

Randomized Cross-Over Controlled Pilot Study of Docosahexaenoic Acid Supplementation on Airway Inflammation and Hyperpnea-Induced Bronchoconstriction in Adults with Asthma

S K Head, T D Mickleborough

Citation

S K Head, T D Mickleborough. *Randomized Cross-Over Controlled Pilot Study of Docosahexaenoic Acid Supplementation on Airway Inflammation and Hyperpnea-Induced Bronchoconstriction in Adults with Asthma*. The Internet Journal of Asthma, Allergy and Immunology. 2013 Volume 9 Number 1.

Abstract

Background: Fish oil supplementation has been shown to attenuate hyperpnea- and exercise-induced bronchoconstriction. In vitro studies suggest that the omega-3 polyunsaturated fatty acid docosahexaenoic acid (DHA), rather than eicosapentaenoic acid, may be the potent component of fish oil.

Objective: Therefore, the main aim of this study was to determine whether DHA supplementation can attenuate hyperpnea-induced bronchoconstriction (HIB) and airway inflammation in adults with asthma compared to placebo.

Methods: Nine subjects (aged 18-30 years) with asthma and HIB participated in a randomized, double-blind, placebo-controlled crossover trial where they received either 4.0 g of 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. Each time, bronchoprovocation was elicited with eucapnic voluntary hyperventilation (EVH), a surrogate for an exercise challenge. Prior to and following the EVH challenge, pulmonary function, exhaled breath condensate (EBC) pH, and EBC concentrations of 8-isoprostane and the DHA metabolites 17S-hydroxydocosahexaenoic acid and protectin D1 were measured. This study was registered at clinicaltrials.gov (study # NCT01200446).

Results: Nine subjects completed the crossover design protocol without any adverse effects. There were no significant changes ($p > 0.05$) in any variables measured (i.e., percent change in forced expiratory volume-1-sec, EBC pH, 8-isoprostane, protectin D1 and 17S-hydroxydocosahexaenoic acid) among the pre-supplementation, placebo, and DHA tests. The effect size (d) for the maximum percent change in FEV1 was 0.08.

Conclusion: The data indicate that supplementation with 4.0 g of DHA for 3 weeks does not attenuate HIB or airway inflammation in asthmatic subjects compared to baseline or placebo. Variations in study design, including time course, dose, and route of administration, should be conducted to further address DHA's role as a potential treatment for HIB.

INTRODUCTION

The Center for Disease Control recently reported that 8.2% of the United States population has asthma [1]. 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 [2]. Moreover, EIB is an indication that a patient's current asthma treatment may be inadequate [3]. As nearly a third of the estimated \$19.7 billion in direct and indirect healthcare costs for asthma in 2007 stemmed from prescription medications [4], there is a growing interest in non-pharmacological alternatives to treat this condition.

A nutritional approach is an appealing alternative as the prevalence of asthma has been linked to societal changes in diet [5]. Moreover, dietary supplement use is already popular in both the general and asthmatic populations, for according to a recent National Health and Nutrition Examination Survey, approximately 50% of people in both of these populations reported "dietary supplement use in the last 30 days." [6]. It is thus important to study these nutritional supplements and their effect on asthma in order to form a scientific basis upon which clinicians can recommend their proper and safe use.

Omega-3 polyunsaturated fatty acid (PUFA)

supplementation is used by 6.7% of asthmatics as nutritional therapy [6]. 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 [7] and in adults with asthma [8,9]. The mechanism of action of the omega-3 PUFAs is thought to involve decreasing the availability of the more pro-inflammatory omega-6 PUFA- derived metabolites (i.e., 4-series leukotrienes and 2-series prostanoids) through competition for common enzymes [5].

The optimal fish oil formula, dose, and duration of treatment 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 [10]. Although there have been several in vivo studies in humans and mice [10,11] as well as in vitro studies on human macrophage cells [12,13] 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 since airway responsiveness is clinically important for patients with asthma.

Studies by Serhan et al. [14] and Levy et al. [15] provide the principle support for DHA as the more potent contributor of fish oil. Serhan et al. [14] demonstrated that a metabolite of DHA, named protectin D1, can actively resolve inflammation by reducing pro-inflammatory signaling. Applying this mechanism to in vivo murine studies, Levy et al. [15] have shown that injecting mice with protectin D1 decreased subsequent bronchoconstriction during a methacholine challenge. Furthermore, 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 the respiratory tissues [15]. In addition, it was shown that during an asthma attack, patients had significantly lower levels of protectin D1 in exhaled breath condensate as compared to healthy individuals [15]. Based on these findings [14,15], it is therefore important to determine whether asthmatics taking DHA supplements 1) increase their exhaled breath condensate concentration of protectin D1 and 2) experience less bronchoconstriction upon provocation.

Therefore, the main aim of this study was to determine the effect of DHA supplementation on hyperpnea-induced

bronchoconstriction (HIB) and airway inflammation in adults with asthma. We hypothesized that DHA supplementation would attenuate HIB and airway inflammation in asthmatic individuals compared to placebo.

MATERIALS AND METHODS

Ethics Statement. 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 testing occurred in the Human Performance Laboratory at Indiana University in Bloomington, IN. All subjects gave their written informed consent before enrolling in the study.

Subjects. Nine subjects (6 male, 3 female) between the ages of 18-30 years with physician-diagnosed asthma and EIB were recruited from a university setting. Mild to moderate asthmatics were included based on their pulmonary function in response to a eucapnic voluntary hyperventilation (EVH) challenge at the first laboratory test. Each subject's forced expiratory volume in one second (FEV1) was measured at rest and following EVH, a surrogate exercise challenge used to diagnose EIB [16]. Subjects who demonstrated a 10-50% change in their FEV1 from pre- to post-EVH 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 his maintenance medication (fluticasone propionate and salmeterol) with the written permission of his physician for four weeks prior to beginning the study [8]. 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. Healthy, non-asthmatic subjects were not recruited to this study to act as a control group; it has been demonstrated that fish oil supplementation does not significantly change pulmonary function or inflammatory mediators in individuals without asthma or EIB [7].

Study Design. This study 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 = 4) or placebo capsules containing a corn and soy oil blend

(n = 5) every day 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.

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). Sealed pill bottles labeled with one of two material numbers were provided. Data collection and initial data analysis was completed before the principle investigator was informed which material number corresponded to each treatment. 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 exercise for 24 hours, caffeine for 8 hours, and their short-acting β_2 -agonist for 6 hours [9]. Before and after bronchoprovocation with EVH, inflammatory markers and pulmonary function were evaluated. Food frequency questionnaires were employed to assess changes in diet between phases of the study. 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 elicited using an 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 H₂O*L⁻¹ air [9]. Subjects were instructed to breathe for 6 minutes at 85% of their maximal voluntary ventilation as estimated by 30 times their resting FEV1 [16]. In order to verify the ventilatory rate, a flow sensor measured ventilation (Vmax 22 Metabolic Measurement Cart, SensorMedics, Yorba Linda, CA) [17].

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) [9]. 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 FEV1 values did not vary by more than 0.15 liters [18]. The largest value of each was recorded. EIB was defined as a greater than 10% decrease in the post-EVH FEV1 from the pre-EVH value [16]. 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 FEV1 [18].

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 [9] according to ATS and European Respiratory Society recommendations [19]. 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 [9]. 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 17S-hydroxydocosahexaenoic 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). The mobile phase and gradient conditions were similar to those used by Lu et al. [20].

Nutrient Intake. The GSEL version of food frequency questionnaires developed by the Nutrient Assessment Shared Resource of the Fred Hutchinson Cancer Research Center 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 referring to their diet during the course of that particular phase. Food frequency questionnaires are a valid and reliable method of collecting dietary data [21].

Data Analysis. 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. 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 ($p \leq 0.05$), 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 $p \leq 0.05$ for all statistical tests. The data is presented as mean and their 95% confidence intervals (CI).

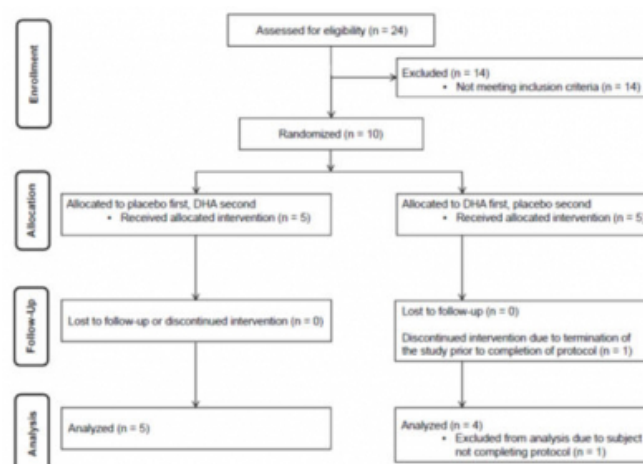
An a priori power analysis was conducted using data from a previous study conducted in our laboratory to determine the number of subjects needed for the present study [8]. Based on the reported maximal post-exercise drop in FEV1 (L) in asthmatics supplemented with fish oil, it was determined that at least 3 subjects would be needed to achieve a power of 0.80. Since this study used DHA only, which is a different fish oil formula, we recruited additional subjects with asthma. After 9 subjects completed the protocol, initial data analysis was performed to calculate the effect size and determine whether to continue the study based on the preliminary results.

RESULTS

Recruitment. Subject recruitment and testing occurred between October 2010 and January 2011. Participant flow through the study was as indicated in figure 1. The estimated number of subjects needed to show a significant difference in the primary outcome measure was 3. Recruitment was suspended after 9 subjects completed the protocol. Initial data analysis was performed indicating that there were no significant differences ($p > 0.05$) between the two treatment arms in any of the dependent variables. The study was thus stopped at that time. One subject who was enrolled and randomized to receive a treatment was immediately removed from the study since the initial analysis showed that data from an additional subject would not have altered the ability of the study to detect significance. This subject was not included in any data analysis. The material codes were then broken, and the statistical analysis was completed.

Figure 1

Participant flow through the study.



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 ($p = 0.76$) between the two treatment periods. The subjects' measurements at the pre-supplementation laboratory visit were considered their baseline values (table 1). The subjects' resting pulmonary function was not significantly different ($p > 0.05$) among the three laboratory visits (table 2).

Table 1

Subject (baseline) characteristics. Values are reported as mean \pm standard error of the mean ($n = 9$). BMI, body mass index; FEV1, forced expiratory volume in one second; FVC, forced vital capacity

Males	6
Females	3
Age, yr (range)	22.36 \pm 1.31 (18-30)
Height, m	1.77 \pm 0.02
Weight, kg	75.77 \pm 2.43
BMI, kg/m² (range)	24.17 \pm 0.86 (22.26-28.35)
Morning Peak Flow, L/min	467.68 \pm 43.61
Evening Peak Flow, L/min	483.17 \pm 46.33
FEV₁/FVC	79.11 \pm 3.44
Percent Predicted FVC, %	110.89 \pm 4.39
Percent Predicted FEV₁, %	103.65 \pm 3.69

Table 2

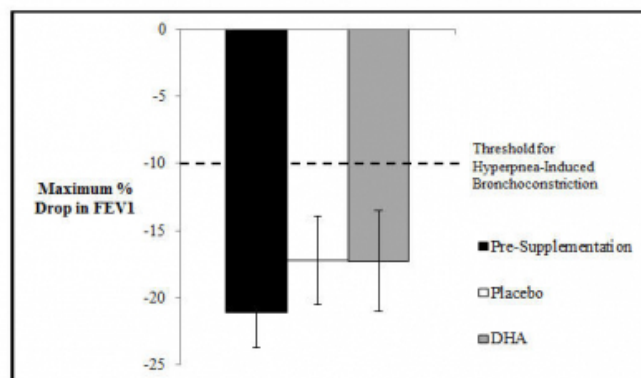
Resting pulmonary function. Percent predicted values are based on age, height, weight, and sex. They are reported as mean \pm standard error of the mean (n = 9). FVC, forced vital capacity; FEV₁, forced expiratory volume in one second; FEF_{25-75%}, forced expiratory flow at 25-75% of the FVC; DHA, docosahexaenoic acid

	Pre-Supplementation	Placebo	DHA
FVC (L)	5.32 \pm 0.33	5.22 \pm 0.38	5.18 \pm 0.30
% predicted	110.89 \pm 4.39	108.59 \pm 5.20	108.04 \pm 4.08
FEV ₁ (L)	4.18 \pm 0.25	4.12 \pm 0.28	4.16 \pm 0.27
% predicted	103.65 \pm 3.69	101.00 \pm 4.14	103.01 \pm 4.04
FEF _{25-75%} (L/min)	4.08 \pm 0.55	3.93 \pm 0.46	4.04 \pm 0.47
% predicted	92.57 \pm 11.68	88.95 \pm 9.56	91.16 \pm 9.65

Pulmonary Function. Subjects were able to obtain between 68.8-88.7% of their individual MVV values at the initial laboratory visit; each subject maintained the percent of his or her MVV achieved at the initial laboratory test at their subsequent laboratory tests. At the pre-supplementation laboratory test, the mean maximum drop in FEV₁ following the eucapnic voluntary hyperventilation (EVH) challenge was 21.07%; 95% CI, -5.49 to 36.65% (n = 9). At the subsequent laboratory tests, the mean maximum drop in FEV₁ remained greater than the diagnostic threshold for EIB; the mean maximum drop in FEV₁ was not significantly different (p = 0.15, η^2 = 0.08) among the pre-supplementation, placebo (17.20%; 95% CI, 2.03 to 36.43%), and DHA supplementation (17.27%; 95% CI, 4.66 to 39.20%) values for the 9 subjects (figure 2). When examined as a change in volume, there were no significant differences (p = 0.48, η^2 = 0) in the values for the maximum drop in FEV₁ among the pre-supplementation (0.87 L; 95% CI, -0.22 to 1.52 L), placebo (0.69 L; 95% CI, 0.07 to 1.45 L), and DHA supplementation (0.79 L; 95% CI, 0.27 to 1.85 L) tests.

Figure 2

The maximum percent change in FEV₁ after the eucapnic voluntary hyperventilation challenge. There were no significant differences (p = 0.15) among the pre-supplementation, placebo, and docosahexaenoic acid (DHA) supplementation phases (n = 9). Error bars express standard error of the mean. FEV₁, forced expiratory volume in one second



No significant differences (p = 0.61, η^2 = 0) were observed in the maximum percent change in FVC among the baseline pre-supplementation (12.23%; 95% CI, -3.82 to 20.64%), placebo (10.49%; 95% CI, 0.62 to 21.60%), and DHA (10.06%; 95% CI, 6.23 to 26.35%) phases. There were also no significant differences (p = 0.06, η^2 = 0.19) in the maximum percent change in FEF_{25-75%} among the pre-supplementation (31.57%; 95% CI, 5.89 to 69.03%), placebo (26.86%; 95% CI, 8.01 to 61.73%), and DHA (26.85%; 95% CI, 13.02 to 66.72%) phases.

Exhaled Breath Condensate. Out of the 9 subjects, only 7 individuals were able to provide enough exhaled breath condensate for pH analysis at each of the laboratory tests. Although a significant difference (p = 0.04, η^2 = 0.20) was detected for the the pre-EVH EBC pH with repeated measures ANOVA, Tukey's post-hoc did not reveal any significant differences among pre-supplementation (6.86; 95% CI, -6.25 to 7.47), placebo (7.15; 95% CI, -6.96 to 7.34), and DHA (6.86; 95% CI, -6.56 to 7.16). The post-EVH exhaled breath condensate (EBC) pH values did not significantly change (p = 0.72; η^2 = 0) among the pre-supplementation (6.86; 95% CI, -6.39 to 7.33), placebo (6.95; 95% CI, -6.26 to 7.64), and DHA (7.01; 95% CI, -6.04 to 7.98) phases.

EBC was also analyzed by liquid chromatography for the oxidative stress marker 8-isoprostane and the DHA metabolites protectin D1 and 17S-hydroxydocosahexaenoic acid at each laboratory test before and after the EVH challenge. For these variables, 7 subjects provided enough

EBC volume for pre-EVH 8-isoprostane analysis while 5 subjects provided enough EBC volume for protectin D1, 17S-hydroxydocosahexaenoic acid, and post-EVH 8-isoprostane analyses. There was no significant difference ($p = 0.51$; $\eta^2 = 0$) in the pre-EVH EBC 8-isoprostane concentration among the pre-supplementation phase (3.08 pg/ μ L; 95% CI, 4.70 to 10.86 pg/ μ L), the placebo treatment (6.16 pg/ μ L; 95% CI, 4.83 to 17.15 pg/ μ L), and DHA treatment (4.48 pg/ μ L; 95% CI, 1.74 to 10.70 pg/ μ L). Also, there was not a significant difference ($p = 0.78$; $\eta^2 = 0$) in the post-EVH EBC 8-isoprostane concentration among the pre-supplementation (2.21 pg/ μ L; 95% CI, 5.09 to 9.51 pg/ μ L), placebo (1.78 pg/ μ L; 95% CI, 2.12 to 5.68 pg/ μ L), and DHA supplementation (3.42 pg/ μ L; 95% CI, 5.10 to 11.94 pg/ μ L) phases. The levels of protectin D1 and 17S-hydroxydocosahexaenoic acid were too low for detection (< 0 pg/ μ L) both pre- and post-EVH at each of the three laboratory tests (table 3).

Table 3

Inflammatory mediators. There were no significant changes in the measures of airway inflammation with DHA supplementation as compared to pre-supplementation or placebo (* $n = 7$, # $n = 5$). Values are reported as mean \pm standard error of the mean. DHA, docosahexaenoic acid; EVH, eucapnic voluntary hyperventilation

Variable	Pre-Supplementation	Placebo	DHA
Pre-EVH Exhaled Breath Condensate pH*	6.86 \pm 0.12	7.15 \pm 0.04	6.86 \pm 0.06
Post-EVH Exhaled Breath Condensate pH*	6.86 \pm 0.09	6.96 \pm 0.13	7.01 \pm 0.19
Pre-EVH 8-Isoprostane Concentration (pg/ μ L)*	3.08 \pm 1.50	6.16 \pm 2.12	4.48 \pm 1.20
Post-EVH 8-Isoprostane Concentration (pg/ μ L)*	2.21 \pm 8.33	1.78 \pm 4.45	3.42 \pm 9.71
Pre-EVH Protectin D1 Concentration (pg/ μ L)*	< 0	< 0	< 0
Post-EVH Protectin D1 Concentration (pg/ μ L)*	< 0	< 0	< 0
Pre-EVH 17S-hydroxydocosahexaenoic acid (pg/ μ L)*	< 0	< 0	< 0
Post-EVH 17S-hydroxydocosahexaenoic acid (pg/ μ L)*	< 0	< 0	< 0

Nutrient Intake and Compliance. Subject adherence to the treatment regimens was assured by finding that pill counts at the end of each supplement period reflected that capsules were consumed regularly. Eight subjects completed a food frequency questionnaire for each phase of the study. Although the subjects' usual diets were expected to vary, the mean daily nutrient intake did not differ significantly among the phases ($p > 0.05$) (table 4).

Table 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 from the subjects' diets are presented here. Please note that DHA from the supplements is not included. SEM, standard error of the mean; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid

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

DISCUSSION

Since it has been shown that fish oil effectively attenuates bronchoconstriction and airway inflammation in elite athletes and asthmatic subjects with EIB [7-9], the main purpose of this study was to determine whether supplementation with docosahexaenoic acid (DHA), an omega-3 fatty acid found in fish oil, can 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 pre- and post-EVH pulmonary function, markers of airway inflammation, or DHA metabolite concentrations in comparison to placebo or baseline values in a group of 9 adults with asthma.

There are several reasons as to why DHA supplementation may not have had a significantly altered pulmonary function or EBC inflammatory markers in the present study. First, it is possible that DHA supplementation, taken in isolation, cannot improve HIB. This may be because eicosapentaenoic acid (EPA) is the more important component of fish oil in terms of attenuating HIB and airway inflammation although there is not a consensus in the literature as to whether this is true. There is substantial evidence to support DHA's effectiveness in reducing inflammation [13,15,22,23]. Levy et al. [15] found that compared to mice injected with saline, mice injected with protectin D1, a metabolite of DHA, 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. Furthermore, bronchoconstriction following exposure of the mice to increasing concentrations of inhaled methacholine was also decreased. When DHA was added *ex vivo* to homogenized lung tissue from these mice, the protectin D1 concentration increased significantly suggesting that DHA can be converted to protectin D1 by respiratory tissues during airway inflammation. Since respiratory DHA levels are reduced in diseases featuring airway inflammation, such as asthma [15], we hypothesized that increasing DHA levels through supplementation would increase the availability of protectin D1 to alleviate airway inflammation and bronchoconstriction. Yokoyama et al. [23] used an atopic asthma mouse model to demonstrate that exposure to aerosolized DHA reduced the airway inflammatory response to a methacholine challenge; the number of cells and percent of eosinophils in the bronchoalveolar lavage were reduced with DHA exposure as compared to exposure with aerosolized saline or soybean oil. In vivo experiments by Weldon et al. [13] similarly showed that DHA has an anti-inflammatory effect. Here, either DHA or EPA was used to treat lipopolysaccharide-stimulated macrophages [13]. Although both DHA and EPA significantly reduced production of the inflammatory cytokines tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and interleukin-6 (IL-6), DHA decreased IL-1 β and IL-6 production to a greater extent than EPA did [13]. In contrast, Mickleborough et al. [12] showed that EPA was more effective than DHA in reducing the inflammatory responses in lipopolysaccharide-stimulated macrophages. Mickleborough et al. [12] demonstrated that treatment with EPA decreased the production of TNF- α , prostaglandin D2, IL-1 β , and leukotriene B4 as compared to treatment with DHA. The discrepancy in the findings between these two studies may be explained by the different cell lines used. Weldon et al. [13] studied human THP-1 macrophages, a monocytic leukemia cell line, whereas Mickleborough et al. [12] 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 used an inappropriate dose of DHA or inadequate time course of supplementation. The DHA supplementation period in the present study was 3 weeks in duration, which is a time course that has been shown to be effective in EIB studies with fish oil supplementation [8,9]. The 4.0 g dose is twice the dose of DHA used in conjunction with EPA in earlier fish oil studies [8,9]. Repeating the measurements after the washout period

may have ensured that the subjects reached their pre-supplementation values prior to starting the second treatment period; however, the results did not show a significant carryover effect, which suggests that the duration of the washout period was sufficient. Since the EBC concentration of the DHA metabolites protectin D1 and 17S-hydroxy-docosahexaenoic acid did not increase with supplementation, it suggests that perhaps a higher dose of DHA is required to have an effect. Levy et al. [15] reported that there were only “trace amounts” of protectin D1 in the EBC of 4 adults during an acute exacerbation of their asthma. In the current study, the concentrations of protectin D1 and 17S-hydroxydocosahexaenoic acid were not detectable at baseline, following placebo supplementation, or following DHA supplementation. Since the levels were below the instrument’s detection ability, differences among the three phases could not be ascertained. Future studies should include a non-asthmatic control group for this parameter to demonstrate significantly greater DHA metabolite levels in a healthy population. Additionally, DHA supplementation was accomplished via oral intake of gel capsules similar to previous fish oil studies [7-9]. However, murine studies demonstrating the effectiveness of DHA administered this omega-3 fatty acid via aerosol [23] or intravenously in its metabolite form [15]. Thus, the efficacy of DHA may have been affected by the means of administration.

Third, the data variability in our subject population was greater than expected. There were 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 [8] 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. [8] study, it was determined that 3 subjects would be needed to show a significant reduction in the maximum drop in FEV1 volume with DHA supplementation compared to placebo. However, we were unable to demonstrate a significant difference in this measure or any other pulmonary function measure in the 9 subjects in the present study. The coefficients of variation for the maximum drop in FEV1 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. [8] 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. This lack of reproducibility may have impaired our ability to detect statistically significant differences between treatments. 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 administering DHA should be attempted. Although intravenous administration of DHA may be problematic in humans, aerosolized DHA may be a viable option [23]. Furthermore, it would also be worthwhile to conduct a similar study using pure EPA instead of DHA.

CONCLUSIONS

In conclusion, this is the first study to evaluate the effect of DHA supplementation on airway responsiveness and inflammation in adults with asthma and HIB. Although no significant changes in pulmonary function, markers of inflammation, or DHA metabolite concentrations were demonstrated in this pilot study, future research should address the time-course, dose, and administration route to further elucidate the anti-inflammatory potential of pure DHA supplementation in adults with asthma. A better understanding of an appropriate omega-3 polyunsaturated fatty acid regimen is necessary in order to advise asthmatics seeking an alternative to pharmacologic treatment.

ACKNOWLEDGEMENTS

Martek Biosciences Corporation (Columbia, MD) generously provided the active docosahexaenoic acid and placebo capsules. Liquid chromatography analysis was completed by Loubna Hammad of the Metacyt Biochemical Analysis Facility at Indiana University, Bloomington, IN.

References

References

1. Akinbami LJ, Moorman JE, Liu X: Asthma prevalence, health care use, and mortality: United States, 2005-2009. National Health Statistics Report; 2011.
2. Houghlum JE: Asthma medications: basic pharmacology and use in the athlete. *J Athl Train*; 2000; 35: 179-187.
3. Leff JA, Busse WW, Pearlman D, Bronsky EA, Kemp J, Hendeles L, Dockhorn R, Kundu S, Zhang J, Seidenberg BC, Reiss TF: Montelukast, a leukotriene-receptor antagonist, for the treatment of mild asthma and exercise-induced bronchoconstriction. *N Engl J Med*; 1998; 339: 147-152.
4. American Lung Association: Trends in asthma morbidity and mortality. Epidemiological and Statistics Unit Research and Program Services Division 2009.
5. Mickleborough TD: A nutritional approach to managing exercise-induced asthma. *Exerc Sport Sci Rev*; 2008; 36: 135-144.
6. Ma J, Xiao L: Use of dietary supplements among US adults with asthma. *Nutrition and Dietary Supplements*; 2011; 3: 9-18.
7. Mickleborough TD, Murray RL, Ionescu AA, Lindley MR: Fish oil supplementation reduces severity of exercise-induced bronchoconstriction in elite athletes. *Am J Respir Crit Care Med*; 2003; 168: 1181-1189.
8. Mickleborough TD, Lindley MR, Ionescu AA, Fly AD: Protective effect of fish oil supplementation on exercise-induced bronchoconstriction in asthma. *Chest*; 2006; 129: 39-49.
9. Tecklenburg-Lund S, Mickleborough TD, Turner LA, Fly AD, Stager JM, Montgomery GS: Randomized controlled trial of fish oil and montelukast and their combination on airway inflammation and hyperpnea-induced bronchoconstriction. *PLoS One*; 2010; 5: e13487.
10. Sierra S, Lara-Villoslada F, Comalada M, Olivares M, Xaus J: Dietary eicosapentaenoic acid and docosahexaenoic acid equally incorporate as decosahexaenoic acid but differ in inflammatory effects. *Nutrition*; 2008; 24: 245-254.
11. Kew S, Mesa MD, Tricon S, Buckley R, Minihane AM, Yaqoob P: Effects of oils rich in eicosapentaenoic and docosahexaenoic acids on immune cell composition and function in healthy humans. *Am J Clin Nutr*; 2004; 79: 674-681.
12. Mickleborough TD, Tecklenburg SL, Montgomery GS, 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*; 2009; 28: 71-77.
13. Weldon SM, Mullen AC, Loscher CE, Hurley LA, Roche HM: Docosahexaenoic acid induces an anti-inflammatory profile in lipopolysaccharide-stimulated human THP-1 macrophages more effectively than eicosapentaenoic acid. *J Nutr Biochem*; 2007; 18: 250-258.
14. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, 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*; 2002; 196: 1025-1037.
15. Levy BD, Kohli P, Gotlinger K, Haworth O, Hong S, Kazani S, Israel E, Haley KJ, Serhan CN: Protectin D1 is generated in asthma and dampens airway inflammation and hyperresponsiveness. *J Immunol*; 2007; 178: 496-502.
16. Anderson SD, Argyros GJ, Magnussen H, Holzer K: Provocation by eucapnic voluntary hyperpnoea to identify exercise induced bronchoconstriction. *Br J Sports Med*; 2001; 35: 344-347.
17. Rundell KW, Spiering BA, Baumann JM, Evans TM: Effects of montelukast on airway narrowing from eucapnic voluntary hyperventilation and cold air exercise. *Br J Sports Med*; 2005; 39: 232-236.
18. 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, Wanger J: Standardisation of spirometry. *Eur Respir J*; 2005; 26: 319-338.
19. 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, Wirtz H: Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J*;

2005; 26: 523-548.

20. Lu Y, Hong S, Tjonahen E, Serhan CN: Mediator-lipidomics: databases and search algorithms for PUFA-derived mediators. *J Lipid Res*; 2005; 46: 790-802.

21. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE: Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol*; 1985; 122: 51-65.

22. Horrocks LA, Yeo YK: Health benefits of docosahexaenoic acid (DHA). *Pharmacol Res*; 1999; 40: 211-225.

23. Yokoyama A, Hamazaki T, Ohshita A, Kohno N, Sakai K, Zhao GD, Katayama H, Hiwada K: Effect of aerosolized docosahexaenoic acid in a mouse model of atopic asthma. *Int Arch Allergy Immunol*; 2000; 123: 327-332.

Author Information

S. K. Head, PhD

Human Performance and Exercise Biochemistry Laboratory, Department of Kinesiology, Indiana University
Bloomington, IN, USA

T. D. Mickleborough, PhD

Indiana University School of Medicine, Department of Cellular and Integrative Physiology
Indianapolis, IN, USA
tmickleb@indiana.edu