Protective Effect of Fish Oil Supplementation on Exercise-Induced Bronchoconstriction in Asthma

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Background: **Previous research has demonstrated that fish oil supplementation has a protective effect on exercise-induced bronchoconstriction (EIB) in elite athletes, which may be attributed to its antiinflammatory properties. Since EIB in asthma involves proinflammatory mediator release, it is feasible that fish oil supplementation may reduce the severity of EIB in asthmatic subjects.**
Study objectives: **To determine the efficacy of fish oil supplementation on severity of EIB in subjects with asthma.**
Design: **Randomized, double-blind, crossover study.**
Setting: **Lung function and exercise testing in a university research laboratory.**
Patients and measurements: **Sixteen asthmatic patients with documented EIB entered the study on their normal diet and then received either fish oil capsules containing 3.2 g of eicosapentaenoic acid and 2.0 g of docohexaenoic acid (fish oil diet, n = 8) or placebo capsules (placebo diet, n = 8) daily for 3 weeks. At the beginning of the study (normal diet) and at the end of each treatment phase, the following pre-exercise and postexercise measures were assessed: (1) pulmonary function; (2) induced sputum differential cell count percentage and proinflammatory eicosanoid metabolite (leukotriene C_4 [LTC_4]-leukotriene E_4 [LTE_4] and prostaglandin D_2 [PGD_2]) and cytokine (interleukin [IL]-1β and tumor necrosis factor [TNF]-α) concentrations; and (3) eicosanoid metabolites leukotriene B_4 (LTB_4) and leukotriene B5 (LTB_5) generation from activated polymorphonuclear leukocytes (PMNLs).**
Results: **On the normal and placebo diet, subjects exhibited EIB. However, the fish oil diet improved pulmonary function to below the diagnostic EIB threshold, with a concurrent reduction in bronchodilator use. Induced sputum differential cell count percentage and concentrations of LTC_4-LTE_4, PGD_2, IL-1β, and TNF-α were significantly reduced before and following exercise on the fish oil diet compared to the normal and placebo diets. There was a significant reduction in LTB_4 and a significant increase in LTB_5 generation from activated PMNLs on the fish oil diet compared to the normal and placebo diets.**
Conclusion: **Our data suggest that fish oil supplementation may represent a potentially beneficial nonpharmacologic intervention for asthmatic subjects with EIB.**

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Key words: cytokines; diet; eicosanoids; exercise-induced asthma; polyunsaturated fatty acids

Abbreviations: AA = arachidonic acid; ANOVA = analysis of variance; AUC_{0-60} = area under the curve of the percentage fall in postexercise FEV_1 plotted against time for 60 min; CI = confidence interval; DHA = docosahexanoic acid; EIB = exercise-induced bronchoconstriction; EPA = eicosapentanoic acid; IL = interleukin; LA = linoleic acid; LTB_4 = leukotriene B_4; LTB_5 = leukotriene B_5; LTC_4 = leukotriene C_4; LTE_4 = leukotriene E_4; PGD_2 = prostaglandin D_2; PGF_2 = prostaglandin F_2; PMNL = polymorphonuclear leukocyte; PUFA = polyunsaturated fatty acid; TNF = tumor necrosis factor

Exercise-induced bronchoconstriction (EIB) refers to the transient narrowing of the airways that can occur during and following vigorous exercise, resulting in a postexercise decrement in lung function.[1] Exercise is a powerful trigger of asthma symptoms and may result in asthmatic patients avoiding physical activity, leading to detrimental consequences to their health. Approximately 80% of

individuals with asthma and a high prevalence of nonatopic elite athletes are hyperresponsive to exercise and experience EIB.[2]

The mechanism responsible for EIB in patients with asthma is not completely understood. However, it is generally accepted that exercise-induced hyperpnea plays an important role as an initiating stimulus through airway surface effects of water loss, and include mucosal cooling and dehydration.[1] It has been suggested that transient dehydration causes an increase in airway surface liquid osmolarity that would activate proinflammatory mediators, such as histamine and the arachidonic acid (AA) metabolites leukotrienes and prostaglandins from resident airway cells, resulting in bronchial smooth-muscle contraction and subsequent airway obstruction.[3] Alternatively, it has been suggested that airway cooling primarily affects the bronchial vasculature, such that rapid rewarming of the airways following exercise may lead to vascular hyperemia and airway edema,[4] which would contribute further to the airway narrowing.

There is accumulating evidence that dietary modification has potential to reduce the prevalence and incidence of asthma[5] and EIB.[6] A possible contributing factor to the increased incidence of asthma in Western societies may be the consumption of a proinflammatory diet.[7] In the typical Western diet, 20-fold to 25-fold more omega (n)-6 polyunsaturated fatty acids (PUFAs) than n-3 PUFAs are consumed, which results in the release of proinflammatory AA metabolites.[8] Eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) are n-3 PUFAs derived from fish oil that competitively inhibit n-6 PUFA AA metabolism and thus reduce the generation of proinflammatory four-series leukotrienes and two-series prostaglandins[9] and production of cytokines from inflammatory cells.[10] Since asthma and EIB are both mediator-driven inflammatory processes, it is possible that the high content of EPA and DHA found in fish oil might reduce airway inflammation. Thus, fish oil supplementation may prove to be a useful intervention for primary prevention of EIB.

We have shown[11] in elite athletes with EIB that supplementing the diet with fish oil capsules containing 3.2 g of EPA and 2.2 g of DHA for 3 weeks reduced the fall in FEV_1 at 15 min after exercise by approximately 80%, as well as a $>$ 20% reduction in bronchodilator use. In addition, the fish oil diet resulted in a significant suppression of proinflammatory eicosanoids leukotriene E_4 (LTE_4), 9α, 11β-prostaglandin F_2 (PGF_2), and cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β.

However, intervention study[12] findings have been equivocal with regard to the clinical effect of n-3 PUFA supplementation in asthma. Prior to the present study, only one other study[13] has evaluated the effect of fish oil supplementation on the airway response to exercise in patients with asthma, and demonstrated no significant change in postexercise lung function despite a suppression of neutrophil leukotriene B_4 (LTB_4) generation.

Therefore, the aim of the present study was to examine more fully the effect of fish oil supplementation in asthmatic patients who experience EIB. In particular, we sought to characterize airway inflammation directly via induced sputum by measuring differential airway cell counts and fluid phase mediators, and to assess tetraene and pentaene leukotriene generation from activated polymorphonuclear cells (PMNLs). We hypothesized that fish oil supplementation will lessen airway inflammation, severity of EIB, medication usage, and improve postexercise pulmonary function in asthmatic subjects.

Materials and Methods

Subjects

Sixteen subjects (10 men and 6 women; mean age ± SD, 23 ± 1.6 years; body mass index, 23.05 ± 2.2 kg/m^2 [normal diet]) with both physician-diagnosed asthma and documented EIB were recruited from a population of university students and the local community and indicated they were recreationally active. All subjects had clinically treated mild-to-moderate persistent asthma, with an FEV_1 $>$ 70% of predicted.[14] A group of nonasthmatic (control) subjects was not included in the present study, as it has been shown that fish oil supplementation does not alter pulmonary function or inflammatory mediator generation in this population.[11]

All subjects had a history of shortness of breath, chest tightness, and intermittent wheezing after exercise, relieved by bronchodilator therapy (n = 7, salbutamol; n = 9, terbutaline). All subjects were asked to withhold taking their maintenance medications (informed consent was obtained from each subject and their physician) prior to participation in the study. Inhaled corticosteroids (n = 5, budesonide), 5-lipoygenase inhibitors (n = 3, zileuton), and leukotriene receptor D_4 antagonists (n = 3, montelukast; n = 5, zafirlukast) were withheld for 4 weeks prior to the start of the study. Short-acting β_2-agonists

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were discontinued 12 h prior to exercise testing. The subjects were also asked to refrain from coffee/alcohol and physical exercise 8 h and 24 h, respectively, prior to the exercise challenge.

Subjects were also excluded if they had a history of taking n-3 PUFA supplements or supplements with antioxidants above the levels recommended for adequate intake, or regularly consumed more than one fish meal per week. Subjects were asked not to eat fish during the course of the study. Each subject completed a health questionnaire and gave written informed consent before enrollment in the study. The local Institutional Research Ethics Committee approved the study protocol.

Study Protocol

The study was conducted as a randomized, double-blind, placebo-controlled crossover trial over 8 consecutive weeks, with each subject serving as their own control. All subjects (n = 16) entered the study on their normal diet (phase 1), after which they were randomly assigned to receive either 20 capsules per day of a triglyceridic oil containing approximately 18% EPA (EPAX 3000 TG; Pronova Biocare; Lysaker, Norway; 160 mg of EPA per gram of triglyceride) and 12% DHA (100 mg of DHA per gram of triglyceride), with 1 to 2 mg of tocopherol per gram of triglyceride added, in 1,000-mg soft gel capsules (n = 8, fish oil diet) equaling 3.2 g of EPA and 2.0 g of DHA or identical placebo (n = 8, placebo diet) capsules containing olive oil for 3 weeks (phase 2). Thereafter, they followed a 2-week washout period (normal diet; phase 3) and then switched to the alternative diet for the remaining 3 weeks (phase 4). All subjects were asked to record bronchodilator use during the last 2 weeks on the normal diet and during the last 2 weeks of each dietary treatment period. Dietary cards were recorded for the duration of the study.

At an initial screening test on the normal diet and at the end of each 3-week treatment phase, pulmonary function was assessed before exercise and at 1, 5, 10, 15, 30, 45, and 60 min after exercise. The screening test was conducted to examine all subjects for the presence of EIB, as indicated by a drop > 10% in postexercise FEV_1 compared with pre-exercise values.[15] At the beginning of the study (normal diet) and at the end of each treatment period, all subjects reported to the laboratory and had venous blood drawn from the antecubital vein before exercise for neutrophil fatty acid analysis. Additional blood was drawn for the determination leukotriene LTB_4 and leukotriene B_5 (LTB_5) production prior to exercise and at 15 min and 60 min following exercise. All subjects underwent sputum induction 48 h prior to exercise in order to establish baseline values, and at 1 h and 24 h after exercise for the determination of sputum differential cell counts and sputum supernatant proinflammatory mediator concentration (leukotriene C_4 [LTC_4]-LTE_4, prostaglandin 2 [PGD_2], IL-1β, and TNF-α). At the end of the 2-week washout period, all subjects reported to the laboratory to have additional blood drawn to verify that neutrophil fatty acid composition and pulmonary function had returned to baseline values established at the beginning of the study on the normal diet.

Exercise Challenge Test

Each subject ran on motorized treadmill, which was elevated 1% per minute until volitional fatigue. Each subject wore a nose clip during the exercise bout and inspired compressed dry air as described previously.[11] Breath-by-breath analysis of expired gases was accomplished by indirect open circuit calorimetry (Vmax 22 metabolic cart; SensorMedics; Yorba Linda, CA). During the exercise test, heart rate was continuously monitored by ECG (Quinton Q710 Stress Test Monitor; Quinton Instrument Company; Seattle, WA), and arterial oxygen saturation was estimated using pulse oximetry.[15]

Pulmonary Function Tests

Spirometry was performed by all subjects in the sitting position using a calibrated computerized spirometer (Superspiro; Micro Medical; Rochester, Kent, UK). Subjects were required to perform three acceptable FVC maneuvers according to American Thoracic Society recommendations.[16] The maximum percentage fall in FEV_1 from the baseline (pre-exercise) value was calculated using the following equation: (pre-exercise FEV_1 – lowest postexercise FEV_1)/(pre-exercise FEV_1). In addition, the bronchoconstrictor response to exercise was also assessed as the area under the curve of the percentage fall in postexercise FEV_1 plotted against time for 60 min (AUC_{0-60}). The AUC_{0-60} was calculated using trapezoidal integration.

Induced-Sputum Production and Processing

Prior to sputum induction, all subjects inhaled 200 μg of salbutamol to minimize bronchoconstriction during the induction procedure. Sputum was induced by inhalation of 3%, 4%, and 5% hypertonic saline solution in sequence for 5 min (DeVilbiss 65 ultrasonic nebulizer; DeVilbiss; Somerset, PA), and the subjects were encouraged to cough and expectorate sputum into sterile containers. FEV_1 was measured after each nebulization. Nebulizations were stopped if a fall in FEV_1 of > 20% compared to baseline values occurred or if troublesome symptoms appeared.

Sputum was examined as previously described.[17] Differential counts were expressed as corrected percentages after subtraction of squamous cells. Sputum eosinophilia was defined as a sputum differential eosinophils count > 2%. To ensure good cell viability, sputum was processed within 2 h of collection.[18]

Induced-Sputum Fluid Phase Measurements

The concentrations of inflammatory mediators in sputum supernatant were determined by competitive immunoassays for PGD_2 (Cayman Chemical; Ann Arbor, MI), cysteinyl leukotrienes (LTC_4-LTE_4), and by sandwich enzyme-linked immunosorbent assay for IL-1β and TNF-α using previously described methods.[17] Because PGD_2 is a relatively unstable compound, we measured PGD_2 methoxime, a stable derivative of PGD_2. The intra-assay and interassay coefficient of variability was 5 to 10% and 3 to 15%, respectively, across the range of mediators measured.

Ex vivo *PMNL LTB_4 and LTB_5 Quantification*

Although several immunoassay quantification techniques for LTB_4 and/or LTB_5 have been developed, they do not allow the simultaneous quantification of LTB_4 and LTB_5 because of the cross-reactivity between these structurally closely related compounds.[19] Due to the difficulty in isolating individual cell types from induced sputum, PMNLs were isolated from venous blood in order to measure the amount of LTB_4 and LTB_5 generated by activated PMNLs using reverse-phase high-performance liquid chromatography.

Purified preparations of PMNLs were prepared as previously described with minor modifications.[19] Briefly, heparinized blood was mixed with 2 mL of 6% dextran (Sigma Chemical Company; St. Louis, MO). The supernatant was layered on a Ficoll-Paque (Pharmacia LKB; Uppsala, Sweden) and centrifuged at 400*g* for 30 min at 25°C. The PMNL fraction was then added to 0.5 mL of distilled H_2O, and contaminating erythrocytes were removed by hypotonic lysis and washed twice (Eagle MEM solution; Life Technologies; Chagrin Falls, OH) and resuspended at a concentration of 2×10^{10} cells/L. Cell suspensions (1×10^7 cells) were

incubated with 25 μmol/L of calcium ionophore A23187 (Sigma Chemical Company), and dissolved in 50 μL of 1% dimethylsulfoxide (by volume) for 10 min at 37°C. The reaction was terminated by adding 1 mL of cold ethanol, and then 50 ng of prostaglandin B_2 was added as an internal standard.

After centrifugation at 1,000*g* for 5 min at 4°C of the ethanolic solution, the supernatant was purified and extracted by using octadecylsilyl silica microcolumns cartridges (C18 Sep-Pak; Waters Corporation; Milford, MA). Briefly, the ethanolic solution was acidified with 2 mol/L citrate per liter to pH 3 and then applied to a C18 Sep-Pak. After washing this column with 10 mL of H_2O and 15% (by volume) of ethanol (10 mL), leukotrienes and prostaglandin B_2 were eluted with 3 mL of methanol. The methanol fraction was evaporated to dryness under a stream of argon. The residue was dissolved in 100 μL of methanol, and a 20-μL aliquot sample was used for the determination of LTB_4 and LTB_5 using reverse-phase high-performance liquid chromatography (HPLC System Model 510; Waters Associates). A Bondasphere 5 μ C18 column (Waters Corporation, Milford, MA) was used under ambient conditions, and methanol/water/acetic acid (70:30:0.01, by volume) was used for separation as the mobile phase. Solvents were purified by distillation before use. Identification of peaks was made by retention times compared with those of synthetic LTB_4 and LTB_5 (Cayman Chemical Company) and ultraviolet-absorbance spectrum of each peak.

Neutrophil Phospholipid Fatty Acid Analysis

Neutrophils were purified from venous blood by means of dextran sedimentation, and the phospholipid fatty acid composition of neutrophil phospholipids was determined using gas chromatography as previously described.[11]

Statistical Analysis

Data were analyzed using statistical software (SPSS version 12; SPSS; Chicago, IL). A repeated-measures analysis of variance (ANOVA) was used to analyze the data, with both treatment and time as within-subject effects. Where a significant *F* ratio was found ($p < 0.05$), a Fisher protected least-square difference *post hoc* test was used to detect differences in group means ($p < 0.05$). IS differential cell counts were analyzed nonparametrically using the Friedman repeated-measures ANOVA on ranks and described as median and interquartile range. Induced-sputum supernatant mediator concentrations (corrected for sputum dilution) were normally distributed.

Correlations between induced-sputum cell counts, sputum supernatant mediator concentrations, and the severity of EIB were calculated using Spearman rank-order correlation coefficients and Pearson product moment correlation coefficient. All reported p values were considered significant at the 0.05 level. Pulmonary function and neutrophil phospholipid fatty acid composition are expressed as mean and 95% confidence intervals (CIs). Data were analyzed for the presence of carry-over effects between treatments using a 2 × 2 ANOVA.

Results

Subjects

Bronchodilator use (total number of doses/puffs) was significantly reduced ($p < 0.05$) during the last 2 weeks of the fish oil diet (45 puffs; 95% CI, 34 to 51 puffs) compared to the normal diet (61 puffs; 95% CI, 53 to 68 puffs) and placebo diet (65 puffs; 95% CI, 56 to 72 puffs). There was no significant difference ($p > 0.05$) in bronchodilator use between the normal and placebo diets. A 2 × 2 ANOVA that was used to test for the presence of carryover effects between diets indicated that none were present for all measured variables ($p > 0.05$); this was further supported by postexercise pulmonary function and neutrophil phospholipid fatty acid composition measured at the end of the 2-week washout period, returning to baseline levels established at the beginning of the study on the normal diet.

Pulmonary Function and Exercise Challenge Test

No significant difference ($p > 0.05$) was observed in baseline (pre-exercise) pulmonary function between diets (Table 1). The percentage change in pre-exercise to postexercise FEV_1, as a result of diet, is shown in Figure 1. Subjects demonstrated EIB on the normal and placebo diets with a significant ($p < 0.05$) decline of 22.4% (− 0.57 L; 95% CI, − 0.24 to − 0.79 L) and 21.3% (− 0.52 L; 95% CI, − 0.28 to − 0.71 L), respectively, at 15 min after exercise. Reductions in the postexercise decline in $FEV_1 > 10\%$ occurred for up to 60 min on the

Table 1—*Pre-exercise (Baseline) Pulmonary Function

Variables	Diet		
	Normal	Placebo	Fish Oil
FVC, % predicted*	3.67 (3.54–3.85)†	3.76 (3.64–3.88)	3.69 (3.54–3.76)
	86.5 (78.9–93.2)	87.6 (83.2–96.8)	87.1 (80.1–94.6)
FEV_1, % predicted*	3.09 (2.94–3.21)	3.14 (2.99–3.26)	3.05 (2.91–3.19)
	88.2 (82.1–97.9)	89.4 (84.4–98.5)	87.7 (81.9–93.4)
FEV_1/FVC, % predicted*	84.2 (78.4–86.3)	83.5 (76.6–87.9)	82.6 (75.6–88.3)
	89.6 (82.5–93.7)	88.3 (81.8–95.6)	85.4 (79.3–96.2)
$FEF_{25–75\%}$, % predicted	2.93 (2.84–3.04)	2.98 (2.82–3.05)	2.89 (2.74–2.98)
	90.3 (85.8–96.7)	91.7 (86.4–97.8)	88.8 (82.6–94.3)

*Data are presented as mean (95% CI). There were no significant differences for any variable between diet ($p > 0.05$). $FEF_{25–75\%}$ = forced expiratory flow at 25 to 75% of FVC.

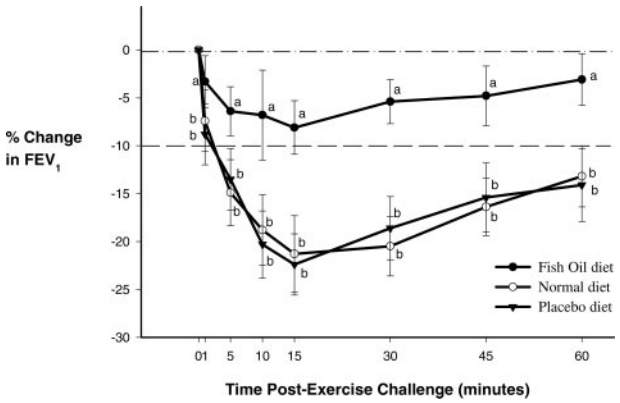

FIGURE 1. The percentage change in FEV_1 from before to after exercise across the three diets. Reductions in FEV_1 > 10% represent a positive diagnosis of EIB. Letters a and b refer to comparisons by diet within respective time period. Different letters designate a significant difference ($p < 0.05$).

normal and placebo diets. The percentage fall in FEV_1 on the fish oil diet decreased by only 8.1% (− 0.19 L; 95% CI, − 0.09 to − 0.29 L), which is indicative of an attenuated EIB response. Similar changes as a result of diet were observed for FVC and forced expiratory flow at 25 to 75% of FVC. The severity of EIB, as determined by the AUC_{0-60}, was significantly greater ($p < 0.05$) on the normal diet (1,024; 95% CI, 978 to 1,070) and placebo diet (1,045.1; 95% CI, 997 to 1,093) compared to the fish oil diet (328.3; 95% CI, 315 to 342). No significant difference ($p > 0.05$) in peak aerobic capacity, peak minute ventilation, or time to exhaustion was observed between diets.

Induced-Sputum Differential Cell Counts and Fluid Phase Mediator Concentrations

Prior to supplementation, there was no significant difference ($p > 0.05$) in induced-sputum differential cell count between the n-3 PUFA and placebo diet (Table 2). However, following the supplementation period at before exercise and at 60 min and 24 h after exercise, the fish oil diet induced a significant reduction ($p < 0.05$) in the percentage of eosinophils, neutrophils, and a significant increase ($p < 0.05$) in the percentage of macrophages, compared to the placebo and normal diets (Table 2).

The sputum supernatant inflammatory mediator concentrations are shown in Figure 2. There was no significant change ($p > 0.05$) in presupplementation mediator concentration between groups. However, significantly lower ($p < 0.05$) sputum supernatant LTC_4-LTE_4 (Fig 2, *top left*), PGD_2 (Fig 2, *top right*), IL-1β (Fig 2, *bottom left*), and TNF-α (Fig 2, *bottom right*) concentrations were observed following fish oil supplementation before exercise and at 1 h and 24 h after exercise compared to the placebo and normal diets. Following the fish oil supplementation period, pre-exercise sputum LTC_4-LTE_4, PGD_2, IL-1β, and TNF-α concentrations significantly decreased ($p < 0.05$) by 74.4% (− 3.2 ng/mL; 95% CI, − 1.2 to − 4.6 ng/mL), 93.8% (− 0.15 ng/mL; 95% CI, − 0.08 to − 0.26 ng/mL), 77.1% (− 3.7 ng/mL; 95% CI, − 1.9 to − 4.8 ng/mL), and 95.4% (− 41.3 pg/mL; 95% CI, − 27.6 to 54.2 pg/mL), respectively, compared to the placebo diet. A significant reduction ($p < 0.05$) in these mediators on the fish oil diet was also observed at 1 h and 24 h after exercise compared to the placebo and normal diets.

The percentage of postexercise sputum eosinophils from subjects on the placebo diet was positively correlated with the severity of EIB (percentage fall in FEV_1, $r = 0.73$, $p = 0.0041$; 95% CI, 0.51 to 0.89; and AUC_{0-60}, $r = 0.68$, $p = 0.0034$; 95% CI, 0.54 to 0.77) and for the normal diet (percentage fall in FEV_1, $r = 0.76$, $p = 0.0028$; 95% CI, 0.62 to 0.84; and AUC_{0-60}, $r = 0.72$, $p = 0.0031$; 95% CI, 0.64 t0 0.81). There was also a positive correlation on the placebo diet between the percentage of sputum neutrophils and the severity of EIB ($r = 0.69$, $p = 0.0045$; 95% CI, 0.54 to 0.85), AUC_{0-60} ($r = 0.64$, $p = 0.0051$; 95% CI, 0.49 to 0.79), and for the normal diet (percentage fall in FEV_1, $r = 0.72$, $p = 0.0034$; 95% CI, 0.61 to 0.81; and AUC_{0-60}, $r = 0.71$, $p = 0.0041$; 95% CI, 0.62 to 0.84).

Ex Vivo *PMNL LTB_4 and LTB_5 Generation*

The amount of LTB_4 and LTB_4 generated by PMNLs obtained from the asthmatic patients before and after treatment (before and after exercise) is shown in Figure 3 (*top left*, and *top right*). There was no significant difference ($p < 0.05$) in presupplementation LTB_4 and LTB_5 production generated by PMNLs. Following fish oil supplementation before exercise, the amount of LTB_4 generated by PMNLs before was significantly reduced ($p < 0.05$) by 131.2% (− 31.0 ng × 10^7 cells; 95% CI, − 15.7 to − 52.4 ng × 10^7 cells) compared to the placebo diet before exercise (Fig 3, *top left*). The fish oil diet induced a significant increase ($p < 0.05$) before exercise in the amount of LTB_5 generated from PMNLs by 156.3% (10 ng × 10^7 cells; 95% CI, 3.9 to 15.5 ng × 10^7 cells) compared to the placebo diet (Fig 3, *top right*).

Neutrophil Phospholipid Fatty Acid Analysis

Neutrophil phospholipid PUFA content is expressed as a percentage of total phospholipid fatty acid content and is shown in Table 3. No significant changes ($p > 0.05$) were observed in neutrophil membrane content when comparing before and after

Table 2—*Induced-Sputum Differential Cell Counts

Variables	Diet		
	Fish Oil Diet	Normal Diet	Placebo Diet
Total cell count, $\times 10^6$/mL			
Before supplementation	2.4 (0.4–4.5)[a]	N/A	2.7 (0.2–4.9)[a]
After supplementation			
Before exercise	1.9 (0.2–3.8)[a]	2.6 (0.4–5.0)[a]	2.9 (0.5–5.2)[a]
60 min after exercise	2.2 (0.2–4.2)[a]	3.6 (0.3–5.5)[a]	3.8 (0.1–6.0)[a]
24 h after exercise	2.0 (0.1–4.7)[a]	3.2 (0.2–5.9)[a]	3.4 (0.2–6.2)[a]
Viability, %			
Before supplementation	78.9 (65.2–86.4)[a]	N/A	77.5 (63.4–84.6)[a]
After supplementation			
Before exercise	81.2 (64.3–87.9)[a]	79.5 (64.1–85.4)[a]	80.9 (66.5–86.8)[a]
60 min after exercise	77.9 (64.3–85.7)[a]	78.6 (62.6–87.4)[a]	79.7 (64.7–88.5)[a]
24 h after exercise	80.4 (65.8–87.6)[a]	77.8 (62.3–89.6)[a]	80.7 (64.7–89.6)[a]
Eosinophils, %			
Before supplementation	7.6 (0.7–16.9)[a]	N/A	8.3 (0.7–19.3)[a]
After supplementation			
Before exercise	6.2 (2.3–24.5)[a]	11.9 (3.7–29.7)[b]	15.8 (1.9–26.5)[b]†
60 min after exercise	9.1 (1.7–22.1)[a]	16.8 (3.2–46.3)[b]	14.6 (3.4–47.6)[b]†
24 h after exercise	10.8 (0.7–24.3)[a]	18.6 (1.5–29.4)[b]	13.5 (1.9–28.3)[b]†
Neutrophils, %			
Before supplementation	30.3 (6.8–59.1)[a]	N/A	34.1 (8.6–64.4)[a]
After supplementation			
Before exercise	32.7 (8.2–52.5)[a]	45.7 (9.3–68.9)[b]	43.2 (10.9–79.3)[b]†
60 min after exercise	43.2 (6.5–65.2)[a]†	51.2 (12.1–76.3)[b]	53.3 (13.9–85.3)[b]†
24 h after exercise	36.3 (9.1–54.7)[a]	46.2 (6.8–65.2)[b]	45.8 (13.2–62.3)[b]†
Lymphocytes, %			
Before supplementation	0.82 (0.2–1.3)[a]	N/A	0.79 (0.2–1.5)[a]
After supplementation			
Before exercise	0.61 (0.1–1.8)[a]	0.84 (0.2–1.7)[b]	0.86 (0.3–1.6)[b]
60 min after exercise	0.69 (0.2–2.1)[a]	0.96 (0.4–2.3)[b]	0.99 (0.5–2.7)[b]
24 h after exercise	0.74 (0.2–1.7)[a]	0.87 (0.1–1.8)[b]	0.85 (0.3–1.9)[b]
Macrophages, %			
Before supplementation	56.7 (8.5–86.5)[a]	N/A	52.4 (11.4–84.6)[a]
After supplementation			
Before exercise	47.8 (2.7–77.8)[a]†	29.6 (4.3–68.3)[b]	33.6 (3.1–61.2)[b]†
60 min after exercise	43.6 (5.4–70.4)[a]†	24.7 (3.6–43.7)[b]	27.8 (4.3–54.2)[b]†
24 h after exercise	50.3 (5.6–82.1)[a]†	29.8 (5.9–56.8)[b]	31.3 (6.2–62.7)[b]†
Bronchial epithelial cells, %			
Before supplementation	1.6 (0.2–18.4)[a]	N/A	1.8 (0.3–16.4)[a]
After supplementation			
Before exercise	1.9 (0.1–15.2)[a]	2.2 (0.1–20.2)[a]	2.1 (0.2–17.1)[a]
60 min after exercise	1.7 (0.3–13.4)[a]	2.4 (0.2–17.4)[a]	2.3 (0.3–16.7)[a]
24 h after exercise	1.8 (0.2–16.2)[a]	2.1 (0.3–18.9)[a]	2.0 (0.1–19.4)[a]

*Data are expressed as median (interquartile range). N/A = not available.

†Postsupplementation values significantly different ($p < 0.05$) to presupplementation (baseline) values (established at the beginning of the study) within diet. Values with different letters (a, b, c) are significantly different ($p < 0.05$) between diets; the same letters are not significantly different ($p > 0.05$) between diets.

supplementation values for linoleic acid (LA), AA, EPA, and DHA on the placebo diet. However, following fish oil supplementation the neutrophil phospholipid content of EPA and DHA significantly increased ($p < 0.05$), while the neutrophil phospholipid content of LA and AA was significantly reduced ($p < 0.05$).

Discussion

This study has demonstrated that a diet supplemented with fish oil ameliorates the severity of exercise-induced airway narrowing in subjects with mild-to-moderate persistent asthma. The fish oil diet improved pulmonary function to below the diagnostic EIB threshold of a 10% fall in postexercise FEV_1, and reduced the fall in FEV_1 at 15 min after exercise by approximately 64%. This improvement in postexercise pulmonary function on the fish oil diet was accompanied by a > 31% reduction in bronchodilator use.

Using the relatively noninvasive technique of sputum induction, we have shown for the first time that a diet supplemented with fish oil reduces airway

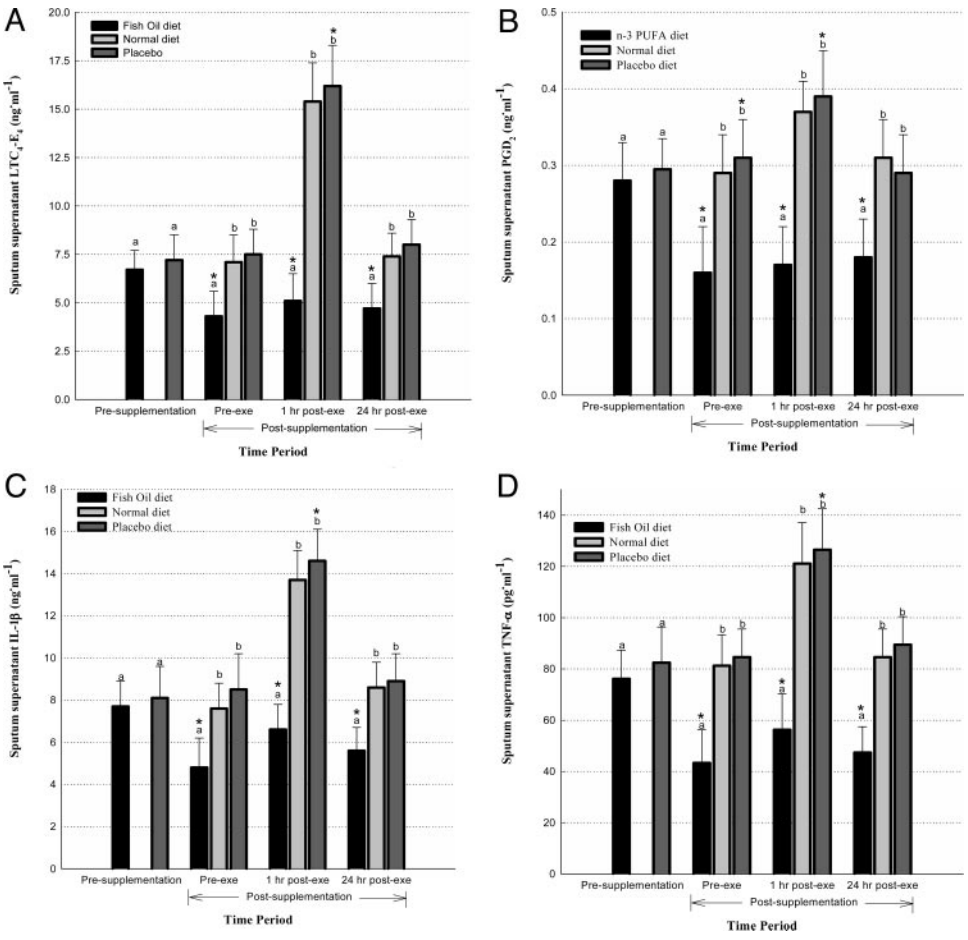

FIGURE 2. *Top left*: Mean sputum LTC_4-LTE_4 concentration. *Indicates significant difference ($p < 0.05$) compared with presupplementation value within diet. A difference in letter (a to b) designates a significant differences ($p < 0.05$) across diet within time. *Top right*: Mean sputum PGD_2 concentration. *Indicates significant difference ($p < 0.05$) compared with presupplementation value within diet. A difference in letter (a to b) designates significant differences ($p < 0.05$) across diet within time. *Bottom left*: Mean sputum IL-1β concentration. *Indicates significant difference ($p < 0.05$) compared with presupplementation value within diet. A difference in letter (a to b) designates significant differences ($p < 0.05$) across diet within time. *Bottom right*: Mean sputum TNF-α concentration. *Indicates significant difference ($p < 0.05$) compared with presupplementation value within diet. A difference in letter (a to b) designates significant differences ($p < 0.05$) across diet within time. exe = exercise.

inflammation in asthmatic subjects with EIB. Specifically, we found that sputum differential eosinophil and neutrophil cell counts and sputum supernatant concentrations of proinflammatory eicosanoids LTC_4-LTE_4, and PGD_2 and cytokines IL-1β and TNF-α were significantly reduced on the fish oil diet. In addition, the fish oil diet decreased LTB_4 and increased LTB_5 generation from activated PMNLs obtained from venous blood.

In the present study dietary, compliance was monitored by measuring incorporation of EPA and DHA into the cell membranes of neutrophils. Dietary enrichment with 3.2 g of EPA and 2.0 g of DHA caused a significant increase in the EPA and DHA content and a reduction of AA and LA content of neutrophil phospholipid in the asthmatic subjects. It has been shown previously that supplementing the diet with fish oil, providing > 2.4 g (EPA plus DHA)/d results in an inhibition of leukotriene production,[20–23] and a suppression of TNF-α synthesis and circulating levels of eosinophils in asthmatic subjects.[24]

EPA and DHA, derived from fish oil, can cause dual inhibition of cyclooxygenase-2 and 5-lipoxyeganse pathways for metabolism of AA. EPA is a much less preferred substrate compared with AA for both pathways, and generally by substrate competition inhibits release of AA-derived eicosanoids, thus reducing the generation of proinflammatory "tetraene" four-series leukotrienes and two-series pro-

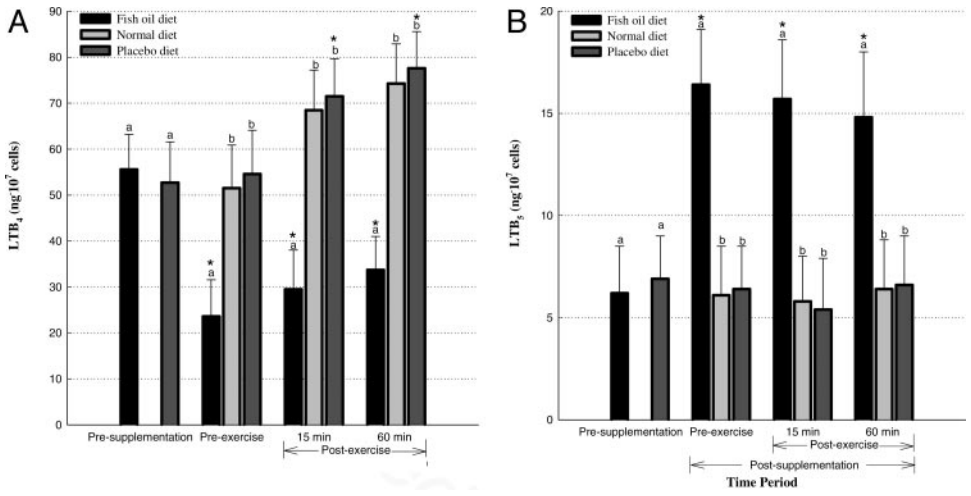

FIGURE 3. *Left*: Mean LTB_4 generation by PMNLs. *Indicates significant difference ($p < 0.05$) compared with presupplementation value within diet. A difference in letter (a to b) designates significant differences ($p < 0.05$) across diet within time. *Right*: Mean LTB_5 generation by PMNLs. *Indicates significant difference ($p < 0.05$) compared with presupplementation value within diet. A difference in letter (a to b) designates significant differences ($p < 0.05$) across diet within time.

stanoids.[9] The present study has shown an attenuation of the four-series cysteinyl leukotrienes obtained from induced sputum and LTB_4 generation from activated PMNLs on the fish oil diet. Eosinophils, mast cells and basophils can directly synthesize the 4-series cysteinyl LTs (LTC_4-LTE_4), which can increase vascular permeability and contract smooth-muscle cells, causing bronchoconstriction and vasoconstriction, and may directly increase eosinophilic airway inflammation[25]; the "pentaene" five-series cysteinyl leukotrienes are equiactive with their tetraene counterparts in constricting nonvascular smooth muscle.[26] LTB_4 is a potent chemoattractant and activator of neutrophils without any significant effect on airway smooth muscle.[27]

In the present study, the amount of LTB_5 generated from activated PMNLs was markedly increased following fish oil supplementation. LTB_5, the 5,12,-dihydroxy derivative of EPA formed from LTA_5, is a weak and partial antagonist compared with LTB_4 in eliciting chemotactic and aggregating responses in PMNLs.[28] Indeed, the chemotactic activity of PMNLs was found to be 10-fold to 100-fold lower than that of LTB_4, while its aggregating property was found to be 20-fold weaker.[28] Differences in binding affinities of LTB_4 and LTB_5 to the leukotriene B receptors T1 and T2 have been suggested to explain their differences.[29] Consuming fish oil results in partial replacement of AA in inflammatory cell membranes by EPA and thus demonstrates a potentially beneficial antiinflammatory effect of n-3 PUFA.[9,10]

The increase in PGD_2 after exercise on the placebo and normal diet is highly indicative of mast-cell activation.[27] Direct evidence of mast-cell activation following exercise in asthmatics has been shown by an increase in urinary excretion of the PGD_2 metabolite 9α, 11-β PGF_2.[30,31] The present study has shown that a fish oil diet significantly reduces spu-

Table 3—*Fatty Acid Composition of Neutrophil Extracts Expressed as a Percentage of Total Fatty Acid Content Before and After Dietary Supplementation

Diet	18:2† LA	20:4† AA	20:5† EPA	22:6† DHA
Normal diet	15.7 (13.9–17.5)	22.3 (20.4–24.2)	0.18 (0.13–0.23)	2.16 (1.6–2.8)
Placebo diet				
Before supplementation	16.9 (15.1–18.7)	23.4 (21.7–25.1)	0.16 (0.11–0.20)	2.18 (1.5–2.9)
After supplementation	17.3 (15.5–19.1)	22.6 (20.8–24.4)	0.15 (0.11–0.22)	2.20 (1.4–3.0)
Fish oil diet				
Before supplementation	16.8 (14.9–18.6)	23.9 (21.9–25.9)	0.16 (0.12–0.21)	2.24 (1.3–3.2)
After supplementation	8.2 (6.3–10.0)‡	13.1 (11.1–15.1)‡	4.01 (3.1–4.9)‡	3.32 (2.5–4.2)‡

*Data are presented as mean (95% CI).
†Ratio represents number of carbon-carbon double bonds.
‡$p < 0.05$ compared to presupplementation period.

tum PGD_2 concentration in asthmatic subjects with EIB, while our previous work[11] has demonstrated that fish oil supplementation causes a significant decrease in urinary 9α, 11-β PGF_2 following exercise in elite athletes with EIB. Taken together these findings suggest that fish oil supplementation suppresses mast-cell activation in subjects with EIB. PGD_2 has similar effects on airway smooth muscle as the cysteinyl leukotrienes, although less potent, and is primarily responsible for neutrophil activation and increasing vascular permeability.[27]

This study confirms our previous findings[11] in elite athletes with EIB that dietary enrichment with fish oil capsules ameliorates the synthesis of plasma levels of proinflammatory cytokines IL-1β and TNF-α, and corroborates work[24] in asthmatics that fish oil supplementation reduces circulating levels of TNF-α. Although it is known that fish oil ingestion effectively enhances cellular concentrations of EPA and DHA, it is not presently known whether EPA, DHA, or both are involved in the suppression of cytokine production. However, it is known that the four-series leukotrienes, in particular LTB_4, enhances the production of IL-1β and TNF-α.[32] These cytokines have been shown to stimulate collagenases and increase the expression of adhesion molecules.[33] It is possible that the effect of n-3 PUFA on proinflammatory cytokines is independent of eicosanoid activity. This appears to be a distinct possibility, with regulation of the transcription factor nuclear factor κB being involved.[34]

In the present study, a higher percentage of eosinophils and neutrophils were found in induced sputum following exercise on the placebo and normal diet compared to the fish oil diet. There was a significant correlation between the degree of eosinophilic and neutrophilic activation in the asthmatic airways following exercise and the severity of EIB on the placebo and normal diet, and thus supports prior studies[35,36] that eosinophilic airway inflammation is an important determinant of the bronchoconstrictor response to exercise in asthmatics. We observed sputum neutrophilia following exercise at all time points on the placebo diet and at 60 min after exercise on the fish oil diet. It is possible that the observed sputum neutrophilia after exercise on the placebo and fish oil diet may be related to the sputum induction procedure itself.[37] However, the increase in both sputum eosinophils and neutrophils may also be related to the fact that while asthmatic inflammation is associated with airway eosinophilic infiltration,[38] hyperpnea may be associated with neutrophilic inflammation.[39]

While some studies of fish oil supplementation in asthma reveal limited clinical impact, other studies have shown significant improvements in asthma symptoms.[12] Apart from the present study, only one other study[13] has evaluated the efficacy of fish oil ingestion on the airway response to exercise in patients with asthma; these authors found no effect of fish oil supplementation on pulmonary function (specific airways conductance) following exercise, despite a significant increase in n-3 PUFA neutrophil content, a significant suppression in neutrophil chemotaxis, and a 50% inhibition of LTB_4 production by ionophore-stimulated neutrophils.

The data from the present study stand in marked distinction to those reported by Arm and coworkers.[13] The negative findings observed by Arm and coworkers[13] may be due to methodologic and statistical limitations of their study. These authors[13] exercised a cohort of mild asthmatics at a very low exercise intensity (80% of predicted maximal oxygen consumption for 8 min) at ambient temperature and humidity. The exercise intensity level may have been too low an intensity to detect changes in pulmonary function following exercise.[3] It is generally accepted that inhaling cold dry air at high ventilation rates initiates EIB. Rundell and coworkers[40] have shown that of 23 subjects who tested positive for EIB in cold-dry air, 18 subjects (78%) tested negative in ambient conditions (21°C and 50% relative humidity). In addition, Evans et al[41] recently demonstrated that dryness of the test conditions rather than a cold temperature is essential to the EIB response. This suggests that the exercise protocol performed in ambient conditions in the study by Arm et al[13] may have been less sensitive to identifying changes in airway hyperresponsiveness following exercise due to inadequate environmental stress. In addition, there was an uneven distribution of corticosteroid use among the asthma patients; asthma medications capacity to improve asthma symptoms can mask the benefits linked to fish oil supplementation. Further, an assessment of the numbers used in the airway response to exercise in the study by Arm et al[13] (five subjects receiving placebo and six subjects receiving fish oil supplementation) suggests insufficient patients to detect a statistical difference and avoid a type I error.

No significant change in peak aerobic capacity, peak minute ventilation, or time to exhaustion was observed between diets. There are various unanswered questions regarding this observation. Although, most asthmatics can complete exercise without bronchoconstriction occurring, it is possible that bronchoconstriction during exercise may be more common among subjects with more severe asthma than the subjects used in the present study.[42] In addition, bronchoconstricting mediators, such as leukotrienes, prostaglandin, and histamine may not have

much of an effect during exercise because of prevailing bronchodilating mediators such as nitric oxide and PGE_2.[42]

Since, the cysteinyl leukotrienes are, overall, the most important proinflammatory mediators causing EIB in subjects with asthma,[43] an important question is how dietary fish oil supplementation fits in with the available armamentarium (*eg*, cystienyl leukotriene type 1 receptor antagonists and 5-lipoxygenase inhibitors) to decrease the expression of leukotrienes, and whether fish oil supplementation may be additive, or used in its own right to block the EIB response. For example, it is possible that a combination of fish oil supplementation and a cysteinyl LT_1 receptor antagonist may provide a greater antiinflammatory effect against developing EIB that either agent alone? Future work should address this issue.

In summary, this study has shown that fish oil supplementation may represent a potentially beneficial nonpharmacologic intervention in asthmatic patients with EIB. The fish oil diet reduced airway inflammation and the severity of EIB with a concomitant decrease in bronchodilator use. Since the results of the present study differ with those of Arm and colleagues,[13] and given that the present study was conducted in a relatively small group of subjects, such results require reproduction.

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