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Fish Oil Supplementation Reduces Severity of Exercise-Induced Bronchoconstriction in Elite Athletes

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ABSTRACT

Exercise-induced bronchoconstriction (EIB) in elite athletes may respond to dietary modification reducing the need for pharmacological treatment. Ten elite athletes with EIB and ten elite athletes without EIB (control) participated in a randomised double-blind crossover study. Subjects entered the study on their normal diet (NORMAL diet), and then received either fish oil capsules containing 3.2 g eicosapentaenoic acid and 2.2 g docohexaenoic acid (n-3 polyunsaturated fatty acid (PUFA) diet; n=5) or placebo capsules containing olive oil (PLACEBO diet; n=5) taken daily for 3-wk. Diet had no effect on pre-exercise pulmonary function in either group and had no effect on post-exercise pulmonary function in control subjects. However, the n-3 PUFA diet improved post-exercise pulmonary function in EIB subjects compared to the NORMAL diet and PLACEBO diet. FEV_1 decreased by 3 ± 2% on n-3 PUFA diet, 14.5 ± 5% on PLACEBO diet and 17.3 ± 6% on NORMAL diet at 15 min post-exercise. LTE_4, 9α, 11β-PGF_2, LTB_4, TNF-α and IL-1β all significantly decreased on the n-3 PUFA diet compared to NORMAL and PLACEBO diet and following the exercise challenge. These data suggest that dietary fish oil supplementation has a markedly protective effect in suppressing EIB in elite athletes and this may be attributed to their anti-inflammatory properties.

Keywords: exercise, asthma, omega-3 polyunsaturated fatty acids, diet, eicosanoids, cytokines, exercise-induced asthma

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INTRODUCTION

Exercise-induced bronchoconstriction (EIB) is a condition characterized by transient airway narrowing during (1) or following (2,3) exercise, resulting in decrements in post-exercise pulmonary function. A high prevalence of EIB and asthma-like symptoms, such as wheezing, chest tightness abnormal breathlessness, cough and/or sputum production have been reported in the elite athlete population (4-10). Collectively, these data suggest that EIB is more prevalent in elite athletes compared with non-elite athletes and the general population. This relatively high incidence of EIB in elite athletes may be due to exercise hyperventilation, prolonged exposure to allergens and bronchial irritants and excessive inhalation of cold, dry air (7,11).

The mechanisms responsible for bronchial hyperreactivity following exercise in patients with asthma have been extensively investigated (12,13). However, EIB in elite athletes is less understood, and likely involves multiple mechanisms. It has been suggested that transient dehydration of the airways activates inflammatory mediator release, such as histamine, neuropeptides and arachidonic acid metabolites (leukotrienes and prostaglandins) from airway cells (2,13,14), resulting in bronchial smooth muscle contraction. On the other hand, it has been suggested that rapid rewarming of the airways following exercise leads to vascular hyperemia and airway edema (12), which would contribute further to the bronchoconstriction. The possibility also exists that repetitive high-intensity exercise itself may contribute to the development of EIB by the release of inflammatory cytokines (15). Recent evidence of airway remodelling in cross-country skiers (16-18), and the fact that EIB in athletes do not respond well to pharmacological prophylaxis (17), suggests a pathology different from that found in asthma.

Treatment of EIB almost exclusively involves the use of pharmacological medications. However, there is accumulating evidence that dietary modification can modify the severity of exercise-induced asthma and EIB (19-24). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are omega-3 (n-3) polyunsaturated fatty acids (PUFA) derived from fish oil that competitively inhibit n-6 PUFA arachidonic acid metabolism and thus reduce the generation of inflammatory 4-series leukotriene and 2-series prostaglandin mediators (25) and the production of cytokines from inflammatory cells (26). Consuming fish oil results in partial replacement of arachidonic acid in inflammatory cell membranes by EPA (25,26). This response alone is a potentially beneficial anti-inflammatory effect of n-3 PUFA. It has been demonstrated that supplementing the diet with n-3 PUFA has reduced arachidonic acid concentrations in neutrophils and neutrophil chemotaxis, reduced LT generation (25,27) and reduced airway late response to allergen exposure (28). These data are consistent with the proposed pathway by which dietary intake of n-3 PUFA modulates lung disease. However, clinical data on the effect of fish oil supplementation in asthma has been equivocal. While no clinical improvement in asthmatic symptoms has been observed in some interventional studies (29-32), other studies have demonstrated an improvement in asthmatic status following n-3 PUFA supplementation (28, 33-37). To date only one study has evaluated the effect of fish oil supplementation on the airway response to exercise in patients with asthma (29). The study demonstrated no significant change after 10 weeks of fish oil supplementation in the maximal post-exercise fall in airway conductance compared to pre-supplementation values.

Since inflammatory mediators have been implicated in the development of EIB in elite athletes (16-18,38), it seems reasonable to suggest that manipulating dietary n-3 PUFA consumption will influence the severity of EIB. Therefore, the aim of the present study was to investigate the effects of high dietary n-3 PUFA ingestion on pulmonary function and proinflammatory mediator and cytokine production in non-asthmatic elite athletes with EIB. We hypothesized that a diet high in n-3 PUFA will improve pulmonary function and reduce several proinflammatory markers in athletes with EIB. Some of the results from preliminary analysis of data from this study have been previously reported in the form of an abstract (39).

METHODS

Subjects

Ten subjects with clinically diagnosed EIB and ten subjects with no history, signs, or symptoms of EIB (control) were recruited from a population of university students and sporting teams throughout Cardiff, United Kingdom, who were either ranked at the collegiate or national level in their particular sport (n=10, triathletes; n=5, cross-country running; n=5, track running). Flyers were posted and visits to sporting teams were made in order to identify potential EIB subjects. All EIB subjects had a history of shortness of breath and intermittent wheezing following exercise, relieved by inhaled bronchodilator therapy (n=6, salbutamol and n=4, terbutaline), but were otherwise free of atopic asthma as diagnosed by their physician. Subjects were not selected if they had a doctor diagnosis of asthma and a history of respiratory complications. All EIB subjects reported by questionnaire having worse asthma-like symptoms after exercise and increased bronchodilator usage in the winter compared to the summer months. An initial test was conducted in order to screen all subjects for the presence of EIB, as indicated by a drop of greater than 10% in post-exercise FEV_1 compared to pre-exercise values (7,40). Control subjects were free of EIB using the same criteria. Each subject completed a health questionnaire and gave written informed consent to participate before enrolment in the study, approved by the University of Wales Institute Cardiff Research Ethics Committee. Table 1 indicates that the two groups were well matched according to age, physical characteristics and fitness level.

Study design and protocol

The study was conduced as a randomised double-blind crossover trial over 7 consecutive weeks during the months of November and December. All subjects entered the study on their normal (NORMAL) diet (**phase 1**), after which they were randomly assigned to either receive 18 capsules of Max-EPA (Seven Seas Ltd, Hull, England), which consisted of 3.2 g eicosapentaenoic acid (EPA) and 2.2 g of docosahexaenoic acid (DHA) (n=5, n-3 PUFA diet) or identical placebo (n=5, PLACEBO) capsules (Seven Seas Ltd, Hull, England) containing olive oil for 3 weeks (**phase 2**). Thereafter, they followed a 2 week washout period (NORMAL diet) and then switched to the alternative diet for the remaining 3 weeks (**phase 3**). Dietary cards were recorded throughout the study period. In addition, all EIB 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.

At the beginning of the study (**phase 1**; NORMAL diet) and at the end of each treatment period (**phase 2 and 3**) all subjects reported to the laboratory and had venous blood drawn prior to exercise for neutrophil fatty acid analysis and for the determination of leukotriene (LTB_4) and cytokine production (TNF-α and IL-1β). Additional blood was collected at 15 and 60 min post-exercise for the determination of LTB_4, TNF-α and IL-1β concentration. A single urine sample was collected prior to exercise and at 15, 60 and 120 min post-exercise for the determination of urinary LTE_4 concentration. Pulmonary function was assessed pre-exercise and at 1, 5, 10, 15, 30, 45 and 60 min post-exercise. At the end of the 2 week washout period all subjects reported to the laboratory to have additional venous blood drawn in order to verify that neutrophil fatty acid composition and leukotriene and cytokine concentrations had returned to baseline levels established at the beginning of the study on the NORMAL diet. (Additional detail on the study design is provided in an online supplement).

Exercise challenge test

All subjects were instructed to avoid both coffee and strenuous physical exertion during the 24 hours prior to the exercise challenge and EIB subjects were instructed to withhold their pulmonary medications for the appropriate time. At an initial screening test conducted on the NORMAL diet and at the end of each treatment period, each subject was required to run on a motorized treadmill (Woodway ELG 2, Rhein, Germany), which was elevated 1% per minute, until volitional exhaustion (40). Each subject wore a nose-clip during the exercise bout in order to promote mouth breathing, as nasal breathing decreases the water loss from the airways (41). In addition, each subject inspired compressed dry air (relative humidity < 10%) at room temperature (22°C) collected in a 150 litre Douglas bag (Cranlea & Co., Birmingham, UK) attached to the inspiratory port of a two-way breathing valve connected to a mouthpiece (42, 43). During the exercise test heart rate was continuously monitored by ECG (Pulmolab EX670, Morgan Medical Ltd, Gillingham, Kent, UK) and breath-by-breath analysis of expired gases was accomplished by indirect open circuit calorimetry (Pulmolab EX670, Morgan Medical Ltd, Gillingham, Kent, UK) (*see* online supplement for further details on the exercise challenge test).

Pulmonary function tests

Pulmonary function tests were conduced on all subjects using a Superspiro computerised spirometer (Micro Medical Ltd, Rochester, Kent, UK). Subjects were required to perform three acceptable forced vital capacity manoeuvres according to the American Thoracic Standardisation of Spirometry (44) (*see* online supplement for further details)

Urinary LTE_4 and 9α, 11β-PGF_2 quantification

Urinary LTE_4 and was measured by a modified HPLC/radioimmunoassay originally described by Tagari et al (45), and used clinically to determine changes in urinary LTE_4 levels in EIB subjects after exercise challenge (46). Cross-reactivity of the LTE_4 antibody against an array of related compounds was: LTE_4, 100%; LTE_5, 78.14%; LTC_4 and LTD_4, 18.12%; LTB_4, 6-trans-LTB_4, 20-OH-LTB_4, PGD_2 and < 0.01% for TXB_2, etc. Urinary 9α, 11β-PGF_2 analysis was performed by enzyme immunoassay (EIA). Cross-reactivity of the 9α, 11β-PGF_2 antibody against an array of related compounds was: 9α, 11β-PGF_2, 100%, PGF_2, 0.24%; PGE_2 and

TXB_2, 0.21%; PGD_2, 0.01% and <0.01% for LTB_4, PGA_1, PGA_2, etc. (additional detail on the method for making these measurements is provided in an online supplement)

Ex vivo whole blood LTB_4 analysis

In order to stimulate *ex vivo* LTB_4 formation, whole blood was incubated with 50μM calcium ionophore A23187 (free acid, molecular weight: 523.6) in dimethyl sulfoxide (DMSO) at 37^oC for 30 min. The plasma LTB_4 concentration was determined using a competition-based EIA, as described by Pradelles and coworkers, with minor modifications (47). Cross-reactivity if the LTB_4 antibody against an array of related compounds was: LTB_4, 100%; 6-trans-LTB_4, 25.0%; LTB_5, 14.58%; 5(S), 12 (S)-DiHETE, 6%; LTD_4, 0.96%; 20-hydroxy-LTB_4, 0.50%; LTE_4, 0.30% and 0.20% for LTC_4, etc. (additional detail on the method for making these measurements is provided in an online supplement).

Inflammatory cytokine analysis

Circulating immunoreactive TNF-α, IL-1β and their soluble receptors were determined by an enzyme-linked immunosorbent assay (ELISA) (R& D Systems, Europe Ltd, Abingdon, Oxford, UK). The ELISA used for the determination of IL-1β is specific for the measurement of natural and recombinant human IL-1β (100%). This ELISA does not cross-react with human IL-1α, IL-1RA, IL-2, IL-3, IL-4, IL-6, IL-7, LI-8, TNF-α, ILNα or IFNγ, rhIL-1α, rhIL-1 sRI, rmIL-1α, rhIL-1ra, rhIL-1 sRII, rmIL-1β. Likewise, the This ELISA used for the determination of TNF-α is specific for the measurement of natural and recombinant human TNF-α. This ELISA does not cross-react with human IL-1β, IL-1α, IL-2-13, TNF-β, IFNγ, rhsTNF RI, rhsTNF R11, rhTNF-β, rmTNF-α, rrTNF-α and rpTNF-α, etc. (additional detail on the method for making these measurements is provided in an online supplement)

Neutrophil phospholipid fatty acid analysis

Neutrophils were purified from 10 mL of anticoagulated venous blood to more than 95% by means of dextran sedimentation (Pharmacia, Milton Keynes, Bucks, UK) and centrifugation on a cushion of Lymphoprep (Nyegaard, Birmingham) (48) and stored under argon at –70°C before extraction of phospholipids using the method developed by Bligh and Dyer (49). Fatty acid composition was analysed by gas chromatography (50) (additional detail on the method for making these measurements is provided in an online supplement).

Statistical analysis

Data were analysed using the SPSS version 11 statistical software (SPSS Inc., Chicago, USA). The data were assessed for normality using the Kolmogorov-Smirnov test and Levene's test was used to test for homogeneity of variance between groups. A 2-way repeated measures ANOVA was used to analyse the data with both treatment and time as "within-subject" effects, while a 2-way ANOVA was used to analyse "between-subject" effects. Mauchly's test was conducted to determine if sphericity was violated. If sphericity was violated, the repeated measures ANOVA was corrected using the Greenhouse-Geiser correction factor.
Pairwise comparisons, with a Bonferroni adjustment (used in order to maintain an overall Type I error rate of 5%), were used to isolate differences in group means: dividing alpha by the number of pairwise comparisons to be made. The percentage change in urinary LTE_4 excretion, LTB_4 and TNF-α and IL-1β production were calculated using the following formula:

$$\frac{[\text{Post-challenge value} - \text{pre-challenge value}]}{[\text{Pre-challenge value}]} \times 100$$

Correlations between urinary LTE_4 excretion following exercise and pulmonary function were calculated using the Pearson product moment correlation. On all diets, the percentage (%) change in urinary LTE_4 excretion was correlated with the maximal decrease in post-exercise FEV_1. Data are expressed as mean ± SD.

RESULTS

Subjects

All EIB and control subjects who entered the trial completed it. There were no significant differences ($p>0.017$) in bronchodilator use (total number of doses/puffs) between the NORMAL diet (58 ± 16 puffs) and PLACEBO diet (55 ± 17 puffs). However, bronchodilator usage significantly declined ($p<0.05$) to 39 ± 13 puffs during the last 2 weeks on the n-3 PUFA diet. A 2 x 2 ANOVA used to test for the presence of carry-over effects indicated that none were present ($p>0.05$) for all measures of lung function and inflammatory markers. This was further supported by inflammatory mediator and cytokine levels measured at the end of the 2 week washout period returning to baseline values established at the beginning of the study (NORMAL diet).

Pulmonary Function

Pre- and post-exercise pulmonary function values for EIB and control subjects are shown in Table 2 and E1 (online data supplement) respectively. No significant difference ($p>0.017$) was observed in pre-exercise (baseline) pulmonary function among diets in either group. The differential effect of the percentage change in FEV_1 pre- to post- exercise in control and EIB subjects is shown in Figure 1. No significant differences ($p>0.017$) in the percentage change in FEV_1 pre- to post-exercise were observed for the control subjects on any diet. EIB subjects demonstrated a significant ($p<0.017$) percent change in FEV_1 pre- to post-exercise on the NORMAL and PLACEBO diet. However, on the n-3 PUFA diet the EIB subjects (Figure 1) demonstrated no significant difference ($p>0.017$) in the percentage change in FEV_1 pre- to post-exercise. FEV_1 decreased by 3 ± 2% on n-3 PUFA diet, 14.5 ± 5% on PLACEBO diet and 17.3 ± 6% on NORMAL at 15 min post-exercise. Similar patterns were observed for FVC.

Inflammatory Markers

Mean (SD) urinary LTE_4 and 9α, 11β-PGF_2 levels and plasma levels of LTB_4, TNF-α and IL-1β for EIB and control subjects are shown in figures 2, 3, 4, 5 and 6 respectively. No significant changes ($p>0.013$) in inflammatory markers as result of exercise or any treatment were observed in the control subjects. While no significant difference ($p>0.05$) was observed between pre-PLACEBO supplementation and post-PLACEBO supplementation at pre-exercise in LTE_4, 9α, 11β-PGF_2, LTB_4, TNF- α and IL-1β values, post-exercise values increased significantly ($p<0.017$) compared to pre-exercise values on the PLACEBO diet and NORMAL diet in EIB subjects. However, on the n-3 PUFA diet urinary LTE_4 excretion (figure 2) was significantly reduced ($p<0.017$) post-supplementation at pre-exercise and 15-min post-exercise by 19.4 pg/mg creatinine and 13.1 pg/mg creatinine respectively, compared to the mean pre-supplementation LTE_4 concentration (56.9 ± 13.3 pg/mg creatinine). Mean urinary excretion of 9α, 11β-PGF_2 (figure 3) on the n-3 PUFA diet decreased significantly ($p<0.017$ post-supplementation at pre-exercise by 16.8 ng/mg mmol creatinine^{-1} and by 13.9 ng/mg mmol creatinine^{-1} at 15-min post-exercise compared to the pre-supplementation level (53.2 + 12.4 ng/mg mmol creatinine^{-1}).The n-3 PUFA supplementation resulted in a significant reduction in pre-exercise values ($p<0.017$) for LTB_4, IL-1β and TNF-α production (figures 4, 5 and 6 respectively) of 17.7 ± 6.7%, 22.6 ± 6.3% ($p<0.017$) and 22.5 ± 6.7% ($p<0.001$) respectively, and a significant reduction ($p<0.05$) in IL-1β of 15.1 + 4.7% at 15 min post-exercise compared to pre- n-3 PUFA supplementation values. In addition, n-3 PUFA post-supplementation LTE_4, LTB_4, TNF-α and IL-1β levels were significantly reduced ($p<0.017$) compared to the NORMAL and PLACEBO diet at all respective time points.

There was no significant correlation between the maximal fall in FEV_1 and post-exercise change in urinary LTE_4 excretion on the NORMAL and PLACEBO diets in EIB subjects (Pearson correlation coefficient: $r = -0.357$, $p = 0.676$, and $r = -0.394$, $p = 0.613$ respectively) or the inhibitory effect of the n-3 PUFA diet on urinary LTE_4 excretion and the protective effect on the maximal fall in FEV_1 (Pearson's correlation coefficient: r = - 0. 228) in EIB subjects.

Neutrophil phospholipid fatty acid content

The fatty acid content of the neutrophil phospholipid was assessed in EIB (Table 3) and control subjects (Table E2; online data supplement) and expressed as a percentage of total fatty acid content. No significant differences (p>0.025) were observed in EIB and control subjects in Neutrophil membrane content for linoleic acid (LA), arachidonic acid (AA), EPA and DHA comparing pre- and post-PLACEBO supplementation values. However after the n-3 PUFA supplementation period EPA content significantly increased (p<0.025) to 3.79 ± 2.1%, while AA and LA content significantly decreased (p<0.001) to 11.9 ± 4.2% and 5.9 ± 2.7% respectively of total Neutrophil fatty acid content in the EIB subjects (Table 4). In the control group (Table 5??), EPA content significantly increased (p<0.025) to 4.10 ± 1.8%, while AA and LA were significantly reduced (p<0.025) to 11.6 ± 3.4 and 5.4 ± 2.3% respectively of total Neutrophil fatty acid content following n-3 PUFA supplementation. No significant changes (p>0.025) were observed in DHA content following n-3 PUFA consumption in either the EIB or control group.

DISCUSSION

The present study has demonstrated for the first time that 3 weeks of dietary n-3 PUFA supplementation markedly reduces the severity of EIB in elite athletes. The airway response to exercise was used to assess changes in non-specific bronchial responsiveness during dietary supplementation with n-3 PUFA. The n-3 PUFA diet significantly improved post–exercise pulmonary function to below the diagnostic limit of a 10% post-exercise fall in FEV_1 in conjunction with a significant decrease in bronchodilators drug use. In addition, the increase in tissue phospholipid n-3 PUFA concentration in EIB subjects was coincident with a significant suppression of the proinflammatory eicosanoids LTE_4, PGD_2 metabolite 9α, 11β-PGF_2 and LTB_4 and proinflammatory cytokines TNF-α and IL-1β.

Verification of diet compliance was accomplished by neutrophil phospholipid fatty acid analysis. Dietary enhancement with 3.2 g of EPA for 3 weeks produced a considerable increase in EPA content of neutrophil phospholipid in both EIB and control subjects, thus confirming dietary compliance with n-3 PUFA supplementation. The dose of EPA selected for the present study has previously been shown to have anti-inflammatory potential as shown by its effect on leukocyte function (25, 29). The potential anti-inflammatory effect of n-3 PUFA stems from its active ingredient, EPA, which is a competitive substrate with arachidonic acid for the generation of inflammatory mediators. The derivatives of arachidonic acid (an n-6 PUFA) are LTB_4, a potent neutrophil chemoattractant and proinflammatory mediator, and the cysteinyl series of leukotrienes (LTC_4, LTD_4 and LTE_4), which produce potent smooth muscle contraction and bronchoconstriction (38). Arachidonic acid is the progenitor of LTB_4 via the 5-lipoxygenase enzymatic pathway. EPA, the n-3 homologue of AA, can inhibit AA metabolism competitively via these enzymatic pathways and, thus, can suppress production of the n-6 eicosanoid mediators. Thus, increasing dietary n-3 fats can shift the balance of the eicosanoids produced to a less inflammatory mixture by reducing the production of proinflammatory leukotrienes.

This study supports data from prior reports that urinary concentrations of LTE_4 increase after exercise in adults with mild asthma (51), and following exercise in asthmatic children, but not in non-asthmatic children (52). Post-exercise increases in urinary LTE_4 (51, 52) and reduced post-exercise bronchoconstriction with cys-LT_1 receptor antagonist treatment, thereby blocking the action of cysteinyl-leukotrienes on their receptors in human airways (38, 53), provide compelling evidence for cysteinyl-leukotriene involvement in EIB. In addition, the n-3 PUFA diet markedly blunted urinary LTE_4 excretion post-exercise in the EIB subjects, which is in agreement with von Scacky et al (54) who observed a 35% reduction in urinary LTE_4 after dietary supplementation of n-3 PUFA in healthy volunteers. The results of the present study have shown that incorporation of n-3 PUFA into neutrophil phospholipid was accompanied by a reduction in LTB_4 release in EIB subjects following exercise. This corroborates other studies which have shown that increased EPA content in neutrophil membrane phospholipids has attenuated the neutrophil chemotactic activity and the generation of leukotriene B products in patients with asthma (27-29, 32), and inhibits the 5-lipoxygenase pathway of neutrophils and moncycytes and attenuates the LTB_4-mediated functions of neutrophils *in vitro* (25). LTB_4 has been implicated in the pathogenesis of exercise-induced asthma. Arm and colleagues (55) observed increased synthesis of LTB_4 by neutrophils stimulated *in vitro* by unopsonozed zymosan and calcium ionophore isolated from patients with asthma following exercise, while Sugoro and colleagues (56) observed an improvement in pulmonary function and a decrease in urinary concentration of LTB_4 following exercise after treatment with a leukotriene antagonist in subjects with exercise-induced asthma.

Whilst the present study has had shown a diminution of the AA “4-series” tetraene sulfidopeptide leukotrienes, the EPA derived “5 series” pentaene sulfidopeptide leukotrienes LTC_5, LTD_5 and LTE_5 are biologically identical to their tetraene counterparts in causing bronchoconstriction (57). It has been suggested that changing to production of pentaene rather than tetraene sulfidopeptides with dietary manipulation may not result in a major difference in biological response (airway reactivity) if there is no reduction in total sulfidopeptide leukotriene production.(31). However, although the present study did not measure the pentaene sulfidopeptides, we have clearly shown that the bronchoconstrictor response to exercise and the tetraene sulfidopeptide leukotrienes are markedly reduced on the n-3 PUFA diet. This suggests that the tetraene leukotrienes are important in elite athletes with EIB and the EPA derived pentaene leukotrienes may have diminished biological capacity, the reason for which is unknown but may be related to dosage and duration of the n-3 PUFA diet.

Significant increases following exercise in the PGD_2 urinary metabolite 9α, 11β-PGF_2 was observed in the elite athletes with EIB, which confirms previous findings of increased urinary 9α, 11β-PGF_2 concentrations after exercise in asthmatic patients (58, 59). Although 9α, 11β-PGF_2, the initial metabolite of PGD_2 is a marker of mast cell activation and a potent bronchoconstrictor eosinophils can also generate PGD_2 albeit in small amounts (59, 60). It has been shown that degranulation of mast cells occurs upon exposure to a hyperosmotic stimuli in vitro (61), and it has been suggested that hyperosmolarity of the airway lining fluid occurs during hyperpnea with cold dry air (62). The n-3 PUFA diet in the present study suppressed urinary 9α, 11β-PGF_2 generation following exercise, suggesting that mast cell activation is an important determinant of EIB in elite athletes.

Dietary enrichment with n-3 PUFA in the present study resulted in significant attenuation in the production of proinflammatory cytokines TNF-α and IL-1β in EIB subjects. It has been shown that dietary supplementation with n-3 PUFA results in decreased monocyte synthesis of TNF-α and IL-1β in healthy subjects (26, 63). However, Hodge et al. (30) while demonstrating reductions in TNF-α production after fish oil supplementation observed no effect on the clinical severity of asthma. These cytokines have proinflammatory activity that can stimulate the synthesis of collagenases (64) and increase the expression of adhesion molecules necessary for leukocyte extravasation (65) and both cytokines have been implicated in the pathogenesis of asthma (30, 66). TNF-α increases the responsiveness of human bronchial tissue *in vitro* (67), and increases airway responsiveness *in vivo* in healthy normal subjects (68). Our findings that plasma levels of TNF-α are increased in elite athletes with EIB are in agreement with Sue-Chu et al. (16). These authors reported a greater macroscopic inflammatory index in the proximal airways of skiers than in healthy nonathletic subjects, which was even greater in skiers with hyperresponsive airways and in those with ski-induced asthma. Such changes were accompanied by increased lymphocyte cell count in bronchoalveolar lavage (BAL) samples in elite cross-country skiers with EIB. TNF-α was above the detectable threshold in 40% of skiers and was not detected in the healthy control subjects.

To our knowledge this is the first study to assess the effect of n-3 PUFA supplementation on pulmonary function and inflammatory mediator production in elite athletes with EIB. However, Arm et al. (29) attempted to determine the effect of fish oil supplementation on pulmonary function following exercise in patients with asthma. After 10 weeks of daily supplementation with 3.2 g EPA and 2.2 g DHA, subjects underwent a histamine challenge, exercise challenge and blood neutrophil studies. Although there was a significant increase in n-3 PUFA neutrophil content and a 50-percent inhibition of total LTB synthesis (LTB_4 and LTB_5), there was no detectable change in the clinical outcome (e.g., histamine response, exercise response, specific conductance of the airway or symptoms scores). The divergent findings between the present study and Arm et al's (29) study are difficult to reconcile, especially since their study had a longer duration supplementation period with an identical fish oil dosage as the current study. However, although it has been suggested that all individuals who exhibit EIB by demonstrating reductions in post-exercise pulmonary function are asthmatic to some degree (69), recent evidence of airway remodelling in cross-country skiers with EIB (16, 18, 70), and the fact it has been shown that inhaled corticosteroids appear to have no effect on airway inflammatory markers or obstructive symptoms in athletes with EIB (17), indicates a different pathphysiology in EIB compared to common asthma. Evidence of this concept comes from Sue-Chu et al. (70) who reported a higher frequency of lymphoid aggregates in endobronchial biopsies from a population of young elite cross-country ski athletes, with asthma-like symptoms, compared to healthy young control subjects. An increase in the number of neutrophils has been observed in the sputum of elite swimmers after training (11) and an increased neutrophil concentration in BALF has been observed in a canine model of hyperpnea with cold dry air (71, 72), providing further evidence that the inflammatory processes in athletes with EIB may be different to individuals with common asthma, although these conclusions ate highly speculative. No measures of inflammatory cells and mediators in the airway lumen via sputum induction were made in the present study. However, future work should be directed towards assessing clinically useful markers of airway inflammation such as eosinophils, neutrophils and soluble cell makers in order to gain a greater insight into the heterogeneity of EIB in elite athletes (73).

It has been proposed that the increased bronchial hyperresponsiveness documented in elite athletes may be due to repetitive airway trauma of the epithelium with consequent remodelling (2), due to exposure to cold/dry air at high ventilation rates which renders these athletes to severe thermal and osmotic stimuli (16, 72, 74). Karjalainen et al. (18) reported an increase in the expression of extracellular matrix protein, tenascin, in the proximal airways of cross country skiers with EIB, which may reflect ongoing healing and repair and airway remodelling after tissue injury, due to repeated exposure of the airways to inadequately conditioned air. Further Davies and colleagues (71) has recently observed in a canine model of hyperventilation histological changes commonly associated with airway dysfunction in asthmatic patients, suggesting that repeated exercise in cold weather can cause airway remodelling and morphological changes similar to that seen in asthma. However, in contrast to asthma the airway damage caused by the repeated hyperpnea challenge with cold dry air was reversible with rest.

In conclusion, the present study has shown that supplementing the diet with n-3 PUFA represents a potentially beneficial treatment for elite athletes with EIB. Dietary modification of EIB with marine oils or highly enriched sources of n-3 PUFA has the potential of optimising the additive effects of drug-diet combinations in elite athletes with EIB. The use of pharmacological treatment use could be decreased in athletes with EIB in concert with increased fish-oil ingestion if both the drug and fish oil are exerting their therapeutic effects through the same molecular actions, e.g. LTE_4 and LTB_4 production. This might also apply to new drugs or new treatment modalities that aim to suppress cytokine concentrations. Thus, the possibility exists for drug-diet interactions that confer greater anti-inflammatory benefits than either agent alone or similar anti-inflammatory effects with less toxicity. The differences between reports on the effect of fish oil supplementation in allergic asthma and exercise-induced bronchial hyperreactivity are probably methodological. The small number of studies and the different methods used for the assessment of bronchial hyperreactivity call for further trials before the benefits of fish oil supplementation can be assessed (75). In addition, due to the fact the present study's findings are in contrast with those of Arm et al. (29) further reproduction of these findings are warranted.

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Figure Legends

Figure 1. The percent change in FEV_1 from pre- to post-exercise in subjects with EIB and control subjects across the three diets. Reductions in FEV_1 in excess of 10% represents abnormal pulmonary function. Letters $^{(a,b)}$ refer to comparisons by diet within respective time period. Different letters designate significant difference ($p<0.017$).

Figure 2. Mean urinary 9α, 11β-PGF_2 excretion (ng/mg mmol·creatinine^{-1}). * Indicates significant difference ($p<0.013$) compared to respective pre-supplementation value within diet. Letters $^{(a,b)}$ designate significant difference ($p<0.017$) compared to respective time point between diet.

Figure 3. Mean urinary LTE_4 excretion (pg/mg creatinine). * Indicates significant difference ($p<0.013$) compared to respective pre-supplementation value within diet. Letters $^{(a,b)}$ designate significant difference ($p<0.017$) compared to respective time point between diet.

Figure 4. Mean plasma levels of LTB_4 (ng/ml). * Indicates significant difference ($p<0.013$) compared to respective pre-supplementation value within diet. Letters $^{(a,b)}$ designate significant difference ($p<0.017$) compared to respective time point between diet.

Figure 5. Mean plasma levels of IL-1β (pg/ml).). * Indicates significant difference ($p<0.013$) compared to respective pre-supplementation value within diet. Letters $^{(a,b)}$ designate significant difference ($p<0.017$) compared to respective time point between diet.

Figure 6. Mean plasma levels of TNF-α (pg/ml). * Indicates significant difference ($p<0.013$) compared to respective pre-supplementation value within diet. Letters $^{(a,b)}$ designate significant difference ($p<0.017$) compared to respective time point between diet.

TABLE 1. SUBJECT CHARACTERISTICS

Group	Gender (M, F)	Age (Years)	Body mass (kg)	Height (cm)	Peak O_2 uptake ($ml \cdot kg^{-1} \cdot min^{-1}$) NORMAL diet
EIB (n=10)	5, 5	23.2 ± 1.9	66.7 ± 4.5	169.3 ± 4.6	61.7 ± 7.9
Control (n=10)	5, 5	22.4 ± 1.7	68.4 ± 3.4	171.4 ± 3.4	62.9 ± 8.6

Values are mean ± SD

TABLE 2. PRE-EXERCISE (BASELINE) PULMONARY FUNCTION FOR CONTROL AND EIB SUBJECTS.

	Diet		
	Normal	Placebo	n-3 PUFA
EIB subjects			
FVC (L)	4.97 ± 0.31	4.85 ± 0.33	4.97 ± 0.30
% predicted*	99.4 ± 9.7	100.4 ± 7.4	98.6 ± 8.0
FEV$_1$ (L)	4.10 ± 0.30	4.01 ± 0.31	4.01 ± 0.29
% predicted*	98.6 ± 8.4	99.8 ± 7.9	100.8 ± 8.9
FEV$_1$/FVC	82.5 ± 2.0	82.7 ± 3.0	80.7 ± 3.0
% predicted*	92.4 ± 3.6	92.6 ± 3.2	91.4 ± 2.4
Control subjects			
FVC (L)	4.90 ± 0.27	4.99 ± 0.32	5.00 ± 0.33
% predicted*	102.2 ± 5.1	103.0 ± 6.7	102.6 ± 7.8
FEV$_1$ (L)	4.34 ± 0.21	4.31 ± 0.29	4.35 ± 0.22
% predicted*	102.8 ± 6.2	103.1 ± 5.6	104.4 ± 5.4
FEV$_1$/FVC	88.6 ± 3.0	86.4 ± 3.0	87.0 ± 2.0
% predicted*	97.6 ± 2.6	96.1 ± 2.8	96.8 ± 2.1

Definition of abbreviations: FVC, forced vital capacity; FEV$_1$, forced expiratory volume in 1-sec.

Values are mean ± SD. There were no significant differences for any variables among diets ($p>0.017$) or groups ($p>0.025$). *reference values (76

TABLE 3. FATTY ACID COMPOSITION OF NEUTROPHIL EXTRACTS EXPRESSED AS A PERCENTAGE OF TOTAL FATTY ACID CONTENT BEFORE AND AFTER THE 3 WEEK DIETARY SUPPLEMENTATION PERIOD IN EIB SUBJECTS.

	18:2	20:4	20:5	22:6
Diet	Linoleic Acid	Arachidonic Acid	Eicosapentaenoic Acid	Docosahexaenoic Acid
NORMAL (n=10)	10.2 ± 3.2	17.2 ± 4.3	0.23 ± 0.19	2.49 ± 1.6
PLACEBO (n=10)				
Before	9.5 ± 2.8	16.6 ± 4.1	0.26 ± 0.21	2.46 ± 1.7
After	8.9 ± 2.6	16.0 ± 3.8	0.27 ± 0.20	2.55 ± 1.9
n-3 PUFA (n=10)				
Before	9.8 ± 2.4	17.8 ± 4.6	0.29 ± 0.19	2.52 ± 1.8
After	5.9 ± 2.7*	11.9 ± 4.2*	3.79 ± 2.1*	3.61 ± 2.1

Values are mean ± SD. *p<0.025 compared to pre-supplementation period

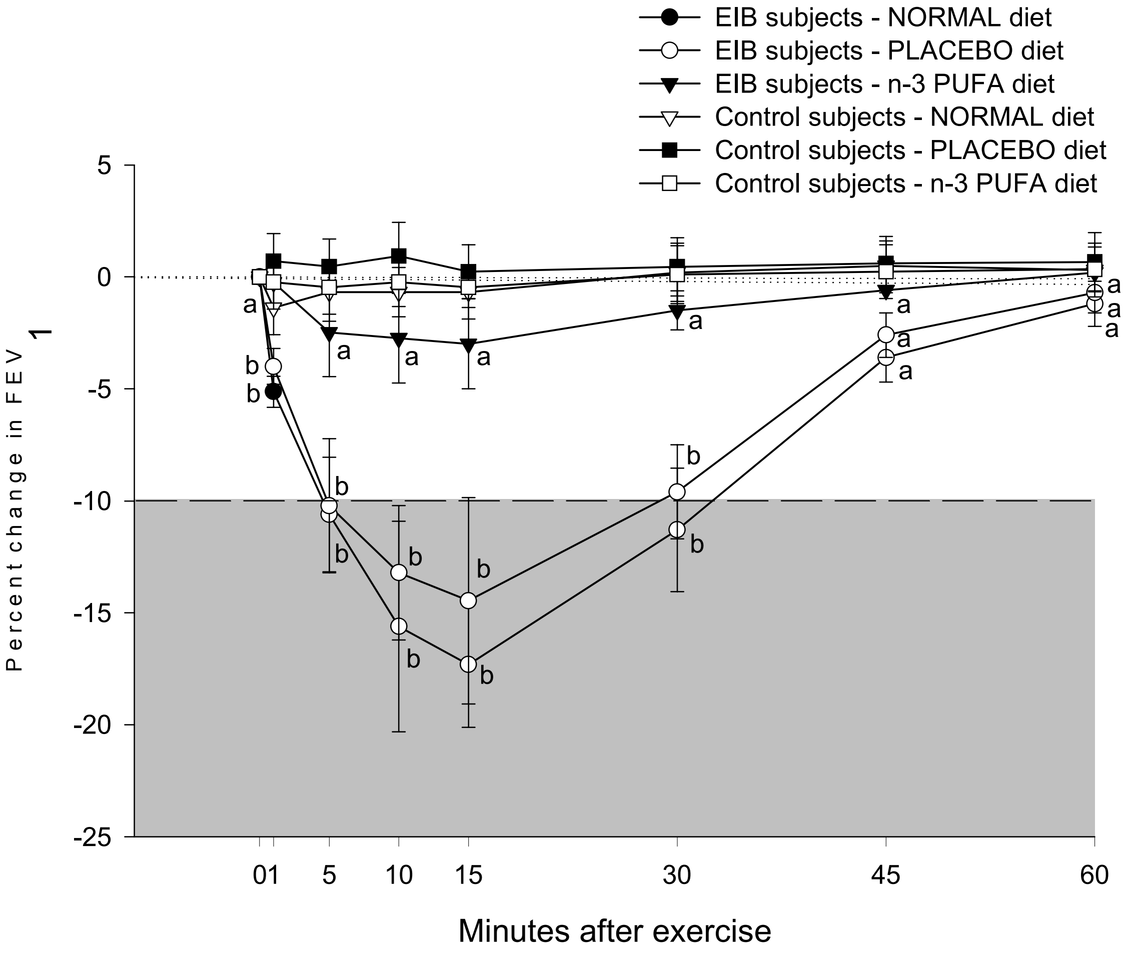

EIB subjects - NORMAL diet
EIB subjects - PLACEBO diet
EIB subjects - n-3 PUFA diet
Control subjects - NORMAL diet
Control subjects - PLACEBO diet
Control subjects - n-3 PUFA diet
Percent change in FEV1
5
0
-5
-10
-15
-20
-25
01
5
10
15
30
45
60
Minutes after exercise
a
b
b
a
a
a
a
a
a
a
a
a
b
b
b
b
b
b
b
b

Figure 1

Figure 2

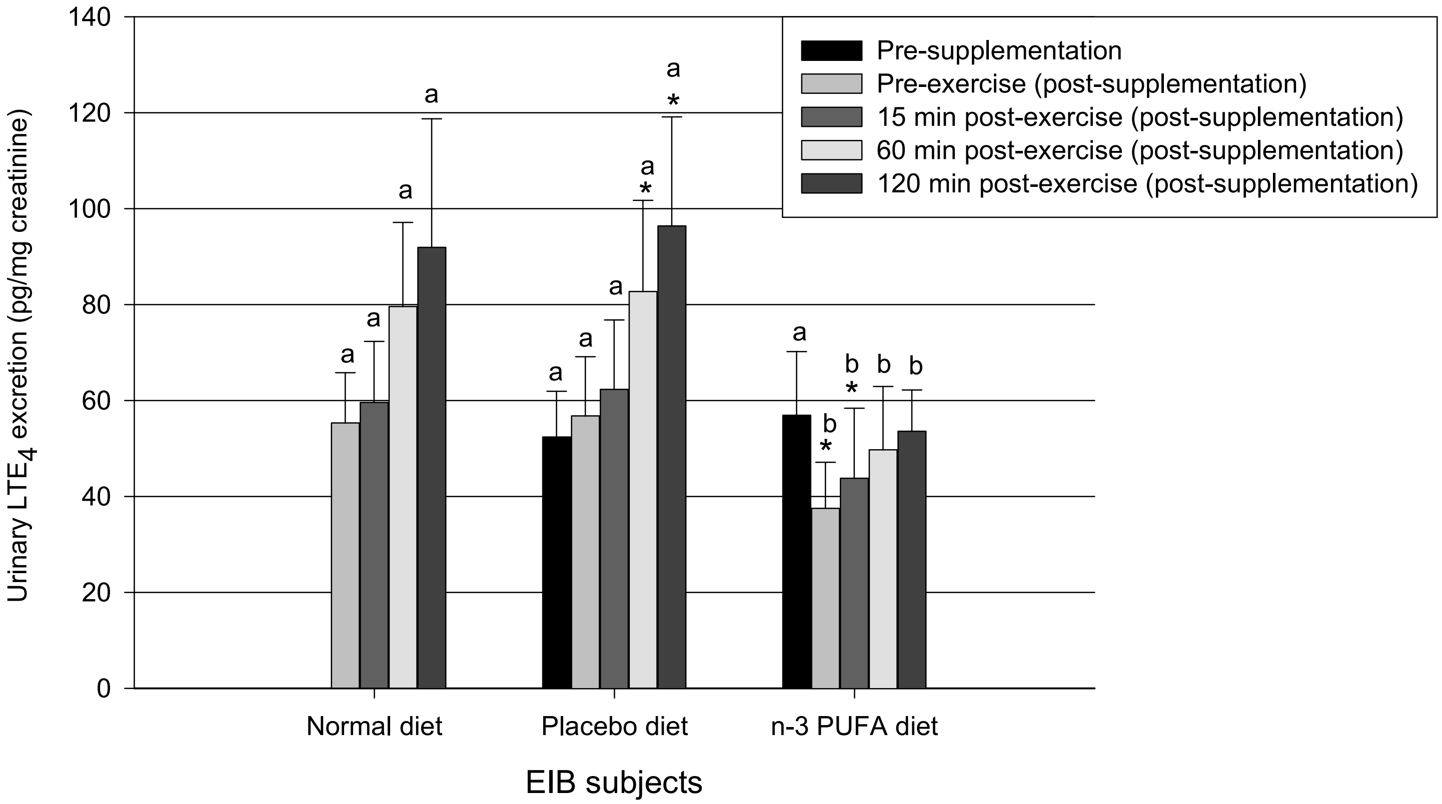

Urinary LTE4 excretion (pg/mg creatinine)
0
20
40
60
80
100
120
140
Pre-supplementation
Pre-exercise (post-supplementation)
15 min post-exercise (post-supplementation)
60 min post-exercise (post-supplementation)
120 min post-exercise (post-supplementation)
a
a
a
a
a
a
a
a
a
*
a
*
a
b
*
b
*
b
b
Normal diet
Placebo diet
n-3 PUFA diet
EIB subjects

Figure 3

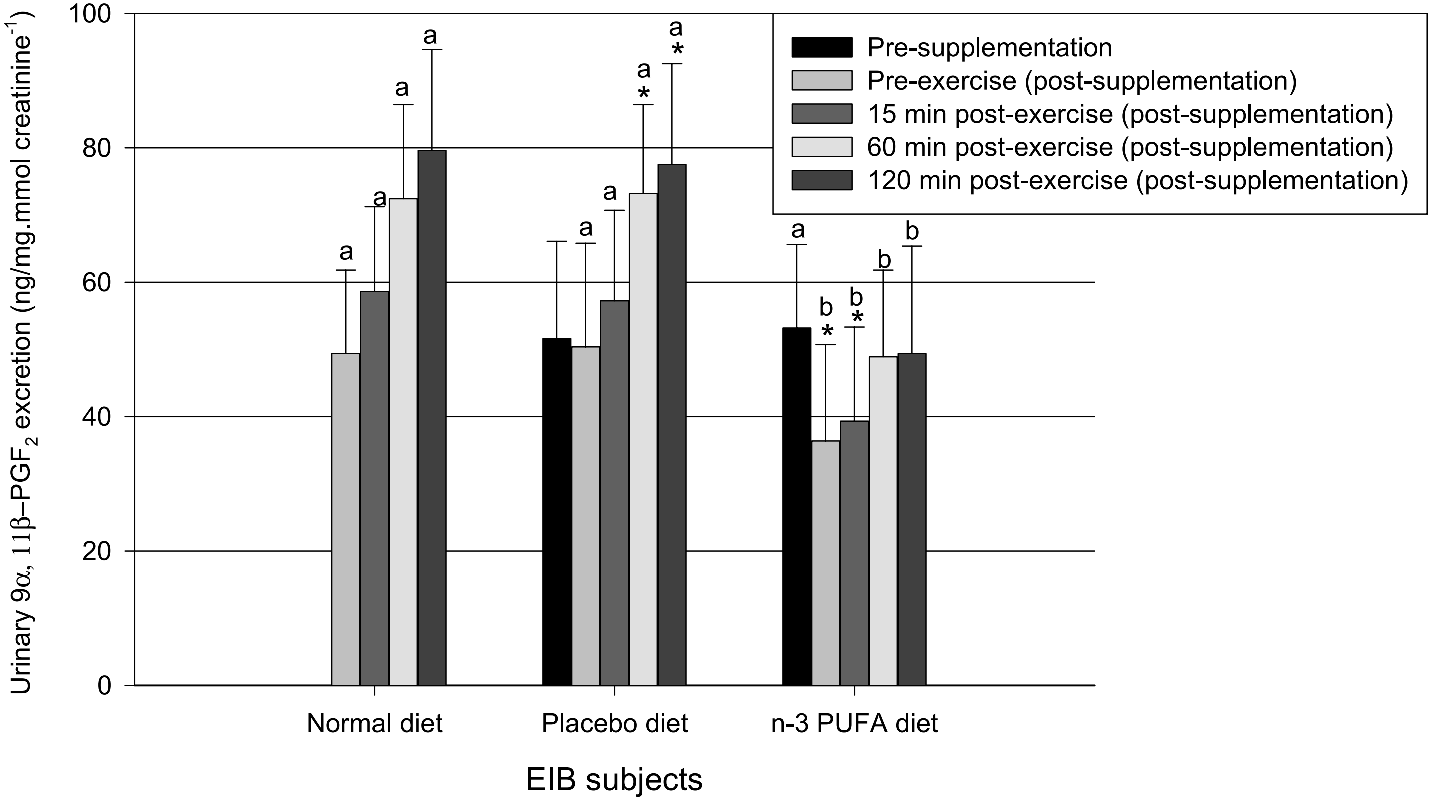

Urinary 9α, 11β–PGF$_2$ excretion (ng/mg.mmol creatinine^{-1})
100
80
60
40
20
0
Pre-supplementation
Pre-exercise (post-supplementation)
15 min post-exercise (post-supplementation)
60 min post-exercise (post-supplementation)
120 min post-exercise (post-supplementation)
a
a
a
a
a
a
a *
a *
a
b *
b *
b
b
Normal diet
Placebo diet
n-3 PUFA diet
EIB subjects

Figure 4

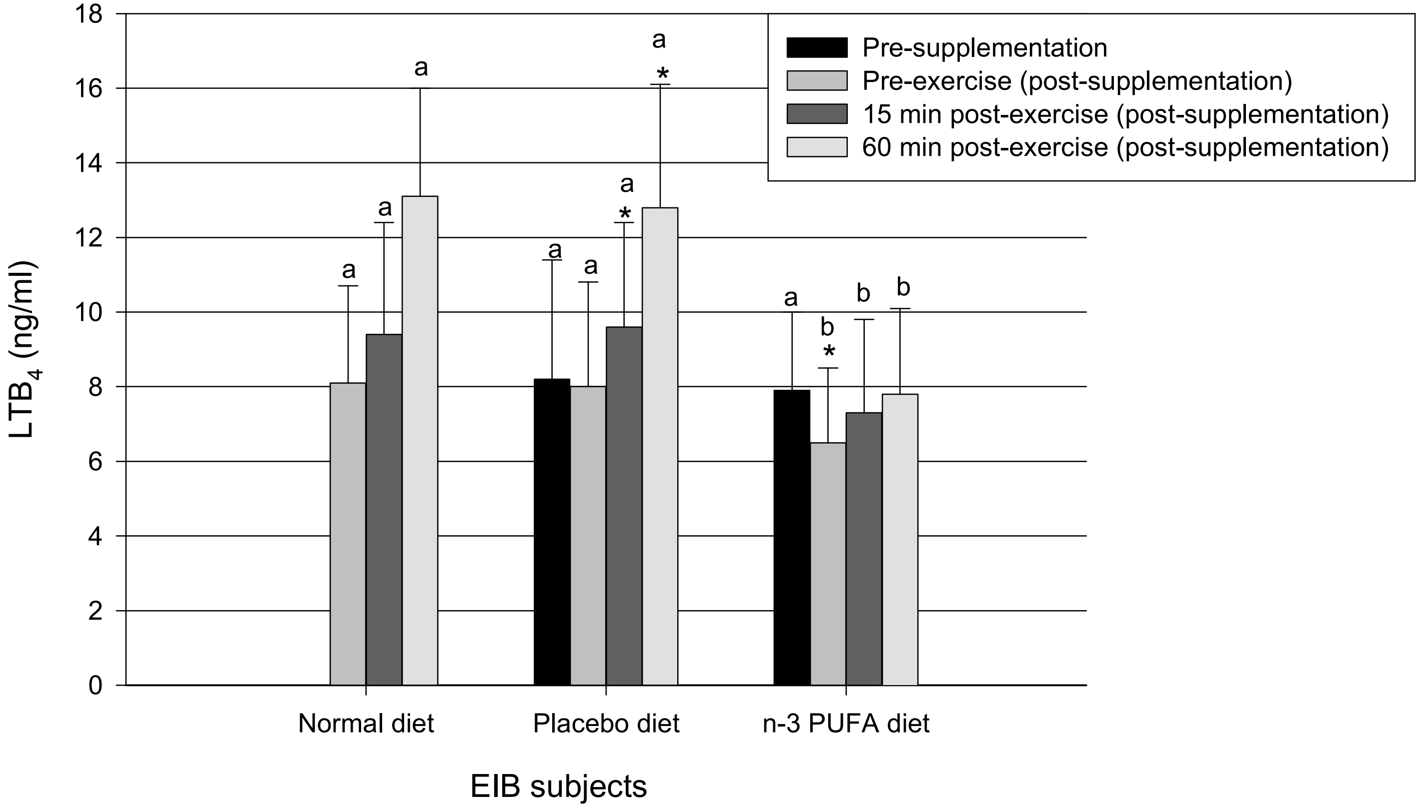

18
16
14
12
10
8
6
4
2
0
LTB$_4$ (ng/ml)
Normal diet
Placebo diet
n-3 PUFA diet
EIB subjects
Pre-supplementation
Pre-exercise (post-supplementation)
15 min post-exercise (post-supplementation)
60 min post-exercise (post-supplementation)
a
a
a
a
a
a
a
a
*
*
a
b
*
b
b

Figure 5

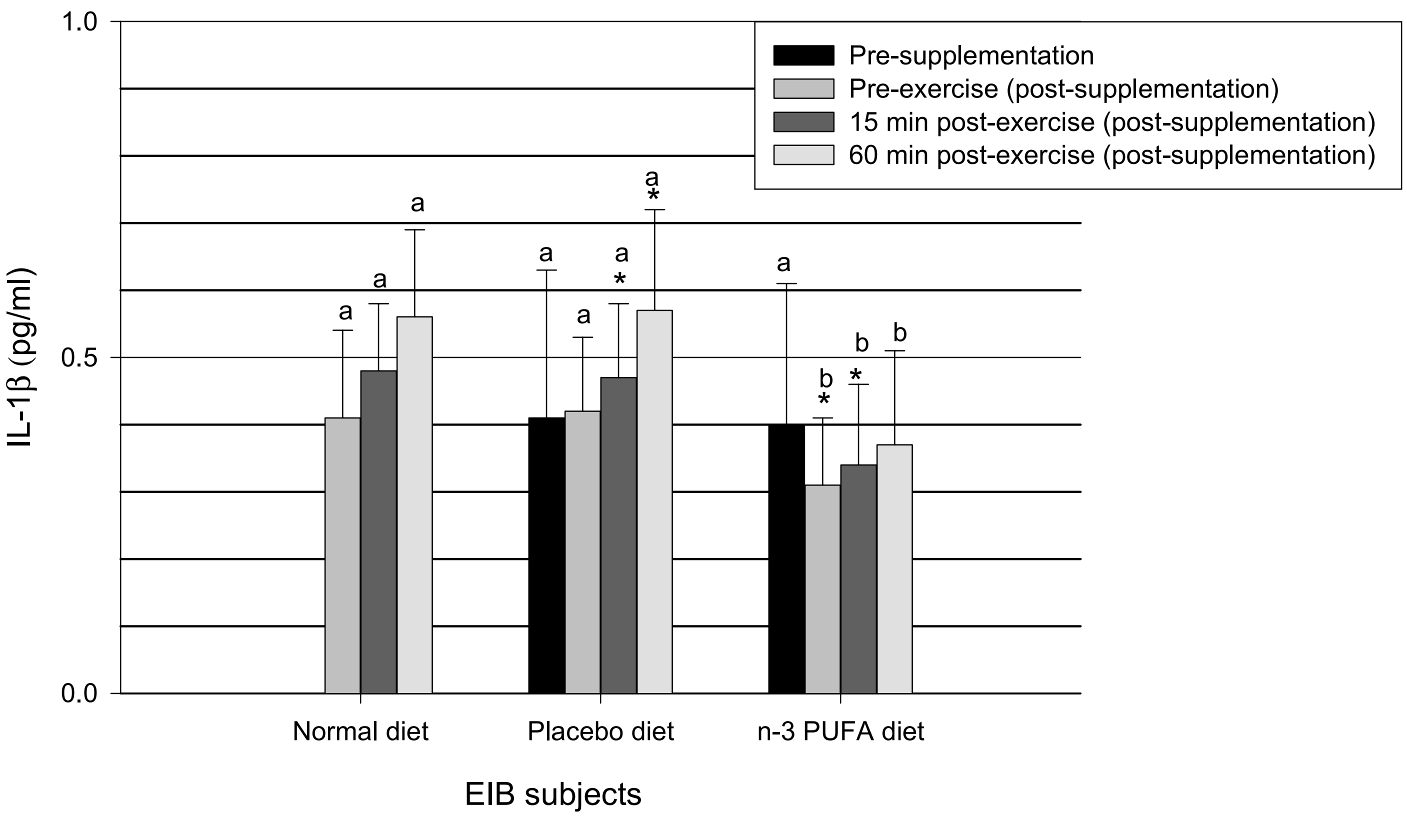
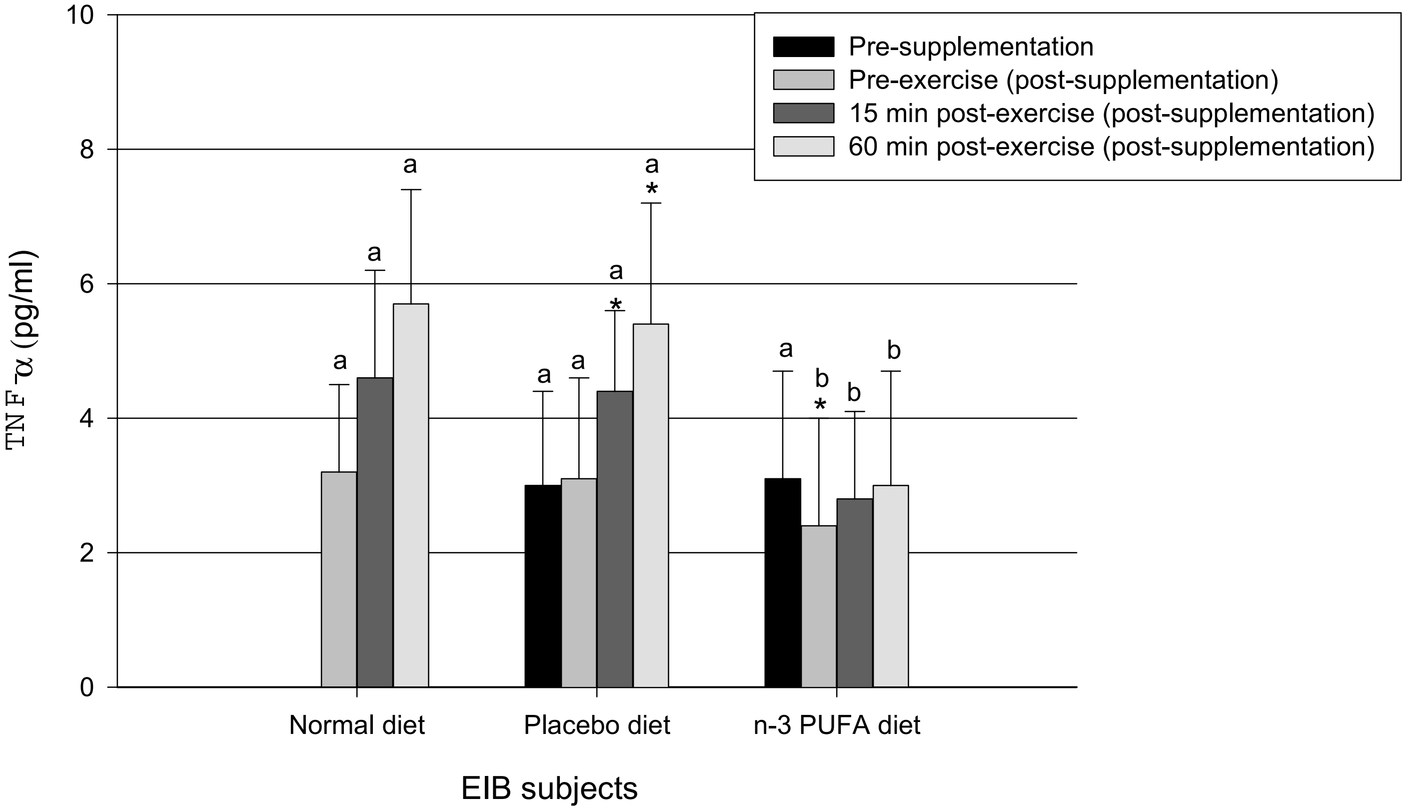

1.0
0.5
0.0
IL-1β (pg/ml)
Pre-supplementation
Pre-exercise (post-supplementation)
15 min post-exercise (post-supplementation)
60 min post-exercise (post-supplementation)
a
a
a
a
a
a
*
a
*
a
b
*
b
*
b
Normal diet
Placebo diet
n-3 PUFA diet
EIB subjects

Figure 6


10
8
6
4
2
0
TNF-α (pg/ml)
Pre-supplementation
Pre-exercise (post-supplementation)
15 min post-exercise (post-supplementation)
60 min post-exercise (post-supplementation)
a
a
a
a
a
a
*
a
*
a
a
b
*
b
b
Normal diet
Placebo diet
n-3 PUFA diet
EIB subjects

Fish Oil Supplementation Reduces Severity of Exercise-Induced Bronchoconstriction in Elite Athletes

Timothy D. Mickleborough, Rachael L. Murray, Alina A. Ionescu

and Martin R. Lindley

METHODS

Study design and protocol

At the beginning of the study (**phase 1**; NORMAL diet) and at the end of each treatment period (**phase 2 and 3**) all subjects reported to the laboratory and had a total of 20 mL of venous blood drawn from the antecubital vein in heparinized tubes approximately 90 min prior to an exercise challenge. One 10 ml sample was collected for fatty acid determination and two 5 ml blood samples were collected for the determination of leukotriene (LTB_4) and cytokine production (TNF-α and IL-1β). Following the exercise challenge, two further 5 ml blood samples were collected at 15 min and 60 min. A single urine sample was collected prior to exercise and at 15, 60 and 120 min post-exercise for the determination of urinary LTE_4 concentration. All subjects were water loaded (800ml) before the exercise-challenge to enhance diuresis.

Exercise challenge test

The rationale for using an exercise challenge to volitional fatigue is that a clinical exercise challenge routinely used for diagnosing exercise-induced asthma (e.g. exercising for 6 min at 85-90% PMHR and then terminating the test) may not be sensitive enough to detect EIB in elite athletes (40)

Pulmonary function tests

The maximum percentage fall in FEV_1 from the baseline (pre-exercise) value was calculated using the following equation:

$$\frac{[\text{Pre-exercise } FEV_1 - \text{lowest post-exercise } FEV_1]}{[\text{Pre-exercise } FEV_1]} \times 100$$

Spirometry was performed with the subject in the sitting position while breathing room air, with the nose being occluded by a clip. The pulmonary function technician and spirometer were the same throughout the study.

Urinary LTE_4 quantification

Urinary LTE_4 was measured by a modified HPLC/radioimmunoassay originally described by Tagari et al (45), and used clinically to determine changes in urinary LTE_4 levels in EIB subjects after exercise challenge (46). HPLC separation was performed as previously described (77), and fractions were assayed in a competitive-binding RIA using a commercially available peptidyl leukotriene antibody (Cascade Biochem, Reading, Berkshire, UK). A C-18 Sep-Pak light column (Waters) was used to remove proteins and organic contaminants during the preassay preparation. The RIA between-day precision, expressed as coefficient of variation (CV), was 6.3%, 8.7% and 13.8% for RIA samples containing 104.5, 35.4, and 9.8 pg LTE_4 respectively. Overall recovery of ^3H-LTE_4 used as an internal standard was 63%. Interday assay precision determined from analysis of pooled urine from healthy control subjects was 13.1% and 11.6% for urine samples containing 54.2 and 20.4 pg LTE_4/mg creatinine respectively.

Ex vivo whole blood LTB_4 analysis

In order to stimulate *ex vivo* LTB_4 formation, whole blood was incubated with 50μM calcium ionophore A23187 (free acid, molecular weight: 523.6) in dimethyl sulfoxide (DMSO) at 37°C for 30 min. The samples were then chilled in an ice bath for at least 15 min, centrifuged at 1000 x *g* for 10 min at 4°C using a refrigerated centrifuge and the obtained plasma transferred into storage tubes. The samples were kept frozen at –20°C until batch analysis. The plasma LTB_4 concentration was determined using a competition-based enzyme-linked immunoassay (Neogen Corp., Lansing, MI), as described by Pradelles and coworkers, with minor modifications (47).

Inflammatory cytokine analysis

Circulating immunoreactive TNF-α, IL-1β and their soluble receptors were determined by an enzyme-linked immunosorbent assay (ELISA) (R& D Systems, Europe Ltd, Abingdon, Oxford, UK).). Intra- and interassay variation was less than 10% in all assays, and no samples were below the sensitivity for each assay.

Neutrophil phospholipid fatty acid analysis

Neutrophils were purified from 10 mL of anticoagulated venous blood to more than 95% by means of dextran sedimentation (Pharmacia, Milton Keynes, Bucks, UK) and centrifugation on a cushion of Lymphoprep (Nyegaard, Birmingham) (48). A portion of 1 x 10^7 cells was resuspended in 500µl Hanks' balanced salt solution without calcium or magnesium and stored under argon at –70°C before extraction of phospholipids using the method developed by Bligh and Dyer (49). Using a steady stream of nitrogen they were resuspended in boron trifluoride (Sigma, Dorset, UK) and heated to 100°C under nitrogen for 90 min to esterify the fatty acids. Samples were then cooled, extracted into hexane (BDH, Dorset, Poole, UK) and stored at –70°C under nitrogen until analysis for fatty acid composition by gas chromatography (50).

TABLE E1. POST-EXERCISE PULMONARY FUNCTION IN CONTROL AND EIB SUBJECTS.

	Control			EIB		
Diet	FVC (L)	FEV$_1$ (L)	FEV$_1$/FVC (%)	FVC (L)	FEV$_1$ (L)	FEV$_1$/FVC (%)
NORMAL						
1min	4.83 ± 0.33	4.29 ± 0.29	88.8 ± 2	4.63. ± 0.33*^{a}	3.89 ± 0.32*^{a}	84.0 ± 3^{a}
5min	4.93 ± 0.32	4.31 ± 0.32	87.4 ± 3	4.27 ± 0.31*^{a}	3.66 ± 0.32*^{a}	85.7 ± 3*^{a}
10min	4.93 ± 0.33	4.31 ± 0.32	87.4 ± 2	4.06 ± 0.34*^{a}	3.45 ± 0.32*^{a}	85.0 ± 2*^{a}
15min	4.90 ± 0.32	4.32 ± 0.33	88.2 ± 2	3.95 ± 0.32*^{a}	3.39 ± 0.29*^{a}	85.8 ± 3*^{a}
30min	4.91 ± 0.33	4.35 + 0.31	88.5 ± 3	4.30 ± 0.31*^{a}	3.63 ± 0.28*	84.4 ± 3*
45min	4.93 ± 0.31	4.36 + 0.32	88.4 ± 2	4.62 ± 0.33*^{a}	3.95 ± 0.31	85.5 ± 2*
60min	4.94 ± 0.30	4.34 + 0.34	87.9 ± 3	4.88 + 0.30^{a}	4.05 ± 0.30	83.0 ± 3
PLACEBO						
1min	4.99 ± 0.32	4.34 ± 0.28	87.0 ± 3	4.62 ± 0.30*^{a}	3.85 ± 0.28*^{a}	83.3 ± 3^{a}
5min	5.07 ± 0.27	4.32 ± 0.26	85.2 ± 3	4.28 ± 0.28*^{a}	3.60 ± 0.25*^{a}	84.1 ± 2*^{a}
10min	5.02 ± 0.32	4.35 ± 0.28	86.7 ± 2	4.20 ± 0.33*^{a}	3.48 ± 0.28*^{a}	82.9 ± 3^{a}
15min	5.00 ± 0.36	4.32 ± 0.27	86.4 ± 3	4.05 ± 0.27*^{a}	3.43 ± 0.28*^{a}	84.7 ± 3*^{a}
30min	5.00 ± 0.29	4.33 ± 0.29	86.6 ± 2	4.26 ± 0.30*^{a}	3.62 ± 0.27*	85.0 ± 3*
45min	5.06 ± 0.30	4.34 ± 0.31	85.8 ± 3	4.52 ± 0.28*^{a}	3.90 ± 0.27	86.3 ± 2*
60min	5.04 ± 0.32	4.34 ± 0.32	86.1 ± 3	4.79 ± 0.31^{a}	3.98 ± 0.29	83.1 ± 3
n-3 PUFA						
1min	5.02 ± 0.30	4.34 ± 0.31	86.5 ± 3	4.95 ± 0.29*^{b}	4.00 ± 0.28*^{a}	80.8 ± 3*^{a}
5min	5.03 ± 0.33	4.33 ± 0.28	86.1 ± 2	4.86 ± 0.31*^{b}	3.91 ± 0.29*^{b}	80.5 ± 2^{a}
10min	5.02 ± 0.30	4.34 ± 0.31	86.4 ± 3	4.88 ± 0.28*^{c}	3.90 ± 0.31*^{b}	80.0 ± 3^{a}
15min	5.02 ± 0.32	4.33 ± 0.27	86.3 ± 3	4.81 ± 0.29*^{b}	3.89 ± 0.27*^{b}	80.1 ± 2^{a}
30min	5.08 ± 0.29	4.35 ± 0.29	85.6 ± 2	4.86 ± 0.30^{b}	3.95 ± 0.31	81.3 ± 3
45min	5.05 ± 0.28	4.36 ± 0.30	86.3 ± 3	4.92 ± 0.28^{b}	3.98 ± 0.29	81.0 ±3
60min	5.04 ± 0.30	4.37 ± 0.28	86.7 ± 2	4.95 ± 0.30^{b}	4.00 ± 0.27	81.0 ± 3

Values are ± SD. There were no significant differences in control subjects for post-exercise values by time ($p>0.025$) or diet ($p>0.017$). For EIB subjects, *$p<0.025$ compared to respective pre-exercise value; and letters $^{(a,b,c)}$ refer to comparisons by diet for the post-exercise time period within specific variable; different letters designate significant difference ($p<0.017$).

TABLE E2. FATTY ACID COMPOSITION OF NEUTROPHIL EXTRACTS EXPRESSED AS A PERCENTAGE OF TOTAL FATTY ACID CONTENT BEFORE AND AFTER THE 3 WEEK DIETARY SUPPLEMENTATION PERIOD IN CONTROL SUBJECTS.

	18:2	20:4	20:5	22:6
Diet	Linoleic Acid	Arachidonic Acid	Eicosapentaenoic Acid	Docosahexaenoic Acid
NORMAL (n=10)	9.8 ± 3.0	16.7 ± 3.6	0.26 ± 0.21	2.67 ± 1.8
PLACEBO (n=10)				
Before	9.4 ± 2.7	15.9 ± 3.8	0.28 ± 0.19	2.45 ± 1.9
After	8.7 ± 3.0	15.1 ± 3.5	0.31 ± 0.21	2.79 ± 16
n-3 PUFA (n=10)				
Before	9.5 ± 2.3	16.0 ± 4.0	0.25 ± 0.19	2.49 ± 1.5
After	5.4 ± 2.3*	11.6 ± 3.4*	4.10 ± 1.8*	3.83 ± 2.3

Values are mean ± SD. *p<0.025 compared to pre-supplementation period