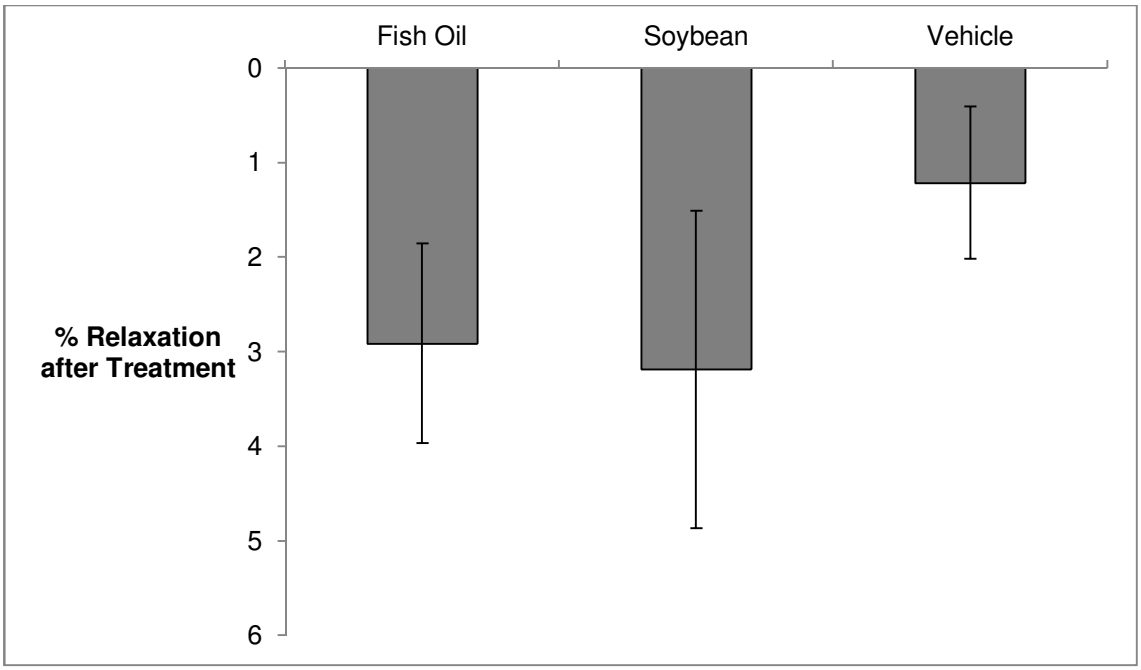

Figure 4-33. ***Acute exposure to fish oil did not significantly relax the canine tracheal smooth muscle response to 10^{-7} M 5-hydroxytryptamine.*** *Following contraction of the tissue with 10^{-7} 5-hydroxytryptamine, fish oil (n = 7), soybean oil (n = 4), or control with vehicle (n = 7) treatments were administered to the tissue baths. There were no significant differences ($p > 0.05$) in the percent relaxation among the fish oil, soybean oil, and vehicle treatments. Error bars represent standard error of the mean.*

Discussion

A series of experiments were undertaken to test the hypothesis that the treatment of canine tracheal smooth muscle tissue with fish oil would reduce arachidonic acid content and increase EPA and DHA content in smooth muscle cell membranes and would be associated with a decrease in the airway smooth muscle responsiveness to a contractile agonist. Although significant changes in both omega-3 polyunsaturated fatty acid content and smooth muscle contractility were demonstrated, these alterations did not occur in concert and therefore are likely unrelated.

Lipid analysis revealed significant incorporation of EPA and DHA in smooth muscle strips incubated for at least 24 hours in fish oil medium as compared to tissue incubated in control medium. Previous studies that have assessed fatty acid

composition following fish oil supplementation have reported the concentration of arachidonic acid, EPA, and DHA in murine lung tissue (102), human neutrophils (47, 61), and human plasma phospholipids (47) but not canine airway smooth muscle (table 4-3). While these studies supplemented the diet with fish oil for weeks, the trend of changes in the lipid profile between treated and control samples are similar to the current study. Notably, examination of the comparative concentrations of the fatty acids indicates that the degree of incorporation seems to vary by the tissue type as well as by the duration of exposure to omega-3 fatty acids.

	Arachidonic Acid (%)	EPA (%)	DHA (%)
Current Study			
Control	16.38 ± 3.69	0.00 ± 0.00	0.21 ± 0.02
Fish Oil	8.90 ± 2.96	5.97 ± 1.12	2.37 ± 0.12
Kew et al., 2004 (Plasma Phospholipids)			
Placebo Supplementation	12.0 ± 2.6	1.3 ± 0.7	7.3 ± 1.6
EPA Supplementation	10.2 ± 1.0	7.2 ± 1.9	7.6 ± 1.1
DHA Supplementation	10.2 ± 2.2	4.5 ± 1.3	13.5 ± 3.1
Kew et al., 2004 (Neutrophils)			
Placebo Supplementation	14.1 ± 1.8	0.6 ± 0.3	2.5 ± 0.9
EPA Supplementation	12.7 ± 2.1	3.0 ± 1.1	2.7 ± 0.9
DHA Supplementation	12.2 ± 1.5	1.8 ± 0.5	5.3 ± 1.1
Mickleborough et al., 2006 (Neutrophils)			
Normal Diet	22.3 ± 0.97	0.18 ± 0.03	2.16 ± 0.33
Placebo Supplementation	22.6 ± 0.92	0.15 ± 0.04	2.2 ± 0.41
Fish Oil Supplementation	13.1 ± 1.02	4.01 ± 0.45	3.32 ± 0.45

Table 4-3. ***Comparison of the arachidonic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) fatty acid composition reported in the current study and the literature.*** *Two* in vivo *studies determined the percent contribution of arachidonic acid, EPA, and DHA to the total fatty acid composition in the subjects' neutrophils and/or plasma phospholipids following supplementation with placebo, EPA-rich, DHA-rich, or fish oil capsules. Healthy subjects were supplemented for 4 weeks in the Kew et al. (47, 61) study while asthmatic subjects with exercise-induced bronchoconstriction were supplemented for 3 weeks in the Mickleborough et al. (61) study. Both studies used olive oil as the placebo treatment. For the current study, canine tracheal smooth muscle strips were incubated in either control or fish oil media for 1 day. Data are reported as the mean percent contribution to total fatty acid composition ± standard error of the mean for the current study and the Kew et al. study. Data are reported as the mean percent contribution to total fatty acid composition ± standard deviation for the Mickleborough et al. study.*

Following 15 hours of incubation in fish oil media, there was not a significant change in arachidonic acid, EPA, or DHA as compared to smooth muscle strips incubated in soybean oil, control with vehicle, or control media. Additionally, following 4 hours of incubation in fish oil media, there was a statistically significant increase in DHA, but not EPA or arachidonic acid, as compared to tissue incubated in control with vehicle or control media; there was not a significant difference in arachidonic acid, EPA, or DHA

composition between tissues incubated in fish oil and soybean oil. Because the percent contribution of DHA to the total fatty acid composition after 4 hours of incubation in fish oil (0.14 ± 0.02%) was similar to that for the tissues incubated in control media for 1 day (0.21 ± 0.02%), this statistically significant difference is likely not physiologically significant. Moreover, although there was not a significant difference between the soybean oil and fish oil treated tissues after 4 hours of incubation, the percent contribution of DHA to the total fatty acid composition was greater with soybean oil (0.39 ± 0.09%) than with fish oil (0.14 ± 0.02%), which should not be due to differences in fatty acid incorporation as soybean oil does not contain either EPA or DHA.

In contrast to the tissue samples that were incubated for a longer time course, the tissues incubated for 4 and 15 hours were contracted in the dose response experiments prior to and following their incubation. Thus, although it may be that these shorter time points did not permit adequate time for lipid incorporation, it is also possible that the lipids that were incorporated were subsequently metabolized to produce ATP for the contraction experiments. This is a feasible explanation as it has been shown that vascular smooth muscle can utilize a variety of endogenous and exogenous substrates for energy production, and at least at rest, endogenous substrates are preferentially oxidized by vascular smooth muscle (21). Furthermore, Odessey et al. (73) determined that for resting vascular smooth muscle, endogenous lipids account for approximately 77% of oxygen consumption when exogenous glucose is present and nearly 100% of oxygen consumption when exogenous glucose is absent. Thus, despite the presence of glucose in the PSS in the tissue baths of the current study, the smooth muscle strips may have oxidized endogenous lipids during the contraction experiments.

Since lipid metabolism was recognized as a potential confounding factor for our experiments, we reduced the washout periods as much as possible and maintained the treatments in the tissue baths. Allen et al. (7) reported that exogenous acetate provided

the major source for ATP production via the citric acid cycle both at rest and during contraction for vascular smooth muscle; importantly, they demonstrated that the muscle's endogenous substrates were not oxidized when exogenous acetate was present. However, longer chain fatty acids, such as EPA (C20) and DHA (C22), are not as readily oxidized as short chain fatty acids when added exogenously to resting vascular smooth muscle (21). Moreover, it was noted by Odessey, et al. (73) that when incubating vascular smooth muscle with longer chain fatty acids, it is the endogenous lipids that are oxidized. Nevertheless, these findings may not hold true for contracting airway smooth muscle. Thus, in the future, lipid analysis should be performed in tandem on tissue that has only been incubated in the particular treatment and on tissue that was similarly incubated but also used in contraction experiments. This would help elucidate whether the lipids were incorporated at the shorter time points and then metabolized or simply not incorporated at all.

The effect of chronic exposure of fish oil on canine tracheal smooth muscle was tested after a range of incubation periods. Following a 24-hour incubation in fish oil medium, there was no significant change in the smooth muscle's contractile response to half-logarithmic doses of either ACh or 5-HT as compared to tissue incubated in control with vehicle medium. Although significant omega-3 fatty acid incorporation was demonstrated at this time point, there was not an associated reduction in contractility as expected. Since lipid analysis was only performed on tissue that had been incubated for 24 hours and not contracted, it is possible that the dose response experiment tissues metabolized their newly incorporated lipids during the contractions. Additionally, differences in contractility between tissues incubated in fish oil or control with vehicle media may have been masked by the deterioration of the post-incubation response to the contractile agonists for both sets of tissues; specifically, observable contractions

were not elicited until the fourth or fifth dose of the agonist post-incubation as opposed to typically the second or third dose of the agonist pre-incubation.

Interestingly, increased smooth muscle contractility was observed at the highest six doses of ACh following 15 hours of incubation in fish oil medium as compared to incubation in control with vehicle medium. The maximum force generated by the fish oil-treated tissues was also significantly greater than that of the control with vehicle-treated tissues. These results were contrary to the hypothesis. Therefore, this set of experiments showed that, if anything, fish oil improved or maintained the tracheal smooth muscle's condition compared to the control with vehicle treatment. Furthermore, as there was not a significant difference in the contractility between tissues incubated in soybean oil and those incubated in control with vehicle, it is likely that the differences with fish oil treatment are due to the presence of the omega-3 fatty acids it contains and not just that fact that the tissue was incubated with oil.

The shorter incubation periods of 2 and 4 hours did not demonstrate any changes in contractility, maximum force generation, or the ED 50 among the treatments. These tissues received their treatments while remaining in their tissue baths. Thus, the incubation media (PSS plus treatment) was different from that for the 15 and 24 hour incubations in an incubator (DMEM plus treatment). Again, for the tissues incubated for 4 hours, it is unknown whether the fish oil lipids were incorporated and then metabolized or never incorporated. Lipid analysis was not performed on the tissues incubated for 2 hours. The post-incubation contractions were very similar to the pre-incubation contractions for this time point.

The limited literature on chronic exposure to fish oil also shows variable results for airway smooth muscle contractility. Morin et al. (69) showed that following a 48-hour incubation with tumor necrosis factor-α (TNF-α) to induce bronchial hyperactivity either in the presence or absence of the EPA metabolite 17(18)-epoxyeicosatetraenoic acid

(17(18)-EpETE), there was a significant difference in the tension produced by the human bronchial smooth muscle tissues in response to methacholine or histamine; the tissues incubated in the presence of 17(18)-EpETE developed less tension. Although not reported as a significant difference, the figures in the Morin et al. (69) study show that incubation with TNF-α and the EPA metabolite was associated with increased tension to higher doses of methacholine and histamine compared to the control tissues incubated with neither TNF-α nor the EPA metabolite; this was a trend similar to the significant effects seen with our tissues incubated for 15 hours. Another study examined the effect of omega-3 and omega-6 fatty acid enriched diets on airway smooth muscle contractility. Here, Abeywardena et al. (4) fed guinea pigs different diets containing a range of omega-3 and omega-6 fatty acids. They did not observe any significant differences in bronchial and tracheal smooth muscle contractility in response to a range of agonists (histamine, carbechol, leukotriene C_4, and prostaglandin-2α) among the high, moderate, and low omega-6:omega-3 fatty acid ratio diets. Fatty acid incorporation in the lung tissue was measured; however, there was not a significant change in the percent composition of EPA and DHA. This contrasts the significant changes in EPA and DHA composition shown in murine lung tissue following dietary supplementation with fish oil in mice (102).

Significant relaxation was observed when fish oil was introduced into the baths of smooth muscle strips contracted with either 10^{-6} M ACh or 10^{-6} M 5-HT. Even though the percent relaxation was 7.58 ± 0.09% for tissues contracted with 10^{-6} M ACh and 9.07 ± 2.19% for tissues contracted with 10^{-6} M 5-HT, these minor changes may be physiologically significant if they are occurring in the small airways throughout the lungs as this would magnify the overall effect of the changes. When the acute exposure experiment was conducted with a lower dose (10^{-7} M) of ACh or 5-HT, significant relaxation was not observed following the administration of fish oil to the baths. In the

case of 5-HT, this may have been because most of the smooth muscle strips were not able to maintain a stable contraction throughout the duration of the experiment making it difficult to detect changes.

The literature also reports both significant relaxation and no significant changes following acute treatment of airway smooth muscle with omega-3 fatty acids. Hichami et al. (39) demonstrated significant relaxation of the basal tone of guinea pig bronchial smooth muscle with DHA although no such relaxation occurred when the smooth muscle was first contracted with carbamylcholine. In contrast, Morin et al. (69) reported a concentration-dependent relaxing effect of acute treatment with the EPA metabolite 17(18)-EpETE on both resting and contracted human bronchial smooth muscle.

In addition to more fully coordinating lipid analysis with the contraction experiments, future studies in this area should focus on creating a model that better mimics asthma. For example, interleukin-13 could be used to induce hyperresponsiveness of the canine tracheal smooth muscle just as Morin et al. (69) used TNF-α to induce hyperresponsiveness in human bronchial smooth muscle. Airway hyperresponsiveness is a classic feature of asthma. Furthermore, as it is known that the presence of epithelium affects airway reactivity (48), tissue with and without epithelium could be tested to determine if fish oil treatment affects them differently. It has been shown that the vascular smooth muscle of some vessels is relaxed by fish oil treatment in an endothelium-dependent manner (101). If the presence of epithelium is shown to be important to the responsiveness of tissue treated with fish oil, this would afford a possible reason for the somewhat variable response of subjects with asthma to fish oil as the airway epithelium is impaired to varying degrees in asthma (48). Moreover, as the clinical application of fish oil is through chronic supplementation, studies using airway smooth muscle tissue obtained from animal models fed fish oil instead of incubating the tissue in fish oil would more closely resemble the practical situation.

Overall, these experiments suggest that fish oil exposure can be associated with changes in airway smooth muscle contractility. However, these changes do not seem to be related to EPA and DHA incorporation. On the other hand, these lipid analysis results may have been confounded by endogenous substrate usage as proposed above. Moreover, in the case of 15 hours of chronic exposure, fish oil incubation was surprisingly associated with increased force generation. Nevertheless, minor, but significant, relaxation of ACh- and 5-HT-stimulated contractions were observed with acute exposure to fish oil. Thus, these results encourage additional research on the impact of fish oil on airway smooth muscle as *in vivo* fish oil supplementation studies purport significant reductions in exercise-induced bronchoconstriction in human subjects.

Acknowledgements

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CHAPTER 5

DISCUSSION

Summary of Findings

According to the most recent report from the Center for Disease Control (CDC), the prevalence of asthma in the United States is increasing after remaining stable for several years (6). Consequently, the economic burden of asthma from healthcare costs for prescription medications and emergency room visits along with indirect costs related to missed days of school and work for children and adults, respectively, is an important issue. Thus, the optimal management of asthma is imperative.

Given that the prevalence of asthma seems to be linked to societal changes in diet, nutritional strategies may offer a viable alternative to traditional pharmaceutical regimens (77). Specifically, a relatively high intake of omega-6 polyunsaturated fatty acids (PUFAs) in comparison to omega-3 PUFA intake may be contributing to chronic inflammatory diseases such as asthma (40, 57). Accordingly, omega-3 PUFA supplementation has reportedly reduced exercise-induced bronchoconstriction (EIB) in asthmatics treated with fish oil (61, 93). Therefore, the inclusion of dietary modification or supplementation in an asthma management plan may reduce reliance on medications that may have side effects or reduced efficacy over time (60).

Our primary objective was to determine the effects of novel nutritional strategies on hyperpnea-induced bronchoconstriction (HIB) in asthmatic individuals. HIB can identify EIB by using a rapid breathing challenge instead of an exercise protocol (9). Although our laboratory has previously demonstrated that fish oil alleviates EIB and HIB (61, 62, 93), several important questions remain unanswered. In particular, fish oil's use in combination with other nutritional supplements, the optimal fish oil formula, and fish oil's effect on airway smooth muscle responsiveness have not been fully explored. In attempt to address these issues, the following specific aims were undertaken:

1. **Determine the effect of fish oil and antioxidant supplementation and their combination on hyperpnea-induced bronchoconstriction (HIB) and airway inflammation in asthmatic individuals.** When taken in isolation, fish oil supplementation significantly attenuated HIB while vitamin C supplementation seemed to positively affect airway inflammation as measured by the fraction of exhaled nitric oxide (F_ENO). The combination treatment also significantly reduced HIB and may have affected F_ENO; however, the effects of the combination treatment were not greater than that of either treatment alone as hypothesized.
2. **Determine the effects of docosahexaenoic acid (DHA), a component of fish oil, on hyperpnea-induced bronchoconstriction (HIB) and airway inflammation in asthmatic individuals.** Although previous *in vitro* studies suggested that DHA can alleviate inflammation on its own and may be the more potent component of fish oil (52, 98), there were no significant changes in pulmonary function, airway inflammation, DHA metabolite concentrations, or levels of 8-isoprostane, a marker of oxidative stress, with DHA supplementation in the current *in vivo* study.
3. **Determine whether fish oil is associated with a reduction in the contractility of canine tracheal smooth muscle.** A series of experiments tested the impact of chronic fish oil incubation and acute fish oil administration on canine tracheal smooth muscle contractility. Following 15 hours of incubation, tissue treated with fish oil generated a greater maximum force in response to acetylcholine than tissue treated with vehicle. However, significant relaxation of smooth muscle strips contracted with 10^{-6} M acetylcholine or 10^{-6} M 5-hydroxytryptamine was observed following the acute administration of fish oil as compared to smooth muscle strips acutely receiving vehicle or control treatments.

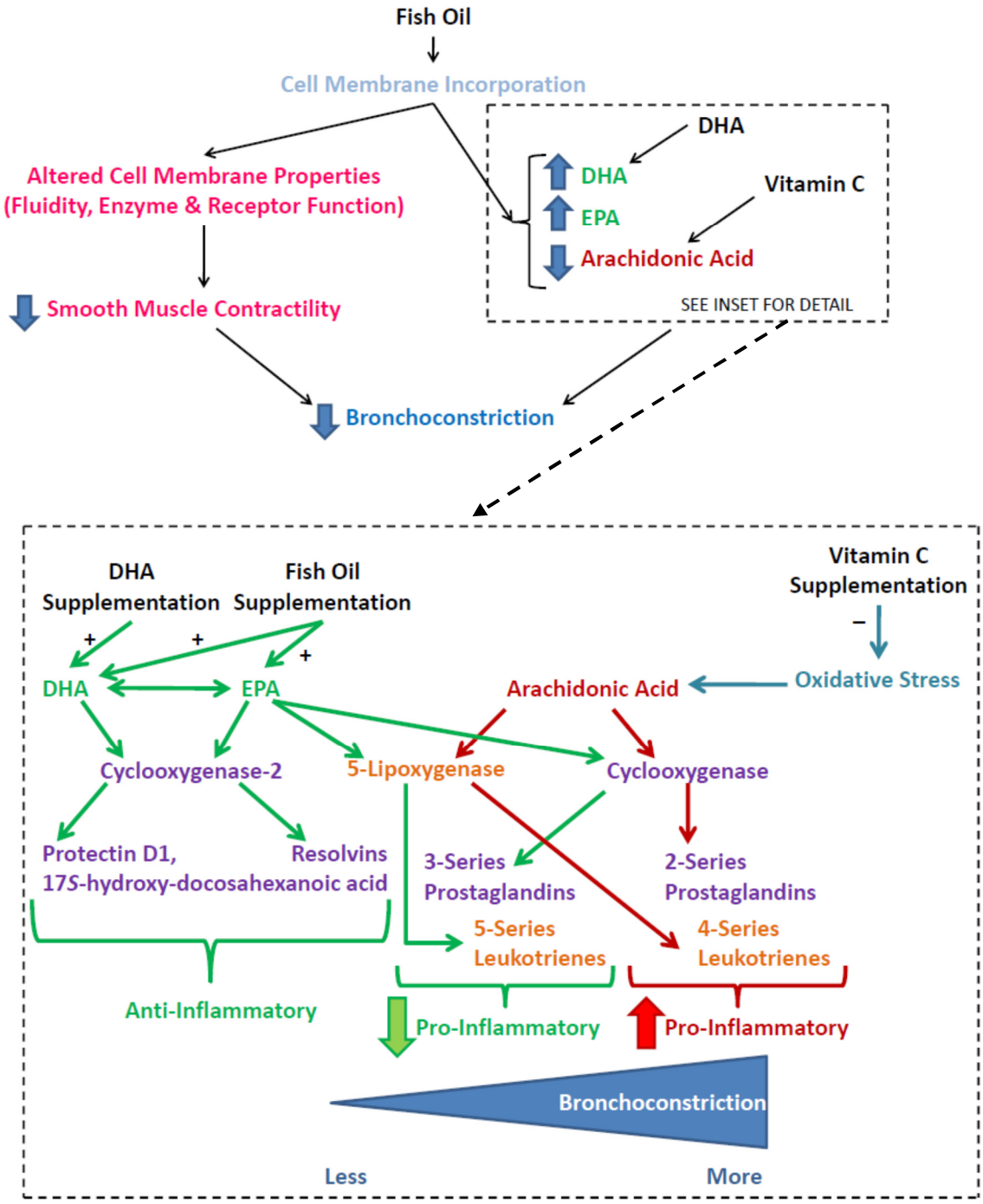

Figure 5-1. ***Proposed mechanism of how omega-3 fatty acids reduce airway inflammation and constriction in hyperpnea-induced bronchoconstriction.*** *The results from these experiments confirmed that fish oil reduces hyperpnea-induced bronchoconstriction and suggest that fish oil may influence smooth muscle contractility. However, the action of vitamin C, and thus an additive effect with the combination treatment, may have been masked by the inclusion of subjects who did not respond to vitamin C supplementation.*

Clinical Implications

The research presented here has important clinical implications. First and foremost, it is clear from the *in vivo* studies that the fish oil formula in supplements is an essential consideration. We have confirmed that fish oil containing 3.2 g EPA and 2.0 g DHA is effective in attenuating hyperpnea-induced bronchoconstriction while fish oil containing 4.0 g DHA is not. Therefore, to continue to move the field of research on the clinical benefits of omega-3 fatty acid supplementation forward, it is imperative that the optimal fish oil formula be determined and subsequently standardized. This is a vital step in helping patients reap the full advantages of fish oil, whether they are trying to treat pulmonary, cardiovascular, or chronic inflammatory conditions, because differences in the supplements' contents may affect digestion or absorption and thus alter their effects (70). Commercial fish oil can contain omega-3 fatty acids in different synthetic compounds such as ethyl esters that may be absorbed differently than their natural triglyceride form found in fish (5). Ethyl ester derivatives of fish oil have been shown to have reduced bioavailability compared to natural fish oil (28). Importantly, the supplements provided by Nordic Naturals (Watsonville, CA) and Martek Biosciences Corporation (Columbia, MD) for these studies did not contain ethyl esters; instead, they contained re-esterified triglycerides, which have been shown to yield greater omega-3 fatty acid incorporation than ethyl ester forms of fish oil (72). In fact, one study demonstrated that the bioavailability of re-esterified triglycerides was greater than that of natural fish oil (28).

The lack of any significant effect of DHA supplementation on pulmonary function, airway inflammation, or the levels of the metabolites tested indicates that DHA did not attenuate HIB or airway inflammation in asthmatic subjects. Therefore, it appears that DHA is not a potent supplement on its own, and it suggests that either EPA is the more

effective component of fish oil or that the combination of EPA and DHA is necessary to improve HIB.

Furthermore, the *in vivo* study on the combination of nutritional supplements signifies that similar to prescription asthma medications, nutritional strategies may not be equally effective for all patients. Despite the encouraging results for vitamin C supplementation reducing exercise–induced bronchoconstriction reported by Tecklenberg et al. (94), the current study did not demonstrate a significant improvement in HIB following vitamin C supplementation. However, four individuals within the group of seven subjects receiving only vitamin C for the first supplementation period did demonstrate improvements in their post-challenge pulmonary function at the laboratory test. This suggests that vitamin C is an effective supplement in a subset of the asthma population, which supports an earlier finding of Cohen et al. (22) that only nine of twenty asthmatic subjects exhibited improved post-exercise pulmonary function following a single 2000 mg dose of vitamin C compared to placebo. Moreover, if vitamin C supplementation is only effective in a specific subset of asthmatics, it would help explain the variable results described in a meta-analysis of the literature pertaining to randomized, placebo-controlled studies on treating asthma with vitamin C (46); the studies examined also demonstrated incongruent protocols that may have added to their differences.

Unfortunately, the *in vivo* study on the combination of nutritional supplements did not demonstrate an additive effect for fish oil and vitamin C supplementation as hypothesized. Because of the noted overall lack of significant improvements with vitamin C in this study, the potential benefits of this combination treatment in a more homogenous, vitamin C-responsive asthmatic population should not be ruled out. Furthermore, the possibility that other combinations of nutritional supplements may be

effective should continue to be considered as the underlying principles advocating this strategy remain.

Because the basis of fish oil's *in vivo* effects are still being elucidated, the *in vitro* study on canine airway smooth muscle has important clinical implications as well. The experiments using an acute administration of fish oil demonstrated that fish oil has a significant relaxing effect on tracheal smooth muscle strips that were contracted with 10^{-6} M acetylcholine or 10^{-6} M 5-hydroxytryptamine. These statistically significant changes in the smooth muscle contraction of the large airways may be clinically significant if similar changes occur at the level of the small airways because a minor percent relaxation as exhibited here experimentally would be magnified. Additionally, the chronic exposure to fish oil experiments and lipid analysis suggest that the mechanism by which fish oil supplementation affects airway smooth muscle responsiveness does not require the incorporation of omega-3 fatty acids; however, it is possible that these results were confounded by the oxidation of endogenous lipids during airway smooth muscle contraction experiments. Although lipid incorporation of EPA and DHA was shown to be significant following 24 hours of incubation in fish oil medium, there was no associated change in the airway smooth muscle contractility at this time point. Furthermore, there was a significant difference in the responsiveness to acetylcholine between tissues incubated for 15 hours in fish oil and those incubated in control with vehicle media despite no concurrent significant changes in the EPA and DHA composition according to the subsequent lipid analysis. Surprisingly, these fish oil-treated tissues displayed a greater contractility during the course of the dose response experiment as compared to the control with vehicle-treated tissues. Even though this was contrary to the hypothesis for this aim, these results may simply indicate that the fish oil treatment maintained the physiologic condition of the tissue samples better than the control with vehicle treatment. Because there was a significant difference between 15 hours of incubation in fish oil and

control with vehicle media but not between 15 hours of incubation in soybean oil and control with vehicle media, it suggests that the omega-3 fatty acids EPA and DHA, which are absent from soybean oil, have an important role in airway smooth muscle responsiveness.

Future Directions and Proposed Studies

Additional research to further examine asthma management through nutritional means is necessary. As dietary supplements are widely used among asthmatics (54), it is important to formally test their efficacy to educate physicians and patients on their proper use. This should lead to improved safety in terms of understanding potential side effects. Moreover, it is critical to distinguish effective strategies from unsubstantiated claims; otherwise, patients may have the false impression that their asthma is being properly managed. Similarly, although nutritional modifications have the potential to reduce spending on prescription drugs for asthma, it would be just as imprudent for patients to spend money on supplements that are not supported by sound research.

Future studies on nutritional supplements for treating asthma should strive to encompass both basic science and clinical trials. Basic science will be vital to explain the effectiveness of current nutritional strategies as well as propose new approaches. However, large-scale clinical trials will be of the utmost importance. Even though basic science research can suggest possible treatments, it is the results from the practical application of these suggestions that ultimately matter. For example, an understanding of the negative effects reactive oxygen species (ROS) can have on the airways along with associated findings that ROS production may be increased and antioxidant defenses diminished in asthma supports the use of antioxidant supplementation in asthmatics. Yet, the findings from clinical trials employing this strategy are inconclusive (46). Nevertheless, these negative results can help structure future research at the bench and bedside.

With this in mind, future studies should address some of the negative findings of the current studies. Research assessing vitamin C supplementation, whether alone or in combination with other nutrients or medications, should plan to screen subjects for vitamin C responsiveness. Regarding DHA supplementation, an increase in dosage, a change in the method of administration, or an extended time course may yield more positive results. Lastly, alterations in the protocol for the canine airway smooth muscle study involving fish oil exposure should be undertaken. To determine whether the omega-3 fatty acids were incorporated during incubation in fish oil and subsequently metabolized during contraction experiments, lipid analysis should be performed on tissue that has only been incubated in the particular treatment as well as on tissue that was similarly incubated but also used in contraction experiments. Moreover, treating the tissue with interleukin-13 would induce hyperresponsiveness to better imitate the airway smooth muscle conditions in asthma. In addition, the role of airway epithelium in the smooth muscle's response to fish oil should be addressed given the importance of the epithelium in airway reactivity (15, 48) and the endothelium-dependent findings for vascular smooth muscle treated with fish oil (101).

Additionally, some important issues stemming from these studies remain unresolved. Determining the optimal formula for fish oil supplementation is essential at this juncture. In order to better compare and build upon the data from different studies on omega-3 fatty acid supplementation, a standard fish oil formula will be needed. With the negative results obtained for the DHA supplementation and HIB study, it may be prudent to begin with a similar study involving EPA supplementation. Should EPA be effective in reducing HIB in subjects with asthma, it could be concluded that it is the more potent component of fish oil and the standardization of the optimal formula could proceed from there. Moreover, experiments involving the metabolites of the omega-3 fatty acids may be a prudent course of action. Morin et al. (69) showed that an EPA

metabolite relaxed human bronchial smooth muscle tissue *in vitro* while Levy et al. (52) showed that a DHA metabolite reduced methacholine-induced bronchoconstriction in mice *in vivo*. Other combinations of two or more nutritional supplements should also be investigated. Biltagi et al. (17) demonstrated that a combination of fish oil, ascorbic acid, and zinc, which is a cofactor in prostaglandin synthesis, was more effective than any one supplement alone in treating children with moderately persistent asthma. Finally, whether the benefits of nutrition are better achieved through supplementation or dietary modification is of interest. Several articles have reviewed the existing literature on epidemiologic studies examining nutrient intake and respiratory health (25, 34, 56, 79); the results generally indicate that a healthy diet is related to better pulmonary function and symptoms. Furthermore, it has been pointed out that it was the low rate of cardiovascular disease among populations who regularly consume fish that spurred the fish oil supplementation trend (71). Although subsequent research has demonstrated the benefits of supplementation, it may be that dietary modification is more efficacious.

Concluding Remarks

The central goal of these studies was to investigate the effects of omega-3 polyunsaturated fatty acid-based nutritional strategies on hyperpnea-induced bronchoconstriction (HIB) in asthmatic individuals and on isolated canine airway smooth muscle's responsiveness to contractile agonists. Results from *in vivo* studies demonstrated that fish oil containing 3.2 g EPA and 2.0 g DHA improved post-eucapnic voluntary hyperventilation (EVH) pulmonary function although there was no further significant improvement from adding vitamin C to the regimen; however, subjects who were unresponsive to vitamin C supplementation may have obscured the potential additive effect of the combination treatment. Supplementation with fish oil containing 4.0 g DHA did not improve the post-EVH pulmonary function of asthmatics with HIB indicating that DHA is not the most effective element in fish oil. *In vitro* experiments

demonstrated that lipid incorporation of omega-3 fatty acids may not be necessary for fish oil to affect airway smooth muscle responsiveness. With acute exposure to fish oil, the airway smooth muscle tissue exhibited significant relaxation of agonist-induced contractions. Overall, these studies have confirmed that fish oil represents a viable treatment modality for asthmatic individuals with HIB and suggest that fish oil may influence airway smooth muscle contractility.

APPENDIX A

INSTITUTIONAL REVIEW BOARD DOCUMENTS FOR CHAPTER 2

INDIANA UNIVERSITY BLOOMINGTON INSTITUTIONAL REVIEW BOARD (IRB) REVIEW

DOCUMENTATION OF REVIEW AND APPROVAL (DRA)

IRB STUDY NUMBER: 0910000751
(IRB Office will assign)

SECTION I: INVESTIGATOR INFORMATION

Principal Investigator: Head, Sally K **Department:** HPER/Kinesiology
(Last, First, Middle Initial — must have faculty/staff status or faculty sponsor must sign)

Building/Room No.: HPER 112 Phone: 812-855-4632 E-Mail: skhead@indiana.edu

Contact Information:

Name: Dr. Timothy Mickleborough Address: HPER 112 Phone: 812-855-0753

Fax: 812-855-3193 E-Mail: tmickleb@indiana.edu

STUDENT PROTOCOLS ONLY: Name of the Student: Sally Head Phone: **812-855-4632**

E-Mail: **skhead@indiana.edu**

Protocol Title: **Comparative Effects of Fish Oil and Ascorbic Acid Supplementation on Exercise-Induced Bronchoconstriction and Airway Inflammation in Asthma**

Sponsor/Funding Agency: N/A PI on Grant:

Sponsor Protocol #/Grant #: Project Duration: From: 11/09 – 11/10

Sponsor Type: ☐ Federal; ☐ State; ☐ Industry*; ☐ Not-for-Profit; ☒ Unfunded; ☐ Internally Funded

Grant Title (if different from project title):

SECTION II: TYPE OF REVIEW

☐ Expedited Review
☒ Full Board Review

SECTION III: SPECIAL SUBJECT POPULATIONS INVOLVED IN THE RESEARCH

☐ Children
☐ Cognitively Impaired
☐ Economically/ Educationally Disadvantaged
☐ Human Fetuses (or Fetal Tissue) or Neonates
☐ Pregnant Women
☐ Prisoners

SECTION IV: DOCUMENTS INCLUDED WITH RESEARCH SUBMISSION

☒ Informed Consent Document(s), dated:
# of consent document(s):
☒ Summary Safeguard Statement (SSS), dated:
☐ Authorization(s), dated:
☒ Protocol, dated:
☐ Other, description:

☐ Assent Document(s), dated:
# of assent document(s):
☒ Recruitment Materials, dated:
☒ Advertisement(s), dated:
☒ Surveys, Questionnaires, dated:

You only need to list document dates if they are required by the investigator or sponsor.

SECTION V: INVESTIGATOR STATEMENT OF COMPLIANCE

By submitting this form, I assure the Board that all procedures performed under the project will be conducted in strict accordance with those federal regulations, Indiana University policies that govern research involving human subjects. I acknowledge that I have the resources required to conduct research in a way that will protect the rights and welfare of participants. I agree to submit *any* deviation from the project (e.g. change in principal investigator, research methodology, subject recruitment procedures, etc.) to the Board in the form of an amendment for IRB approval prior to implementation.

Signature of Investigator: received by email Date: October 26, 2009

SECTION VI: IRB APPROVAL

This research project, including all documents included with the submission (e.g., informed consent statement, authorization, and/or waiver of authorization) has been reviewed and approved by the Indiana University Bloomington Institutional Review Board for a maximum of a one year period beyond the final approval date unless otherwise indicated as follows:

Authorized IRB Signature: IRB Approval Date: 12/14/2009

INDIANA UNIVERSITY BLOOMINGTON INSTITUTIONAL REVIEW BOARD (IRB) REVIEW

CONTINUING REVIEW

STATUS: ONGOING – OPEN TO ENROLLMENT

IRB Study No. 0910000751

SECTION I: INVESTIGATOR INFORMATION

Principal Investigator: Head, Sally, K **Department:** HPER/Kinesiology
(Last, First, Middle Initial)
Building/Room No.: HPER 112 Phone: 812-855-4632 E-Mail: skhead@indiana.edu

Faculty Sponsor: Mickleborough, Timothy, D **Department:** HPER/Kinesiology
(Last, First, Middle Initial)
Building/Room No.: HPER 112 Phone: 812-855-3193 E-Mail: tmickleb@indiana.edu

Project Title: **Comparative Effects of Fish Oil and Ascorbic Acid Supplementation on Exercise-Induced Bronchoconstriction and Airway Inflammation in Asthma**

Sponsor/Funding Agency: N/A

SECTION II: CURRENT STUDY STATUS

☒ **ONGOING – OPEN TO ENROLLMENT**
Date study was initiated: _____
Projected date of completion: _____

(Select one below)
☐ Enrollment of new participants or review of records/specimens continues
☐ No participants have been enrolled to date (Skip Sections III and IV)

☐ **Please check here if the study is currently suspended (temporarily) and indicate the reason(s) for the suspension:**
_____

SECTION III: SUBJECT SUMMARY

☐ Check here if your study utilizes records or specimens versus interaction with human subjects. When the form asks for the number of subjects, document the number of records/specimens that have been reviewed or collected.

1. SUBJECT SUMMARY TABLE

		On-Site
Since last IRB review	Total number of subjects **CONSENTED**	33
	Total number of subjects who **FAILED SCREENING** (e.g. found ineligible to participate)	9
	Total number of subjects who have **WITHDRAWN** from the study	10
Since beginning of study	Total number of subjects **CONSENTED**	33
	Total number of subjects who **FAILED SCREENING** (e.g. found ineligible to participate)	9
	Total number of subjects who have **WITHDRAWN** from the study	10
Number of **ACTIVE** subjects		7
Number of subjects who have **COMPLETED** the study		7

CONTINUING REVIEW

STATUS: ONGOING – OPEN TO ENROLLMENT

If necessary, please provide further explanation regarding the subject summary: _____

2. **WITHDRAWAL.**

If any subjects have withdrawn from the study since the last IRB review, please state the reasons: Nine subjects cited personal reasons for withdrawing. Their explanations included that they were too busy to continue in the study, had to go out of town, had a cold and no longer wanted to participate, or did not comply with the study requirements. One subject was unreachable by phone or email following the familiarization session when he signed the informed consent.

3. **JUSTIFICATION FOR STUDY CONTINUATION**

Have subjects accrued in the study since the last IRB review?

☒ Yes

☐ No, justify study continuation: _____

4. **Vulnerable Populations.** Are any of the subjects who have consented or enrolled in the study members of a vulnerable population **which have not previously been approved for enrollment by the IRB**? This includes children, pregnant women and human fetuses, prisoners, cognitively impaired individuals, and students.

☒ No

☐ Yes. Please indicate which population(s) have consented or enrolled:

☐ Children ☐ Pregnant Women and Human Fetuses

☐ Prisoners ☐ Economically/Educationally Disadvantaged

☐ Cognitively Impaired ☐ Students

Please note that you must submit an amendment to the IRB to request the inclusion of these subjects.

5. **For studies employing waivers of assent:**

a. State the number of assent waivers that were employed since the last IRB review: _____

b. Explain the circumstances surrounding each assent waiver employed: _____

SECTION IV: ETHNIC/RACIAL REPORTING REQUIRED FOR FEDERALLY-SPONSORED STUDIES

SUBJECT ACCRUAL

Ethnic Category	**Sex/Gender**			**Total**
	Females	**Males**	**Unknown or Not Reported**	
Hispanic or Latino				
Not Hispanic or Latino				
Unknown (Individuals Not Reporting Ethnicity)	17	16		33
Ethnic Category Total of All Subjects*				
Racial Categories				
American Indian/Alaska Native				
Asian				
Native Hawaiian or Other Pacific Islander				
Black or African American				
White				
More Than One Race				
Unknown or Not Reported	17	16		33
Racial Categories Total of All Subjects*	**17**	**16**		**33**

If ETHNIC and RACIAL category totals are not equal, please explain: _____

INDIANA UNIVERSITY BLOOMINGTON INSTITUTIONAL REVIEW BOARD (IRB) REVIEW

CONTINUING REVIEW

STATUS: ONGOING – OPEN TO ENROLLMENT

Have there been any unexpected problems recruiting participants, especially subjects in a particular category (including children and women)?

☒ No.
☐ Yes. Please explain: _____

SECTION V: SUMMARY OF EVENTS

V.A. **Since the last IRB review**, did any unanticipated problems, including adverse events, protocol deviations, or subject complaints, or noncompliance occur that required prompt reporting to the IRB?

☒ No.
☐ Yes. Were these events reported previously to the IRB, if applicable?
  ☐ No. Please explain why these events were not previously reported: _____
  ☐ Yes. Please attach a **summary** of these events.

V.B. **Since the last IRB review**, did any unanticipated problems, including adverse events, protocol deviations, or subject complaints, or noncompliance occur that did **not** require prompt reporting to the IRB?

☒ No.
☐ Yes. Please attach a **summary** of these events..

V.C. Is there a Data Safety Monitoring Board for this study?

☐ No.
☒ Yes. Provide the most recent monitoring report if it has not already been provided to the IRB or explain why one cannot be provided: **This continuing review serves as the monitoring report.**

V.D. Based on the above information, do you feel the validity of the data is affected?

☒ No.
☐ Yes. Explain:

V.E. Based on the above information, do you feel there is an increase in risk to subjects or others or in the frequency or severity of adverse events, protocol deviations, problems, complaints, etc. since the last IRB review?

☒ No.
☐ Yes. Explain: _____

SECTION VI: SUMMARY

VI.A. Describe the progress of the research, including any preliminary observations and information about study results or trends: Seven subjects have completed the protocol and seven more subjects are currently active. Final data analysis will not be available until all subjects have completed the study; however, thus far, it appears that fish oil alone and the combination of fish oil and vitamin C consistently improve pulmonary function above baseline. Improvement in pulmonary function with vitamin C alone was observed in 2 of the 5 subjects receiving this treatment; there was not a significant change in pulmonary function in the other 3 subjects.

If no progress description is provided, please explain why: _____

VI.B. Have subjects experienced any **direct** benefit(s) from their participation in the study?

☐ No.
☒ Yes.
Please explain: All seven subjects who have completed the study experienced improved pulmonary function after the surrogate exercise challege following supplementation with the combination of fish oil and vitamin C.

VI.C. Has any recent literature related to this research study been published or presented since the last IRB review?

☒ No.
☐ Yes. Please attach a copy or explain why one cannot be provided: _____

INDIANA UNIVERSITY BLOOMINGTON INSTITUTIONAL REVIEW BOARD (IRB) REVIEW

CONTINUING REVIEW

STATUS: ONGOING – OPEN TO ENROLLMENT

VI.D. Have there been any audits from federal agencies conducted since the last IRB review that identified unanticipated problems involving risks to subjects or others or noncompliance?

☒ No.
☐ Yes. Attach the report(s).

VI.E. Do you believe the balance of risks and benefits presented to the subjects has changed based on all of the information provided on this form and any attachments?

☒ No.
☐ Yes. Explain:_____

SECTION VII: REQUIRED ATTACHMENTS

All of the following documents must be included with your continuing review submission. Please check the appropriate boxes as they apply to your study.

☒ Continuing review form*
☒ Summary safeguard statement (SSS) (must be document version date of 06/05 or later)
☐ Recruitment checklist, if your study is subject to HIPAA and your study documentation includes a recruitment checklist
☒ Informed consent document(s), unless the IRB previously approved a waiver of consent
# of consent documents: 1
☐ Check here if a waiver of assent was approved by the IRB
☐ Assent document(s), if your study is enrolling children or cognitively impaired individuals and the IRB previously approved an assent document
# of assent documents: _____
☐ Check here if a waiver of assent was approved by the IRB
☐ Authorization(s), if your study is subject to HIPAA and the IRB previously approved an authorization
# of authorizations: _____
☐ Check here if a waiver of authorization was approved by the IRB
☒ Advertisement(s), if the IRB previously approved an advertisement(s) for the study
# of advertisements: 1
☒ Protocol
☒ Other, description: Scripts for email and phone responses to potential subjects

Include the following documents, as applicable:

☐ Publications, if you answered YES to VI.C. above
☐ Audit reports, if you answered YES to VI.D above
☐ Summaries, if you indicated in Section V that summaries are attached
☐ DSMB report, if the study includes a DSMB and you are submitting the most recent DSMB report
☐ Interim findings, if there are any to report
☐ Multi-center trial reports, if there are any available

NOTES:

- No changes to previously approved study documents are allowed at the time of continuing review unless requested by the IRB.
- Incomplete submissions will result in a processing delay, which could result in study expiration.

Your submission of this form certifies that this study has been and will continue to be conducted in full compliance with the IRB-approved protocol, HHS/FDA regulations and the IUB policies governing human subject research. You also certify that the information contained on or with this form is accurate.

INDIANA UNIVERSITY BLOOMINGTON INSTITUTIONAL REVIEW BOARD (IRB) REVIEW

CONTINUING REVIEW

STATUS: ONGOING – OPEN TO ENROLLMENT

Signature of Principal Investigator:_____ Date:_____

SECTION VII: IRB APPROVAL

***** For Office Use Only *****

Type of review: ☑ Full Board

☐ Expedited, Category:_____

IRB Reviewer:

☑ Check here to confirm that the most recent informed consent statement has been reviewed and no additional information needs to be provided to subjects based on any new findings.

STATUS OF STUDY: ONGOING, Open to Enrollment

This continuing review has been reviewed and approved as meeting the criteria for IRB approval as outlined in 45 CFR 46.111(a) by the IU Bloomington IRB. Based on the criteria for determining the frequency of continuing review and the level of risk, this study will expire on: 11/18/2011. If the study is not re-approved prior to that date all research activities must cease on that date, including enrollment of new subjects, intervention/interaction with current participants, and analysis of identified data.

Authorized IRB Signature:_____ IRB Approval Date: 12/4/10

INDIANA UNIVERSITY BLOOMINGTON

INFORMED CONSENT STATEMENT

Comparative Effects of Fish Oil and Ascorbic Acid Supplementation on Exercise-Induced Bronchoconstriction and Airway Inflammation in Asthma

You are invited to participate in a research study of the effect of dietary supplementation on asthma and exercise-induced bronchoconstriction (EIB). You were selected as a possible subject because you identified yourself as having asthma. We ask that you read this form and ask any questions you may have before agreeing to be in the study.

The study is being conducted by Sally Head, an MD/PhD candidate in the Department of Cellular and Integrative Physiology at the Indiana University School of Medicine. It is not funded by any agency and is being conducted under the supervision of Dr. Timothy Mickleborough in the Department of Kinesiology at Indiana University Bloomington.

STUDY PURPOSE

The purpose of this study is to determine the roles of two different dietary supplements in alleviating signs and symptoms of exercise-induced bronchconstriction (EIB). Fish oil and ascorbic acid (vitamin C) have each been shown to have positive effects on lung function and inflammation related to EIB. Our goal is to compare these nutritional interventions as well as to determine if the combination of supplements is better than either alone.

NUMBER OF PEOPLE TAKING PART IN THE STUDY:

If you agree to participate, you will be one of thirty subjects with EIB who will be participating in this research.

PROCEDURES FOR THE STUDY:

If you agree to be in the study, you will do the following things:

You will be asked to volunteer a total of about 7 ½ hours of your time over 10-12 weeks, plus an additional 5 minutes per day throughout the study. You will need to come to the laboratory a total of 4 times. The first visit is simply a familiarization session lasting about 30 min to introduce you to the study and allow you to ask questions. If you decide to participate in the study, you will be given materials regarding the study and asked to complete some at-home logs and peak flow tests at home (about 5 min/day) prior to the supplementation part of the study. You will be given a log book to record your daily medication use and a diary to record your daily symptoms during the course of the study. Each week throughout the study, the primary investigator will email you to check-in with you about your asthma symptoms, peak flows, and completing your at-home logs.

The other 3 visits will require about 1 hour each and you will be asked to do some lung function tests and a simulated exercise test involving only heavy breathing. You will be asked to

abstain from exercise for at least 24 hours before coming to the laboratory, not to drink caffeinated beverages 8 hours before the test, and not to take any inhaler medication in the 6 hours preceding the laboratory test. However, if you need to use your inhaler within the 6 hours prior to your scheduled test session, you should do so, and we will reschedule your session. If you are on other medications for your asthma you will be asked to refrain from using them for 2-4 weeks before the study with the permission of your doctor. You will be asked to limit your fish consumption to 1 fish meal per week throughout the course of the study. You will also be asked to avoid vitamin C-rich foods during the course of the study. You will be encouraged to drink 6-8 glasses of water per day during the course of the study. You will also receive 8 unannounced phone calls throughout the 10-12 week study to complete 24-hour dietary recall interviews, which will last about 30 minutes each.

Familiarization Session & Study Entry Procedures:

For the familiarization session, you will meet with the primary investigator in the exercise physiology labs in HPER (room 076) for approximately 30 minutes. The details of the study will be explained to you and you will have the opportunity to ask any questions that you may have. You will be asked to complete a questionnaire which asks about medications you are taking and whether you have allergies to the supplements to be given in the study. This questionnaire will take about 5 minutes to complete. If you are currently taking medications for your asthma, you will be asked to stop taking them before you start the study. Depending upon the type of medication you are taking, you will have to stop taking it 2-4 weeks before you start the study with permission from your doctor. You will be given a form that needs to be completed by your doctor and brought back to your first lab testing session. On this form, your physician will set limitations on your rescue inhaler use and peak flow measures such that if you exceed these values you will be removed from the study for your safety. If you are not currently taking any asthma maintenance medications, you will not need to have a doctor fill out the permission form. Dr. Greg Montgomery, a pulmonologist, will set the limitations on your rescue inhaler use and peak flow measures such that if you exceed these values you will be removed from the study for your safety. If you are taking a corticosteroid inhaler such as Advair®, Flovent ®, Azmacort®, Pulmicort®, or Beclovent®, you will need to stop taking it for 4 weeks before the study and during the study. If you are taking a leukotriene receptor antagonist or 5-lipoxygenase inhibitor such as Singulair®, Accolate®, Zyflo®, or Ultair®, you will be asked to stop taking it for 2 weeks before the study and during the study. If you are taking theophylline, Theo-Dur®, Uni-Dur®, Bronkodyl® , Elixophyllin® , Slo-bid®, Slo-Phyllin® , Theo-24® , Theolair®, or Uniphyl®, you will be asked to stop taking it 1 week before the study and during the study. You may use your rescue inhaler anytime during the course of the study. You will be asked not to use it 6 hours before any testing session in the lab, but may use it if you need to. If you do use your inhaler within 6 hours of a lab session, you will need to reschedule your test. If you do not drop in pulmonary function by at least 10%, you will not be eligible for the study.

You will be talked through the lab testing procedures for the simulated exercise test, lung function tests, exhaled nitric oxide tests, and exhaled breath condensate procedures, which will all occur on the three lab testing days.

You will also receive information on the tasks to be completed at home, including peak flow readings, recording rescue inhaler usage, recording daily symptoms, and dietary recall interviews. You will receive a peak flow meter to take with you. Instructions for its use will be provided to you and reviewed. You will receive a log book to record your peak flow (the fastest speed a person can blow air out of their lungs), FEV_1 (the amount of air blown out in the first second of a forced exhalation), and rescue inhaler use every day for the duration of the study. You will also receive a daily symptom diary containing 4 questions about daytime symptoms and 1 question about nighttime symptoms that is to be completed every day throughout the study. The 24-hour dietary recall interview procedure will be explained to you. Between the familiarization session and first lab testing day, you will receive 2 unannounced phone calls from a professional trained to interview you about everything you have had to eat and drink over the previous 24-hour period. These dietary

recall interviews will last about 30 minutes each.

You will not receive any supplements at this time. If you qualify and choose to participate in the study, you will receive either active or placebo (inactive) ascorbic acid (vitamin C) and fish oil pills for two separate 3-week periods. During the supplementation periods, you will take 2ascorbic acid pills (active or placebo) and 10 fish oil pills (active or placebo) per day.

Lab Testing Sessions (approximately 1.5 hours each)

First Testing (following 2-4-week run-in period)

You will come to the lab in HPER 076. You should not have exercised for 24 hours before arriving and should not have had any caffeine for 8 hours before arriving. You will be asked to return your peak flow/rescue inhaler logs and daily symptom diaries from the run-in period (time between the familiarization session and first lab testing session) at this time. You will have your height and weight measured. You will perform an *exhaled nitric oxide test* that will measure the amount of nitric oxide in your exhaled breath, which indicates the presence of inflammation in the lungs. This procedure requires that you inhale to a full lung and then exhale immediately while wearing a nose clip. This will be followed by a *pulmonary function test* where you will have to wear a nose clip while you inhale and exhale maximally through a mouthpiece connected to a computerized instrument that measures lung volumes. If your FEV_1 (the amount of air blown out in the first second of a forced exhalation) is less than 60% of your predicted value, you will be excluded from the study. Next, you will do the *exhaled breath condensate procedure* where you will have the breath that you breathe out collected. You sill sit in a chair, wear a nose clip, and breathe through a mouthpiece for 10 minutes such that you are breathing in normal room air and the breath you breathe out will be collected in a tube on the other side of the mouthpiece. Following these pre-tests, a simulated exercise test called *eucapnic voluntary hyperventilation (EVH)* will be performed. For the EVH test, you will sit in a chair and breathe gases from a large bag through a mouthpiece while wearing a nose clip. The bag is supplied with gas from a tank with air that has the same amount of oxygen as regular room air, but the air will be drier and will contain 5% carbon dioxide to help you breathe at the levels you need to breathe and to keep you from fainting. You will be asked to breathe at a fast and deep rate like you would during exercise, but you will just be sitting in a chair. You will breathe this air at the high rate for 6 minutes. You may stop the test at any time if you become too uncomfortable. You may use your inhaler if necessary, but this may mean that we would need to re-test you if you still wish to participate in the study. This method is routinely used in laboratories for the diagnosis of exercise-induced asthma.

Immediately after the EVH test, you will do the *exhaled breath condensate procedure* for 5 minutes. A break will be taken for the 5 minute post-EVH *pulmonary function test*. You will then return to do the final 5 minutes of the *exhaled breath condensate procedure*. Additional *pulmonary function tests* will be performed at 10, 15, and 20 minutes post-EVH. If your FEV_1 (the amount of air blown out in the first second of a forced exhalation) drops by more than 10% of your pre-EVH value, you will qualify to continue in the study. You will perform a post-EVH *exhaled nitric oxide test*.

If you qualify and choose to continue in the study, you will be given a set fish oil pills (active or placebo) and ascorbic acid pills (active or placebo), a new peak flow/rescue inhaler log book, and a new daily symptom diary. You will be reminded of the instructions received during your familiarization session for taking the pills and filling out the peak flow/rescue inhaler log and daily symptom diary. You will also be reminded that you will receive two phone calls for 24-hour dietary recall interviews, lasting approximately 30 minutes each, during the 3-week supplementation period.

Second Testing (following 3-week supplementation period)

The procedures for the second lab testing session will be the same as at the first lab testing session. You will be asked to return your peak flow/rescue inhaler logs and daily symptom diaries from the first supplementation period as well as any leftover pills at this time.

You will have your weight measuredand perform pre- and post-EVH exhaled nitric oxide tests, EBC procedures, and pulmonary function tests as before. You will be given the pills for the second supplementation period but instructed to wait 2 weeks before taking this set of pills. The primary investigator will contact you by phone and/or email (whichever you prefer) to remind you when to begin taking the pills. This set of pills will include fish oil pills (active or placebo) and ascorbic acid pills (active or placebo). You will be given a new peak flow/rescue inhaler log book and a new daily symptom diary. You will be reminded to fill out the peak flow/rescue inhaler log and daily symptom diary throughout the washout (2 weeks) and second supplementation period (3 weeks). You will be informed that you will receive two phone calls for 24-hour dietary recall interviews during the 2-week washout period and two phone calls for 24-hour dietary recall interviews during the 3-week supplementation period. Each call will last approximately 30 minutes.

Third Testing (following a 2-week washout and a 3-week supplementation period)

The procedures for the third lab testing session will be the same as at the first and second lab testing sessions. You will be asked to return your peak flow meter, peak flow/rescue inhaler logs, daily symptom diaries, and any leftover pills at this time. You will have your weight measured and perform pre- and post-EVH exhaled nitric oxide tests, EBC procedures, and pulmonary function tests as before.

RISKS OF TAKING PART IN THE STUDY:

While on the study, the risks are:

1. The exercise simulation test, EVH, may induce bronchospasm (rapid narrowing of the airways).
2. Headache, transient light-headedness, or fainting may occur during the pulmonary function tests, nitric oxide procedure, or the exhaled breath procedure.
3. Contamination or infection from mouthpieces is unlikely.
4. There are no known risks of taking fish oil or ascorbic acid for subjects who are not allergic.

To minimize the risks listed above, the following measures will be employed:

1. Subjects who have severe and moderate asthma will be excluded from the study to avoid the greatest risk of bronchospasm. These subjects will be identified at the first lab testing session (when they have been off their medication for 2-4 weeks depending on the type of medication) by a resting FEV_1 (the amount of air blown out in the first second of a forced exhalation) less than 60% of their predicted value based on age, sex, height, and weight.
2. All subjects will be required to bring their bronchodilators (rescue inhalers) with them to all testing sessions.
3. If severe wheezing begins during EVH, the EVH test will be immediately stopped and the subject will be given their bronchodilator and oxygen if necessary.
4. The subject will be seated and carefully monitored during EVH, pulmonary function tests, the nitric oxide procedure, and the exhaled breath procedure.
5. To avoid the risk of infection, disposable mouthpieces are used for most testing procedures, and when rubber mouthpieces are used, they are cleansed in a detergent solution and disinfected following each use.6. Subjects who are allergic to fish oil or ascorbic acid will be excluded from the study.
7. Subjects will be instructed to report any adverse effects to the principal investigator.
8. Subjects will be instructed to discontinue the study procedures and return to their medications if they reach the rescue inhaler usage and peak flow measurement limitations set by their own physicians or Dr. Montogomery.

BENEFITS OF TAKING PART IN THE STUDY:

The benefits to participation that are reasonable to expect are a better understanding of how fish oil and ascorbic acid supplementation affect the signs and symptoms of exercise-induced bronchoconstriction. Subjects will have access to all their own data regarding their own personal lung function and markers of inflammation, which they can share with their personal physician.

ALTERNATIVES TO TAKING PART IN THE STUDY:

An alternative to participating in the study is to choose not to participate.

CONFIDENTIALITY

Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. Your identity will be held in confidence in reports in which the study may be published and in databases in which results may be stored.

Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the study investigator and his/her research associates, the IUB Institutional Review Board or its designees, the study sponsor, Dr. Timothy Mickleborough, and (as allowed by law) state or federal agencies, specifically the Office for Human Research Protections (OHRP) who may need to access your medical and/or research records.

COSTS

Taking part in this study will lead to no added costs to you.

PAYMENT

You will be paid $150 for taking part in this study. You will not receive any compensation for the 30 minute familiarization session where you will learn about the study, receive study materials, and fill out the informed consent, health questionnaire, and asthma medication questionnaire. You will receive $10 at the first actual testing session, $25 after the 2nd testing session, and $115 after the last testing session. You will be compensated for the completion of each session. If you withdraw from the study, you will be compensated for the completed tests.

COMPENSATION FOR INJURY

In the event of physical injury resulting from your participation in this research, necessary medical treatment will be provided to you and billed as part of your medical expenses. Costs not covered by your health care insurer will be your responsibility. Also, it is your responsibility to determine the extent of your health care coverage. There is no program in place for other monetary compensation for such injuries. However, you are not giving up any legal rights or benefits to which you are otherwise entitled.

CONTACTS FOR QUESTIONS OR PROBLEMS

For questions about the study or a research-related injury, contact the researcher Sally Head at 812-855-4632.

For questions about your rights as a research participant or to discuss problems, complaints or concerns about a research study, or to obtain information, or offer input, contact the IUB Human Subjects office, 530 E Kirkwood Ave, Carmichael Center, 203, Bloomington IN 47408, 812-856-4242 or by email at iub_hsc@indiana.edu

VOLUNTARY NATURE OF STUDY

Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. Your decision whether or not to participate in this study will not affect your current or future relations with the investigator(s).

SUBJECT'S CONSENT

In consideration of all of the above, I give my consent to participate in this research study.

I will be given a copy of this informed consent document to keep for my records. I agree to take part in this study.

Subject's Printed Name:_____

Subject's Signature:_____**Date**:_____
(must be dated by the subject)

Printed Name of Person Obtaining Consent:_____

Signature of Person Obtaining Consent:_____**Date**:_____

Form date: December 2, 2010

Email for Response to Potential Subjects

Thank you for your interest in our study. I have a few initial questions that you can either respond to by email or by calling me at 812-855-4632.

First, do you take any maintenance medications for asthma? For this study you are required to stop taking certain medications with your doctor's permission. If you take a corticosteroid, such as advair or flovent for example, you need to be off of it for 4 weeks before starting the study. If you take a leukotriene receptor antagonist, such as singulair or accolade for example, you need to be off it for 2 weeks before starting the study. If you take theophylline, you need to be off it for 1 week before starting the study. You can still take your rescue inhaler.

Second, do you have any other medical conditions such as heart disease, hypertension, or high cholesterol, or are you pregnant? Everyone with any of these conditions will not be able to take part in our study.

Third, do you currently take any nutritional supplements, including fish oil and vitamin C? This study requires that you stop taking fish oil and vitamin C. You would need to stop taking these supplements for 2 weeks before beginning the study until the study is over, for a total of 10-12 weeks.

Depending on your responses to these questions, you may meet the criteria to be a part of our study. Here is a little more information about it. We are doing a study to look at nutritional supplements for treating asthma, specifically fish oil and vitamin C. You would take two sets of pills for two 3 week periods and be tested at the beginning of the study and after each of the 3 weeks of pills. Some of the pills are placebo and you might be assigned to a group with a placebo. You will also do a simulated exercise test where you will breathe fast and deep like you would during exercise, but you will be seated in a chair. You will also have some lung function tests done before and after each simulated exercise test. You will also be asked to breathe into a tube for collection of your exhaled breath. You will be asked to provide urine and blood samples. You also need to do peak flow measurements at home during the study.

If you are still interested in participating, please let me know your response to the above questions. If you have any questions, please contact me at skhead@indiana.edu or 812-855-4632.

If you do meet the criteria for participation and are interested in participating, I will contact you about setting up an initial appointment for a half-hour familiarization session.

Thank you,
Sally Head
MD/PhD Student
Indiana University School of Medicine
skhead@indiana.edu

Medication & Allergy Questionnaire

1. What medications do you take on a daily basis for your asthma?

2. What medications do you take just before exercise for your asthma?

3. How many times a week on average do you use your rescue inhaler?

4. Do you take any of medications like the following (circle any that apply): Advair®, Flovent ®, Azmacort®, Pulmicort®, Beclovent® Singulair®, Accolate®, Zyflo®, Ultair®, Theo-Dur®, Uni-Dur®, Bronkodyl® , Elixophyllin® , Slo-bid®, Slo-Phyllin® , Theo-24® , Theolair®, or Uniphyl®

5. Are you allergic to ascorbic acid (vitamin C)?

6. Are you allergic to fish oil?

7. Do you have any other allergies, including seasonal allergies?

8. Have you ever been diagnosed as being atopic (hyperallergic)?

Subject #_____Week #_____

Peak Flow & Rescue Inhaler Log

Record your best of 3 tries for peak flow and FEV_1 both for morning and evening. Also, record the number of puffs you took of your rescue inhaler.

Date	*Peak Flow Morning*	*Peak Flow Evening*	*FEV_1 morning*	*FEV_1 evening*	*# of puffs from rescue inhaler (including pre-exercise)*

Subject #_____Week #_____

Exercise Log

Date	*Type of Exercise*	*Duration*	*Symptoms/ Comments*	*# of puffs of rescue inhaler immediately before exercise*	*# of puffs of rescue inhaler during or after exercise*

Daily Symptom Diary

Date:_____

Daytime symptom diary scale questions

1) How often did you experience asthma symptoms today?

0	1	2	3	4	5	6
None of the time						All of the time

2) How much did your asthma symptoms bother you today?

0	1	2	3	4	5	6
Not at all bothered						Severely bothered

3) How much activity could you do today?

0	1	2	3	4	5	6
More than usual activity						Less than usual activity

4) How often did your asthma affect your activities today?

0	1	2	3	4	5	6
None of the time						All of the time

Nocturnal diary scale question

1) Did you wake up with asthma symptoms (This can be awakening in the middle of the night or on awakening in the morning)?

❑ No ❑ Once ❑ More than once ❑ Awake "all night"

Santanello NC, Barber BL, Reiss TF, Friedman BS, Juniper EF, and Zhang J. Measurement characteristics of two asthma symptom diary scales for use in clinical trials. *Eur Respir J* 10: 646-651, 1997.

Nutrition Coordinating Center (NCC) Standard Introduction Script for Dietary Recalls

If you are conducting the 24-hour recall in person:

A. "Hello, my name is _____. Thank you for agreeing to participate in this study. During this part of your visit, I will be collecting the 24-hour dietary recall, where you tell me everything you had to eat and drink during the past 24 hours. Everything you tell me is confidential and this will take about 30 minutes, OK?"

B. "What we'll do first is make a list of the foods and beverages you had from 12 a.m. yesterday until 12 midnight last night. This includes all meals snacks, beverages, including tap water (and alcoholic beverages), as well as sampling of foods."

C. *If you are collecting supplements during the recall:*
"I'll also ask you about any vitamin, mineral, or other supplements that you may have taken."

D. "I'll ask you questions to help you remember what you ate yesterday, so the information about your diet can be used for *(per study protocol, insert this important research project or study.)*"

E. "I'll need you to tell me an approximate time you had each item. For example, "At 8 a.m. I had this, at 10 a.m. I had that." We'll make a general list at first and then we'll go back and fill it in with more detail. Finally, we'll go through the list once more to make sure we haven't missed anything. We can use these *(refer to amount estimation tools)* to estimate the amount of what you ate yesterday."
"Do you have any questions before we begin?"

F. *If the response is no, reply "OK".*

"Take a moment to think about yesterday, what you did, where you went and so forth. Thinking about the day can help you remember what you did yesterday and when you ate."
"Now let's begin."
Refer to the Quick List window: "After midnight, what was the first time you had something to eat or drink?"

If you are conducting the 24-hour recall by phone:

A. "Hello! My name is *use your first name only* and I am calling for *(per study protocol, insert name of study or institution)* to collect 24-hour dietary recalls. Is this a good time to talk?"

Hesitate and wait for a response.
If the response is "no", ask if you can call back later that day. If they tell you that yesterday was an atypical day (e.g. they were ill), tell them that you will call back on another day. If they sat, "Call tomorrow," explain that you can't make any appointments beyond today, as our calls need to be unannounced. Thank them for their time and tell them we'll try another day.
If the response is "yes", say:
"Thank you for agreeing to participate in this study. Before we begin, I'd like to explain that for the dietary recall, you'll tell me everything you had to eat and drink during the past 24-hours. Everything you tell me is confidential and this will take about 30 minutes, OK?"

Continue with the introduction script starting with section B.

The questions the interviewer will ask are in bold.
After the introduction a quick list of foods eaten the previous day is compiled. The list is assembled by asking the following questions:

After midnight, what was the first time you had something to eat or drink?
What did you have at that time?
Did you have anything else at that time?

These three questions are asked repeatedly, the first question then changes to "When was the next time you had something to eat or drink?"

Once the quick list is compiled it is reviewed with the interviewer asking:

At (time) you had (read all foods). Can you think of anything else you had at that time?
Did you have a beverage with that meal? (If a beverage is not listed.)
Did you have any snacks between means or did you sample food as you prepared for the meal?

Now we will fill in your list with more detail.

At 6:30 am you had breakfast at home. Is this correct?
You said you had coffee, did you add anything to the coffee? (for example milk added)
What type of coffee was it? The computer goes to a food hierarchy to select from the type of coffee; made from ground, made from instant, unknown preparation, from vending machine, dry instant-unprepared.
How much coffee did you drink?
What brand or type of milk was it? The computer goes to a food hierarchy to select from the type of milk; whole, 2% fat or reduced fat, 1 ½%, 1% fat or lowfat, ½% fat, skim, nonfat, or fat free, unknown % fat, reconstituted from dry, unprepared dry powder, buttermilk.
You also had a bagel for breakfast, what type of bagel was it? Again the computer goes through a food hierarchy to determine what kind of bagel was eaten.

This process of asking questions is repeated for all foods the interviewee can recall.
At the end of the interview, the interviewer will ask:
Now we will review the record. Tell me if I have missed anything.
The interviewer will read through the list of foods and amounts.
And concluded with:
Please tell me if the amount of intake was: Usual, considerably more than usual, or considerably less than usual.
Thank you for your time and help in our study.

Study # 0910000751

Do You Have Asthma?

Indiana University Research Study

Who: People who have asthma

What: This study is testing the effects of fish oil (omega-3 fatty acid) supplementation and vitamin C (ascorbic acid) supplementation on asthma. It will take place over 10-12 weeks with 4 total lab visits. The study requires:

- one 30min familiarization visit to the lab
- three 1 hour lab testing visits
- eight 30min dietary recall phone interviews
- daily at home peak flow measurements (2-5 min/day) for 10-12 weeks

You may receive supplements. Participation will involve a simulated exercise test, lung function tests, and exhaled breath test.

When: Whenever your schedule permits

Benefits: You will receive all data and information on how fish oil and vitamin C supplementation can affect your asthma.

Compensation: $150

Who to contact if interested:

Sally Head
073 HPER
855-4632
skhead@indiana.edu

APPENDIX B

INSTITUTIONAL REVIEW BOARD DOCUMENTS FOR CHAPTER 3

INDIANA UNIVERSITY BLOOMINGTON INSTITUTIONAL REVIEW BOARD (IRB) REVIEW

DOCUMENTATION OF REVIEW AND APPROVAL (DRA)

IRB STUDY NUMBER: 1005001346
(IRB Office will assign)

SECTION I: INVESTIGATOR INFORMATION

Principal Investigator: Head, Sally K **Department:** HPER/Kinesiology
(Last, First, Middle Initial — must have faculty/staff status or faculty sponsor must sign)

Building/Room No.: HPER 112 Phone: 812-855-4632 E-Mail: skhead@indiana.edu

Contact Information:

Name: Dr. Timothy Mickleborough Address: HPER 112 Phone: 812-855-0753

Fax: 812-855-3193 E-Mail: tmickleb@indiana.edu

STUDENT PROTOCOLS ONLY: Name of the Student: Sally Head Phone: 812-855-4632

E-Mail: skhead@indiana.edu

Protocol Title: **Docosahexaenoic Acid (DHA) as a Nutritional Treatment for Exercise-Induced Bronchoconstriction and Airway Inflammation in Asthma**

Sponsor/Funding Agency: Department of Kinesiology's AAU/Bell-Updyke-Willett Research Fund PI on Grant: Sally Head

Sponsor Protocol #/Grant #: Project Duration: From: 06/10 – 06/11

Sponsor Type: ☐ Federal; ☐ State; ☐ Industry*; ☐ Not-for-Profit; ☐ Unfunded; ☒ Internally Funded

Grant Title (if different from project title):

SECTION II: TYPE OF REVIEW

☐ Expedited Review
☒ Full Board Review

SECTION III: SPECIAL SUBJECT POPULATIONS INVOLVED IN THE RESEARCH

☐ Children
☐ Cognitively Impaired
☐ Economically/ Educationally Disadvantaged
☐ Human Fetuses (or Fetal Tissue) or Neonates
☐ Pregnant Women
☐ Prisoners

SECTION IV: DOCUMENTS INCLUDED WITH RESEARCH SUBMISSION

☒ Informed Consent Document(s), dated:
# of consent document(s):
☒ Summary Safeguard Statement (SSS), dated:
☐ Authorization(s), dated:
☒ Protocol, dated:
☐ Other, description:
☐ Assent Document(s), dated:
# of assent document(s):
☒ Recruitment Materials, dated:
☒ Advertisement(s), dated:
☒ Surveys, Questionnaires, dated:

You only need to list document dates if they are required by the investigator or sponsor.

SECTION V: INVESTIGATOR STATEMENT OF COMPLIANCE

By submitting this form, I assure the Board that all procedures performed under the project will be conducted in strict accordance with those federal regulations, Indiana University policies that govern research involving human subjects. I acknowledge that I have the resources required to conduct research in a way that will protect the rights and welfare of participants. I agree to submit any deviation from the project (e.g. change in principal investigator, research methodology, subject recruitment procedures, etc.) to the Board in the form of an amendment for IRB approval prior to implementation.

Signature of Investigator: received by email Date: May 12, 2010

SECTION VI: IRB APPROVAL

This research project, including all documents included with the submission (e.g., informed consent statement, authorization, and/or waiver of authorization) has been reviewed and approved by the Indiana University Bloomington Institutional Review Board for a maximum of a one year period beyond the final approval date unless otherwise indicated as follows:

Authorized IRB Signature: IRB Approval Date: Jul. 16, 2010

INDIANA UNIVERSITY BLOOMINGTON

INFORMED CONSENT STATEMENT

Docosahexaenoic Acid (DHA) as a Nutritional Treatment for Exercise-Induced Bronchoconstriction and Airway Inflammation in Asthma

You are invited to participate in a research study of the effect of dietary supplementation on asthma and exercise-induced bronchoconstriction (EIB). You were selected as a possible subject because you identified yourself as having asthma. We ask that you read this form and ask any questions you may have before agreeing to be in the study.

The study is being conducted by Sally Head, an MD/PhD candidate in the Department of Cellular and Integrative Physiology at the Indiana University School of Medicine. It is funded by a grant received from the Department of Kinesiology's AAU/Bell-Updyke-Willett Research Fund and is being conducted under the supervision of Dr. Timothy Mickleborough in the Department of Kinesiology at Indiana University Bloomington.

STUDY PURPOSE

The purpose of this study is to determine the role of a specific component of fish oil, docosahexanoic acid (DHA), in alleviating signs and symptoms of exercise-induced bronchoconstriction (EIB). Fish oil has been shown to have positive effects on lung function and inflammation related to EIB; however, it is not known which component of fish oil is more potent. Our goal is to determine if the supplementation with DHA is better than placebo.

NUMBER OF PEOPLE TAKING PART IN THE STUDY:

If you agree to participate, you will be one of thirty-two subjects with EIB who will be participating in this research.

PROCEDURES FOR THE STUDY:

If you agree to be in the study, you will do the following things:

You will be asked to volunteer a total of about 5 ½ hours of your time over 10-12 weeks, plus an additional 5 minutes per day throughout the study. You will need to come to the laboratory a total of 4 times. The first visit is simply a familiarization session lasting about 30 min to introduce you to the study and allow you to ask questions. If you decide to participate in the study, you will be given materials regarding the study and asked to complete some at-home logs and peak flow tests at home (about 5 min/day) prior to the supplementation part of the study. You will be given a log book to record your daily medication use and a diary to record your daily symptoms during the course of the study. Each week throughout the study, the primary investigator will email you to check-in with you about your asthma symptoms, peak flows, and completing your at-home logs. If you are currently taking asthma maintenance medication, you

will be asked to obtain your physician's approval to stop for the duration of the study. You will be given a form to give to your physician to sign. This must be returned at the first lab testing session.

The other 3 visits will require about 1.5 hours each and you will be asked to do some lung function tests and a simulated exercise test involving only heavy breathing. You will also be asked to fill out a food frequency questionnaire about your eating habits during the preceding phase of the study. You will be asked to abstain from exercise for at least 24 hours before coming to the laboratory, not to drink caffeinated beverages 8 hours before the test, and not to take any inhaler medication in the 6 hours preceding the laboratory test. However, if you need to use your inhaler within the 6 hours prior to your scheduled test session, you should do so, and we will reschedule your session. If you are on other medications for your asthma you will be asked to refrain from using them for 2-4 weeks before the study with the permission of your doctor. You will be asked to limit your fish consumption to 1 fish meal per week throughout the course of the study.

Familiarization Session & Study Entry Procedures:

For the familiarization session, you will meet with the primary investigator in the exercise physiology labs in HPER (room 076) for approximately 30 minutes. The details of the study will be explained to you and you will have the opportunity to ask any questions that you may have. You will be asked to complete a questionnaire which asks about medications you are taking and whether you have allergies to the supplements to be given in the study. This questionnaire will take about 5 minutes to complete. If you are currently taking medications for your asthma, you will be asked to stop taking them before you start the study. Depending upon the type of medication you are taking, you will have to stop taking it 2-4 weeks before you start the study with permission from your doctor. If you need to stop taking asthma medication, you will be given a form that needs to be completed by your doctor and brought back to your first lab testing session. On this form, your physician will set limitations on your rescue inhaler use and peak flow measures such that if you exceed these values you will be removed from the study for your safety. If you are not currently taking any asthma maintenance medications, you will not need to have a doctor fill out the permission form. In this case, Dr. Greg Montgomery, a pulmonologist, will be sent information on your rescue inhaler use and peak flow measures. He will use this information to set the limitations on your rescue inhaler use and peak flow measures such that if you exceed these values you will be removed from the study for your safety. If you are taking a corticosteroid inhaler such as Advair®, Flovent ®, Azmacort®, Pulmicort®, or Beclovent®, you will need to stop taking it for 4 weeks before the study and during the study. If you are taking a leukotriene receptor antagonist or 5-lipoxygenase inhibitor such as Singulair®, Accolate®, Zyflo®, or Ultair®, you will be asked to stop taking it for 2 weeks before the study and during the study. If you are taking theophylline, Theo-Dur®, Uni-Dur®, Bronkodyl® , Elixophyllin® , Slo-bid®, Slo-Phyllin® , Theo-24® , Theolair®, or Uniphyl®, you will be asked to stop taking it 1 week before the study and during the study. You may use your rescue inhaler anytime during the course of the study. You will be asked not to use it 6 hours before any testing session in the lab, but may use it if you need to. If you do use your inhaler within 6 hours of a lab session, you will need to reschedule your test. If you do not drop in pulmonary function by at least 10%, you will not be eligible for the study.

You will be talked through the lab testing procedures for the simulated exercise test, lung function tests, exhaled nitric oxide tests, exhaled breath condensate procedures, and food frequency questionnaire which will all occur on the three lab testing days.

You will also receive information on the tasks to be completed at home, including peak flow readings, recording rescue inhaler usage, recording daily symptoms, and dietary recall interviews. You will receive a peak flow meter to take with you. Instructions for its use will be provided to you and reviewed. You will receive a log book to record your peak flow (the fastest speed a person can blow air out of their lungs), FEV_1 (the amount of air blown out in the first second of a forced exhalation), and rescue inhaler use every day for the duration of the study. You will also receive a daily symptom diary containing 4 questions about daytime symptoms and 1 question about nighttime symptoms that is to be completed every day throughout the study.

You will not receive any supplements at this time. If you qualify and choose to participate in the study, you will receive either active or placebo (inactive) DHA pills for two separate 3-week periods. During the supplementation periods, you will take 8 active DHA pills or 8 placebo DHA pills per day.

Lab Testing Sessions (approximately 1 hour each)

First Testing (following 2-4-week run-in period)

You will come to the lab in HPER 076. You should not have exercised for 24 hours before arriving and should not have had any caffeine for 8 hours before arriving. You will be asked to return your peak flow/rescue inhaler logs and daily symptom diaries from the run-in period (time between the familiarization session and first lab testing session) at this time. You will have your height and weight measured. You will perform an *exhaled nitric oxide test* that will measure the amount of nitric oxide in your exhaled breath, which indicates the presence of inflammation in the lungs. This procedure requires that you inhale to a full lung and then exhale immediately while wearing a nose clip. This will be followed by a *pulmonary function test* where you will have to wear a nose clip while you inhale and exhale maximally through a mouthpiece connected to a computerized instrument that measures lung volumes. Next, you will do the *exhaled breath condensate procedure* where you will have the breath that you breathe out collected. You sill sit in a chair, wear a nose clip, and breathe through a mouthpiece for 10 minutes such that you are breathing in normal room air and the breath you breathe out will be collected in a tube on the other side of the mouthpiece. Following these pre-tests, a simulated exercise test called *eucapnic voluntary hyperventilation (EVH)* will be performed. For the EVH test, you will sit in a chair and breathe gases from a large bag through a mouthpiece while wearing a nose clip. The bag is supplied with gas from a tank with air that has the same amount of oxygen as regular room air, but the air will be drier and will contain 5% carbon dioxide to help you breathe at the levels you need to breathe and to keep you from fainting. You will be asked to breathe at a fast and deep rate like you would during exercise, but you will just be sitting in a chair. You will breathe this air at the high rate for 6 minutes. You may stop the test at any time if you become too uncomfortable. You may use your inhaler if necessary, but this may mean that we would need to re-test you if you still wish to participate in the study. This method is routinely used in laboratories for the diagnosis of exercise-induced asthma.

Immediately after the EVH test, you will do the *exhaled breath condensate procedure* for 5 minutes. A break will be taken for the 5 minute post-EVH *pulmonary function test*. You will then return to do the final 5 minutes of the *exhaled breath condensate procedure*. Additional *pulmonary function tests* will be performed at 10, 15, and 20 minutes post-EVH. If your FEV_1 (the amount of air blown out in the first second of a forced exhalation) drops by more than 10% of your pre-EVH value, you will qualify to continue in the study. However, if your FEV_1 decreases by more than 50% of your pre-EVH value, you will be excluded from the study. You will perform a post-EVH *exhaled nitric oxide test*. You will be asked to fill out a *food frequency questionnaire* about the food and beverages you consumed during the run-in phase.

If you qualify and choose to continue in the study, you will be given a set of active or placebo DHA pills, a new peak flow/rescue inhaler log book, and a new daily symptom diary. You will be reminded of the instructions received during your familiarization session for taking the pills and filling out the peak flow/rescue inhaler log and daily symptom diary.

Second Testing (following 3-week supplementation period)

The procedures for the second lab testing session will be the same as at the first lab testing session. You will be asked to return your peak flow/rescue inhaler logs and daily symptom diaries from the first supplementation period as well as any leftover pills at this time. You will have your weight measured and perform pre- and post-EVH exhaled nitric oxide tests, EBC procedures, and pulmonary function tests as before. You will be asked to fill out a *food frequency questionnaire* about the food and beverages you consumed during the first supplementation phase. You will be given the pills for the second supplementation period but instructed to wait 2 weeks before taking this set of pills. You will also be given a food frequency

questionnaire to take home and fill out about the food and beverages you consumed during the washout phase. The primary investigator will contact you by phone and/or email (whichever you prefer) to remind you when to begin taking the pills and to fill out the food frequency questionnaire (this will take approximately 30 minutes). This set of pills will include either active or placebo DHA. You will be given a new peak flow/rescue inhaler log book and a new daily symptom diary. You will be reminded to fill out the peak flow/rescue inhaler log and daily symptom diary throughout the washout (2 weeks) and second supplementation period (3 weeks).

Third Testing (following a 2-week washout and a 3-week supplementation period)

The procedures for the third lab testing session will be the same as at the first and second lab testing sessions. You will be asked to return your peak flow meter, peak flow/rescue inhaler logs, daily symptom diaries, and any leftover pills at this time. You will have your weight measured and perform pre- and post-EVH exhaled nitric oxide tests, EBC procedures, and pulmonary function tests as before. You will be asked to fill out a food frequency questionnaire about the food and beverages you consumed during the second supplementation phase.

RISKS OF TAKING PART IN THE STUDY:

While on the study, the risks are:

1. The exercise simulation test, EVH, may induce bronchospasm (rapid narrowing of the airways).
2. Headache, transient light-headedness, or fainting may occur during the pulmonary function tests, nitric oxide procedure, or the exhaled breath procedure.
3. Contamination or infection from mouthpieces is unlikely.
4. There are no known risks of taking fish oil for subjects who are not allergic.

To minimize the risks listed above, the following measures will be employed:

1. Subjects who have severe asthma will be excluded from the study to avoid the greatest risk of bronchospasm. These subjects will be identified at the first lab testing session (if they had been taking medication, they will have been off it for 2-4 weeks depending on the type of medication). Subjects whose FEV_1 (the amount of air blown out in the first second of a forced exhalation) drops by more than 50% of their pre-EVH value will be excluded from the study because this is indicative of severe asthma.
2. All subjects will be required to bring their bronchodilators (rescue inhalers) with them to all testing sessions.
3. If severe wheezing begins during EVH, the EVH test will be immediately stopped and the subject will be given their bronchodilator and oxygen if necessary.
4. The subject will be seated and carefully monitored during EVH, pulmonary function tests, the nitric oxide procedure, and the exhaled breath procedure.
5. To avoid the risk of infection, disposable mouthpieces are used for most testing procedures, and when rubber mouthpieces are used, they are cleansed in a detergent solution and disinfected following each use.
6. Subjects who are allergic to fish oil will be excluded from the study.
7. Subjects will be instructed to report any adverse effects to the principal investigator.
8. Subjects will be instructed to discontinue the study procedures and return to their medications if they reach the rescue inhaler usage and peak flow measurement limitations set by their own physicians or Dr. Montogomery.

BENEFITS OF TAKING PART IN THE STUDY:

The benefits to participation that are reasonable to expect are a better understanding of how DHA supplementation affects the signs and symptoms of exercise-induced bronchoconstriction. Subjects will have access to all their own data regarding their own personal lung function and markers of inflammation, which they can share with their personal physician.

ALTERNATIVES TO TAKING PART IN THE STUDY:

An alternative to participating in the study is to choose not to participate.

CONFIDENTIALITY

Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. Your identity will be held in confidence in reports in which the study may be published and in databases in which results may be stored.

Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the study investigator and his/her research associates, the IUB Institutional Review Board or its designees, the study sponsor, Dr. Timothy Mickleborough, and (as allowed by law) state or federal agencies, specifically the Office for Human Research Protections (OHRP) who may need to access your medical and/or research records.

COSTS

Taking part in this study will lead to no added costs to you.

PAYMENT

You will be paid $150 for taking part in this study. You will not receive any compensation for the 30 minute familiarization session where you will learn about the study, receive study materials, and fill out the informed consent, health questionnaire, and asthma medication questionnaire. You will receive $10 after the first actual testing session, $25 after the 2nd testing session, and $115 after the last testing session. You will be compensated for the completion of each session. If you withdraw from the study, you will be compensated for the completed tests.

COMPENSATION FOR INJURY

In the event of physical injury resulting from your participation in this research, necessary medical treatment will be provided to you and billed as part of your medical expenses. Costs not covered by your health care insurer will be your responsibility. Also, it is your responsibility to determine the extent of your health care coverage. There is no program in place for other monetary compensation for such injuries. However, you are not giving up any legal rights or benefits to which you are otherwise entitled.

CONTACTS FOR QUESTIONS OR PROBLEMS

For questions about the study or a research-related injury, contact the researcher Sally Head at 812-855-4632.

For questions about your rights as a research participant or to discuss problems, complaints or concerns about a research study, or to obtain information, or offer input, contact the IUB Human

Subjects office, 530 E Kirkwood Ave, Carmichael Center, 203, Bloomington IN 47408, 812-855-3067 or by email at iub_hsc@indiana.edu

VOLUNTARY NATURE OF STUDY

Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. Your decision whether or not to participate in this study will not affect your current or future relations with the investigator(s).

SUBJECT'S CONSENT

In consideration of all of the above, I give my consent to participate in this research study.

I will be given a copy of this informed consent document to keep for my records. I agree to take part in this study.

Subject's Printed Name:_____

Subject's Signature:_____**Date**:_____

(must be dated by the subject)

Printed Name of Person Obtaining Consent:_____

Signature of Person Obtaining Consent:_____**Date**:_____

Form date: December 2, 2010

Email for Response to Potential Subjects

Thank you for your interest in our study. I have a few initial questions that you can either respond to by email or by calling me at 812-855-4632.

First, are you between 18-40 years old? This is the age range we are testing for this study.

Second, do you take any maintenance medications for asthma? For this study you are required to stop taking certain medications with your doctor's permission. If you take a corticosteroid, such as advair or flovent for example, you need to be off of it for 4 weeks before starting the study. If you take a leukotriene receptor antagonist, such as singulair or accolade for example, you need to be off it for 2 weeks before starting the study. If you take theophylline, you need to be off it for 1 week before starting the study. You can still take your rescue inhaler.

Third, do you have any other medical conditions such as heart disease, hypertension, or high cholesterol, or are you pregnant? Everyone with any of these conditions will not be able to take part in our study.

Fourth, do you currently take any nutritional supplements, including fish oil? This study requires that you stop taking fish oil. You would need to stop taking these supplements for 2 weeks before beginning the study until the study is over, for a total of 10-12 weeks.

Depending on your responses to these questions, you may meet the criteria to be a part of our study. Here is a little more information about it. We are doing a study to look at nutritional supplements for treating asthma, specifically a component of fish oil called docosahexaenoic acid or DHA for short. You would take 8 pills per day for two 3 week periods and be tested at the beginning of the study and after each of the 3 weeks of pills. Some of the pills are placebo and you might be assigned to a group with a placebo. You will also do a simulated exercise test where you will breathe fast and deep like you would during exercise, but you will be seated in a chair. You will also have some lung function tests done before and after each simulated exercise test. You will also be asked to breathe into a tube for collection of your exhaled breath. You will be asked to complete a food frequency questionnaire at each test to assess the nutrients in your diet. You also need to do peak flow measurements at home during the study.

If you are still interested in participating, please let me know your response to the above questions. If you have any questions, please contact me at skhead@indiana.edu or 812-855-4632.

If you do meet the criteria for participation and are interested in participating, I will contact you about setting up an initial appointment for a half-hour familiarization session.

Thank you,
Sally Head
MD/PhD Student
Indiana University School of Medicine
skhead@indiana.edu

Medication & Allergy Questionnaire

1. What medications do you take on a daily basis for your asthma?

2. What medications do you take just before exercise for your asthma?

3. How many times a week on average do you use your rescue inhaler?

4. Do you take any of medications like the following (circle any that apply): Advair®, Flovent ®, Azmacort®, Pulmicort®, Beclovent® Singulair®, Accolate®, Zyflo®, Ultair®, Theo-Dur®, Uni-Dur®, Bronkodyl® , Elixophyllin® , Slo-bid®, Slo-Phyllin® , Theo-24® , Theolair®, or Uniphyl®

5. Are you allergic to fish oil?

6. Are you allergic to corn or soy products?

7. Do you have any other allergies, including seasonal allergies?

8. Have you ever been diagnosed as being atopic (hyperallergic)?

Subject #_____Week #_____

Peak Flow & Rescue Inhaler Log

Record your best of 3 tries for peak flow and FEV_1 both for morning and evening. Also, record the number of puffs you took of your rescue inhaler.

Date	*Peak Flow Morning*	*Peak Flow Evening*	*FEV_1 morning*	*FEV_1 evening*	*# of puffs from rescue inhaler (including pre-exercise)*

Subject #_____Week #_____

Exercise Log

Date	*Type of Exercise*	*Duration*	*Symptoms/ Comments*	*# of puffs of rescue inhaler immediately before exercise*	*# of puffs of rescue inhaler during or after exercise*

Daily Symptom Diary

Date:_____

Daytime symptom diary scale questions

1) How often did you experience asthma symptoms today?

0	1	2	3	4	5	6
None of the time						All of the time

2) How much did your asthma symptoms bother you today?

0	1	2	3	4	5	6
Not at all bothered						Severely bothered

3) How much activity could you do today?

0	1	2	3	4	5	6
More than usual activity						Less than usual activity

4) How often did your asthma affect your activities today?

0	1	2	3	4	5	6
None of the time						All of the time

Nocturnal diary scale question

1) Did you wake up with asthma symptoms (This can be awakening in the middle of the night or on awakening in the morning)?

☐ No ☐ Once ☐ More than once ☐ Awake "all night"

Santanello NC, Barber BL, Reiss TF, Friedman BS, Juniper EF, and Zhang J. Measurement characteristics of two asthma symptom diary scales for use in clinical trials. *Eur Respir J* 10: 646-651, 1997.

Food Questionnaire

This form asks about your usual food intake during _____.

Please use **pencil**.

Answer by filling in the correct oval.

● Yes ○ No

Do not make any other marks on the form. Please use a separate piece of paper to make comments.

SEX
○ Male
○ Female

TODAY'S DATE		
MO	DAY	YEAR

IDENTIFICATION NUMBER

GSEL

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Part I: Usual Food Choices

These questions are about the types of foods you ate during _____.

1. Did you eat chicken or turkey?

- ○ Yes → **When you ate chicken or turkey, how often did you eat the skin?**
  - ○ Almost always
  - ○ Often
  - ○ Sometimes
  - ○ Rarely
  - ○ Never
- ○ No

2. Did you eat beef, pork, ham or lamb?

- ○ Yes → **When you ate beef, pork, ham or lamb, how often did you eat the fat?**
  - ○ Almost always
  - ○ Often
  - ○ Sometimes
  - ○ Rarely
  - ○ Never
- ○ No

3. Did you eat hamburger or other ground meat?

- ○ Yes → **When you ate hamburger or other ground meat, was it usually...** *Mark one or two.*
  - ○ Regular
  - ○ Lean
  - ○ Extra lean
  - ○ Ground chicken or turkey
  - ○ Don't know
- ○ No

4. Did you drink orange, grapefruit or other fruit juices?

- ○ Yes → **Were any of these vitamins or minerals added (specially fortified) to the juices you drank?** *Mark all that apply.*
  - ○ Extra Vitamin C
  - ○ Vitamin E
  - ○ Calcium
  - ○ None
  - ○ Don't know
- ○ No

5. Did you eat cold cereals?

- ○ Yes → **When you ate cold cereal, what type did you usually eat?** *Mark one or two.*
  - ○ Highly fortified cereals (100% of Daily Values) such as Total®, Smart Start® and Product 19®
  - ○ High fiber or bran cereals such as Raisin Bran® and All Bran®
  - ○ Regular granola (not lowfat)
  - ○ All other cereals such as lowfat granola, Cheerios®, Corn Flakes® and Frosted Flakes®
- ○ No

6. Did you put milk (all types), cream or creamer on cereal?

- ○ Yes → **When you put milk, cream or creamer on cereal, what type did you usually use?** *Mark one or two.*
  - ○ Cream or half and half
  - ○ Whole milk
  - ○ 2% milk
  - ○ 1% milk or buttermilk
  - ○ Nonfat or skim milk
  - ○ Soy milk
  - ○ Non-dairy creamer
  - ○ Don't know
- ○ No

7. Did you put milk (all types), cream or creamer in coffee or tea?

- ○ Yes → **When you put milk, cream or creamer in coffee or tea, what type did you usually use?** *Mark one or two.*
  - ○ Cream or half and half
  - ○ Whole milk
  - ○ 2% milk
  - ○ 1% milk or buttermilk
  - ○ Nonfat or skim milk
  - ○ Soy milk
  - ○ Non-dairy creamer
  - ○ Don't know
- ○ No

8. Did you drink milk (all types)? *Also include beverages made with milk, such as lattes, cappuccinos, mochas or hot chocolate.*

- ○ Yes → **When you drank milk or beverages made with milk, was it usually...** *Mark one or two.*
  - ○ Whole milk
  - ○ 2% milk
  - ○ 1% milk or buttermilk
  - ○ Nonfat or skim milk
  - ○ Soy milk
  - ○ Don't know
- ○ No

9. Did you use salad dressing?

- ○ Yes → **When you used salad dressing, what type did you usually use?** *Mark one or two.*
  - ○ Regular, including oil and vinegar
  - ○ Low or reduced fat
  - ○ Fat free or nonfat
- ○ No

10. Did you use mayonnaise?

- ○ Yes → **When you used mayonnaise, what type did you usually use?** *Mark one or two.*
  - ○ Regular
  - ○ Low or reduced fat
  - ○ Fat free or nonfat
- ○ No

11. Did you eat cookies or cakes?

- ○ Yes → **When you ate cookies or cakes, how often were they fig bars, SnackWells®, angel food cakes, or other types of low or nonfat cookies or cakes?**
  - ○ Almost always
  - ○ Often
  - ○ Sometimes
  - ○ Rarely
  - ○ Never
- ○ No

12. **In your household, what kinds of fat were usually used when cooking, for example to flavor vegetables or fry meat?** *Mark up to four.*

- ○ Butter
- ○ Stick margarine
- ○ Tub or liquid margarine
- ○ Lowfat margarine
- ○ Olive oil
- ○ Canola oil
- ○ Other oils such as corn, soybean, peanut and safflower
- ○ Lard, bacon fat or meat drippings
- ○ Didn't use fat or used non-stick spray (such as Pam®)

13. **What kinds of fat did you use at the table, for example on breads, vegetables or potatoes?** *Mark up to four.*

- ○ Butter
- ○ Stick margarine
- ○ Tub or liquid margarine
- ○ Lowfat margarine
- ○ Olive oil
- ○ Sour cream
- ○ Didn't use fat

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Part II: Usual Food Use

These questions are about foods you ate during _____.

14. Mark the column to show how often, on average, you ate the following foods. Mark your usual serving size as small, medium or large.

- A small serving is about one-half (½) the medium serving size or less.
- A large serving is about one-and-a-half (1½) times the medium serving size or more.

EXAMPLE: This person ate spaghetti with meat sauce every Saturday. They usually ate about 1½ cups.

	HOW OFTEN DID YOU EAT THESE FOODS?								→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Spaghetti, lasagna, and other pasta with tomato with meat sauce	○	○	○	●	○	○	○	○	○	1 cup	○	○	●

CEREALS, BREADS, SNACKS

	HOW OFTEN DID YOU EAT THESE FOODS?								→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Cold cereals	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Cooked cereals and grits	○	○	○	○	○	○	○	○	○	1cup	○	○	○
Milk on cereals	○	○	○	○	○	○	○	○	○	½ cup	○	○	○
Pancakes, French toast and waffles	○	○	○	○	○	○	○	○	○	2 pieces	○	○	○
Muffins, scones, croissants and biscuits	○	○	○	○	○	○	○	○	○	1 medium	○	○	○
White breads, including bagels, rolls and English muffins	○	○	○	○	○	○	○	○	○	2 slices or 1 medium	○	○	○
Dark breads, including dark bagels and rolls	○	○	○	○	○	○	○	○	○	2 slices or 1 medium	○	○	○
Cornbread and corn muffins	○	○	○	○	○	○	○	○	○	2 slices or 1 medium	○	○	○
Butter or margarine on breads, cereals, pancakes, etc.	○	○	○	○	○	○	○	○	○	2 pats or 2 teaspoons	○	○	○
Jam, jelly, honey, syrup and sugar (including in coffee, tea and cereal)	○	○	○	○	○	○	○	○	○	2 Tbsp.	○	○	○
Granola bars and cereal bars such as Nutri-Grain Bars®	○	○	○	○	○	○	○	○	○	1 bar	○	○	○
Sports or meal replacement bars such as Power Bars® and Clif Bars®	○	○	○	○	○	○	○	○	○	1 bar	○	○	○

CEREALS, BREADS, SNACKS (continued)

	HOW OFTEN DID YOU EAT THESE FOODS?									→ AMOUNT?			
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Low or nonfat potato chips, tortilla chips, corn chips and pretzels	○	○	○	○	○	○	○	○	○	2 handfuls or 1 sm. bag	○	○	○
Regular potato chips, tortilla chips, corn chips and puffs	○	○	○	○	○	○	○	○	○	2 handfuls or 1 sm. bag	○	○	○
Plain popcorn (no butter) or lowfat microwave popcorn	○	○	○	○	○	○	○	○	○	4 handfuls	○	○	○
Buttered or regular microwave popcorn	○	○	○	○	○	○	○	○	○	4 handfuls	○	○	○
Low or nonfat crackers such as saltines and SnackWells®	○	○	○	○	○	○	○	○	○	6 medium	○	○	○
Regular crackers such as Ritz® and Wheat Thins®	○	○	○	○	○	○	○	○	○	6 medium	○	○	○
Peanut butter, peanuts and other nuts and seeds	○	○	○	○	○	○	○	○	○	2 Tbsp. (spreads) or ¼ cup (nuts)	○	○	○

MEAT, FISH, EGGS

	HOW OFTEN DID YOU EAT THESE FOODS?									→ AMOUNT?			
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Eggs (egg substitute, mark "NEVER")	○	○	○	○	○	○	○	○	○	2 eggs	○	○	○
Bacon and breakfast sausage	○	○	○	○	○	○	○	○	○	3 strips or 2 links	○	○	○
Low or reduced fat hot dogs and sausage	○	○	○	○	○	○	○	○	○	1 hot dog or 2 ounces	○	○	○
Regular hot dogs and sausage such as bratwurst and chorizo	○	○	○	○	○	○	○	○	○	1 hot dog or 2 ounces	○	○	○
Lunch meats such as ham, turkey and lowfat bologna	○	○	○	○	○	○	○	○	○	2 slices	○	○	○
All other lunch meat such as bologna, salami and Spam®	○	○	○	○	○	○	○	○	○	2 slices	○	○	○
Canned tuna, tuna salad and tuna casserole	○	○	○	○	○	○	○	○	○	½ can tuna or 1 cup casserole	○	○	○
Beef, pork, ham and lamb	○	○	○	○	○	○	○	○	○	4 ounces	○	○	○
Ground meat, including hamburgers and meatloaf	○	○	○	○	○	○	○	○	○	1 medium patty or 3 ounces	○	○	○
Liver, chicken liver and organ meats	○	○	○	○	○	○	○	○	○	4 ounces	○	○	○
Fried chicken, including nuggets and tenders	○	○	○	○	○	○	○	○	○	1 large piece or 6 nuggets	○	○	○

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MEAT, FISH, EGGS (continued)

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Chicken and turkey (roasted, stewed, grilled or broiled)	○	○	○	○	○	○	○	○	○	1 large or 2 small pieces	○	○	○
Fried fish, fish sandwich and fried shellfish (shrimp and oysters)	○	○	○	○	○	○	○	○	○	3 ounces or 1 sandwich	○	○	○
Shellfish, not fried (shrimp, lobster, crab and oysters)	○	○	○	○	○	○	○	○	○	3 ounces or 1 cup	○	○	○
White fish (broiled or baked) such as sole, halibut, snapper and cod	○	○	○	○	○	○	○	○	○	4 ounces	○	○	○
Dark fish (broiled or baked) such as salmon, mackerel and bluefish	○	○	○	○	○	○	○	○	○	4 ounces	○	○	○

SPAGHETTI, MIXED DISHES, SOUPS

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Stew, pot pie, curries and casseroles with meat or chicken	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Chili with meat and beans	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Spaghetti, lasagna and other pasta with tomato and meat sauce	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Spaghetti and other pasta with tomato sauce (no meat)	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Spaghetti and other pasta with oil, cheese or cream sauce, including macaroni and cheese	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Asian-style (stir-fried) noodles and rice such as chow mein, fried rice and Pad Thai	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Pizza	○	○	○	○	○	○	○	○	○	2 slices	○	○	○
Tofu, tempeh and products such as tofu hot dogs, soy burgers and tofu cheese	○	○	○	○	○	○	○	○	○	3 ounces, 1 hot dog or 1 burger	○	○	○
Burritos, tacos, tostadas and quesadillas	○	○	○	○	○	○	○	○	○	1 medium	○	○	○
Enchiladas and tamales	○	○	○	○	○	○	○	○	○	1 medium	○	○	○
Vegetable, minestrone and tomato soup	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Cream soups such as chowders, potato and cheese	○	○	○	○	○	○	○	○	○	1 cup	○	○	○

SPAGHETTI, MIXED DISHES, SOUPS (continued)

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Bean soups such as pea, lentil and black bean	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Miso soup	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Ramen noodle soup	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Other soups such as chicken noodle	○	○	○	○	○	○	○	○	○	1 cup	○	○	○

DAIRY PRODUCTS

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Cottage cheese and ricotta cheese	○	○	○	○	○	○	○	○	○	½ cup	○	○	○
Low or reduced fat cheese, including cheese used in cooking	○	○	○	○	○	○	○	○	○	1 slice or ¼ cup shredded	○	○	○
All other cheese (American, cheddar or cream), including cheese used in cooking	○	○	○	○	○	○	○	○	○	1 slice, ¼ cup shredded or 2 Tbsp. cream	○	○	○
Yogurt, all types except frozen	○	○	○	○	○	○	○	○	○	1 cup	○	○	○

VEGETABLES and GRAINS

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Mark all vegetables you ate, including in salads, mixed dishes, sandwiches and stir-fries.													
Green salad (lettuce or spinach)	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Salad dressing (all types)	○	○	○	○	○	○	○	○	○	2 Tbsp.	○	○	○
Fresh tomatoes	○	○	○	○	○	○	○	○	○	1 medium or 4 slices	○	○	○
Carrots	○	○	○	○	○	○	○	○	○	½ cup	○	○	○
Green peppers and green chilies	○	○	○	○	○	○	○	○	○	¼ cup	○	○	○
Red peppers and red chilies	○	○	○	○	○	○	○	○	○	¼ cup	○	○	○

VEGETABLES and GRAINS (continued)

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Mark all vegetables you ate, including in salads, mixed dishes, sandwiches and stir-fries.													
Broccoli	○	○	○	○	○	○	○	○	○	½ cup	○	○	○
Cauliflower, cabbage and Brussels sprouts	○	○	○	○	○	○	○	○	○	½ cup	○	○	○
Green or string beans	○	○	○	○	○	○	○	○	○	½ cup	○	○	○
Green peas	○	○	○	○	○	○	○	○	○	½ cup	○	○	○
Corn and hominy	○	○	○	○	○	○	○	○	○	½ cup	○	○	○
Summer squash and zucchini	○	○	○	○	○	○	○	○	○	½ cup	○	○	○
Winter squash such as acorn, butternut and pumpkin	○	○	○	○	○	○	○	○	○	½ cup	○	○	○
Yams and sweet potatoes	○	○	○	○	○	○	○	○	○	1 medium	○	○	○
Cooked greens such as spinach, mustard greens and collards	○	○	○	○	○	○	○	○	○	½ cup	○	○	○
Onions and leeks	○	○	○	○	○	○	○	○	○	¼ cup	○	○	○
Fresh garlic, including in cooking	○	○	○	○	○	○	○	○	○	1 clove	○	○	○
Avocado and guacamole	○	○	○	○	○	○	○	○	○	¼ medium or ¼ cup	○	○	○
French fries, fried potatoes and hash browns	○	○	○	○	○	○	○	○	○	¾ cup	○	○	○
Potatoes (boiled, baked or mashed)	○	○	○	○	○	○	○	○	○	1 medium or ¾ cup	○	○	○
Refried beans	○	○	○	○	○	○	○	○	○	½ cup	○	○	○
All other beans (baked, lima or chili without meat)	○	○	○	○	○	○	○	○	○	½ cup	○	○	○
Coleslaw	○	○	○	○	○	○	○	○	○	½ cup	○	○	○
Potato, macaroni and pasta salads made with mayonnaise or oil	○	○	○	○	○	○	○	○	○	½ cup	○	○	○
Rice, noodles and other grains (as a side dish)	○	○	○	○	○	○	○	○	○	¾ cup	○	○	○
Butter, margarine, sour cream and other fat added to vegetables, potatoes and rice	○	○	○	○	○	○	○	○	○	1 pat or 1 teaspoon	○	○	○

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SAUCES and CONDIMENTS

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Cheese sauce and cream sauce	○	○	○	○	○	○	○	○	○	1/4 cup	○	○	○
Meat gravies	○	○	○	○	○	○	○	○	○	1/4 cup	○	○	○
Ketchup	○	○	○	○	○	○	○	○	○	2 Tbsp.	○	○	○
Salsa (as dip or on foods)	○	○	○	○	○	○	○	○	○	1/4 cup	○	○	○
Mayonnaise and mayonnaise-type spreads	○	○	○	○	○	○	○	○	○	2 Tbsp.	○	○	○

FRUITS

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Apples, applesauce and pears	○	○	○	○	○	○	○	○	○	1 medium or 1/2 cup	○	○	○
Bananas	○	○	○	○	○	○	○	○	○	1 medium	○	○	○
Peaches, nectarines and plums	○	○	○	○	○	○	○	○	○	1 medium or 1/2 cup	○	○	○
Apricots (fresh, canned or dried)	○	○	○	○	○	○	○	○	○	2 medium or 4 halves	○	○	○
Dried fruit (other than apricots) such as raisins and prunes	○	○	○	○	○	○	○	○	○	1/4 cup	○	○	○
Oranges, grapefruit and tangerines (not juice)	○	○	○	○	○	○	○	○	○	1 orange or 1/2 grapefruit	○	○	○
Berries such as strawberries and blueberries	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Cantaloupe, orange melon and mango (in season)	○	○	○	○	○	○	○	○	○	1/4 melon or 1/2 mango	○	○	○
Watermelon and red melon	○	○	○	○	○	○	○	○	○	1 medium slice	○	○	○
Any other fruit such as grapes, fruit cocktail, pineapple and cherries	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○

SWEETS

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Low or nonfat frozen desserts such as lowfat ice cream, frozen yogurt and sherbet	○	○	○	○	○	○	○	○	○	1 scoop	○	○	○
Ice cream and milkshakes	○	○	○	○	○	○	○	○	○	1 scoop or 1 shake	○	○	○
Pudding, custard and flan	○	○	○	○	○	○	○	○	○	¾ cup	○	○	○
Doughnuts, pies and pastries	○	○	○	○	○	○	○	○	○	1 medium piece or slice	○	○	○
Cookies and cakes	○	○	○	○	○	○	○	○	○	2 med. cookies or 1 piece of cake	○	○	○
Chocolate, candy bars and toffee	○	○	○	○	○	○	○	○	○	1 regular bar or 2 pieces	○	○	○
Other candy such as Lifesavers®, licorice and jelly beans	○	○	○	○	○	○	○	○	○	4 pieces or 12 jellybeans	○	○	○

PLEASE ANSWER THESE THREE IMPORTANT QUESTIONS!

	NEVER or less than once per week	1-2 per week	3-4 per week	5-6 per week	1 per day	2 per day	3 per day	4 per day	5+ per day
	Note that the frequency headings are different.								
How often did you eat foods that were cooked in fat (pan-fried, sautéed, or deep-fried)? *Count all fat such as margarine, butter, oil or lard.*	○	○	○	○	○	○	○	○	○
How often did you eat a serving of vegetables? *Do not count potatoes, salad or beans.*	○	○	○	○	○	○	○	○	○
How often did you eat a serving of fruit? *Do not count juices.*	○	○	○	○	○	○	○	○	○

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BEVERAGES and ALCOHOL

	HOW OFTEN DID YOU DRINK THESE BEVERAGES?									→	AMOUNT?		
	NEVER or less than once per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day	Medium serving size	S	M	L
	Note that the frequency headings are different.												
Milk (all types) as a beverage	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Latte, cappuccino, mocha or hot chocolate	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Coffee (not lattes or mochas)	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Tea (all types)	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Milk, cream or creamer added to tea and coffee	○	○	○	○	○	○	○	○	○	1 Tbsp.	○	○	○
Tomato juice, V-8® and other vegetable juices	○	○	○	○	○	○	○	○	○	¾ cup	○	○	○
Orange juice and grapefruit juice	○	○	○	○	○	○	○	○	○	¾ cup	○	○	○
Other 100% fruit juice such as apple, grape and cranberry	○	○	○	○	○	○	○	○	○	¾ cup	○	○	○
Fruit drinks fortified with Vitamin C such as Hi-C®, Fruitopia® and Kool-Aid®	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Meal replacement drinks and shakes such as Slim•Fast®, Ensure® and Carnation Instant Breakfast®	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Diet soft drinks	○	○	○	○	○	○	○	○	○	12 ounces or 1 can	○	○	○
Regular soft drinks	○	○	○	○	○	○	○	○	○	12 ounces or 1 can	○	○	○
Water (tap, bottled or sparkling)	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Beer (all types)	○	○	○	○	○	○	○	○	○	12 ounce can or bottle	○	○	○
Red wine	○	○	○	○	○	○	○	○	○	1 medium glass (6 oz)	○	○	○
White or rosé wine	○	○	○	○	○	○	○	○	○	1 medium glass (6 oz)	○	○	○
Liquor and mixed drinks	○	○	○	○	○	○	○	○	○	1 shot (1½oz) or 1 mixed drink	○	○	○

THANK YOU!

Please take a moment to fill in any questions you may have skipped.

Study #1005001346

EXERCISE-INDUCED ASTHMA

Indiana University Research Study

Who: People with asthma or exercise-induced asthma between 18-40 years old

What: This study is testing the effects of a specific component of fish oil called docosahaexanoic acid (DHA, an omega-3 fatty acid) on asthma. It will take place over 10-12 weeks with 4 total lab visits. The study requires:

- one 30 min familiarization visit to the lab
- three 1.5 hour lab testing visits
- daily at home peak flow measurements (2-5 min/day) for 10-12 weeks

You may receive supplements. Participation will involve a simulated exercise test, lung function tests, exhaled breath test, and a questionnaire about what you eat. You will need your physician's approval to stop taking asthma maintenance medication (rescue inhaler use is permitted during the study).

When: Whenever your schedule permits

Benefits: You will receive all data and information on how DHA supplementation can affect your asthma.

Compensation: $150

Who to contact if interested:

Sally Head
073 HPER
855-4632
skhead@indiana.edu

APPENDIX C

RAW DATA FOR CHAPTER 2

Maximum Drop in FEV_1 (L)

Subject	Pre-Supplementation	Fish Oil	Fish Oil + Vitamin C
1	-1.13	-0.57	-0.61
4	-0.86	-0.54	-0.84
8	-0.76	-0.56	-0.60
9	-0.36	-0.30	-0.12
13	-0.68	-0.21	-0.07
18	-0.55	-0.15	-0.31
19	-0.28	-0.40	-0.93
Mean	-0.66	-0.39	-0.50
Standard Deviation	0.29	0.17	0.34
Standard Error	0.11	0.07	0.13

Subject	Pre-Supplementation	Vitamin C	Fish Oil + Vitamin C
2	-1.78	-2.28	-0.47
3	-0.89	-0.98	-0.56
7	-1.16	-1.34	-0.54
10	-0.32	-0.23	-0.24
14	-0.41	-0.14	-0.13
17	-0.88	0.08	0.04
20	-0.73	-0.43	-0.45
Mean	-0.88	-0.76	-0.34
Standard Deviation	0.49	0.83	0.23
Standard Error	0.19	0.32	0.09

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	-1.13	-0.61
4	-0.86	-0.84
8	-0.76	-0.60
9	-0.36	-0.12
13	-0.68	-0.07
18	-0.55	-0.31
19	-0.28	-0.93
2	-1.78	-0.47
3	-0.89	-0.56
7	-1.16	-0.54
10	-0.32	-0.24
14	-0.41	-0.13
17	-0.88	0.04
20	-0.73	-0.45
Mean	-0.77	-0.42
Standard Deviation	0.41	0.29
Standard Error	0.11	0.08

Maximum Drop in FEV_1 (%)

Subject	Pre-Supplementation	Fish Oil	Fish Oil + Vitamin C
1	-20.14	-11.45	-11.07
4	-23.89	-15.47	-24.35
8	-17.39	-13.15	-13.61
9	-11.84	-10.83	-3.86
13	-18.99	-5.56	-1.92
18	-11.51	-3.20	-6.35
19	-10.77	-15.94	-37.35
Mean	-16.36	-10.80	-14.07
Standard Deviation	5.07	4.82	12.70
Standard Error	1.92	1.82	4.80

Subject	Pre-Supplementation	Vitamin C	Fish Oil + Vitamin C
2	-46.35	-52.90	-10.02
3	-24.12	-26.06	-15.64
7	-23.72	-25.48	-10.65
10	-10.16	-7.21	-8.51
14	-12.02	-3.99	-3.76
17	-26.99	2.17	1.08
20	-20.98	-12.76	-12.97
Mean	-23.48	-18.03	-8.64
Standard Deviation	11.91	18.65	5.66
Standard Error	4.50	7.05	2.14

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	-20.14	-11.07
4	-23.89	-24.35
8	-17.39	-13.61
9	-11.84	-3.86
13	-18.99	-1.92
18	-11.51	-6.35
19	-10.77	-37.35
2	-46.35	-10.02
3	-24.12	-15.64
7	-23.72	-10.65
10	-10.16	-8.51
14	-12.02	-3.76
17	-26.99	1.08
20	-20.98	-12.97
Mean	-19.92	-11.36
Standard Deviation	9.54	9.86
Standard Error	2.55	2.63

Maximum Drop in FVC (L)

Subject	Pre-Supplementation	Fish Oil	Fish Oil + Vitamin C
1	-0.77	-0.41	-0.44
4	-0.30	-0.14	-0.33
8	-0.64	-0.29	-0.27
9	-0.15	-0.09	-0.18
13	-0.76	-0.35	0.00
18	-0.54	-0.13	-0.28
19	-0.08	-0.17	-0.27
Mean	-0.46	-0.23	-0.25
Standard Deviation	0.29	0.12	0.14
Standard Error	0.11	0.05	0.05

Subject	Pre-Supplementation	Vitamin C	Fish Oil + Vitamin C
2	-1.62	-1.99	-0.28
3	-0.54	-0.43	-0.32
7	-1.01	-0.98	-0.46
10	-0.27	-0.10	-0.09
14	-0.34	-0.02	-0.01
17	-0.15	-0.29	0.16
20	-0.47	-0.13	-0.24
Mean	-0.63	-0.56	-0.18
Standard Deviation	0.52	0.71	0.21
Standard Error	0.20	0.27	0.08

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	-0.77	-0.44
4	-0.30	-0.33
8	-0.64	-0.27
9	-0.15	-0.18
13	-0.76	0.00
18	-0.54	-0.28
19	-0.08	-0.27
2	-1.62	-0.28
3	-0.54	-0.32
7	-1.01	-0.46
10	-0.27	-0.09
14	-0.34	-0.01
17	-0.15	0.16
20	-0.47	-0.24
Mean	-0.55	-0.22
Standard Deviation	0.41	0.17
Standard Error	0.11	0.05

Maximum Drop in FVC (%)

Subject	Pre-Supplementation	Fish Oil	Fish Oil + Vitamin C
1	-11.16	-6.65	-6.38
4	-5.75	-2.80	-6.65
8	-10.79	-4.87	-4.52
9	-3.79	-2.36	-4.63
13	-18.72	-8.22	0.00
18	-8.53	-2.05	-4.42
19	-1.76	-4.97	-7.96
Mean	-8.64	-4.56	-4.94
Standard Deviation	5.65	2.32	2.55
Standard Error	2.14	0.88	0.96

Subject	Pre-Supplementation	Vitamin C	Fish Oil + Vitamin C
2	-27.69	-34.37	-4.83
3	-10.31	-8.14	-6.07
7	-14.49	-13.94	-6.50
10	-7.18	-2.61	-2.43
14	-8.08	-0.47	-0.24
17	-3.77	-6.49	3.86
20	-12.95	-3.83	-6.84
Mean	-12.07	-9.98	-3.29
Standard Deviation	7.77	11.61	3.96
Standard Error	2.94	4.39	1.50

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	-11.16	-6.38
4	-5.75	-6.65
8	-10.79	-4.52
9	-3.79	-4.63
13	-18.72	0.00
18	-8.53	-4.42
19	-1.76	-7.96
2	-27.69	-4.83
3	-10.31	-6.07
7	-14.49	-6.50
10	-7.18	-2.43
14	-8.08	-0.24
17	-3.77	3.86
20	-12.95	-6.84
Mean	-10.35	-4.11
Standard Deviation	6.77	3.31
Standard Error	1.81	0.89

Maximum Drop in $FEF_{25-75\%}$ (L/s)

Subject	Pre-Supplementation	Fish Oil	Fish Oil + Vitamin C
1	-2.07	-0.97	-1.04
4	-0.89	-0.86	-0.62
8	-0.83	-0.75	-0.96
9	-0.77	-0.47	-0.27
13	-1.19	-0.27	-0.38
18	-0.64	-0.17	-0.49
19	-0.60	-0.44	-1.33
Mean	-1.00	-0.56	-0.73
Standard Deviation	0.51	0.30	0.39
Standard Error	0.19	0.11	0.15

Subject	Pre-Supplementation	Vitamin C	Fish Oil + Vitamin C
2	-1.70	-2.61	-1.27
3	-0.98	-1.33	-0.73
7	-1.32	-1.67	-0.58
10	-0.66	-0.70	-0.45
14	-0.75	-0.70	-0.41
17	-1.56	0.47	-0.17
20	-2.08	-2.07	-1.34
Mean	-1.29	-1.23	-0.71
Standard Deviation	0.52	1.02	0.44
Standard Error	0.20	0.39	0.17

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	-2.07	-1.04
4	-0.89	-0.62
8	-0.83	-0.96
9	-0.77	-0.27
13	-1.19	-0.38
18	-0.64	-0.49
19	-0.60	-1.33
2	-1.70	-1.27
3	-0.98	-0.73
7	-1.32	-0.58
10	-0.66	-0.45
14	-0.75	-0.41
17	-1.56	-0.17
20	-2.08	-1.34
Mean	-1.15	-0.72
Standard Deviation	0.52	0.40
Standard Error	0.14	0.11

Maximum Drop in $FEF_{25-75\%}$ (%)

Subject	Pre-Supplementation	Fish Oil	Fish Oil + Vitamin C
1	-38.33	-20.73	-20.76
4	-35.74	-33.33	-25.41
8	-24.13	-23.58	-27.75
9	-29.39	-22.27	-12.56
13	-26.50	-6.03	-8.52
18	-15.88	-4.64	-11.53
19	-26.55	-23.53	-64.88
Mean	-28.08	-19.16	-24.49
Standard Deviation	7.47	10.29	19.24
Standard Error	2.82	3.89	7.27

Subject	Pre-Supplementation	Vitamin C	Fish Oil + Vitamin C
2	-63.91	-74.79	-26.40
3	-37.84	-48.19	-30.42
7	-36.46	-39.29	-15.14
10	-18.80	-20.59	-13.39
14	-21.80	-18.67	-11.85
17	-53.42	13.35	-4.04
20	-42.71	-40.91	-27.92
Mean	-39.28	-32.73	-18.45
Standard Deviation	16.09	27.65	9.86
Standard Error	6.08	10.45	3.73

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	-38.33	-20.76
4	-35.74	-25.41
8	-24.13	-27.75
9	-29.39	-12.56
13	-26.50	-8.52
18	-15.88	-11.53
19	-26.55	-64.88
2	-63.91	-26.40
3	-37.84	-30.42
7	-36.46	-15.14
10	-18.80	-13.39
14	-21.80	-11.85
17	-53.42	-4.04
20	-42.71	-27.92
Mean	-33.68	-21.47
Standard Deviation	13.38	15.02
Standard Error	3.58	4.01

Pre-EVH F_ENO (ppb)

Subject	Pre-Supplementation	Fish Oil	Fish Oil + Vitamin C
1	18.8	22.1	21.2
4	63.0	68.8	35.3
8	31.6	67.9	39.1
9	27.7	33.8	32.1
13	17.1	13.1	13.9
18	42.6	42.6	44.3
19	110.1	114.0	166.3
Mean	44.4	51.8	50.3
Standard Deviation	32.9	34.7	52.2
Standard Error	12.4	13.1	19.7

Subject	Pre-Supplementation	Vitamin C	Fish Oil + Vitamin C
2	67.0	61.6	59.0
3	82.6	78.8	99.3
7	47.7	50.2	34.5
10	42.5	34.6	41.2
14	21.0	22.7	23.2
17	353.3	311.3	169.0
20	13.6	19.7	36.2
Mean	89.7	82.7	66.1
Standard Deviation	118.7	103.0	51.8
Standard Error	44.9	38.9	19.6

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	18.8	21.2
4	63.0	35.3
8	31.6	39.1
9	27.7	32.1
13	17.1	13.9
18	42.6	44.3
19	110.1	166.3
2	67.0	59.0
3	82.6	99.3
7	47.7	34.5
10	42.5	41.2
14	21.0	23.2
17	353.3	169.0
20	13.6	36.2
Mean	67.0	58.2
Standard Deviation	86.9	50.6
Standard Error	23.2	13.5

Post-EVH F_ENO (ppb)

Subject	Pre-Supplementation	Fish Oil	Fish Oil + Vitamin C
1	13.9	18.4	16.8
4	66.5	61.5	33.6
8	27.2	44.1	30.2
9	36.4	39.4	34.9
13	10.9	9.2	10.0
18	42.0	44.1	41.3
19	87.8	89.6	124.7
Mean	40.7	43.8	41.6
Standard Deviation	28.0	26.7	38.2
Standard Error	10.6	10.1	14.4

Subject	Pre-Supplementation	Vitamin C	Fish Oil + Vitamin C
2	45.2	43.8	58.7
3	75.2	62.1	73.9
7	34.3	33.9	28.2
10	37.3	28.1	33.9
14	17.1	16.0	13.8
17	221.8	305.7	152.0
20	30.1	18.4	22.9
Mean	65.8	72.6	54.8
Standard Deviation	71.1	104.0	47.7
Standard Error	26.9	39.3	18.0

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	13.9	16.8
4	66.5	33.6
8	27.2	30.2
9	36.4	34.9
13	10.9	10.0
18	42.0	41.3
19	87.8	124.7
2	45.2	58.7
3	75.2	73.9
7	34.3	28.2
10	37.3	33.9
14	17.1	13.8
17	221.8	152.0
20	30.1	22.9
Mean	53.3	48.2
Standard Deviation	53.5	42.1
Standard Error	14.3	11.2

Pre-EVH Exhaled Breathe Condensate pH

Subject	Pre-Supplementation	Fish Oil	Fish Oil + Vitamin C
1	7.10	7.52	7.24
4	6.39	6.98	6.63
8	6.88	7.24	6.41
9	7.41	7.30	7.01
13	7.03	6.70	6.68
18	6.74	7.20	6.82
19	6.87	7.30	7.15
Mean	6.92	7.18	6.85
Standard Deviation	0.32	0.26	0.30
Standard Error	0.12	0.10	0.11

Subject	Pre-Supplementation	Vitamin C	Fish Oil + Vitamin C
2	-	7.13	6.96
3	7.12	7.13	7.07
7	7.16	6.96	7.21
10	7.04	6.92	6.79
14	7.21	6.84	6.89
17	6.54	7.11	6.53
20	7.23	6.50	6.91
Mean	7.05	6.94	6.91
Standard Deviation	0.26	0.23	0.21
Standard Error	0.11	0.09	0.08

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	7.10	7.24
4	6.39	6.63
8	6.88	6.41
9	7.41	7.01
13	7.03	6.68
18	6.74	6.82
19	6.87	7.15
2	-	6.96
3	7.12	7.07
7	7.16	7.21
10	7.04	6.79
14	7.21	6.89
17	6.54	6.53
20	7.23	6.91
Mean	6.98	6.88
Standard Deviation	0.29	0.25
Standard Error	0.08	0.07

Post-EVH Exhaled Breathe Condensate pH

Subject	Pre-Supplementation	Fish Oil	Fish Oil + Vitamin C
1	6.78	7.50	7.28
4	6.59	6.93	6.33
8	7.14	7.08	6.02
9	6.99	7.04	6.51
13	6.81	7.40	6.92
18	6.97	7.24	6.63
19	6.68	7.32	7.05
Mean	6.85	7.22	6.68
Standard Deviation	0.19	0.21	0.44
Standard Error	0.07	0.08	0.17

Subject	Pre-Supplementation	Vitamin C	Fish Oil + Vitamin C
2	7.34	7.24	6.98
3	7.37	7.16	7.25
7	7.23	7.01	7.54
10	6.83	6.89	6.95
14	6.76	6.79	6.89
17	6.79	7.47	7.31
20	7.40	6.86	7.45
Mean	7.10	7.06	7.20
Standard Deviation	0.29	0.24	0.26
Standard Error	0.11	0.09	0.10

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	6.78	7.28
4	6.59	6.33
8	7.14	6.02
9	6.99	6.51
13	6.81	6.92
18	6.97	6.63
19	6.68	7.05
2	7.34	6.98
3	7.37	7.25
7	7.23	7.54
10	6.83	6.95
14	6.76	6.89
17	6.79	7.31
20	7.40	7.45
Mean	6.98	6.94
Standard Deviation	0.27	0.44
Standard Error	0.07	0.12

Daily Symptom Score

Subject	Pre-Supplementation	Fish Oil	Washout	Fish Oil + Vitamin C
1	0.75	0.75	0.75	0.75
4	1.83	1.63	1.52	1.50
8	0.74	0.39	0.39	0.39
9	1.27	0.72	0.30	0.33
13	1.24	0.85	0.84	0.92
18	1.56	1.46	1.20	0.84
19	0.56	0.51	0.54	0.62
Mean	1.13	0.90	0.79	0.76
Standard Deviation	0.47	0.47	0.44	0.39
Standard Error	0.18	0.18	0.17	0.15

Subject	Pre-Supplementation	Vitamin C	Washout	Fish Oil + Vitamin C
2	2.78	2.24	2.22	2.03
3	1.23	0.43	0.88	2.67
7	1.75	1.86	1.86	1.88
10	1.38	1.27	2.00	1.09
14	2.24	0.66	0.79	0.86
17	0.98	-	2.08	1.33
20	1.04	0.79	0.68	0.75
Mean	1.63	1.21	1.50	1.52
Standard Deviation	0.67	0.72	0.68	0.70
Standard Error	0.25	0.27	0.26	0.27

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	0.75	0.75
4	1.83	1.50
8	0.74	0.39
9	1.27	0.33
13	1.24	0.92
18	1.56	0.84
19	0.56	0.62
2	2.78	2.03
3	1.23	2.67
7	1.75	1.88
10	1.38	1.09
14	2.24	0.86
17	0.98	1.33
20	1.04	0.75
Mean	1.38	1.14
Standard Deviation	0.61	0.67
Standard Error	0.16	0.18

Nightly Symptom Score

Subject	Pre-Supplementation	Fish Oil	Washout	Fish Oil + Vitamin C
1	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00
9	0.62	0.28	0.00	0.00
13	0.06	0.00	0.00	0.00
18	0.00	0.00	0.00	0.00
19	0.00	0.00	0.00	0.00
Mean	0.10	0.04	0.00	0.00
Standard Deviation	0.23	0.10	0.00	0.00
Standard Error	0.09	0.04	0.00	0.00

Subject	Pre-Supplementation	Vitamin C	Washout	Fish Oil + Vitamin C
2	0.53	0.29	0.47	0.55
3	0.38	0.00	0.00	0.27
7	0.29	0.00	0.00	0.00
10	0.00	0.00	0.07	0.00
14	0.00	0.00	0.00	0.00
17	0.25	-	0.42	0.10
20	0.00	0.00	0.07	0.05
Mean	0.21	0.05	0.15	0.14
Standard Deviation	0.21	0.12	0.21	0.21
Standard Error	0.08	0.05	0.08	0.08

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	0.00	0.00
4	0.00	0.00
8	0.00	0.00
9	0.62	0.00
13	0.06	0.00
18	0.00	0.00
19	0.00	0.00
2	0.53	0.55
3	0.38	0.27
7	0.29	0.00
10	0.00	0.00
14	0.00	0.00
17	0.25	0.10
20	0.00	0.05
Mean	0.15	0.07
Standard Deviation	0.22	0.16
Standard Error	0.06	0.04

Bronchodilator Use (number of puffs per day)

Subject	Pre-Supplementation	Fish Oil	Washout	Fish Oil + Vitamin C
1	0.00	0.00	0.00	0.00
4	2.15	0.00	0.29	0.20
8	0.00	0.00	0.00	0.00
9	0.15	0.22	0.00	0.29
13	0.35	0.05	0.00	0.00
18	0.00	0.00	0.14	0.00
19	0.00	0.00	0.15	0.05
Mean	0.38	0.04	0.08	0.08
Standard Deviation	0.79	0.08	0.11	0.12
Standard Error	0.30	0.03	0.04	0.04

Subject	Pre-Supplementation	Vitamin C	Washout	Fish Oil + Vitamin C
2	4.73	3.42	1.93	1.79
3	0.18	0.14	-	0.37
7	0.06	0.00	0.33	0.32
10	0.23	0.00	0.00	0.09
14	0.71	0.50	0.00	0.00
17	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00
Mean	0.84	0.58	0.38	0.37
Standard Deviation	1.73	1.27	0.77	0.65
Standard Error	0.65	0.48	0.32	0.24

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	0.00	0.00
4	2.15	0.20
8	0.00	0.00
9	0.15	0.29
13	0.35	0.00
18	0.00	0.00
19	0.00	0.05
2	4.73	1.79
3	0.18	0.37
7	0.06	0.32
10	0.23	0.09
14	0.71	0.00
17	0.00	0.00
20	0.00	0.00
Mean	0.61	0.22
Standard Deviation	1.32	0.47
Standard Error	0.35	0.13

Morning FEV_1 (L)

Subject	Pre-Supplementation	Fish Oil	Washout	Fish Oil + Vitamin C
1	5.55	5.58	5.56	5.78
4	3.03	2.51	2.49	2.96
8	4.28	4.23	4.20	4.17
9	2.75	3.03	3.02	2.99
13	3.04	3.62	3.19	3.55
18	4.37	4.17	4.43	4.49
19	2.53	2.50	2.52	2.63
Mean	3.65	3.66	3.63	3.80
Standard Deviation	1.11	1.11	1.14	1.10
Standard Error	0.42	0.42	0.43	0.42

Subject	Pre-Supplementation	Vitamin C	Washout	Fish Oil + Vitamin C
2	3.42	3.09	3.24	3.29
3	3.47	3.42	-	3.53
7	5.15	5.68	5.52	5.52
10	3.03	3.06	2.98	3.12
14	2.87	3.00	2.89	2.52
17	2.24	2.49	2.51	2.50
20	2.30	2.02	2.46	2.40
Mean	3.21	3.25	3.27	3.27
Standard Deviation	0.98	1.17	1.14	1.09
Standard Error	0.37	0.44	0.47	0.41

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	5.55	5.78
4	3.03	2.96
8	4.28	4.17
9	2.75	2.99
13	3.04	3.55
18	4.37	4.49
19	2.53	2.63
2	3.42	3.29
3	3.47	3.53
7	5.15	5.52
10	3.03	3.12
14	2.87	2.52
17	2.24	2.50
20	2.30	2.40
Mean	3.43	3.53
Standard Deviation	1.03	1.09
Standard Error	0.28	0.29

Evening FEV_1 (L)

Subject	Pre-Supplementation	Fish Oil	Washout	Fish Oil + Vitamin C
1	5.57	5.75	5.55	5.71
4	2.49	2.42	2.61	2.92
8	4.32	4.20	4.19	4.21
9	2.79	2.98	3.03	2.99
13	3.06	3.63	3.26	3.55
18	4.21	4.16	4.43	4.47
19	2.58	2.62	2.67	2.66
Mean	3.57	3.68	3.68	3.79
Standard Deviation	1.15	1.15	1.09	1.08
Standard Error	0.44	0.44	0.41	0.41

Subject	Pre-Supplementation	Vitamin C	Washout	Fish Oil + Vitamin C
2	3.19	3.04	3.21	3.34
3	3.29	3.40	-	3.38
7	5.56	6.48	6.03	6.11
10	3.12	3.13	3.05	3.15
14	2.88	3.19	2.84	2.83
17	2.72	2.80	2.68	2.67
20	2.25	2.07	2.49	2.40
Mean	3.29	3.44	3.39	3.41
Standard Deviation	1.06	1.41	1.32	1.24
Standard Error	0.40	0.53	0.54	0.47

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	5.57	5.71
4	2.49	2.92
8	4.32	4.21
9	2.79	2.99
13	3.06	3.55
18	4.21	4.47
19	2.58	2.66
2	3.19	3.34
3	3.29	3.38
7	5.56	6.11
10	3.12	3.15
14	2.88	2.83
17	2.72	2.67
20	2.25	2.40
Mean	3.43	3.60
Standard Deviation	1.08	1.14
Standard Error	0.29	0.30

Morning PEF (L/min)

Subject	Pre-Supplementation	Fish Oil	Washout	Fish Oil + Vitamin C
1	692.20	714.76	703.69	741.25
4	310.15	265.62	287.86	303.96
8	592.63	611.15	605.00	612.95
9	323.23	407.67	415.87	416.00
13	382.00	425.65	409.94	402.52
18	579.08	606.95	635.71	650.82
19	287.46	292.45	263.54	280.10
Mean	452.39	474.89	474.51	486.80
Standard Deviation	164.47	172.03	174.40	180.66
Standard Error	62.16	65.02	65.92	68.28

Subject	Pre-Supplementation	Vitamin C	Washout	Fish Oil + Vitamin C
2	377.53	314.82	273.18	325.80
3	450.27	446.36	-	410.68
7	601.47	593.50	572.07	569.32
10	360.91	352.71	377.14	393.68
14	414.47	329.20	357.40	390.15
17	252.77	279.00	260.00	265.24
20	256.14	192.22	304.86	298.00
Mean	387.65	358.26	357.44	378.98
Standard Deviation	120.18	128.91	114.73	99.92
Standard Error	45.42	48.72	46.84	37.76

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	692.20	741.25
4	310.15	303.96
8	592.63	612.95
9	323.23	416.00
13	382.00	402.52
18	579.08	650.82
19	287.46	280.10
2	377.53	325.80
3	450.27	410.68
7	601.47	569.32
10	360.91	393.68
14	414.47	390.15
17	252.77	265.24
20	256.14	298.00
Mean	420.02	432.89
Standard Deviation	142.40	151.00
Standard Error	38.06	40.36

Evening PEF (L/min)

Subject	Pre-Supplementation	Fish Oil	Washout	Fish Oil + Vitamin C
1	691.60	732.52	707.00	707.16
4	253.77	236.43	279.00	323.25
8	590.63	612.30	606.43	614.05
9	347.54	418.44	418.87	411.43
13	380.71	419.00	404.44	412.38
18	570.77	596.70	635.79	661.45
19	303.00	302.20	310.46	311.57
Mean	448.29	473.94	480.28	491.61
Standard Deviation	167.39	179.39	168.51	165.21
Standard Error	63.27	67.80	63.69	62.44

Subject	Pre-Supplementation	Vitamin C	Washout	Fish Oil + Vitamin C
2	350.40	325.83	290.13	316.65
3	411.40	416.70	-	387.31
7	645.80	662.00	593.08	599.05
10	390.23	377.09	384.86	387.26
14	424.29	411.65	399.70	436.30
17	335.69	360.30	280.15	293.19
20	269.64	202.68	314.50	307.65
Mean	403.92	393.75	377.07	389.63
Standard Deviation	118.82	138.64	116.70	105.98
Standard Error	44.91	52.40	47.64	40.06

Subject	Pre-Supplementation	Fish Oil + Vitamin C
1	691.60	707.16
4	253.77	323.25
8	590.63	614.05
9	347.54	411.43
13	380.71	412.38
18	570.77	661.45
19	303.00	311.57
2	350.40	316.65
3	411.40	387.31
7	645.80	599.05
10	390.23	387.26
14	424.29	436.30
17	335.69	293.19
20	269.64	307.65
Mean	426.10	440.62
Standard Deviation	141.35	143.46
Standard Error	37.78	38.34

Dietary Data: Pre-Supplementation

Subject	Total Grams	Energy (kcal)	Total Fat (g)	Total Carbohydrate (g)	Total Protein (g)	Total Dietary Fiber (g)
1	6472.03	4265.87	107.15	617.43	237.15	57.89
4	4647.08	1663.85	72.42	159.34	96.53	14.73
8	3997.98	3424.01	64.25	427.30	101.08	31.78
9	2440.58	2095.23	91.46	261.63	65.96	19.28
13	2250.57	3520.34	142.17	450.49	120.24	19.34
18	4283.80	3224.07	178.77	271.54	136.24	15.56
19	2374.25	1863.75	80.33	202.15	84.00	7.47
Mean	3780.90	2865.30	105.22	341.41	120.17	23.72
Standard Deviation	1549.71	989.20	41.52	162.92	56.44	16.75
Standard Error	585.73	373.88	15.69	61.58	21.33	6.33

Subject	Linoleic Acid (g)	Linolenic Acid (g)	Arachidonic Acid (g)	Eicosapentaenoic Acid (EPA) (g)	Docosahexaenoic Acid (DHA) (g)
1	16.13	3.27	0.08	0.01	0.02
4	17.08	0.83	0.23	0.07	0.18
8	11.98	2.20	0.20	0.07	0.09
9	19.68	1.79	0.14	0.00	0.03
13	37.05	4.38	0.15	0.02	0.05
18	31.62	3.16	0.39	0.04	0.13
19	13.74	2.00	0.18	0.01	0.04
Mean	21.04	2.52	0.20	0.03	0.07
Standard Deviation	9.53	1.17	0.10	0.03	0.06
Standard Error	3.60	0.44	0.04	0.01	0.02

Dietary Data: Pre-Supplementation

Subject	Total Vitamin A Activity (International Units)	Vitamin E (Total Alpha-Tocopherol) (mg)	Vitamin C (ascorbic acid) (mg)	Lycopene (mcg)	Zinc (mg)	Selenium (mcg)	Sodium (mg)	Caffeine (mg)
1	14168.74	35.78	197.13	36289.35	41.51	259.49	6191.33	21.84
4	5243.08	10.97	49.08	2573.85	9.62	137.50	3940.92	0.00
8	4049.29	9.24	107.90	15441.63	10.87	142.48	5017.03	178.78
9	4862.07	14.33	81.74	875.37	13.44	115.40	5111.90	1.24
13	2864.92	10.79	89.63	6372.32	11.16	170.66	5764.29	29.13
18	1302.02	8.30	35.81	0.00	16.13	171.93	5219.02	4.22
19	2871.09	5.65	108.83	1149.49	10.80	99.82	4378.08	55.24
Mean	5051.60	13.58	95.73	8957.43	16.22	156.76	5088.94	41.49
Standard Deviation	4238.61	10.15	52.60	13184.78	11.36	52.45	765.64	63.65
Standard Error	1602.04	3.84	19.88	4983.38	4.29	19.82	289.39	24.06

Dietary Data: Pre-Supplementation

Subject	Total Grams	Energy (kcal)	Total Fat (g)	Total Carbohydrate (g)	Total Protein (g)	Total Dietary Fiber (g)
2	1402.53	1151.52	46.82	144.20	40.78	
3	3870.35	2453.97	69.57	258.42	82.50	13.90
7	7390.70	4696.12	179.30	556.08	231.62	38.20
10	5218.69	2196.79	62.95	306.38	104.60	27.00
14	5485.37	2768.74	65.72	431.63	88.79	30.01
17	4254.78	4642.34	159.93	683.63	134.00	23.10
20	3204.59	940.37	24.69	127.32	54.63	6.81
Mean	4403.86	2692.84	87.00	358.24	105.27	23.17
Standard Deviation	1893.32	1503.85	58.70	209.04	63.64	11.32
Standard Error	715.61	568.40	22.19	79.01	24.05	4.28

Subject	Linoleic Acid (g)	Linolenic Acid (g)	Arachidonic Acid (g)	Eicosapentaenoic Acid (EPA) (g)	Docosahexaenoic Acid (DHA) (g)
2	6.95	0.77	0.07	0.00	0.01
3	13.39	1.20	0.12	0.00	0.03
7	16.43	1.95	0.35	0.02	0.10
10	12.25	1.62	0.10	0.04	0.18
14	19.56	1.98	0.05	0.01	0.02
17	32.48	4.13	0.23	0.01	0.05
20	3.01	0.44	0.16	0.02	0.10
Mean	14.87	1.73	0.16	0.02	0.07
Standard Deviation	9.55	1.21	0.11	0.01	0.06
Standard Error	3.61	0.46	0.04	0.00	0.02

Dietary Data: Pre-Supplementation

Subject	Total Vitamin A Activity (International Units)	Vitamin E (Total Alpha-Tocopherol) (mg)	Vitamin C (ascorbic acid) (mg)	Lycopene (mcg)	Zinc (mg)	Selenium (mcg)	Sodium (mg)	Caffeine (mg)
2	1297.90	2.08	8.75	0.00	5.29	49.24	1488.50	87.78
3	5076.58	5.33	87.80	822.54	9.50	158.09	3681.60	2.08
7	10118.45	9.00	331.71	8179.87	30.73	258.50	8912.32	29.95
10	9092.69	9.87	75.42	12507.51	13.95	197.99	3019.12	196.31
14	4181.72	10.18	109.37	36585.68	14.89	138.54	5696.71	0.00
17	3659.30	9.62	323.39	11357.92	17.10	180.92	6765.00	68.54
20	1721.18	2.47	9.05	2565.84	4.95	93.63	1273.73	80.08
Mean	5021.12	6.94	135.07	10288.48	13.77	153.85	4405.28	66.39
Standard Deviation	3412.59	3.57	136.88	12630.34	8.83	68.87	2837.51	67.58
Standard Error	1289.84	1.35	51.74	4773.82	3.34	26.03	1072.48	25.54

Dietary Data: Fish Oil Treatment

Subject	Total Grams	Energy (kcal)	Total Fat (g)	Total Carbohydrate (g)	Total Protein (g)	Total Dietary Fiber (g)
1	6862.94	3874.01	104.56	559.13	210.56	66.02
4	2823.79	3548.07	123.78	512.22	108.13	23.74
8	2972.81	2900.06	123.66	323.83	128.22	17.99
9	2147.84	1798.58	59.60	270.95	48.11	10.79
13	2804.93	1466.93	32.99	224.82	68.72	11.06
18	3015.64	2361.45	69.82	307.55	86.62	23.15
19	1999.86	2132.44	75.58	289.16	81.45	14.37
Mean	3232.54	2583.08	84.29	355.38	104.55	23.87
Standard Deviation	1650.27	895.51	34.27	127.77	53.44	19.31
Standard Error	623.74	338.47	12.95	48.29	20.20	7.30

Subject	Linoleic Acid (g)	Linolenic Acid (g)	Arachidonic Acid (g)	Eicosapentaenoic Acid (EPA) (g)	Docosahexaenoic Acid (DHA) (g)
1	14.18	2.62	0.18	0.16	0.16
4	37.73	4.32	0.09	0.01	0.02
8	18.16	2.40	0.08	0.01	0.03
9	11.06	1.91	0.03	0.00	0.01
13	7.18	0.76	0.10	0.01	0.03
18	4.08	0.74	0.07	0.00	0.01
19	16.86	1.92	0.10	0.01	0.03
Mean	15.61	2.10	0.09	0.03	0.04
Standard Deviation	10.99	1.22	0.05	0.06	0.05
Standard Error	4.15	0.46	0.02	0.02	0.02

Dietary Data: Fish Oil Treatment

Subject	Total Vitamin A Activity (International Units)	Vitamin E (Total Alpha-Tocopherol) (mg)	Vitamin C (ascorbic acid) (mg)	Lycopene (mcg)	Zinc (mg)	Selenium (mcg)	Sodium (mg)	Caffeine (mg)
1	28813.23	45.72	868.11	1029.20	35.81	183.02	3598.65	12.51
4	2823.39	19.41	76.22	8977.69	10.43	158.33	4818.07	60.53
8	2350.15	8.15	26.31	2081.01	12.82	159.59	7699.91	1.02
9	1693.40	5.92	90.91	7596.13	5.50	74.68	2785.74	57.09
13	3745.11	3.62	25.56	2798.50	7.84	92.86	3480.10	34.87
18	3963.80	4.87	123.46	9286.28	14.31	107.58	3198.10	0.55
19	3179.91	12.07	74.14	9528.95	15.10	105.47	3641.99	31.70
Mean	6652.71	14.25	183.53	5899.68	14.54	125.93	4174.65	28.32
Standard Deviation	9803.09	14.88	303.86	3761.96	9.99	40.66	1674.42	24.79
Standard Error	3705.22	5.63	114.85	1421.89	3.78	15.37	632.87	9.37

Dietary Data: Vitamin C Treatment

Subject	Total Grams	Energy (kcal)	Total Fat (g)	Total Carbohydrate (g)	Total Protein (g)	Total Dietary Fiber (g)
2	1575.23	1513.55	58.28	167.95	79.11	7.23
3	3618.28	3318.07	139.51	306.48	140.86	26.60
7	4928.50	3864.61	154.80	450.76	176.40	28.13
10	3393.17	1349.07	48.14	188.08	58.62	27.73
14	5226.28	4007.65	167.90	491.98	136.46	32.20
17	2514.11	3031.59	111.25	400.71	118.31	13.27
20	2895.67	1005.57	41.79	88.43	57.61	7.97
Mean	3450.18	2584.30	103.10	299.20	109.62	20.45
Standard Deviation	1295.54	1262.82	53.31	155.18	45.59	10.56
Standard Error	489.67	477.30	20.15	58.65	17.23	3.99

Subject	Linoleic Acid (g)	Linolenic Acid (g)	Arachidonic Acid (g)	Eicosapentaenoic Acid (EPA) (g)	Docosahexaenoic Acid (DHA) (g)
2	14.72	0.76	0.07	0.00	0.00
3	25.62	1.69	0.11	0.04	0.19
7	25.77	2.17	0.33	0.02	0.09
10	12.91	1.27	0.03	0.00	0.01
14	52.24	5.12	0.19	0.02	0.07
17	17.66	1.57	0.37	0.02	0.08
20	4.21	0.56	0.17	0.01	0.04
Mean	21.88	1.88	0.18	0.02	0.07
Standard Deviation	15.34	1.53	0.13	0.01	0.06
Standard Error	5.80	0.58	0.05	0.01	0.02

Dietary Data: Vitamin C Treatment

Subject	Total Vitamin A Activity (International Units)	Vitamin E (Total Alpha-Tocopherol) (mg)	Vitamin C (ascorbic acid) (mg)	Lycopene (mcg)	Zinc (mg)	Selenium (mcg)	Sodium (mg)	Caffeine (mg)
2	971.31	7.09	20.86	1253.18	12.92	105.31	2730.60	76.23
3	2429.76	15.40	33.42	9720.70	22.50	211.26	4375.97	23.68
7	13385.26	16.30	140.18	5853.55	25.48	280.55	6694.95	0.00
10	8939.23	16.23	145.89	4281.07	8.02	86.51	2216.45	192.62
14	7438.03	16.00	71.37	20513.85	11.56	211.33	9286.85	84.06
17	3789.28	7.41	49.13	4704.19	13.15	170.09	4935.30	7.50
20	2228.61	3.08	31.81	4072.33	6.56	71.79	1892.97	23.81
Mean	5597.35	11.64	70.38	7199.84	14.31	162.41	4590.44	58.27
Standard Deviation	4495.54	5.59	52.17	6394.29	7.10	77.49	2677.73	67.57
Standard Error	1699.15	2.11	19.72	2416.81	2.68	29.29	1012.09	25.54

Dietary Data: Washout

Subject	Total Grams	Energy (kcal)	Total Fat (g)	Total Carbohydrate (g)	Total Protein (g)	Total Dietary Fiber (g)
1	6738.55	4322.55	108.56	615.30	181.95	56.24
4	2495.90	4667.49	239.29	510.90	143.82	43.17
8	2217.97	2148.50	88.56	247.33	89.78	11.30
9	2271.60	1678.50	45.07	267.88	55.15	7.25
13	3760.37	1588.24	52.92	162.47	107.09	11.51
18	5444.49	2852.05	37.80	419.90	95.41	37.26
19	1485.14	1477.67	49.77	211.88	49.16	10.25
Mean	3487.72	2676.43	88.85	347.95	103.19	25.28
Standard Deviation	1938.92	1329.20	71.13	169.64	47.15	19.83
Standard Error	732.84	502.39	26.89	64.12	17.82	7.49

Subject	Linoleic Acid (g)	Linolenic Acid (g)	Arachidonic Acid (g)	Eicosapentaenoic Acid (EPA) (g)	Docosahexaenoic Acid (DHA) (g)
1	15.47	1.56	0.14	0.17	0.18
4	72.59	7.84	0.27	0.04	0.11
8	16.20	1.95	0.10	0.01	0.03
9	4.43	0.41	0.06	0.01	0.01
13	4.55	0.72	0.14	0.12	0.39
18	8.58	1.39	0.06	0.01	0.02
19	7.89	0.87	0.04	0.00	0.01
Mean	18.53	2.11	0.11	0.05	0.11
Standard Deviation	24.30	2.58	0.08	0.07	0.14
Standard Error	9.19	0.98	0.03	0.02	0.05

Dietary Data: Washout

Subject	Total Vitamin A Activity (International Units)	Vitamin E (Total Alpha-Tocopherol) (mg)	Vitamin C (ascorbic acid) (mg)	Lycopene (mcg)	Zinc (mg)	Selenium (mcg)	Sodium (mg)	Caffeine (mg)
1	16232.92	19.57	388.05	10719.81	27.28	230.56	5614.01	58.11
4	5840.83	34.37	267.05	2541.01	16.19	207.88	6546.54	33.52
8	940.03	6.20	6.68	1474.90	7.37	135.02	4700.91	0.00
9	2647.65	1.94	92.44	334.49	9.28	62.76	2334.06	0.00
13	10202.07	6.41	78.14	14737.65	14.12	114.00	3664.91	28.42
18	12428.52	4.82	117.27	108.58	11.16	128.61	4278.86	118.40
19	2719.05	2.56	32.77	0.00	11.91	91.37	2568.76	30.09
Mean	7287.30	10.84	140.34	4273.78	13.90	138.60	4244.01	38.36
Standard Deviation	5769.57	11.94	137.57	5958.36	6.58	60.49	1537.22	40.68
Standard Error	2180.69	4.51	52.00	2252.05	2.49	22.86	581.01	15.38

Dietary Data: Washout

Subject	Total Grams	Energy (kcal)	Total Fat (g)	Total Carbohydrate (g)	Total Protein (g)	Total Dietary Fiber (g)
2	1563.82	1699.08	69.91	184.67	82.07	7.83
3	1848.02	832.57	41.14	101.42	19.94	11.42
7	3955.87	3617.07	132.51	441.73	161.09	25.44
10	3579.67	1906.43	58.31	259.75	81.36	31.64
14	5183.77	1548.42	21.54	278.82	44.95	22.76
17	2124.80	2261.71	77.81	324.88	75.32	8.51
20	2942.11	1050.32	29.87	126.38	66.72	7.90
Mean	3028.29	1845.08	61.59	245.38	75.92	16.50
Standard Deviation	1302.05	920.46	37.42	118.86	43.78	9.89
Standard Error	492.13	347.90	14.14	44.93	16.55	3.74

Subject	Linoleic Acid (g)	Linolenic Acid (g)	Arachidonic Acid (g)	Eicosapentaenoic Acid (EPA) (g)	Docosahexaenoic Acid (DHA) (g)
2	14.62	1.95	0.11	0.01	0.03
3	5.29	1.72	0.01	0.00	0.00
7	22.12	2.06	0.39	0.02	0.10
10	10.13	0.64	0.05	0.00	0.01
14	3.94	0.50	0.04	0.01	0.02
17	15.01	1.43	0.19	0.01	0.04
20	5.43	0.58	0.19	0.02	0.11
Mean	10.93	1.27	0.14	0.01	0.04
Standard Deviation	6.67	0.68	0.13	0.01	0.04
Standard Error	2.52	0.26	0.05	0.00	0.02

Dietary Data: Washout

Subject	Total Vitamin A Activity (International Units)	Vitamin E (Total Alpha-Tocopherol) (mg)	Vitamin C (ascorbic acid) (mg)	Lycopene (mcg)	Zinc (mg)	Selenium (mcg)	Sodium (mg)	Caffeine (mg)
2	1105.39	4.61	20.83	9400.74	8.39	95.12	2956.64	103.95
3	11763.36	6.69	92.08	5757.85	3.25	24.91	2221.56	2.60
7	10258.31	15.57	183.79	14545.46	19.76	250.09	13064.01	93.60
10	6165.54	7.61	99.02	17660.28	11.77	119.96	3139.36	193.27
14	1150.17	2.74	121.63	1521.47	5.06	65.26	3096.61	0.00
17	2000.66	5.25	73.47	7238.54	8.48	102.65	3547.09	2.50
20	2335.92	4.46	16.18	3675.00	6.36	117.30	1879.76	111.10
Mean	4968.48	6.71	86.72	8542.76	9.01	110.76	4272.15	72.43
Standard Deviation	4487.52	4.22	58.19	5807.29	5.47	69.87	3918.90	73.66
Standard Error	1696.12	1.59	21.99	2194.95	2.07	26.41	1481.20	27.84

Dietary Data: Combination Treatment

Subject	Total Grams	Energy (kcal)	Total Fat (g)	Total Carbohydrate (g)	Total Protein (g)	Total Dietary Fiber (g)
1	6019.51	3661.00	105.60	508.71	190.56	41.58
4	3023.41	6089.73	290.42	726.33	162.35	44.43
8	4145.99	3186.62	142.08	332.69	145.79	15.15
9	2576.31	1882.51	69.39	225.82	92.43	15.07
13	3504.45	2382.62	80.32	317.07	99.49	18.87
18	2755.43	3176.94	92.86	422.56	97.13	21.35
19	996.49	1088.79	27.27	177.88	42.36	13.17
Mean	3288.80	3066.89	115.42	387.29	118.59	24.23
Standard Deviation	1546.79	1596.92	84.72	186.54	50.26	13.13
Standard Error	584.63	603.58	32.02	70.50	19.00	4.96

Subject	Linoleic Acid (g)	Linolenic Acid (g)	Arachidonic Acid (g)	Eicosapentaenoic Acid (EPA) (g)	Docosahexaenoic Acid (DHA) (g)
1	15.45	3.01	0.14	0.19	0.62
4	87.97	10.97	0.19	0.04	0.07
8	23.70	2.31	0.16	0.02	0.04
9	12.09	1.74	0.13	0.03	0.05
13	12.17	1.22	0.06	0.00	0.02
18	11.76	1.20	0.08	0.00	0.01
19	3.31	0.26	0.04	0.00	0.01
Mean	23.78	2.96	0.12	0.04	0.12
Standard Deviation	28.94	3.64	0.06	0.07	0.22
Standard Error	10.94	1.38	0.02	0.03	0.08

Dietary Data: Combination Treatment

Subject	Total Vitamin A Activity (International Units)	Vitamin E (Total Alpha-Tocopherol) (mg)	Vitamin C (ascorbic acid) (mg)	Lycopene (mcg)	Zinc (mg)	Selenium (mcg)	Sodium (mg)	Caffeine (mg)
1	8181.36	25.17	244.77	9663.33	29.44	245.59	4800.75	17.24
4	13918.26	25.00	189.89	6812.64	18.19	254.35	8541.90	16.36
8	2389.40	10.79	14.06	8722.83	15.05	193.06	7374.84	1.53
9	11846.07	6.15	91.40	1600.53	13.53	110.05	3350.62	9.04
13	4579.65	7.13	68.46	12195.17	12.42	137.57	4241.15	71.27
18	3191.84	7.29	126.57	17966.38	14.04	148.78	4926.53	126.48
19	2547.68	1.46	43.67	16802.36	6.03	64.64	1413.61	12.03
Mean	6664.89	11.86	111.26	10537.61	15.53	164.86	4949.91	36.28
Standard Deviation	4715.17	9.44	82.14	5700.67	7.15	69.97	2393.43	45.93
Standard Error	1782.17	3.57	31.05	2154.65	2.70	26.44	904.63	17.36

Dietary Data: Combination Treatment

Subject	Total Grams	Energy (kcal)	Total Fat (g)	Total Carbohydrate (g)	Total Protein (g)	Total Dietary Fiber (g)
2	1288.60	1339.55	49.93	136.54	83.06	6.14
3	1957.10	1923.28	63.77	282.38	60.23	34.73
7	4126.06	3226.20	116.75	367.30	177.13	19.51
10	4408.36	1985.22	68.94	262.40	82.16	26.80
14	6453.09	2480.62	119.04	198.94	57.34	20.46
17	2457.68	2573.80	90.15	353.51	97.30	13.80
20	3925.75	768.91	36.23	68.09	44.37	8.78
Mean	3516.66	2042.51	77.83	238.45	85.94	18.60
Standard Deviation	1754.73	817.42	32.02	110.54	44.11	10.05
Standard Error	663.22	308.96	12.10	41.78	16.67	3.80

Subject	Linoleic Acid (g)	Linolenic Acid (g)	Arachidonic Acid (g)	Eicosapentaenoic Acid (EPA) (g)	Docosahexaenoic Acid (DHA) (g)
2	4.03	0.55	0.12	0.00	0.01
3	14.06	1.92	0.14	0.25	0.81
7	16.22	1.31	0.47	0.02	0.09
10	20.06	3.26	0.14	0.01	0.04
14	33.81	3.91	0.16	0.04	0.09
17	21.76	2.58	0.10	0.04	0.05
20	2.74	0.69	0.26	0.27	0.96
Mean	16.10	2.03	0.20	0.09	0.29
Standard Deviation	10.72	1.28	0.13	0.12	0.41
Standard Error	4.05	0.48	0.05	0.04	0.15

Dietary Data: Combination Treatment

Subject	Total Vitamin A Activity (International Units)	Vitamin E (Total Alpha-Tocopherol) (mg)	Vitamin C (ascorbic acid) (mg)	Lycopene (mcg)	Zinc (mg)	Selenium (mcg)	Sodium (mg)	Caffeine (mg)
2	548.14	2.53	16.19	6636.71	14.45	97.04	2906.57	90.09
3	7289.11	9.01	78.39	0.00	14.97	99.10	4454.68	0.00
7	6486.54	8.87	192.08	14582.54	24.14	276.45	5606.55	0.00
10	13327.20	16.35	117.19	4095.71	10.20	118.60	3513.29	199.02
14	14400.34	13.22	132.63	32989.45	7.45	71.39	4674.90	21.81
17	4446.47	6.66	74.41	5527.30	10.30	141.54	4572.68	5.00
20	2759.07	5.41	10.20	162.73	4.83	76.73	1928.91	244.35
Mean	7036.70	8.86	88.73	9142.06	12.33	125.84	3951.08	80.04
Standard Deviation	5184.41	4.69	64.76	11599.65	6.32	70.60	1243.40	102.54
Standard Error	1959.52	1.77	24.48	4384.26	2.39	26.68	469.96	38.76

APPENDIX D

RAW DATA FOR CHAPTER 3

Maximum Drop in FEV_1 (L)

Subject	Pre-Supplementation	Placebo	DHA
201	-1.00	-1.10	-0.77
202	-1.24	-1.15	-1.26
203	-0.60	-0.72	-1.78
204	-0.42	-0.23	-0.36
205	-0.54	-0.81	-0.50
206	-1.10	-0.48	-0.71
207	-0.86	-0.27	-0.18
208	-1.38	-1.15	-1.24
212	-0.67	-0.33	-0.28
Mean	-0.87	-0.69	-0.79
Standard Deviation	0.33	0.38	0.54
Standard Error	0.11	0.13	0.18

Maximum Drop in FEV1 (%)

Subject	Pre-Supplementation	Placebo	DHA
201	-19.65	-20.11	-15.10
202	-33.60	-32.76	-34.52
203	-21.43	-25.90	-31.27
204	-10.34	-6.10	-9.11
205	-10.69	-16.14	-10.20
206	-26.51	-11.29	-15.40
207	-18.82	-5.86	-3.80
208	-30.07	-27.06	-28.05
212	-18.51	-9.57	-7.95
Mean	-21.07	-17.20	-17.27
Standard Deviation	7.94	9.81	11.20
Standard Error	2.65	3.27	3.73

Maximum Drop in FVC (L)

Subject	Pre-Supplementation	Placebo	DHA
201	-0.68	-0.86	-0.22
202	-0.59	-0.67	-0.49
203	-0.47	-0.69	-1.10
204	-0.55	-0.05	-0.20
205	-0.63	-0.83	-0.67
206	-0.60	-0.38	-0.20
207	-0.88	-0.26	-0.19
208	-1.05	-0.93	-0.96
212	-0.50	-0.31	-0.37
Mean	-0.66	-0.55	-0.49
Standard Deviation	0.19	0.31	0.35
Standard Error	0.06	0.10	0.12

Maximum Drop in FVC (%)

Subject	Pre-Supplementation	Placebo	DHA
201	-9.34	-11.23	-3.19
202	-10.77	-13.06	-9.40
203	-11.49	-17.25	-28.06
204	-11.00	-1.08	-4.17
205	-10.23	-13.67	-11.19
206	-6.96	-6.82	-3.66
207	-19.13	-5.58	-3.97
208	-19.59	-18.13	-18.15
212	-11.60	-7.58	-8.71
Mean	-12.23	-10.49	-10.06
Standard Deviation	4.28	5.66	8.31
Standard Error	1.43	1.89	2.77

Maximum Drop in $FEF_{25-75\%}$ (L/s)

Subject	Pre-Supplementation	Placebo	DHA
201	-1.19	-1.36	-1.23
202	-1.28	-1.29	-1.23
203	-0.68	-0.68	-0.57
204	-0.41	-0.57	-0.62
205	-0.65	-0.90	-0.26
206	-1.47	-0.75	-2.03
207	-0.35	0.30	0.19
208	-3.35	-2.21	-2.63
212	-1.15	-0.70	-0.39
Mean	-1.17	-0.91	-0.97
Standard Deviation	0.91	0.68	0.90
Standard Error	0.30	0.23	0.30

Maximum Drop in $FEF_{25-75\%}$ (%)

Subject	Pre-Supplementation	Placebo	DHA
201	-32.16	-32.85	-29.71
202	-53.33	-53.31	-51.46
203	-35.60	-36.96	-36.59
204	-10.76	-15.83	-16.23
205	-13.29	-18.67	-5.92
206	-46.37	-21.37	-40.36
207	-4.88	3.66	2.99
208	-58.57	-48.46	-53.67
212	-29.11	-17.99	-10.66
Mean	-31.57	-26.86	-26.85
Standard Deviation	19.12	17.79	20.35
Standard Error	6.37	5.93	6.78

Pre-Eucapnic Voluntary Hyperventilation F_ENO (ppb)

Subject	Pre-Supplementation	Placebo	DHA
201	26.97	32.67	21.47
202	110.85	82.57	144.23
203	118.43	71.90	108.07
204	20.10	24.73	23.63
205	16.23	12.37	11.33
206	203.60	76.17	188.33
207	25.50	21.67	32.70
208	85.33	54.53	78.80
212	50.20	12.70	12.03
Mean	73.02	43.26	68.96
Standard Deviation	62.87	28.25	64.93
Standard Error	20.96	9.42	21.64

Post-Eucapnic Voluntary Hyperventilation F_ENO (ppb)

Subject	Pre-Supplementation	Placebo	DHA
201	30.63	29.43	14.77
202	86.30	60.10	109.07
203	119.00	66.13	98.60
204	19.03	26.27	23.60
205	12.57	8.87	9.17
206	155.37	76.13	161.63
207	23.13	17.50	33.60
208	81.40	33.87	79.43
212	44.10	9.20	8.97
Mean	63.50	36.39	59.87
Standard Deviation	50.02	25.09	54.61
Standard Error	16.67	8.36	18.20

Pre-Eucapnic Voluntary Hyperventilation Exhaled Breath Condensate pH

Subject	Pre-Supplementation	Placebo	DHA
201	6.66	7.12	6.93
202	7.11	7.29	6.82
203	7.02	-	7.23
204	6.56	7.11	6.95
205	7.41	7.01	7.08
206	6.95	7.12	6.79
207	6.78	7.26	6.59
208	6.58	7.14	6.83
212	6.83	6.68	-
Mean	6.88	7.09	6.90
Standard Deviation	0.28	0.19	0.19
Standard Error	0.09	0.07	0.07

Post-Eucapnic Voluntary Hyperventilation Exhaled Breath Condensate pH

Subject	Pre-Supplementation	Placebo	DHA
201	6.48	6.75	7.30
202	7.11	7.35	7.25
203	6.92	-	7.15
204	6.59	7.47	7.33
205	7.07	7.10	7.67
206	6.95	6.79	6.45
207	6.85	6.63	6.58
208	6.99	6.60	6.46
212	6.38	6.74	-
Mean	6.82	6.93	7.02
Standard Deviation	0.27	0.33	0.46
Standard Error	0.09	0.12	0.16

Daily Symptom Score

Subject	Pre-Supplementation	Placebo	Washout	DHA
201	1.59	1.60	-	-
202	2.05	0.68	0.65	0.33
203	1.56	0.71	0.93	1.23
204	0.73	0.25	0.80	0.73
205	0.05	0.03	0.00	0.03
206	1.14	0.83	0.93	1.07
207	0.76	0.64	0.63	0.48
208	0.75	0.68	0.75	0.79
212	1.91	1.46	1.25	1.00
Mean	1.17	0.76	0.74	0.70
Standard Deviation	0.66	0.50	0.36	0.41
Standard Error	0.22	0.17	0.12	0.14

Nightly Symptom Score

Subject	Pre-Supplementation	Placebo	Washout	DHA
201	0.00	0.00	-	-
202	0.07	0.00	0.00	0.04
203	0.15	0.00	0.00	0.00
204	0.00	0.00	0.00	0.00
205	0.00	0.00	0.00	0.00
206	0.21	0.00	0.00	0.00
207	0.00	0.00	0.00	0.00
208	0.08	0.00	0.00	0.00
212	0.82	0.00	0.08	0.14
Mean	0.15	0.00	0.01	0.02
Standard Deviation	0.26	0.00	0.03	0.05
Standard Error	0.09	0.00	0.01	0.02

Bronchodilator Use (number of puffs per day)

Subject	Pre-Supplementation	Placebo	Washout	DHA
201	1.13	0.60	-	-
202	0.36	0.00	0.00	0.19
203	0.10	0.00	0.00	0.00
204	0.00	0.00	0.00	0.00
205	0.00	0.00	0.00	0.00
206	0.29	0.15	0.21	0.32
207	0.00	0.00	0.00	0.00
208	0.31	0.09	0.00	0.30
212	0.27	0.05	0.00	0.00
Mean	0.27	0.10	0.03	0.10
Standard Deviation	0.35	0.20	0.08	0.14
Standard Error	0.12	0.07	0.03	0.05

Morning FEV_1 (L)

Subject	Pre-Supplementation	Placebo	Washout	DHA
201	5.45	5.49	-	-
202	3.09	2.60	2.75	2.73
203	1.96	1.47	1.54	2.02
204	3.46	3.30	2.80	2.83
205	4.07	4.12	3.96	4.12
206	4.24	4.10	4.10	4.18
207	3.03	3.46	4.03	3.09
208	4.44	4.65	4.58	4.68
212	3.48	3.26	3.42	3.43
Mean	3.69	3.61	3.40	3.38
Standard Deviation	1.00	1.17	0.99	0.89
Standard Error	0.33	0.39	0.33	0.30

Evening FEV_1 (L)

Subject	Pre-Supplementation	Placebo	Washout	DHA
201	5.80	5.87	-	-
202	2.89	2.61	2.52	2.60
203	2.44	1.54	1.64	2.44
204	3.73	3.36	2.62	2.84
205	4.08	4.18	4.08	4.20
206	4.29	4.23	4.30	4.26
207	3.03	3.37	3.82	3.19
208	4.53	4.69	4.60	4.84
212	3.28	3.40	3.46	3.55
Mean	3.79	3.69	3.38	3.49
Standard Deviation	1.03	1.24	1.03	0.87
Standard Error	0.34	0.41	0.34	0.29

Morning PEF (L/min)

Subject	Pre-Supplementation	Placebo	Washout	DHA
201	636.13	635.15	-	-
202	367.00	370.05	376.43	373.57
203	267.22	318.63	295.47	312.64
204	415.31	402.36	374.79	368.68
205	505.74	525.10	515.27	531.56
206	572.43	585.30	577.77	567.73
207	467.29	504.00	540.46	488.33
208	634.23	648.91	621.71	674.75
212	343.82	358.60	403.62	434.10
Mean	467.68	483.12	463.19	468.92
Standard Deviation	130.83	125.05	115.86	120.24
Standard Error	43.61	41.68	38.62	40.08

Evening PEF (L/min)

Subject	Pre-Supplementation	Placebo	Washout	DHA
201	697.56	698.50	-	-
202	332.07	366.00	340.17	349.75
203	363.95	317.84	270.68	306.66
204	423.08	407.86	369.64	357.71
205	516.84	534.30	535.09	560.78
206	585.64	605.65	603.67	600.80
207	479.47	503.16	522.46	502.90
208	642.46	652.18	638.86	686.20
212	307.45	380.95	418.38	428.19
Mean	483.17	496.27	462.37	474.12
Standard Deviation	138.98	136.28	132.14	135.51
Standard Error	46.33	45.43	44.05	45.17

8-Isoprostane Concentration (pg/µL)

Subject	Pre-Supplementation		Placebo		DHA	
	Pre	Post	Pre	Post	Pre	Post
201	1.43	0.00	6.68	4.19	9.79	10.39
202	0.00	0.00	3.74	1.07	1.88	0.00
204	0.00	2.44	1.63	0.00	6.37	2.02
205	10.56	-	2.24	1.62	1.28	1.78
206	3.98	8.60	13.90	0.04	1.36	0.00
207	5.61	0.00	1.00	3.61	5.02	4.67
208	0.00	-	13.94	13.74	5.67	27.30
Mean	3.08	2.21	6.16	3.47	4.48	6.59
Standard Deviation	3.96	3.73	5.61	4.82	3.17	9.81
Standard Error	1.50	1.67	2.12	1.82	1.20	3.71

Protectin D1 Concentration (pg/µL)

Subject	Pre-Supplementation		Placebo		DHA	
	Pre-EVH	Post-EVH	Pre-EVH	Post-EVH	Pre-EVH	Post-EVH
201	< 0	< 0	< 0	< 0	< 0	< 0
202	< 0	< 0	< 0	< 0	< 0	< 0
204	< 0	< 0	< 0	< 0	< 0	< 0
205	< 0	-	< 0	< 0	< 0	< 0
206	< 0	< 0	< 0	< 0	< 0	< 0
207	< 0	< 0	< 0	< 0	< 0	< 0
208	< 0	-	< 0	< 0	< 0	< 0

17*S*-Hydroxy-Docosahexaenoic Acid Concentration (pg/µL)

Subject	Pre-Supplementation		Placebo		DHA	
	Pre-EVH	Post-EVH	Pre-EVH	Post	Pre-EVH	Post-EVH
201	< 0	< 0	< 0	< 0	< 0	< 0
202	< 0	< 0	< 0	< 0	< 0	< 0
204	< 0	< 0	< 0	< 0	< 0	< 0
205	< 0	-	< 0	< 0	< 0	< 0
206	< 0	< 0	< 0	< 0	< 0	< 0
207	< 0	< 0	< 0	< 0	< 0	< 0
208	< 0	-	< 0	< 0	< 0	< 0

Dietary Data: Pre-Supplementation

Subject	Total Grams	Energy (kcal)	Total Fat (g)	Total Carbohydrate (g)	Total Protein (g)	Total Dietary Fiber (g)
201	7099.63	4711.06	158.70	617.26	230.56	64.89
202	3783.10	2232.19	50.43	322.15	65.24	22.67
203	2587.24	2192.12	94.14	232.51	107.81	27.28
204	2143.08	1408.92	66.34	163.50	51.00	20.27
205	4463.34	4999.96	210.59	640.23	155.62	30.18
206	1342.55	1559.86	64.80	158.67	78.05	12.32
207	3155.40	3766.07	149.85	425.81	174.74	24.72
208	2905.10	1421.32	45.69	159.04	75.44	17.02
212	1178.92	1127.69	41.41	131.77	59.39	10.33
Mean	3184.26	2602.13	98.00	316.77	110.87	25.52
Standard Deviation	1811.68	1497.30	60.59	200.48	62.32	16.16
Standard Error	603.89	499.10	20.20	66.83	20.77	5.39

Dietary Data: Pre-Supplementation

Subject	Linoleic Acid (g)	Linolenic Acid (g)	Arachidonic Acid (g)	Eicosapentaenoic Acid (EPA) (g)	Docosahexaenoic Acid (DHA) (g)
201	28.70	3.60	0.48	0.02	0.09
202	9.87	1.11	0.05	0.00	0.01
203	17.13	1.53	0.24	0.04	0.08
204	17.19	1.18	0.12	0.01	0.03
205	38.63	4.75	0.18	0.04	0.07
206	9.98	1.31	0.12	0.00	0.02
207	21.94	2.22	0.25	0.06	0.09
208	7.76	0.81	0.15	0.01	0.04
212	6.75	0.74	0.14	0.01	0.04
Mean	17.55	1.92	0.19	0.02	0.05
Standard Deviation	10.70	1.38	0.12	0.02	0.03
Standard Error	3.57	0.46	0.04	0.01	0.01

Dietary Data: Pre-Supplementation

Subject	Total Vitamin A Activity (International Units)	Vitamin E (Total Alpha-Tocopherol) (mg)	Vitamin C (ascorbic acid) (mg)	Lycopene (mcg)	Zinc (mg)	Selenium (mcg)	Sodium (mg)	Caffeine (mg)
201	51570.77	58.07	510.71	13821.27	31.31	313.51	8324.48	2.79
202	7622.10	18.06	87.39	13387.01	10.97	84.73	3115.62	3.55
203	13734.50	17.48	101.72	11173.57	14.96	148.20	4321.82	174.64
204	3716.97	19.96	77.40	2792.82	6.10	79.19	1839.65	11.81
205	7624.55	28.24	164.30	13667.89	18.67	271.95	7861.19	142.15
206	5533.29	6.99	56.10	8227.38	10.82	102.11	3223.10	48.80
207	5143.27	17.16	113.37	15264.53	24.57	212.20	6897.74	16.60
208	10744.47	9.91	104.72	5519.08	8.72	98.97	2964.98	172.48
212	2810.80	5.71	30.36	5237.50	8.20	74.47	2109.33	48.17
Mean	12055.64	20.18	138.45	9899.01	14.92	153.93	4517.55	69.00
Standard Deviation	15209.36	15.85	144.55	4560.34	8.44	90.29	2509.94	73.10
Standard Error	5069.79	5.28	48.18	1520.11	2.81	30.10	836.65	24.37

Dietary Data: Placebo Supplementation

Subject	Total Grams	Energy (kcal)	Total Fat (g)	Total Carbohydrate (g)	Total Protein (g)	Total Dietary Fiber (g)
201	6765.48	4543.44	169.98	536.34	231.66	51.06
202	2056.53	1182.31	34.59	142.43	44.05	14.84
203	3676.24	2567.37	99.90	301.43	113.61	27.32
204	2107.03	1163.72	36.01	171.26	42.99	15.59
205	3701.03	2555.67	98.23	335.46	92.57	19.19
206	968.50	1075.53	46.01	117.48	53.15	9.32
207	2731.63	3339.44	142.04	367.08	143.56	23.67
208	2434.72	1277.03	45.05	137.68	64.39	13.87
212	1585.11	1581.33	49.00	204.11	81.02	19.31
Mean	2891.81	2142.87	80.09	257.03	96.33	21.57
Standard Deviation	1704.05	1205.76	50.01	139.31	60.78	12.29
Standard Error	568.02	401.92	16.67	46.44	20.26	4.10

Dietary Data: Placebo Supplementation

Subject	Linoleic Acid (g)	Linolenic Acid (g)	Arachidonic Acid (g)	Eicosapentaenoic Acid (EPA) (g)	Docosahexaenoic Acid (DHA) (g)
201	22.10	3.18	0.56	0.01	0.05
202	6.53	0.53	0.03	0.01	0.01
203	14.66	1.79	0.21	0.03	0.12
204	8.03	0.97	0.16	0.01	0.04
205	16.09	1.82	0.12	0.01	0.03
206	10.83	1.23	0.06	0.00	0.01
207	25.96	2.69	0.17	0.05	0.07
208	6.94	0.69	0.15	0.01	0.03
212	7.86	0.88	0.19	0.02	0.05
Mean	13.22	1.53	0.18	0.02	0.05
Standard Deviation	7.05	0.92	0.15	0.02	0.03
Standard Error	2.35	0.31	0.05	0.01	0.01

Dietary Data: Placebo Supplementation

Subject	Total Vitamin A Activity (International Units)	Vitamin E (Total Alpha-Tocopherol) (mg)	Vitamin C (ascorbic acid) (mg)	Lycopene (mcg)	Zinc (mg)	Selenium (mcg)	Sodium (mg)	Caffeine (mg)
201	64984.94	24.48	320.97	16013.10	34.34	300.25	7490.73	8.68
202	10226.71	8.25	92.22	6032.92	6.71	48.96	1791.69	2.03
203	16022.46	14.60	197.50	12333.07	15.68	144.75	4218.60	160.77
204	6138.41	14.13	87.30	2190.94	5.41	53.98	1756.03	12.96
205	2917.27	12.03	103.19	3614.98	10.32	116.13	4104.52	206.36
206	2251.56	5.90	36.42	5090.72	7.37	63.02	2128.48	53.80
207	4899.61	17.17	101.80	15118.77	20.28	184.52	6299.31	8.80
208	4212.94	7.63	76.63	5344.13	8.13	77.87	2410.88	125.00
212	2478.03	7.39	31.41	5405.74	9.78	129.37	3346.17	6.23
Mean	12681.33	12.40	116.38	7904.93	13.11	124.32	3727.38	64.96
Standard Deviation	20112.68	5.96	90.45	5155.96	9.26	80.33	2038.05	78.53
Standard Error	6704.23	1.99	30.15	1718.65	3.09	26.78	679.35	26.18

Dietary Data: Washout

Subject	Total Grams	Energy (kcal)	Total Fat (g)	Total Carbohydrate (g)	Total Protein (g)	Total Dietary Fiber (g)
201	-	-	-	-	-	-
202	2882.23	1299.28	32.65	173.73	44.62	18.40
203	3197.90	2229.40	93.10	220.79	113.19	18.46
204	1930.81	1485.60	41.44	230.98	56.38	23.94
205	2374.76	2080.69	81.74	292.40	52.67	12.75
206	880.39	811.93	34.72	87.17	36.57	6.42
207	2143.02	2550.93	116.34	252.65	120.03	14.32
208	3201.98	1183.96	38.62	119.14	59.43	13.65
212	1586.49	1600.99	53.73	132.81	74.06	7.59
Mean	2274.70	1655.35	61.54	188.71	69.62	14.44
Standard Deviation	814.56	586.11	31.51	71.87	31.04	5.82
Standard Error	287.99	207.22	11.14	25.41	10.97	2.06

Dietary Data: Washout

Subject	Linoleic Acid (g)	Linolenic Acid (g)	Arachidonic Acid (g)	Eicosapentaenoic Acid (EPA) (g)	Docosahexaenoic Acid (DHA) (g)
201	-	-	-	-	-
202	6.28	0.58	0.04	0.00	0.01
203	13.05	1.53	0.35	0.09	0.32
204	8.14	1.08	0.19	0.02	0.09
205	20.32	2.50	0.05	0.01	0.01
206	8.13	1.01	0.03	0.00	0.00
207	19.19	1.90	0.22	0.06	0.09
208	5.15	0.57	0.27	0.00	0.02
212	7.88	0.71	0.14	0.01	0.03
Mean	11.02	1.23	0.16	0.02	0.07
Standard Deviation	5.87	0.69	0.12	0.03	0.11
Standard Error	2.07	0.24	0.04	0.01	0.04

Dietary Data: Washout

Subject	Total Vitamin A Activity (International Units)	Vitamin E (Total Alpha-Tocopherol) (mg)	Vitamin C (ascorbic acid) (mg)	Lycopene (mcg)	Zinc (mg)	Selenium (mcg)	Sodium (mg)	Caffeine (mg)
201	-	-	-	-	-	-	-	-
202	6616.50	7.86	79.59	7392.08	6.83	57.03	2108.48	4.92
203	8901.72	10.54	114.69	9610.77	13.45	140.28	3550.94	158.23
204	13163.91	22.37	162.01	1905.87	8.05	64.54	1915.61	14.86
205	2736.51	11.74	83.05	9253.64	6.18	97.17	2951.41	85.07
206	5999.92	4.37	21.22	4085.15	5.36	40.31	1578.35	57.86
207	3309.85	12.86	57.68	5351.26	17.17	149.31	4451.82	12.60
208	14491.77	6.12	63.74	5213.52	8.14	84.00	2294.48	353.55
212	3063.08	12.80	29.56	6646.79	11.15	102.57	2622.11	8.84
Mean	7285.41	11.08	76.44	6182.39	9.54	91.90	2684.15	86.99
Standard Deviation	4559.55	5.53	45.67	2596.20	4.08	38.67	944.76	119.86
Standard Error	1612.04	1.96	16.15	917.90	1.44	13.67	334.02	42.38

Dietary Data: DHA Supplementation

Subject	Total Grams	Energy (kcal)	Total Fat (g)	Total Carbohydrate (g)	Total Protein (g)	Total Dietary Fiber (g)
201	6195.58	3746.27	118.65	514.40	175.22	58.18
202	1582.56	1439.59	35.54	198.76	60.58	19.90
203	2649.15	1939.18	76.97	218.93	92.28	19.74
204	1889.39	1437.29	44.77	204.27	56.00	21.87
205	2623.45	1921.24	72.19	286.76	41.07	11.25
206	1240.35	1272.81	45.89	144.18	54.84	9.26
207	2369.41	2328.40	106.06	222.21	113.14	13.27
208	4168.53	1367.68	40.67	155.62	65.28	17.59
212	1623.51	1593.54	47.04	187.10	75.64	10.64
Mean	2704.66	1894.00	65.31	236.91	81.56	20.19
Standard Deviation	1568.77	773.84	30.24	111.93	41.28	14.96
Standard Error	522.92	257.95	10.08	37.31	13.76	4.99

Dietary Data: DHA Supplementation

Subject	Linoleic Acid (g)	Linolenic Acid (g)	Arachidonic Acid (g)	Eicosapentaenoic Acid (EPA) (g)	Docosahexaenoic Acid (DHA) (g)
201	17.74	2.16	0.44	0.01	0.05
202	8.04	0.92	0.10	0.07	0.20
203	11.99	1.21	0.17	0.04	0.06
204	8.73	1.09	0.24	0.02	0.10
205	18.62	2.35	0.04	0.00	0.01
206	11.03	1.43	0.07	0.00	0.01
207	14.56	1.65	0.19	0.01	0.04
208	6.37	0.71	0.14	0.01	0.04
212	7.04	0.81	0.11	0.01	0.02
Mean	11.57	1.37	0.17	0.02	0.06
Standard Deviation	4.55	0.58	0.12	0.02	0.06
Standard Error	1.52	0.19	0.04	0.01	0.02

Dietary Data: DHA Supplementation

Subject	Total Vitamin A Activity (International Units)	Vitamin E (Total Alpha-Tocopherol) (mg)	Vitamin C (ascorbic acid) (mg)	Lycopene (mcg)	Zinc (mg)	Selenium (mcg)	Sodium (mg)	Caffeine (mg)
201	48047.47	21.18	332.92	7294.08	26.41	238.53	5842.59	7.56
202	6968.95	9.67	47.63	14993.36	7.50	97.53	3065.22	3.70
203	11077.38	15.02	78.81	11149.61	13.66	133.53	3253.59	141.43
204	13482.97	12.05	131.22	2017.23	6.25	72.31	2033.72	6.47
205	1650.69	8.79	71.70	2359.42	5.15	57.51	2169.47	106.07
206	2109.61	15.50	57.25	3517.52	11.83	72.01	2197.95	60.34
207	4029.20	9.64	55.33	6378.76	15.53	123.17	3691.23	8.06
208	6798.72	7.48	95.96	5943.88	7.79	78.92	2593.96	383.18
212	3416.67	16.76	40.56	6614.26	12.46	98.58	2755.26	9.59
Mean	10842.41	12.90	101.27	6696.46	11.84	108.01	3067.00	80.71
Standard Deviation	14512.54	4.51	91.23	4190.93	6.53	54.87	1176.74	124.18
Standard Error	4837.51	1.50	30.41	1396.98	2.18	18.29	392.25	41.39

APPENDIX E

RAW DATA FOR CHAPTER 4

Post-24 Incubation: Control with Vehicle Treatment Force (% of Pre-Incubation Maximum Force)

	Dose of Acetylcholine (- log M)										
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**	**4.5**	**4**
1	0.00	0.06	-0.06	0.19	3.37	9.19	26.08	49.26	69.77	85.57	96.96
2	0.00	0.09	0.09	0.34	2.05	9.39	27.92	53.54	76.94	92.66	101.79
3	0.00	0.00	0.35	0.35	0.83	5.38	21.05	41.10	60.56	77.41	92.61
4	0.00	0.00	0.00	0.07	0.92	6.24	23.24	43.07	62.90	79.19	93.70
5	0.00	0.55	1.00	1.37	3.65	17.88	44.62	68.61	83.67	91.33	93.98
6	0.00	0.31	1.03	3.21	16.55	43.23	70.42	89.45	98.66	103.72	104.76
7	0.00	0.00	0.16	0.78	6.60	30.15	57.11	78.01	90.75	97.90	100.08
Mean	0.00	0.14	0.37	0.90	4.85	17.35	38.64	60.44	77.61	89.68	97.70
Standard Deviation	0.00	0.21	0.46	1.11	5.52	14.32	19.17	18.55	14.27	9.61	4.63
Standard Error of the Mean	0.00	0.08	0.18	0.42	2.09	5.41	7.25	7.01	5.39	3.63	1.75

Post-24 Incubation: Fish Oil Treatment Force (% of Pre-Incubation Maximum Force)

	Dose of Acetylcholine (- log M)										
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**	**4.5**	**4**
1	0.00	0.06	0.06	0.94	1.00	5.97	22.95	44.91	65.38	81.75	94.41
2	0.00	0.00	-0.07	0.43	1.35	6.45	22.04	41.46	60.31	74.91	83.56
3	0.00	0.00	0.00	0.32	1.86	6.40	20.50	42.14	61.91	77.80	90.19
4	0.00	0.13	0.13	0.25	1.50	8.27	25.06	50.13	74.94	94.36	109.52
5	0.00	-0.36	-0.43	0.36	3.75	25.38	53.64	76.93	93.80	104.04	108.44
6	0.00	0.24	0.79	1.74	6.58	28.21	52.06	73.85	89.22	98.18	102.85
7	0.00	0.18	0.36	1.43	10.98	38.30	63.93	85.36	99.20	106.96	110.89
Mean	0.00	0.03	0.12	0.78	3.86	17.00	37.17	59.25	77.82	91.14	99.98
Standard Deviation	0.00	0.20	0.38	0.60	3.70	13.36	18.55	18.73	16.15	12.95	10.70
Standard Error of the Mean	0.00	0.07	0.14	0.23	1.40	5.05	7.01	7.08	6.10	4.89	4.04

Post-24 Incubation: Maximum Force Generated (% of Pre-Incubation Maximum Force)

Tissue	Vehicle	Fish Oil
1	96.96	94.41
2	101.79	83.56
3	92.61	90.19
4	93.70	109.52
5	93.98	108.44
6	104.76	102.85
7	100.08	110.89
Mean	97.70	99.98
Standard Deviation	4.63	10.70
Standard Error of the Mean	1.75	4.04

Post-24 Incubation: Effective Dose 50 (- log M Acetylcholine)

Tissue	Vehicle	Fish Oil
1	5.48	5.41
2	5.53	5.47
3	5.31	5.38
4	5.36	5.37
5	5.94	5.97
6	6.33	6.01
7	6.12	6.17
Mean	5.73	5.68
Standard Deviation	0.40	0.35
Standard Error of the Mean	0.15	0.13

Post-24 Incubation: Control with Vehicle Treatment Force (% of Pre-Incubation Maximum Force)

	Dose of 5-Hydroxytryptamine (- log M)										
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**	**4.5**	**4**
1	0.00	-0.22	-0.11	0.87	12.28	42.50	66.20	70.43	63.37	55.65	53.04
2	0.00	-0.16	-0.62	-0.16	7.49	39.16	60.22	54.45	45.40	41.65	42.75
3	0.00	0.53	3.20	30.09	71.77	93.87	102.26	97.47	87.48	80.83	76.70
4	0.00	0.00	0.57	10.51	52.98	73.86	80.82	76.56	70.31	62.50	56.11
5	0.00	-0.45	0.68	24.04	60.32	78.57	86.85	85.71	79.59	74.04	68.71
6	0.00	-0.24	0.48	10.79	45.82	72.12	80.48	74.42	62.79	50.06	41.58
Mean	0.00	-0.09	0.70	12.69	41.78	66.68	79.47	76.51	68.16	60.79	56.48
Standard Deviation	0.00	0.34	1.32	12.20	26.20	21.47	14.99	14.51	14.69	14.75	14.01
Standard Error of the Mean	0.00	0.14	0.54	4.98	10.69	8.76	6.12	5.92	6.00	6.02	5.72

Post-24 Incubation: Fish Oil Treatment Force
(% of Pre-Incubation Maximum Force)

	Dose of 5-Hydroxytryptamine (- log M)										
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**	**4.5**	**4**
1	0.00	0.00	0.47	1.49	16.34	47.71	70.21	76.47	72.92	68.35	68.07
2	0.00	-0.08	0.00	1.41	9.96	37.33	69.65	84.31	83.29	74.82	70.90
3	0.00	0.44	1.40	26.21	66.17	83.70	90.80	91.50	87.29	81.68	81.33
4	0.00	0.15	1.92	17.78	50.96	77.55	91.57	95.79	92.11	77.01	76.78
5	0.00	-0.56	-0.28	12.58	60.59	86.65	98.25	101.40	100.00	84.21	83.93
Mean	0.00	-0.01	0.70	11.89	40.80	66.59	84.10	89.89	87.12	77.21	76.20
Standard Deviation	0.00	0.37	0.93	10.70	25.92	22.51	13.25	9.76	10.09	6.19	6.72
Standard Error of the Mean	0.00	0.16	0.42	4.79	11.59	10.07	5.93	4.36	4.51	2.77	3.00

Post-24 Incubation: Maximum Force Generated (% of Pre-Incubation Maximum Force)

Tissue	Vehicle	Fish Oil
1	70.43	76.47
2	60.22	84.31
3	102.26	91.50
4	80.82	95.79
5	86.85	101.40
6	80.48	
Mean	80.18	89.89
Standard Deviation	14.33	9.76
Standard Error of the Mean	5.85	4.36

Post-24 Incubation: Effective Dose 50 (- log M 5-Hydroxytryptamine)

Tissue	**Vehicle**	**Fish Oil**
1	6.68	6.68
2	6.73	6.48
3	7.34	7.29
4	7.19	7.11
5	7.30	7.13
6	7.19	
Mean	7.07	6.94
Standard Deviation	0.29	0.34
Standard Error of the Mean	0.12	0.15

Post-15 Incubation: Control Treatment Force
(% of Pre-Incubation Maximum Force)

	Dose of Acetylcholine (- log M)										
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**	**4.5**	**4**
1	0.00	-0.21	-0.21	1.04	5.62	19.69	40.78	58.74	71.15	80.10	85.30
2	0.00	0.12	0.31	0.75	3.43	13.67	32.21	50.50	62.48	67.54	67.98
3	0.00	-0.06	0.00	0.28	2.49	11.22	28.47	45.77	58.98	67.05	71.75
4	0.00	0.00	0.08	0.62	3.39	16.41	38.29	57.63	67.10	67.33	62.56
Mean	0.00	-0.03	0.05	0.67	3.73	15.25	34.94	53.16	64.93	70.51	71.90
Standard Deviation	0.00	0.14	0.21	0.32	1.33	3.64	5.62	6.13	5.32	6.40	9.70
Standard Error of the Mean	0.00	0.07	0.11	0.16	0.67	1.82	2.81	3.07	2.66	3.20	4.85

Post-15 Incubation: Control with Vehicle Treatment Force (% of Pre-Incubation Maximum Force)

	Dose of Acetylcholine (- log M)										
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**	**4.5**	**4**
1	0.00	-0.24	-0.16	-0.24	0.49	5.83	18.96	33.47	45.95	55.67	62.80
2	0.00	-0.09	0.00	0.28	1.94	13.24	31.11	46.76	58.52	66.67	71.94
3	0.00	-0.27	-0.09	1.79	16.77	41.52	60.81	73.81	82.51	83.50	83.32
4	0.00	0.88	2.96	8.72	21.20	38.08	53.84	64.64	70.16	72.16	70.96
5	0.00	0.07	0.07	0.39	1.31	7.73	23.26	39.32	50.00	55.24	56.68
6	0.00	0.00	0.05	1.04	2.70	14.78	33.77	51.76	64.68	70.64	71.84
7	0.00	-0.07	-0.07	0.07	2.40	10.30	24.42	39.03	49.54	55.33	57.16
Mean	0.00	0.04	0.39	1.72	6.69	18.79	35.17	49.83	60.19	65.60	67.82
Standard Deviation	0.00	0.39	1.13	3.16	8.53	14.71	16.04	14.73	13.17	10.81	9.54
Standard Error of the Mean	0.00	0.15	0.43	1.19	3.22	5.56	6.06	5.57	4.98	4.09	3.61

Post-15 Incubation: Soybean Oil Treatment Force (% of Pre-Incubation Maximum Force)

	Dose of Acetylcholine (- log M)										
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**	**4.5**	**4**
1	0.00	-0.45	0.15	5.65	26.28	49.10	68.45	81.85	89.98	93.30	93.22
2	0.00	0.13	1.86	11.97	31.52	53.46	74.34	89.63	96.94	99.73	99.07
3	0.00	-0.16	0.47	3.57	19.53	44.19	65.74	82.79	89.92	92.09	91.63
4	0.00	-0.31	0.63	7.85	31.24	55.26	75.35	87.28	93.56	96.39	97.17
5	0.00	-0.22	0.00	4.29	25.87	48.12	56.91	68.96	75.54	78.42	78.20
6	0.00	-0.33	0.25	7.77	34.88	60.91	80.83	94.30	103.47	106.28	103.31
7	0.00	0.63	3.07	11.66	28.93	50.27	69.08	82.37	90.51	91.86	89.06
8	0.00	0.13	0.19	0.76	2.40	12.65	31.18	50.92	66.86	77.17	82.16
9	0.00	0.75	1.71	3.54	7.45	15.96	29.46	44.40	57.58	66.79	72.58
10	0.00	0.00	0.16	0.85	4.35	15.85	32.40	48.80	61.23	68.14	71.33
Mean	0.00	0.02	0.85	5.79	21.24	40.58	58.37	73.13	82.56	87.02	87.77
Standard Deviation	0.00	0.41	1.02	3.98	12.16	18.34	19.92	18.57	16.03	13.52	11.20
Standard Error of the Mean	0.00	0.13	0.32	1.26	3.85	5.80	6.30	5.87	5.07	4.28	3.54

Post-15 Incubation: Fish Oil Treatment Force
(% of Pre-Incubation Maximum Force)

	Dose of Acetylcholine (- log M)										
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**	**4.5**	**4**
1	0.00	-0.42	-0.34	0.68	9.68	32.85	53.14	67.66	77.16	83.36	84.72
2	0.00	0.14	0.29	0.57	2.93	17.51	40.60	59.19	72.19	81.13	85.85
3	0.00	-0.39	0.29	4.55	27.62	54.07	76.36	92.05	100.87	105.23	107.36
4	0.00	-0.23	1.04	8.24	28.65	52.67	72.62	85.15	93.85	92.92	91.76
5	0.00	-0.16	0.24	6.05	26.37	50.16	69.92	84.11	93.31	98.39	100.32
6	0.00	-0.39	-0.26	1.29	15.44	38.48	60.10	75.80	84.94	90.48	91.63
7	0.00	-0.22	0.00	4.29	25.87	48.12	56.91	68.96	75.54	78.42	78.20
8	0.00	-0.33	0.25	7.77	34.88	60.91	80.83	94.30	103.47	106.28	103.31
9	0.00	0.63	3.07	11.66	28.93	50.27	69.08	82.37	90.51	91.86	89.06
10	0.00	0.07	0.29	0.43	1.64	11.62	29.58	47.90	62.72	72.20	77.05
11	0.00	0.11	0.28	0.83	4.02	16.39	34.82	53.85	68.92	78.49	83.44
Mean	0.00	-0.11	0.47	4.21	18.73	39.37	58.54	73.76	83.95	88.98	90.25
Standard Deviation	0.00	0.32	0.94	3.85	12.26	17.29	17.35	15.52	13.52	11.29	9.93
Standard Error of the Mean	0.00	0.10	0.28	1.16	3.70	5.21	5.23	4.68	4.08	3.40	2.99

Post-15 Incubation: Maximum Force Generated (% of Pre-Incubation Maximum Force)

Tissue	Control	Vehicle	Soybean Oil	Fish Oil
1	85.2982	62.80	93.2982	84.7199
2	67.9775	71.94	99.734	85.847
3	71.7523	83.50	92.093	107.364
4	67.3344	72.16	97.1743	93.8515
5		56.68	78.4183	100.323
6		71.84	106.281	91.6345
7		57.16	91.8626	78.4183
8			82.1632	106.281
9			72.5763	91.8626
10			71.3287	77.0492
11				83.4433
Mean	73.0906	68.01	88.493	90.9813
Standard Deviation	8.36848	9.66	11.809	10.361
Standard Error of the Mean	4.18424	3.65	3.73432	3.12396

Post-15 Incubation: Effective Dose 50 (- log M Acetylcholine)

Tissue	Control	Vehicle	Soybean Oil	Fish Oil
1	5.9399	5.55	6.5532	6.2615
2	5.9396	5.84	6.6028	5.9202
3	5.7819	6.46	6.4473	6.5119
4	6.115	6.57	6.6508	6.6271
5		5.84	6.6721	6.5047
6		5.93	6.6586	6.3369
7		5.85	6.6182	6.6721
8			5.7392	6.6586
9			5.7432	6.6182
10			5.8993	5.7473
11				5.8145
Mean	5.9441	6.01	6.35847	6.33391
Standard Deviation	0.13608	0.37	0.39718	0.35181
Standard Error of the Mean	0.06804	0.14	0.1256	0.10608

Post-4 Incubation: Control Treatment Force (% of Pre-Incubation Maximum Force)

	Dose of Acetylcholine (- log M)								
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**
1	0.00	0.00	0.18	11.92	35.32	53.81	70.29	83.87	93.87
2	0.00	0.40	2.61	18.79	37.69	57.39	74.17	85.93	93.07
3	0.00	0.00	4.56	24.42	44.20	63.71	80.14	91.23	98.28
Mean	0.00	0.13	2.45	18.38	39.07	58.30	74.87	87.01	95.07
Standard Deviation	0.00	0.23	2.20	6.26	4.60	5.01	4.96	3.80	2.81
Standard Error of the Mean	0.00	0.13	1.27	3.61	2.65	2.90	2.86	2.19	1.62

Post-4 Incubation: Control with Vehicle Treatment Force
(% of Pre-Incubation Maximum Force)

	Dose of Acetylcholine (- log M)								
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**
1	0.00	0.00	0.00	13.45	32.05	51.63	71.81	87.93	97.73
2	0.00	-0.18	0.91	9.15	26.35	45.47	65.78	85.45	99.54
3	0.00	0.47	5.22	16.79	33.02	50.93	68.47	82.37	92.54
4	0.00	1.04	23.75	38.96	55.52	69.79	81.35	91.15	97.29
5	0.00	0.43	5.18	27.86	50.86	66.74	79.59	90.28	97.62
Mean	0.00	0.35	7.01	21.24	39.56	56.91	73.40	87.44	96.94
Standard Deviation	0.00	0.48	9.66	12.09	12.81	10.69	6.83	3.60	2.62
Standard Error of the Mean	0.00	0.21	4.32	5.41	5.73	4.78	3.05	1.61	1.17

Post-4 Incubation: Soybean Oil Treatment Force (% of Pre-Incubation Maximum Force)

	Dose of Acetylcholine (- log M)								
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**
1	0.00	0.25	5.11	21.30	39.98	60.65	79.83	94.77	102.12
2	0.00	-0.42	-0.34	10.14	28.13	47.72	66.55	82.77	92.82
3	0.00	0.27	0.69	14.42	37.09	58.65	78.02	94.23	103.85
4	0.00	0.21	5.37	27.28	51.77	71.43	86.57	97.64	104.73
5	0.00	15.38	25.41	34.62	45.33	56.32	66.62	73.35	77.47
6	0.00	0.25	9.27	26.31	50.93	69.06	81.53	90.73	96.04
Mean	0.00	2.66	7.59	22.34	42.20	60.64	76.52	88.91	96.17
Standard Deviation	0.00	6.24	9.40	8.98	9.02	8.68	8.21	9.19	10.28
Standard Error of the Mean	0.00	2.55	3.84	3.67	3.68	3.54	3.35	3.75	4.20

Post-4 Incubation: Fish Oil Treatment Force (% of Pre-Incubation Maximum Force)

	Dose of Acetylcholine (- log M)								
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**
1	0.00	0.83	2.28	21.70	39.25	58.67	76.53	90.76	97.20
2	0.00	0.87	4.87	24.57	45.45	61.26	76.52	87.88	96.21
3	0.00	0.48	1.75	9.79	26.97	47.57	66.27	83.29	96.58
4	9.10	17.06	27.01	43.70	60.95	75.83	88.44	97.16	102.94
5	0.00	2.82	17.00	39.73	59.33	74.45	87.07	95.72	100.00
Mean	1.82	4.41	10.58	27.90	46.39	63.56	78.96	90.96	98.59
Standard Deviation	4.07	7.13	11.08	13.85	14.22	11.77	9.06	5.69	2.85
Standard Error of the Mean	1.82	3.19	4.95	6.19	6.36	5.26	4.05	2.54	1.28

Post-4 Incubation: Maximum Force Generated (% of Pre-Incubation Maximum Force)

Tissue	Control	Vehicle	Soybean Oil	Fish Oil
1	93.865	97.73	102.1171	97.1963
2	93.0653	99.54	92.82095	96.2121
3	98.2803	92.54	103.8462	96.5792
4		97.29	104.7261	102.938
5		97.62	77.47253	100
6			96.0371	
Mean	95.0702	96.94	96.16998	98.5852
Standard Deviation	2.80863	2.62	10.27937	2.85205
Standard Error of the Mean	1.62156	1.17	4.196534	1.27548

Post-4 Incubation: Effective Dose 50 (- log M Acetylcholine)

Tissue	Control	Vehicle	Soybean Oil	Fish Oil
1	6.6907	6.57	6.7526	6.7868
2	6.8028	6.35	6.5239	6.9487
3	6.9232	6.63	6.6565	6.4335
4		7.18	7.015	7.3082
5		7.08	7.2609	7.1936
6			7.0928	
Mean	6.80557	6.76	6.883617	6.93416
Standard Deviation	0.11627	0.35	0.283374	0.34634
Standard Error of the Mean	0.06713	0.16	0.115687	0.15489

Post-4 Incubation: Control Treatment Force (% of Pre-Incubation Maximum Force)

	Dose of Acetylcholine (- log M)								
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**
1	0.00	0.27	17.54	32.73	70.59	91.10	106.83	110.88	112.23
2	0.00	0.56	20.56	51.26	75.07	87.81	94.33	94.88	92.37
3	0.00	0.08	0.17	28.67	64.72	84.99	96.01	99.41	98.22
Mean	0.00	0.30	12.75	37.55	70.13	87.97	99.06	101.72	100.94
Standard Deviation	0.00	0.24	11.00	12.04	5.19	3.06	6.79	8.25	10.20
Standard Error of the Mean	0.00	0.14	6.35	6.95	3.00	1.77	3.92	4.76	5.89

Post-4 Incubation: Control with Vehicle Treatment Force (% of Pre-Incubation Maximum Force)

	Dose of Acetylcholine (- log M)								
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**
1	4.24	9.28	18.92	36.43	65.87	85.59	97.70	100.62	99.73
2	1.20	3.00	21.46	43.69	71.16	88.33	97.42	99.83	100.17
Mean	2.72	6.14	20.19	40.06	68.51	86.96	97.56	100.22	99.95
Standard Deviation	2.15	4.44	1.79	5.14	3.74	1.94	0.20	0.56	0.31
Standard Error of the Mean	1.52	3.14	1.27	3.63	2.64	1.37	0.14	0.40	0.22

Post-4 Incubation: Soybean Oil Treatment Force (% of Pre-Incubation Maximum Force)

	Dose of 5-Hydroxytryptamine (- log M)								
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**
1	0.00	4.21	13.36	28.32	56.28	81.55	93.54	98.11	94.77
2	32.31	40.96	48.65	59.74	79.74	95.72	106.90	110.66	109.87
3	0.00	0.28	14.09	33.21	58.30	79.48	90.76	94.03	93.10
Mean	10.77	15.15	25.36	40.42	64.77	85.58	97.07	100.93	99.25
Standard Deviation	18.66	22.44	20.17	16.90	13.00	8.84	8.63	8.66	9.24
Standard Error of the Mean	10.77	12.95	11.64	9.76	7.50	5.10	4.98	5.00	5.33

Post-4 Incubation: Fish Oil Treatment Force (% of Pre-Incubation Maximum Force)

	Dose of 5-Hydroxytryptamine (- log M)								
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**
1	0.00	-0.19	11.03	32.62	62.41	84.66	96.13	100.64	99.42
2	0.00	9.62	17.91	29.81	57.12	76.06	88.55	93.98	93.17
Mean	0.00	4.71	14.47	31.22	59.77	80.36	92.34	97.31	96.30
Standard Deviation	0.00	6.94	4.87	1.99	3.74	6.07	5.36	4.71	4.42
Standard Error of the Mean	0.00	4.91	3.44	1.41	2.64	4.30	3.79	3.33	3.12

Post-4 Incubation: Maximum Force Generated (% of Pre-Incubation Maximum Force)

Tissue	Control	Vehicle	Soybean Oil	Fish Oil
1	112.23	100.62	98.11	100.64
2	94.88	100.17	110.66	93.98
3	99.41		94.03	
Mean	102.17	100.40	100.93	97.31
Standard Deviation	9.00	0.32	8.66	4.71
Standard Error of the Mean	5.20	0.22	5.00	3.33

Post-4 Incubation: Effective Dose 50 (- log M 5-Hydroxytryptamine)

Tissue	Control	Vehicle	Soybean Oil	Fish Oil
1	7.21	7.24	7.14	7.23
2	7.59	7.44	7.23	7.19
3	7.21		7.26	
Mean	7.34	7.34	7.21	7.21
Standard Deviation	0.22	0.14	0.06	0.03
Standard Error of the Mean	0.13	0.10	0.04	0.02

Post-4 Incubation: Control with Vehicle Treatment Force (% of Pre-Incubation Maximum Force)

	Dose of Acetylcholine (- log M)										
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**	**4.5**	**4**
1	0.00	-0.33	-0.11	10.34	29.92	48.39	65.29	79.20	88.77	96.11	99.67
2	0.00	-0.11	0.00	15.50	32.75	50.44	65.17	78.28	87.55	94.76	100.44
Mean	0.00	-0.22	-0.06	12.92	31.34	49.41	65.23	78.74	88.16	95.43	100.05
Standard Deviation	0.00	0.16	0.08	3.65	2.00	1.45	0.08	0.65	0.86	0.95	0.54
Standard Error of the Mean	0.00	0.11	0.06	2.58	1.41	1.02	0.06	0.46	0.61	0.67	0.39

Post-4 Incubation: Soybean Oil Treatment Force (% of Pre-Incubation Maximum Force)

	Dose of Acetylcholine (- log M)										
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**	**4.5**	**4**
1	0.00	-0.14	0.14	3.33	24.20	49.42	69.86	84.49	93.77	100.14	100.72
2	0.00	0.11	0.44	15.56	33.30	51.25	67.57	79.22	87.27	93.36	98.91
3	0.00	-0.15	-0.07	11.68	30.51	46.28	61.98	75.52	85.71	93.97	99.33
Mean	0.00	-0.06	0.17	10.19	29.34	48.98	66.47	79.74	88.92	95.83	99.66
Standard Deviation	0.00	0.15	0.26	6.25	4.66	2.51	4.05	4.51	4.27	3.75	0.95
Standard Error of the Mean	0.00	0.09	0.15	3.61	2.69	1.45	2.34	2.60	2.47	2.17	0.55

Post-4 Incubation: Fish Oil Treatment Force (% of Pre-Incubation Maximum Force)

	Dose of Acetylcholine (- log M)										
Tissue	**9**	**8.5**	**8**	**7.5**	**7**	**6.5**	**6**	**5.5**	**5**	**4.5**	**4**
1	0.00	0.58	0.93	14.25	36.15	54.81	70.57	83.08	92.24	96.18	97.22
2	0.00	0.46	0.69	4.12	23.25	44.90	63.23	77.43	87.51	95.07	99.89
3	0.00	0.00	0.78	14.90	37.44	55.31	71.89	84.20	93.01	97.80	99.61
Mean	0.00	0.35	0.80	11.09	32.28	51.67	68.56	81.57	90.92	96.35	98.91
Standard Deviation	0.00	0.31	0.12	6.04	7.84	5.87	4.67	3.63	2.97	1.37	1.47
Standard Error of the Mean	0.00	0.18	0.07	3.49	4.53	3.39	2.69	2.09	1.72	0.79	0.85

Post-4 Incubation: Maximum Force Generated (% of Pre-Incubation Maximum Force)

Tissue	Vehicle	Soybean Oil	Fish Oil
1	99.67	100.725	97.219
2	100.44	98.9119	99.8855
3		99.3304	99.6114
Mean	100.05	99.6556	98.9053
Standard Deviation	0.54	0.94915	1.46678
Standard Error of the Mean	0.39	0.54799	0.84684

Post-4 Incubation: Effective Dose 50 (- log M Acetylcholine)

Tissue	Vehicle	Soybean Oil	Fish Oil
1	6.51	6.4684	6.7004
2	6.61	6.6538	6.3876
3		6.4931	6.7101
Mean	6.56	6.53843	6.59937
Standard Deviation	0.07	0.10067	0.18346
Standard Error of the Mean	0.05	0.05812	0.10592

Percent Relaxation of Contraction with 10^{-6} M Acetylcholine

Tissue	Control	Vehicle	Soybean Oil	Fish Oil
1	2.57	5.37	4.43	7.51
2	0.80	5.33	7.38	7.77
3		2.20	2.61	7.47
Mean	1.69	4.30	4.81	7.58
Standard Deviation	1.24627	1.82054	2.406545	0.16352
Standard Error of the Mean	0.88125	1.05109	1.389419	0.09441

Percent Relaxation of Contraction with 10^{-7} M Acetylcholine

Tissue	Vehicle	Soybean Oil	Fish Oil
1	2.83	7.86	4.49
2	10.92	3.67	4.52
3	5.93	7.67	2.87
4	3.73	6.46	2.99
Mean	5.85	6.42	3.72
Standard Deviation	3.62386	1.92884	0.913488
Standard Error of the Mean	1.81193	0.96442	0.456744

Percent Relaxation of Contraction with 10^{-6} M 5-Hydroxytryptamine

Tissue	Control	Vehicle	Soybean Oil	Fish Oil
1	1.03	2.16	3.69	6.13
2	2.20	4.48	6.30	4.12
3	1.46	2.21	2.58	6.15
4	1.40	1.56	2.19	7.44
5	2.87	4.55	4.32	21.31
6		5.11	3.98	9.13
7				1.35
8				18.50
9				7.47
Mean	1.79	2.99	3.82	9.03
Standard Deviation	0.73	1.41	1.63	6.96
Standard Error of the Mean	0.33	0.63	0.73	3.11

Percent Relaxation of Contraction with 10^{-7} M 5-Hydroxytryptamine

Tissue	Vehicle	Soybean Oil	Fish Oil
1	1.87	6.69	3.62
2	-1.57	1.73	3.00
3	-0.63	5.10	-2.24
4	2.20	-0.78	0.94
5	0.57		6.28
6	1.08		4.49
7	4.98		4.30
Mean	1.21	3.19	2.91
Standard Deviation	2.13	3.36	2.79
Standard Error of the Mean	0.80	1.68	1.05

REFERENCES

1. American Lung Association: Trends in asthma morbidity and mortality. *Epidemiological and Statistics Unit Research and Program Services Division* 2009.
2. Recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children - 1999. *Am J Respir Crit Care Med* 160: 2104-2117, 1999.
3. **Aarrhus LL, Rimele TJ, and Vanhoutte PM**. Removal of epithlelium causes bronchial supersensitivity to acetycholine and 5-hydroxytryptamine. *Fedn Proc* 43: 995, 1984.
4. **Abeywardena MY, Jablonskis LT, and Head RJ**. Dietary n-3 and n-6 polyunsaturated oils and airway contractility. *Prostaglandins Leukot Essent Fatty Acids* 64: 281-287, 2001.
5. **Ackman RG**. The absorption of fish oils and concentrates. *Lipids* 27: 858-862, 1992.
6. **Akinbami LJ, Moorman JE, and Liu X**. Asthma prevalence, health care use, and mortality: United States, 2005-2009. *National Health Statistics Report* 2011.
7. **Allen TM, and Hardin CD**. Pattern of substrate utilization in vascular smooth muscle using 13C isotopomer analysis of glutamate. *Am J Physiol* 275: H2227-2235, 1998.
8. **Anderson SD**. Provocative challenges to help diagnose and monitor asthma: exercise, methacholine, adenosine, and mannitol. *Curr Opin Pulm Med* 14: 39-45, 2008.
9. **Anderson SD, Argyros GJ, Magnussen H, and Holzer K**. Provocation by eucapnic voluntary hyperpnoea to identify exercise induced bronchoconstriction. *Br J Sports Med* 35: 344-347, 2001.
10. **Anderson SD, and Brannan JD**. Bronchial provocation testing: the future. *Curr Opin Allergy Clin Immunol* 11: 46-52.
11. **Anderson SD, Charlton B, Weiler JM, Nichols S, Spector SL, and Pearlman DS**. Comparison of mannitol and methacholine to predict exercise-induced bronchoconstriction and a clinical diagnosis of asthma. *Respir Res* 10: 4, 2009.
12. **Anderson SD, and Kippelen P**. Exercise-induced bronchoconstriction: pathogenesis. *Curr Allergy Asthma Rep* 5: 116-122, 2005.
13. **Arm JP, Horton CE, Mencia-Huerta JM, House F, Eiser NM, Clark TJ, Spur BW, and Lee TH**. Effect of dietary supplementation with fish oil lipids on mild asthma. *Thorax* 43: 84-92, 1988.
14. **Barnes PJ**. Reactive oxygen species and airway inflammation. *Free Radic Biol Med* 9: 235-243, 1990.
15. **Barnes PJ, Cuss FM, and Palmer JB**. The effect of airway epithelium on smooth muscle contractility in bovine trachea. *Br J Pharmacol* 86: 685-691, 1985.
16. **Baumann JM, Rundell KW, Evans TM, and Levine AM**. Effects of cysteine donor supplementation on exercise-induced bronchoconstriction. *Med Sci Sports Exerc* 37: 1468-1473, 2005.
17. **Biltagi MA, Baset AA, Bassiouny M, Kasrawi MA, and Attia M**. Omega-3 fatty acids, vitamin C and Zn supplementation in asthmatic children: a randomized self-controlled study. *Acta Paediatr* 98: 737-742, 2009.
18. **Bousquet J, Jeffery PK, Busse WW, Johnson M, and Vignola AM**. Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 161: 1720-1745, 2000.

19. **Carpagnano GE, Barnes PJ, Francis J, Wilson N, Bush A, and Kharitonov SA**. Breath condensate pH in children with cystic fibrosis and asthma: a new noninvasive marker of airway inflammation? *Chest* 125: 2005-2010, 2004.
20. **Carpagnano GE, Foschino-Barbaro MP, Resta O, Gramiccioni E, and Carpagnano F**. Endothelin-1 is increased in the breath condensate of patients with non-small-cell lung cancer. *Oncology* 66: 180-184, 2004.
21. **Chace KV, and Odessey R**. The utilization by rabbit aorta of carbohydrates, fatty acids, ketone bodies, and amino acids as substrates for energy production. *Circ Res* 48: 850-858, 1981.
22. **Cohen HA, Neuman I, and Nahum H**. Blocking effect of vitamin C in exercise-induced asthma. *Arch Pediatr Adolesc Med* 151: 367-370, 1997.
23. **Dahlen B, Roquet A, Inman MD, Karlsson O, Naya I, Anstren G, O'Byrne PM, and Dahlen SE**. Influence of zafirlukast and loratadine on exercise-induced bronchoconstriction. *J Allergy Clin Immunol* 109: 789-793, 2002.
24. **de Gouw HW, Hendricks J, Woltman AM, Twiss IM, and Sterk PJ**. Exhaled nitric oxide (NO) is reduced shortly after bronchoconstriction to direct and indirect stimuli in asthma. *Am J Respir Crit Care Med* 158: 315-319, 1998.
25. **Denny SI, Thompson RL, and Margetts BM**. Dietary factors in the pathogenesis of asthma and chronic obstructive pulmonary disease. *Curr Allergy Asthma Rep* 3: 130-136, 2003.
26. **Donohue JF, and Ohar JA**. New combination therapies for asthma. *Curr Opin Pulm Med* 7: 62-68, 2001.
27. **Drazen JM, Silverman EK, and Lee TH**. Heterogeneity of therapeutic responses in asthma. *Br Med Bull* 56: 1054-1070, 2000.
28. **Dyerberg J, Madsen P, Moller JM, Aardestrup I, and Schmidt EB**. Bioavailability of marine n-3 fatty acid formulations. *Prostaglandins Leukot Essent Fatty Acids* 83: 137-141.
29. **ElHalawani SM, Ly NT, Mahon RT, and Amundson DE**. Exhaled nitric oxide as a predictor of exercise-induced bronchoconstriction. *Chest* 124: 639-643, 2003.
30. **Engler MB**. Effects of omega-3, omega-6 and omega-9 fatty acids on vascular smooth muscle tone. *Eur J Pharmacol* 215: 325-328, 1992.
31. **Engler MB**. Vascular relaxation to omega-3 fatty acids: comparison to sodium nitroprusside, nitroglycerin, papaverine, and D600. *Cardiovasc Drugs Ther* 6: 605-610, 1992.
32. **Engler MB, Engler MM, Browne A, Sun YP, and Sievers R**. Mechanisms of vasorelaxation induced by eicosapentaenoic acid (20:5n-3) in WKY rat aorta. *Br J Pharmacol* 131: 1793-1799, 2000.
33. **Engler MB, Karanian JW, and Salem N, Jr.** Docosahexaenoic acid (22:6n3)-induced relaxation of the rat aorta. *Eur J Pharmacol* 185: 223-226, 1990.
34. **Fogarty A, and Britton J**. The role of diet in the aetiology of asthma. *Clin Exp Allergy* 30: 615-627, 2000.
35. **Fujisawa T**. Role of oxygen radicals on bronchial asthma. *Curr Drug Targets Inflamm Allergy* 4: 505-509, 2005.
36. **Gelfand EW**. Is asthma in childhood different from asthma in adults? Why do we need special approaches to asthma in children? *Allergy Asthma Proc* 29: 99-102, 2008.
37. **Gotshall RW, Mickleborough TD, and Cordain L**. Dietary salt restriction improves pulmonary function in exercise-induced asthma. *Med Sci Sports Exerc* 32: 1815-1819, 2000.
38. **Henricks PA, and Nijkamp FP**. Reactive oxygen species as mediators in asthma. *Pulm Pharmacol Ther* 14: 409-420, 2001.

39. **Hichami A, Morin C, Rousseau E, and Khan NA**. Diacylglycerol-containing docosahexaenoic acid in acyl chain modulates airway smooth muscle tone. *Am J Respir Cell Mol Biol* 33: 378-386, 2005.
40. **Horrobin DF**. Low prevalences of coronary heart disease (CHD), psoriasis, asthma and rheumatoid arthritis in Eskimos: are they caused by high dietary intake of eicosapentaenoic acid (EPA), a genetic variation of essential fatty acid (EFA) metabolism or a combination of both? *Med Hypotheses* 22: 421-428, 1987.
41. **Horrocks LA, and Yeo YK**. Health benefits of docosahexaenoic acid (DHA). *Pharmacol Res* 40: 211-225, 1999.
42. **Horvath I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, Becher G, van Beurden WJ, Corradi M, Dekhuijzen R, Dweik RA, Dwyer T, Effros R, Erzurum S, Gaston B, Gessner C, Greening A, Ho LP, Hohlfeld J, Jobsis Q, Laskowski D, Loukides S, Marlin D, Montuschi P, Olin AC, Redington AE, Reinhold P, van Rensen EL, Rubinstein I, Silkoff P, Toren K, Vass G, Vogelberg C, and Wirtz H**. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J* 26: 523-548, 2005.
43. **Houglum JE**. Asthma medications: basic pharmacology and use in the athlete. *J Athl Train* 35: 179-187, 2000.
44. **Janssen LJ, and Killian K**. Airway smooth muscle as a target of asthma therapy: history and new directions. *Respir Res* 7: 123, 2006.
45. **Jenkins HA, Cherniack R, Szefler SJ, Covar R, Gelfand EW, and Spahn JD**. A comparison of the clinical characteristics of children and adults with severe asthma. *Chest* 124: 1318-1324, 2003.
46. **Kaur B, Rowe BH, and Arnold E**. Vitamin C supplementation for asthma. *Cochrane Database Syst Rev* CD000993, 2009.
47. **Kew S, Mesa MD, Tricon S, Buckley R, Minihane AM, and Yaqoob P**. Effects of oils rich in eicosapentaenoic and docosahexaenoic acids on immune cell composition and function in healthy humans. *Am J Clin Nutr* 79: 674-681, 2004.
48. **Knight DA, and Holgate ST**. The airway epithelium: structural and functional properties in health and disease. *Respirology* 8: 432-446, 2003.
49. **Leff JA, Busse WW, Pearlman D, Bronsky EA, Kemp J, Hendeles L, Dockhorn R, Kundu S, Zhang J, Seidenberg BC, and Reiss TF**. Montelukast, a leukotriene-receptor antagonist, for the treatment of mild asthma and exercise-induced bronchoconstriction. *N Engl J Med* 339: 147-152, 1998.
50. **Lepage G, and Roy CC**. Direct transesterification of all classes of lipids in a one-step reaction. *J Lipid Res* 27: 114-120, 1986.
51. **Lepage G, and Roy CC**. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *J Lipid Res* 25: 1391-1396, 1984.
52. **Levy BD, Kohli P, Gotlinger K, Haworth O, Hong S, Kazani S, Israel E, Haley KJ, and Serhan CN**. Protectin D1 is generated in asthma and dampens airway inflammation and hyperresponsiveness. *J Immunol* 178: 496-502, 2007.
53. **Lu Y, Hong S, Tjonahen E, and Serhan CN**. Mediator-lipidomics: databases and search algorithms for PUFA-derived mediators. *J Lipid Res* 46: 790-802, 2005.
54. **Ma J, and Xiao L**. Use of dietary supplements among US adults with asthma. *Nutrition and Dietary Supplements* 3: 9-18, 2011.
55. **Martin N, and Pavord ID**. Asthma in elite athletes. *Clinical Pulmonary Medicine* 17: 155-161, 2010.
56. **McKeever TM, and Britton J**. Diet and asthma. *Am J Respir Crit Care Med* 170: 725-729, 2004.

57. **McKeever TM, Lewis SA, Cassano PA, Ocke M, Burney P, Britton J, and Smit HA**. The relation between dietary intake of individual fatty acids, FEV1 and respiratory disease in Dutch adults. *Thorax* 63: 208-214, 2008.
58. **Menzies D, Nair A, and Lipworth BJ**. Non-invasive measurement of airway inflammation in asthma. *J Asthma* 43: 407-415, 2006.
59. **Mickleborough T, and Gotshall R**. Dietary components with demonstrated effectiveness in decreasing the severity of exercise-induced asthma. *Sports Med* 33: 671-681, 2003.
60. **Mickleborough TD**. A nutritional approach to managing exercise-induced asthma. *Exerc Sport Sci Rev* 36: 135-144, 2008.
61. **Mickleborough TD, Lindley MR, Ionescu AA, and Fly AD**. Protective effect of fish oil supplementation on exercise-induced bronchoconstriction in asthma. *Chest* 129: 39-49, 2006.
62. **Mickleborough TD, Murray RL, Ionescu AA, and Lindley MR**. Fish oil supplementation reduces severity of exercise-induced bronchoconstriction in elite athletes. *Am J Respir Crit Care Med* 168: 1181-1189, 2003.
63. **Mickleborough TD, and Rundell KW**. Dietary polyunsaturated fatty acids in asthma- and exercise-induced bronchoconstriction. *Eur J Clin Nutr* 59: 1335-1346, 2005.
64. **Mickleborough TD, Tecklenburg SL, Montgomery GS, and Lindley MR**. Eicosapentaenoic acid is more effective than docosahexaenoic acid in inhibiting proinflammatory mediator production and transcription from LPS-induced human asthmatic alveolar macrophage cells. *Clin Nutr* 28: 71-77, 2009.
65. **Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, and Wanger J**. Standardisation of spirometry. *Eur Respir J* 26: 319-338, 2005.
66. **Misso NL, Brooks-Wildhaber J, Ray S, Vally H, and Thompson PJ**. Plasma concentrations of dietary and nondietary antioxidants are low in severe asthma. *Eur Respir J* 26: 257-264, 2005.
67. **Montuschi P, Corradi M, Ciabattoni G, Nightingale J, Kharitonov SA, and Barnes PJ**. Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. *Am J Respir Crit Care Med* 160: 216-220, 1999.
68. **Moreira A, Moreira P, Delgado L, Fonseca J, Teixeira V, Padrao P, and Castel-Branco G**. Pilot study of the effects of n-3 polyunsaturated fatty acids on exhaled nitric oxide in patients with stable asthma. *J Investig Allergol Clin Immunol* 17: 309-313, 2007.
69. **Morin C, Sirois M, Echave V, Rizcallah E, and Rousseau E**. Relaxing effects of 17(18)-EpETE on arterial and airway smooth muscles in human lung. *Am J Physiol Lung Cell Mol Physiol* 296: L130-139, 2009.
70. **Nelson GJ, and Ackman RG**. Absorption and transport of fat in mammals with emphasis on n-3 polyunsaturated fatty acids. *Lipids* 23: 1005-1014, 1988.
71. **Nestel PJ**. Effects of N-3 fatty acids on lipid metabolism. *Annu Rev Nutr* 10: 149-167, 1990.
72. **Neubronner J, Schuchardt JP, Kressel G, Merkel M, von Schacky C, and Hahn A**. Enhanced increase of omega-3 index in response to long-term n-3 fatty acid supplementation from triacylglycerides versus ethyl esters. *Eur J Clin Nutr* 65: 247-254.
73. **Odessey R, and Chace KV**. Utilization of endogenous lipid, glycogen, and protein by rabbit aorta. *Am J Physiol* 243: H128-132, 1982.
74. **Orehek J, Douglas JS, and Bouhuys A**. Contractile responses of the guinea-pig trachea in vitro: modification by prostaglandin synthesis-inhibiting drugs. *J Pharmacol Exp Ther* 194: 554-564, 1975.

75. **Powers SK, and Hamilton K**. Antioxidants and exercise. *Clin Sports Med* 18: 525-536, 1999.
76. **Reddy C**. Bronchoprovocation Testing. *Clin Rev Allergy Immunol* 2009.
77. **Reisman J, Schachter HM, Dales RE, Tran K, Kourad K, Barnes D, Sampson M, Morrison A, Gaboury I, and Blackman J**. Treating asthma with omega-3 fatty acids: where is the evidence? A systematic review. *BMC Complement Altern Med* 6: 26, 2006.
78. **Ricciardolo FL**. Multiple roles of nitric oxide in the airways. *Thorax* 58: 175-182, 2003.
79. **Romieu I, and Trenga C**. Diet and obstructive lung diseases. *Epidemiol Rev* 23: 268-287, 2001.
80. **Rooke TW, Rimele TJ, and Vanhoutte PM**. Contractions of the canine coronary artery in calcium-free solution. *Am J Physiol* 247: H259-263, 1984.
81. **Rundell KW, Spiering BA, Baumann JM, and Evans TM**. Effects of montelukast on airway narrowing from eucapnic voluntary hyperventilation and cold air exercise. *Br J Sports Med* 39: 232-236, 2005.
82. **Rundell KW, Wilber RL, Szmedra L, Jenkinson DM, Mayers LB, and Im J**. Exercise-induced asthma screening of elite athletes: field versus laboratory exercise challenge. *Med Sci Sports Exerc* 32: 309-316, 2000.
83. **Santanello NC, Barber BL, Reiss TF, Friedman BS, Juniper EF, and Zhang J**. Measurement characteristics of two asthma symptom diary scales for use in clinical trials. *Eur Respir J* 10: 646-651, 1997.
84. **Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, and Moussignac RL**. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J Exp Med* 196: 1025-1037, 2002.
85. **Shapiro GS, Yegen U, Xiang J, Kottakis J, and Della Cioppa G**. A randomized, double-blind, single-dose, crossover clinical trial of the onset and duration of protection from exercise-induced bronchoconstriction by formoterol and albuterol. *Clin Ther* 24: 2077-2087, 2002.
86. **Sierra S, Lara-Villoslada F, Comalada M, Olivares M, and Xaus J**. Dietary eicosapentaenoic acid and docosahexaenoic acid equally incorporate as decosahexaenoic acid but differ in inflammatory effects. *Nutrition* 24: 245-254, 2008.
87. **Sinha T, and David AK**. Recognition and management of exercise-induced bronchospasm. *Am Fam Physician* 67: 769-774, 675, 2003.
88. **Spannhake EW, and Menkes HA**. Vitamin C--new tricks for an old dog. *Am Rev Respir Dis* 127: 139-140, 1983.
89. **Stelmach I, Grzelewski T, Majak P, Jerzynska J, Stelmach W, and Kuna P**. Effect of different antiasthmatic treatments on exercise-induced bronchoconstriction in children with asthma. *J Allergy Clin Immunol* 121: 383-389, 2008.
90. **Storms WW**. Review of exercise-induced asthma. *Med Sci Sports Exerc* 35: 1464-1470, 2003.
91. **Subbarao P, Duong M, Adelroth E, Otis J, Obminski G, Inman M, Pedersen S, and O'Byrne P M**. Effect of ciclesonide dose and duration of therapy on exercise-induced bronchoconstriction in patients with asthma. *J Allergy Clin Immunol* 117: 1008-1013, 2006.
92. **Tecklenburg-Lund S**. Comparative and synergistic effects of fish oil supplemenetation and a leukotriene receptor antagnoist on exercise-induced bronchoconstriction and airway inflammation in subjects with asthma. In: *Department of Kinesiology*. Bloomington, IN: Indiana University, 2009.

93. **Tecklenburg-Lund S, Mickleborough TD, Turner LA, Fly AD, Stager JM, and Montgomery GS**. Randomized controlled trial of fish oil and montelukast and their combination on airway inflammation and hyperpnea-induced bronchoconstriction. *PLoS One* 5: e13487, 2010.
94. **Tecklenburg SL, Mickleborough TD, Fly AD, Bai Y, and Stager JM**. Ascorbic acid supplementation attenuates exercise-induced bronchoconstriction in patients with asthma. *Respir Med* 101: 1770-1778, 2007.
95. **Tindle HA, Davis RB, Phillips RS, and Eisenberg DM**. Trends in use of complementary and alternative medicine by US adults: 1997-2002. *Altern Ther Health Med* 11: 42-49, 2005.
96. **Trebble TM, Wootton SA, Miles EA, Mullee M, Arden NK, Ballinger AB, Stroud MA, Burdge GC, and Calder PC**. Prostaglandin E2 production and T cell function after fish-oil supplementation: response to antioxidant cosupplementation. *Am J Clin Nutr* 78: 376-382, 2003.
97. **Tsujimura M, Higasa S, Nakayama K, Yanagisawa Y, Iwamoto S, and Kagawa Y**. Vitamin C activity of dehydroascorbic acid in humans--association between changes in the blood vitamin C concentration or urinary excretion after oral loading. *J Nutr Sci Vitaminol (Tokyo)* 54: 315-320, 2008.
98. **Weldon SM, Mullen AC, Loscher CE, Hurley LA, and Roche HM**. Docosahexaenoic acid induces an anti-inflammatory profile in lipopolysaccharide-stimulated human THP-1 macrophages more effectively than eicosapentaenoic acid. *J Nutr Biochem* 18: 250-258, 2007.
99. **Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, and Speizer FE**. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 122: 51-65, 1985.
100. **Xu Z, Harvey K, Pavlina T, Dutot G, Zaloga G, and Siddiqui R**. An improved method for determining medium- and long-chain FAMEs using gas chromatography. *Lipids* 45: 199-208.
101. **Yanagisawa A, and Lefer AM**. Vasoactive effects of eicosapentaenoic acid on isolated vascular smooth muscle. *Basic Res Cardiol* 82: 186-196, 1987.
102. **Yin H, Liu W, Goleniewska K, Porter NA, Morrow JD, and Peebles RS, Jr.** Dietary supplementation of omega-3 fatty acid-containing fish oil suppresses F2-isoprostanes but enhances inflammatory cytokine response in a mouse model of ovalbumin-induced allergic lung inflammation. *Free Radic Biol Med* 47: 622-628, 2009.
103. **Yokoyama A, Hamazaki T, Ohshita A, Kohno N, Sakai K, Zhao GD, Katayama H, and Hiwada K**. Effect of aerosolized docosahexaenoic acid in a mouse model of atopic asthma. *Int Arch Allergy Immunol* 123: 327-332, 2000.
104. **Zhao R, Du L, Huang Y, Wu Y, and Gunst SJ**. Actin depolymerization factor/cofilin activation regulates actin polymerization and tension development in canine tracheal smooth muscle. *J Biol Chem* 283: 36522-36531, 2008.
105. **Zhao Y, Joshi-Barve S, Barve S, and Chen LH**. Eicosapentaenoic acid prevents LPS-induced TNF-alpha expression by preventing NF-kappaB activation. *J Am Coll Nutr* 23: 71-78, 2004.

CURRICULUM VITAE

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Education

University of Notre Dame
May 2006, Bachelor of Science, Biological Sciences, *Summa cum Laude*

Indiana University
September 2011, Doctor of Philosophy, Department of Cellular and Integrative Physiology

Indiana University School of Medicine (IUSM)
Class of 2013, MD Candidate

Grants, Scholarships, Awards

- Indiana Medical Scientist Training Program Grant – IUSM
- Indiana Physiological Society Conference – February 5, 2011 – Indianapolis, IN – Outstanding Abstract
- AAU/Bell-Updyke-Willet Kinesiology Research Fund – Indiana University Department of Kinesiology – March 2010-2011
- School of HPER Travel Grant – Indiana University Department of Kinesiology – May 2010
- The National Dean's List – 2003, 2004, 2006, 2007
- Western New York MENSA Society Scholarship Essay Contest – November 2006
- Outstanding Senior Biological Scientist – University of Notre Dame – May 2006
- Notre Dame Club of Buffalo Scholarship – 2005
- Tylenol Health Sciences Scholarship – 2003
- Biology Department Scholarship – Catawba College – 2002
- First Family Scholarship – Catawba College – 2002
- Varsity Basketball Athletic Scholarship – Catawba College – 2002

Research Experience

- Indiana University – Dept. of Kinesiology – Graduate Student – Mentor: Dr. Timothy Mickleborough – July 2008 – June 2013
- IUSM – Dept. of Cellular and Integrative Physiology – Graduate Student – Mentor: Dr. Susan Gunst – June 2010 – June 2013
- IUSM – Dept. of Pediatric Pulmonology – Graduate Student – Mentor: Dr. Robert Tepper – July 2007
- IUSM – Dept. of Cellular and Integrative Physiology – Graduate Student – Mentor: Dr. Michael Sturek – June 2007
- IUSM – Dept. of Biochemistry and Molecular Biology – Graduate Student – Mentor: Dr. David Ingram – June – July 2008
- University of Notre Dame, IUSM-South Bend – Dept. of Physiology – Undergraduate Student – Mentor: Dr. Kenneth Olson – January 2005 – May 2006
- SUNY-Buffalo – Dept. of Exercise and Nutrition Sciences – Undergraduate – Mentor: Dr. Peter Horvath

Teaching Experience

- University of Notre Dame – Dept. of Biological Sciences – Undergraduate Teaching Assistant – Classical and Molecular Genetics Laboratory – Fall 2005
- University of Notre Dame – Dept. of Biological Sciences – Undergraduate Teaching Assistant – Molecular Genetics Technology – Fall 2004
- Catawba College Tutor – General Chemistry – Fall 2002

Memberships in Professional Organizations and Honor Societies

- American Academy of Family Physicians, 2011
- Indiana Physiological Society, 2011
- American Association for the Advancement of Science, 2010-2011
- American College of Sports Medicine, 2009-2010
- Golden Key International Honour Society, Academic Honor Society, inducted 2008
- Phi Beta Kappa Society, inducted 2006
- Alpha Epsilon Delta, Pre-Medical Honor Society, inducted 2005

Peer Reviewed Publications

- Dombkowski RA, Doellman MM, **Head SK**, and Olson KR. Hydrogen sulfide mediates hypoxia-induced relaxation of trout urinary bladder smooth muscle. *J Exp Biol* 209: 3234-3240, 2006.
- Olson KR, Dombkowski RA, Russell MJ, Doellman MM, **Head SK**, Whitfield NL, and Madden JA. Hydrogen sulfide as an oxygen sensor/transducer in vertebrate hypoxic vasoconstriction and hypoxic vasodilation. *J Exp Biol* 209: 4011-4023, 2006.

Abstracts and Presentations

- **Head SK**, Duke JW, Mickleborough TD. Combining fish oil and vitamin C reduces hyperpnea-induced bronchoconstriction and airway inflammation in adults with asthma. Women in Science Conference, Bloomington, IN: March 2011.
- **Head SK**, Duke JW, Mickleborough TD. Combining fish oil and vitamin C reduces hyperpnea-induced bronchoconstriction and airway inflammation in adults with asthma. Poster presentation at the Indiana Physiological Society Conference, Indianapolis, IN: February 2011.
- **Head SK**, Hornsby WE, McCracken CM, Mattson CD, Stager JM. Highly-active masters swimmers maintain arterial elasticity. Head, Sally K.; Hornsby, Whitney E.; McCracken, Colleen M.; Mattson, Christopher D.; Stager, Joel M. Medicine & Science in Sports & Exercise. 42(5):310, May 2010.
- McCracken, CM, Kitano, K, **Head, SK**, Johnston, JD, Finn, PR, Stager, JM (2010a). Cognitive profiles and neuro-motor properties of physically active masters swimmers. International Symposium on Biomechanics and Medicine in Swimming, Oslo, Norway: June 2010.
- McCracken, CM, **Head, SK**, Mattson, CD, Peirce, A, Johnston, JD, Finn, PR, Stager, JM (2010b). Markers of Cardiovascular Health in Physically Active Masters Swimmers. International Symposium on Biomechanics and Medicine in Swimming, Oslo, Norway: June 2010.
- "H_2S: Tissue Production and Uptake by Red Blood Cells," University of Notre Dame Undergraduate Research Symposium, Notre Dame, IN: April 2006.