

REVIEW

Dietary polyunsaturated fatty acids in asthma- and exercise-induced bronchoconstriction

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Despite progress that has been made in the treatment of asthma, the prevalence and burden of this disease has continued to increase. While pharmacological treatment of asthma is usually highly effective, medications may have significant side effects or exhibit tachyphylaxis. Alternative therapies for treatment that reduce the dose requirements of pharmacological interventions would be beneficial, and could potentially reduce the public health burden of this disease. Ecological and temporal data suggest that dietary factors may have a role in recent increases in the prevalence of asthma. A possible contributing factor to the increased incidence of asthma in Western societies may be the consumption of a proinflammatory diet. In the typical Western diet, 20- to 25-fold more omega (n)-6 polyunsaturated fatty acids (PUFA) than n-3 PUFA are consumed, which promotes the release of proinflammatory arachidonic acid metabolites (leukotrienes and prostanoids). This review will analyze the evidence for the health effects of n-3 PUFA in asthma- and exercise-induced bronchoconstriction (EIB). While clinical data evaluating the effect of omega-3 fatty acid supplementation in asthma has been equivocal, it has recently been shown that fish oil supplementation, rich in n-3 PUFA, reduces airway narrowing, medication use, and proinflammatory mediator generation in nonatopic elite athletes with EIB. These findings are provocative and suggest that dietary fish oil supplementation may be a viable treatment modality and/or adjunct therapy in asthma and EIB.

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Introduction

Asthma is a significant worldwide health problem, with high and increasing incidence in many countries (Burney *et al*, 1990); morbidity—reflected in hospital admission rates (Halfon & Newacheck, 1986), use of medical services, drug use, and trends in mortality rates are substantial (Anderson, 1989; Klauka *et al*, 1991). The incidence of asthma varies by region and by age, but the global burden of asthma can be approximated from measured prevalence (reflecting incidence, duration, persistence, and recurrence of disease). Approximately 20.3 million Americans (6.3 million children)

had asthma in 2001, 73.4 per 1000 population (American Lung Association, 2003), while it is estimated that around 300 million people in the world currently have asthma (Masoli *et al*, 2004). Despite the progress that has been made in the treatment of asthma, it remains a major illness in terms of morbidity, suffering, and cost (National Heart, Lung and Blood Institute, 2002).

Asthma is a chronic inflammatory disorder of the airways and causes recurrent episodes of wheezing, breathlessness, chest tightness, and cough (Barbato *et al*, 2003). Long-term airway remodeling is characteristic of asthma and may be associated with an increase in airway hyper-responsiveness to a variety of stimuli (Barbato *et al*, 2003). Airway responsiveness is the tendency for airways to constrict under the influence of nonsensitizing physical stimuli such as cold air and exercise, chemical substances such as methacholine, or sensitizing agents such as allergens. Airway hyper-responsiveness can be defined as the increase above normal in the degree to which the airways will constrict upon exposure to these stimuli (Boulet, 2003) and it is closely

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related to the underlying mechanisms of asthma as we currently understand them (National Heart, Lung and Blood Institute, 1997).

Exercise is a powerful trigger of asthma symptoms and may result in asthmatic patients avoiding physical activity resulting in detrimental consequences to their physical and social well-being. Approximately 90% of asthmatics and a high proportion of nonatopic elite athletes are hyper-responsive to exercise and experience exercise-induced bronchoconstriction (EIB) (Rundell & Jenkinson, 2002). A characteristic evident in individuals with EIB is a marked decrease in exercise capacity and breathlessness upon exertion. Individuals who demonstrate EIB are often asthmatics; signs and symptoms of EIB include cough, wheeze, dyspnea, shortness of breath, chest tightness, and chest pain normally beginning after a brief period of exercise (5–8 min) and resolving spontaneously within 30–60 min after stopping exercise (Dahlen *et al*, 2001).

Significant numbers of individuals without chronic asthma or allergic rhinitis have diagnosed EIB; the prevalence of EIB in these individuals has not been well studied or reported. The best estimate for the general population is 3–10% (Rupp, 1996); however, estimates of EIB for various athletic populations are generally higher (Weiler *et al*, 1998; Wilber *et al*, 2000; Rundell *et al*, 2005). Estimates for nonathletes range as high as 19.3% in a sample of Australian school children (Haby *et al*, 1995). Thus, the prevalence of EIB is high in the asthmatic population, and of significant numbers in the nonasthmatic population. In individuals with clinically recognized asthma EIB is usually associated with atopy, and frequently with increased numbers of eosinophils in sputum (Kivity *et al*, 2000). Allergen exposure enhances the response to exercise in asthmatics and can increase responsiveness to pharmacological agents. A high prevalence of EIB and asthma-like symptoms have been reported in the nonatopic elite athlete population (Larsson *et al*, 1993; Paul *et al*, 1993; Heir & Oseid, 1994; Sue-Chu *et al*, 1996; Schoene *et al*, 1997; Helenius *et al*, 1998). It should be noted that although the pathogenesis of EIB in nonatopic elite athletes and frank asthma may be different, both conditions reflect active inflammation, remodeling, and hyper-reactivity of the airways (Sue-Chu *et al*, 1999; Barbatto *et al*, 2003).

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 (Anderson & Kippelen, 2005). It has been suggested that transient dehydration causes an increase in airway surface liquid osmolarity, which would activate proinflammatory mediators such as histamine and the arachidonic acid (AA) metabolites leukotrienes (LTs) and prostaglandins (PGs) from resident airway cells, resulting in bronchial smooth muscle contraction and subsequent airway obstruction (Anderson & Daviskas, 2000). 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 (McFadden *et al*, 1986), which would contribute further to the airway narrowing.

While the precise pathophysiological mechanism involved in EIB remains unclear, it does appear that LTs play a role in mediating a portion of this airway narrowing (Anderson & Kippelen, 2005). Agents that act in two distant manners on the LT pathway—namely, cysteinyl LT₁ receptor antagonists and 5-lipoxygenase inhibitors—are able to block a proportion of the bronchoconstrictor response, thus demonstrating that LTs do mediate a part of the exercise-induced airway narrowing response (Horwitz *et al*, 1998). The inability of the LT agents to block more than 50–60% of the exercise-induced narrowing suggests that other mediators such as histamine and PGs are also involved in the EIB response.

Inhaled corticosteroids, long-acting β_2 -agonists and short-acting β_2 -agonists have proven highly effective as medications in relief of symptoms, and have facilitated the management of asthma. In addition, daily medications such as LT receptor antagonists and LT enzyme inhibitors have recently proven effective in asthma therapy (Kemp, 2003). However, these medications are not without real and potential side effects. Prolonged use of some medications may result in reduced efficacy, or tachyphylaxis. For example, daily use of long-acting β_2 -agonists in the management of exercise-induced asthma in children has recently been questioned (Bisgaard, 2000), and reversal of an asthma attack, such as exercise-induced asthma, may be ineffective in a large portion of asthmatics when short-acting β_2 -agonists are used daily (Hancox *et al*, 2002). Therefore, alternative treatment approaches in asthma that focus on manipulation of dietary factors are of real interest since they could potentially reduce the dose requirements of pharmacological medications (Hackman *et al*, 1996; Smit *et al*, 1999; Baker & Ayres, 2000; Fogarty & Britton, 2000a; Devereux & Seaton, 2001; Picado *et al*, 2001; Romieu & Trenga, 2001; Smit, 2001; Denny *et al*, 2003; Mickleborough & Gotshall, 2003), and reduce the public health burden of this disease. The effect of diet on asthma is complex, and likely involves involvement from a variety of nutrients, including flavonoids, vitamins, minerals, and fatty acids (Fogarty & Britton, 2000b; McKeever & Britton, 2004).

The purpose of this review is to examine critically the existing information regarding the relationship between fish oil supplementation in asthma and, in particular, to address the question as to whether supplementing the diet with (n)-3 polyunsaturated fatty acids (PUFA) represents a viable alternative treatment for asthma and EIB.

For this review, the keywords n-3 PUFA, n-6 PUFA, and fish oils were coupled with keywords asthma, airway hyper-responsiveness, exercise-induced asthma, EIB, exercise-induced obstruction, and exercise-induced airway narrowing for a Medline[®], EMBASE, Cochrane Central Register of Controlled Trials, and SPORTDiscus search.

Biological effects of omega-3 and omega-6 fatty acids on airway inflammation

Although the impact of n-3 PUFA on lipid mediator generation has been greatly clarified, the understanding of subcellular effects is still limited. Omega-3 PUFA affects biophysical characteristics of cellular membranes by alteration of the membrane phospholipid composition and may modify the function of membrane-linked enzyme systems and signal transduction pathways. Many of the anti-inflammatory effects of n-3 PUFA appear to be exerted at the level of altered gene expression and have been demonstrated only a limited number of times

in vitro, and thus the extent of these effects *in vivo* is not yet clear.

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), derived from fish oil, competitively inhibit n-6 PUFA arachidonic (AA) metabolism, thus reducing the generation of proinflammatory four-series LTs and two-series prostanoids (PGs and thromboxanes) (Lee *et al*, 1985), and the production of cytokines from inflammatory cells (Endres *et al*, 1989). The EPA-derived metabolites (five-series LTs and three-series prostanoids) have lower biological activity compared with the analogous AA-derived four-series LTs and two-series PGs (Figure 1). Four-series cysteinyl LTs

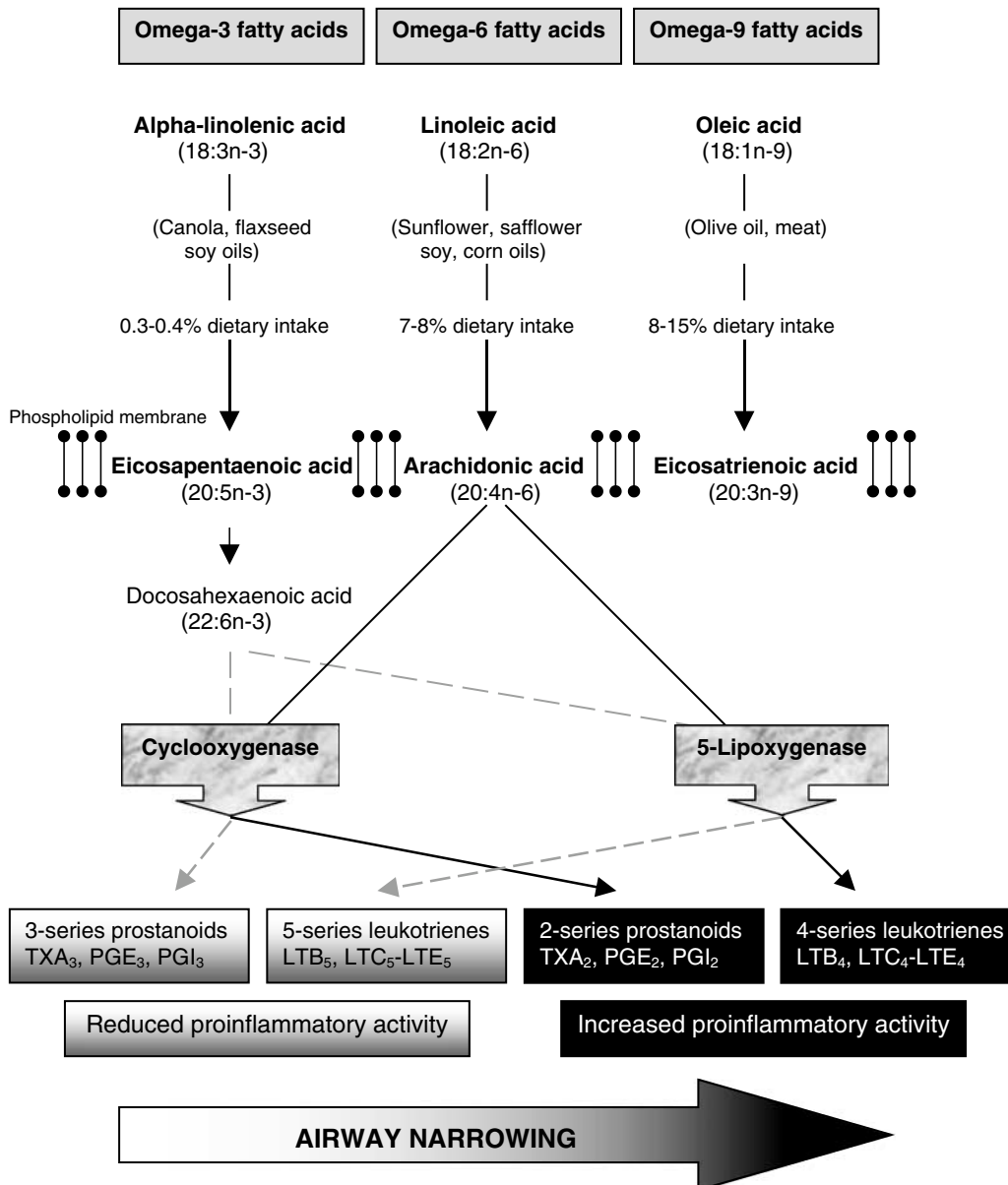


Figure 1 Metabolism of dietary fatty acids after ingestion via the COX and 5-lipoxygenase enzymatic pathways and subsequent effects on inflammatory activity and bronchoconstriction. LT, leukotriene; PG, prostaglandin; TX, thromboxane.

increase vascular permeability and contract smooth muscle cells, causing bronchoconstriction and vasoconstriction (De Caterina & Zampolli, 2004); The bronchoconstrictive and chemotactic potency of LTB₄ is two orders of magnitude higher than the activity of LTB₅ (Heller *et al*, 1998) (Figure 1). Consuming fish oil results in partial replacement of AA in inflammatory cell membranes by EPA (Lee *et al*, 1985; Endres *et al*, 1989) and thus demonstrates a potentially beneficial anti-inflammatory effect of n-3 PUFA. Supplementing the diet with n-3 PUFA has been shown to reduce AA concentrations in neutrophils and neutrophil chemotaxis, reduce LT generation (Lee *et al*, 1985; Payan *et al*, 1986), and reduce airway late response to allergen exposure (Arm *et al*, 1989). These data are consistent with the proposed pathway by which dietary intake of n-3 PUFA modulates lung disease.

Mounting evidence now suggests that fatty acids are not only the precursors of eicosanoids and other lipid mediators but also can modulate signaling molecules and transcription factors such as nuclear factor-kappaB (NF- κ B) (Hwang, 2000;

Liu *et al*, 2001; Jump, 2002). Since macrophages of induced sputum and bronchial epithelial cells from stable asthmatics exhibit increased NF- κ B activity compared with cells from healthy individuals (Hart *et al*, 1998), it has been suggested that NF- κ B plays a pivotal role in the pathogenesis of asthma (Hart *et al*, 1998, 2000; Bureau *et al*, 2000a,b; Zhao *et al*, 2001; Gagliardo *et al*, 2003). Recently, Lee *et al* (2001, 2003) demonstrated that activation of general proinflammatory pathways, such as NF- κ B and cyclooxygenase-2 (COX-2) expression by saturated fatty acids and inhibition of this induction by n-3 PUFA, are mediated through a common signaling pathway derived from toll-like receptor 4 (Tlr-4). If activation of Tlr-4 is modulated by n-3 PUFA, then signaling pathways downstream, and consequent cellular responses (eg inducible nitric oxide, proinflammatory cytokines, tumor necrosis factor (TNF)- α , IL-1 β , and eicosanoids (prostanoids and LTs)) should also be modulated by n-3 PUFA (Lee *et al*, 2001, 2003) (Figure 2). Indeed, It has been demonstrated that proinflammatory cytokine inhibition in

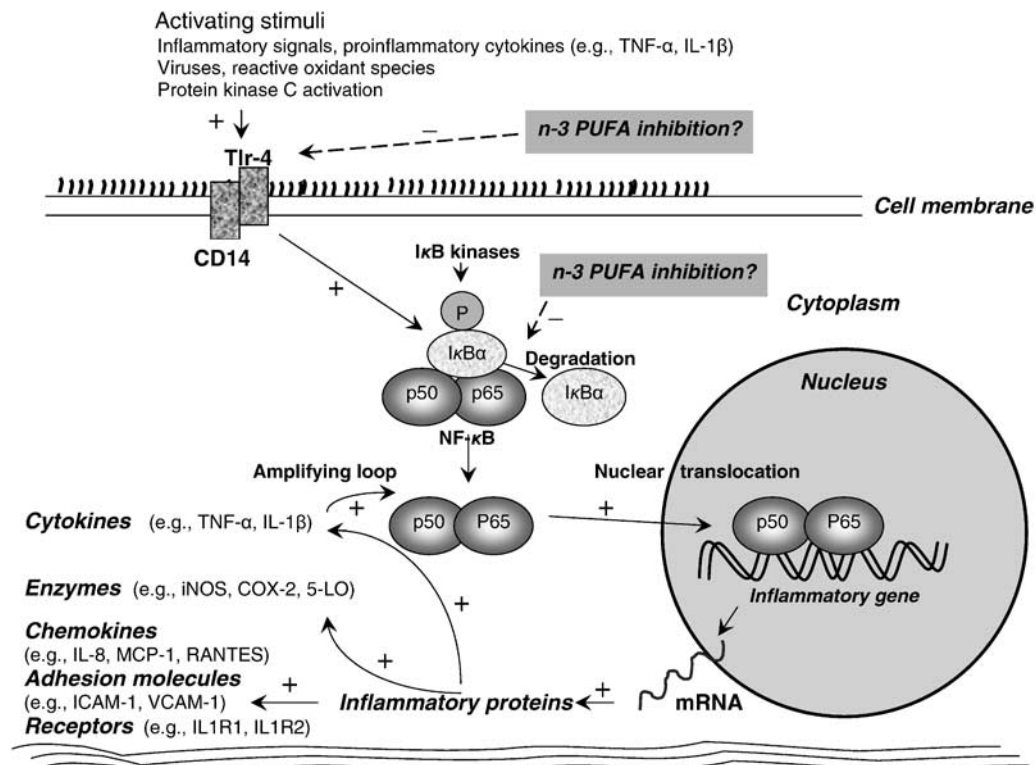


Figure 2 Selected pathways of Tlr-4 and NF- κ B by multiple inflammatory stimuli, resulting in the coordinated expression of genes for several cytokines, enzymes, chemokines, adhesion molecules, and receptors. A schematic representation of signaling cascades for various activating stimuli and activation of NF- κ B (p50/p65). The activation of NF- κ B begins with stimulation of specific receptor families at the cell surface (eg Tlr-4 and CD14), interleukin-1 receptor, and TNF receptor 1) and recruitment of adaptor proteins that leads to specific pathways of transduction controlled by various kinases. This activation promotes the phosphorylation of I κ B. This activation targets I κ B for ubiquitination and degradation. As a consequence, the NF- κ B inhibitory protein is removed and free NF- κ B is rapidly translocated to the nucleus where it binds to specific promoter regions of various genes encoding inflammatory cytokines, enzymes, chemokines, adhesion molecules, and receptors. The cytokines (TNF- α and interleukin (IL)-1 β) are both activated and amplified by NF- κ B. Potential sites of n-3 PUFA inhibition are shown. iNOS, inducible nitric oxide; mRNA, messenger RNA; TNF- α , tumor necrosis factor- α ; COX-2, cyclooxygenase-2; 5-LO, 5-lipoxygenase; IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; RANTES, regulated upon activation, normal T cell expressed and secreted.

murine macrophages by n-3 PUFA is mediated, in part, through inactivation of NF- κ B (Lo *et al*, 1999; Novak *et al*, 2003) and inhibition of COX-2 and PGE₂ expression in blood monocytes with a Tlr-4 agonist (Lee *et al*, 2003). Therefore, since Tlr-4 conveys signals as a part of innate immunity from the endotoxin receptor (CD14) on the surface of macrophages to the inner cell, a downregulation of nuclear transcription factors might be reduced after fish oil ingestion (Molvig *et al*, 1991).

Cell culture studies have demonstrated that AA activates NF- κ B in monocytic cells (Camandola *et al*, 1996). It is possible that this is the mechanism by which AA induces COX-2 and inflammatory cytokines. In contrast, EPA has been shown not to activate NF- κ B in a monocytic cell line (Camandola *et al*, 1996), and to prevent NF- κ B activation by TNF- α in cultured pancreatic cells (Ross *et al*, 1999). This reduced activation of NF- κ B involved decreased degradation of I κ B, perhaps through decreased phosphorylation (Ross *et al*, 1999).

Similarly, it has been shown that EPA decreased endotoxin-induced activation of NF- κ B in human monocytes (Novak *et al*, 2003). This was associated with decreased I κ B phosphorylation. Collectively, these observations suggest direct effects of n-3 PUFA on inflammatory gene expression via inhibition of the proinflammatory transcription factor NF- κ B. *In vitro* experiments in the investigation of the regulatory mechanisms of n-3 PUFA on NF- κ B activity are warranted; specifically, the interaction between n-3 PUFA, inflammatory eicosanoids, and Tlr-4 receptor function in asthma requires further investigation.

Omega-3 PUFA therefore seems to interfere with early inflammatory signal transduction processes and is thus capable of blunting hyperinflammation. Elucidating the mechanism of this modulation could help us to understand how dietary n-3 PUFA achieve their specific effects on airway inflammation.

Omega-3 fatty acids in asthma

A possible contributing factor to the increased incidence of asthma in Western societies may be the consumption of a proinflammatory diet. In the typical Western diet, 20- to 25-fold more n-6 PUFA than n-3 PUFA are consumed (Gadek *et al*, 1999). This predominance of n-6 PUFA is due to the abundance of dietary linoleic acid (18:2n-6), which is present in high concentrations in soy, corn, safflower, and sunflower oils (Figure 1). By contrast, there is a low intake of the n-3 homologue of linoleic acid, α -linolenic acid (18:3n-3), which is present in leafy green vegetables and in flaxseed and canola oils (Figure 1). Once ingested, the 18-carbon fatty acids are desaturated and elongated to 20-carbon n-6 PUFA. Linoleic acid is converted to AA and α -linolenic acid is converted to EPA (20:5n-3) (Figure 1). Compared with linoleic acid, there is little dietary intake of AA and EPA, which are present in meat and fish, respectively. Linoleic

acid and α -linolenic are necessary for a complete diet and cannot be synthesized in vertebrates; therefore, they are essential fatty acids. As a consequence, the relative dietary amounts of n-6 and n-3 PUFA are determinants of the relative cellular amounts of linoleic acid and α -linolenic acid.

There has been increased emphasis on the beneficial effects for cardiovascular health of replacing lard and dairy fats rich in saturated fatty acids. This has led to increased consumption of vegetable oils rich in n-6 PUFA and a simultaneous decrease in consumption of oily fish and leafy vegetables, the major sources of n-3 PUFA. This dietary shift is characterized by a fall in consumption of saturated fats and an increase in n-6 PUFA (Kelley, 2001). The anti-inflammatory properties of n-3 PUFA such as EPA and DHA and generally proinflammatory properties of dietary n-6 PUFA (Broughton *et al*, 1997; Okamoto *et al*, 2000a), such as linoleic acid, suggest that these dietary trends may have predisposed some individuals to inflammatory disorders, including asthma.

Epidemiological studies

The notion that consumption of dietary fatty acids can influence the development and activity of an inflammatory disease such as asthma is attractive in view of the complex metabolic role that fatty acids play in cell metabolism and structure. During the period of increasing asthma prevalence in England and Wales, dietary consumption of fatty acids also changed; this may be due to a reduction in the consumption of animal fat (saturated fat) and an increase in the use of margarine and vegetable oils containing n-6 PUFA, such as linoleic acid (Black & Sharpe, 1997). Support for the hypothesis that fat intake may be important is available from a case-control study showing an association of higher fat intake with adult onset wheeze in Scotland (Bodner *et al*, 1999), and a cohort study from Malmo in which men with asthma had a higher intake of dietary fat (Strom *et al*, 1996). Haby *et al* (2001) assessed the prevalence of asthma and risk factors for asthma in Australian preschool children and found that a high level of PUFA (high n-6, low n-3) was associated with increased risk of recent asthma. Hodge *et al* (1996) found an inverse relationship between weekly oily fish intake over the course of 12 months and the prevalence of asthma in 574 schoolchildren in a cross-sectional study. These studies suggest that consumption of oily fish is associated with a reduced risk of asthma in childhood.

Patal *et al* (2002) demonstrated an association between consumption of oily fish and symptomatic wheeze in individuals with and without physician diagnosed asthma. Takemura *et al* (2002) assessed the relationship between dietary fish intake and the prevalence of asthma among a Japanese childhood population and their results indicated that the frequency of fish intake was positively related to the prevalence of asthma. In a prospective 4-y cohort study of

2531 Norwegian children, the relationship between the introduction of a fish diet during the first year of life and the risk of developing asthma and allergic rhinitis was evaluated (Nafstad *et al*, 2003). This group of researchers found that the introduction of a fish diet was negatively associated with the risk of developing allergic rhinitis and asthma. Additionally, Oddy *et al* (2004) recently investigated whether childhood asthma was associated with the ratio of n-6:n-3 PUFA in the diet (n-6:n-3) using a cross-sectional study design. They found evidence for the promotion of a diet with increased n-3 PUFA (fresh or oily fish at least once a week, whole grain cereals, raw sunflower and flaxseeds, and canola oil) and reduced n-6 PUFA (margarines, vegetable oils, processed foods) to protect children against symptoms of asthma.

Bolte *et al* (2005) recently showed that young adults eating low-fat margarine (rich in n-6 PUFA) alone or in combination with low-fat butter frequently had increased odds for asthma, but not for hay fever, atopic dermatitis, and allergic sensitization. Nagel *et al* (2003) investigated in a prospective study the associations between dietary intake of fatty acids, antioxidants, and hay fever manifestation in adulthood. The data showed that the risk of hay fever increases with an increasing ratio of n-6 vs n-3 PUFA. The study also demonstrated that a high intake of EPA and vitamin E was inversely related to hay fever. Prevalence data from the European Community Respiratory Health Survey (ECRHS) was examined to test the hypothesis that the intake of dietary antioxidants was inversely associated, and mono-unsaturated fatty acids (MUFA) and PUFA were positively correlated with allergic sensitization (Heinrich *et al*, 2001). The findings suggest that a diet high in antioxidants might play a protective, and a high dietary intake of MUFA may promote the development of allergic sensitization. Weiland *et al* (1999) have shown a positive correlation between the intake of trans fatty acids and the prevalence of asthma symptoms in children aged 13–14 y. This is a particularly important finding since it has been reported that trans-fatty acids influence the desaturation (impaired delta-6-desaturase activity) and chain elongation of n-3 and n-6 PUFA into precursors of proinflammatory eicosanoids (ie PGs and LTs).

Recently, de Luis *et al* (2005) and colleagues in a case-controlled study investigated the differences in dietary intake between a population of asthmatic and healthy nonasthmatic subjects. These authors established that asthmatic patients have a lower dietary intake of antioxidant vitamins than nonasthmatic subjects and found a positive correlation between n-3 PUFA and forced expiratory volume in 1 s (FEV₁), which was independent of other dietary habits. Woods *et al* (2004), in a community-based cross-sectional study, sought to determine whether plasma levels of n-3 PUFA, as a measure of dietary intake, was protective against asthma and atopy in young adults. These authors did not find any evidence to suggest that n-3 PUFA are associated with a reduced risk of asthma or atopy; their results suggest that the n-6 PUFA gamma (γ)-linolenic acid (GLA) has the

strongest association with asthma. Owing to the fact that this was a cross-sectional study, the authors were unable to establish a cause and affect relationship for the fatty acid/asthma associations found. Apart from pollutants (Romieu *et al*, 1996) and allergens (Bettiol *et al*, 2002) influencing the onset of asthma, other dietary factors, such as dietary antioxidants, have been shown to have a protective role in asthma (Britton *et al*, 1995; Dow *et al*, 1996; Soutar *et al*, 1997; Grievink *et al*, 1998; Hijazi *et al*, 2000; Shaheen *et al*, 2001; Romieu *et al*, 2002).

Interventional studies

Considering the role of LTs, PGs, and cell-cytokine interactions in airway inflammation, remodeling, and hyper-reactivity in asthma, the potential therapeutic effect of a diet rich in fish oil has been examined repeatedly. 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 (Arm *et al*, 1988; Kirsch *et al*, 1988; Stenius-Aarniala *et al*, 1989; Thien *et al*, 1993; Hodge *et al*, 1998), other studies have demonstrated an improvement in asthmatic status following n-3 PUFA supplementation (Arm *et al*, 1989; Dry & Vincent, 1991; Broughton *et al*, 1997; Villani *et al*, 1998; Nagakura *et al*, 2000; Okamoto *et al*, 2000a,b; Emelyanov *et al*, 2002). Early short-term trials (8 weeks) of up to 4 g/day of EPA in patients with severe asthma showed no clinical benefit, despite demonstrating profound suppression of neutrophil chemotaxis and LT mediator production (Kirsch *et al*, 1988). EPA (6 weeks) of 3 g/day had a deleterious effect on patients with aspirin-intolerant asthma (Picado *et al*, 1988), consistent with the known aspirin-like effect of COX inhibition by EPA. Further studies in milder asthmatics with 3.2 g/day for 10 weeks showed no benefit in either clinical symptoms or bronchial hyper-responsiveness (Arm *et al*, 1988), despite demonstrating attenuation of allergen-induced late-phase bronchoconstriction induced in the laboratory (Arm *et al*, 1989). A more prolonged trial for 6 months with 3.2 g/day of EPA also showed no clinical benefit in patients with pollen-induced asthma and seasonal hay fever (Thien *et al*, 1993). In addition, Stenius-Aarniala *et al* (1989) demonstrated no clinical benefit of 10 weeks of fish oil supplementation in relatively stable asthmatics. However, their method of assessing lung function is open to question since each subject used a Peak Flow Meter at home under no supervision. Surette *et al* (2003) showed no change in baseline pulmonary function occurred in a population of atopic asthmatics, even though daily consumption of dietary GLA and EPA inhibited LT biosynthesis. However, asthma severity and reliance of medication were not assessed. McDonald *et al* (1990) provided 2.7 g EPA and 1.8 g DHA for 10 weeks to 15 nonsmoking asthmatics and found no change in peak expiratory flow rate following fish oil supplementation.

Recently, Broadfield *et al* (2004) evaluated whether a higher intake of n-6 PUFA or a lower intake of n-3 PUFA increased the risk of asthma, by measuring dietary fatty acid intake by a food frequency questionnaire (FFQ) and erythrocyte membrane fatty acids as an objective biomarker of intake. These authors compared individual fatty acid intake estimated by FFQ and by mass spectrometry of fasting erythrocyte cell membranes in 89 cases of asthma and 89 community-matched controls. They found that a higher erythrocyte membrane level of linoleic acid, an n-6 PUFA, was associated with a decreased risk of asthma. However, as the authors acknowledge, major limitations to their study design may include overmatching of the cases and controls, since social class is strongly associated with diet. This would have resulted in odds ratios that underestimated the true difference between asthmatics and controls. In addition, the limitations in FFQ in introducing measurement error have recently been highlighted (Bingham *et al*, 2003).

In contrast, Dry and Vincent (1991) have shown positive results using a small placebo-controlled trial of low-dose EPA (1 g/day) for 12 months in 12 adult asthmatics; after 9 months, a small but significant improvement of 23% was found in FEV₁. However, no details were given of concurrent medication use or confirmation of compliance by leukocyte membrane phospholipid analysis. Hodge *et al* (1998) demonstrated that dietary supplementation with n-3 PUFAs over 6 months increased plasma levels of these fatty acids and reduced stimulated TNF- α and circulating eosinophils, with a concurrent improvement in peak expiratory flow and reduced medication use in asthmatic children (Hodge *et al*, 1998). Nagakura *et al* (2000) showed that dietary supplementation with fish oil (84 mg EPA and 36 mg DHA per day) over 10 months decreased asthma scores and reduced acetylcholine thresholds during an acetylcholine inhalation test in children with bronchial asthma. Okamoto *et al* (2000a, b) observed suppression of LTB₄ and LTC₄ generation by leukocytes and improvement in respiratory function following 4 weeks of perilla seed oil (n-3 PUFA)-rich supplementation in asthmatic subjects. Payan *et al* (1986) found that high doses, compared to low doses, of EPA ethyl ester taken daily for 8 weeks increased LTB₅ generation, and reduced AA, LTB₄, and PGE₂ generation by polymorphonuclear (PMN) and mononuclear leukocytes in asthmatic patients. These authors did not report pulmonary function scores, medication use, or asthma symptom scores. Villani *et al* (1998) observed a significant improvement in FEV₁ with a concomitant reduction in airway resistance after only 30 days supplementation with 3 g/day of n-3 PUFA in seven atopic patients.

Masuev (1997a) observed significant attenuation of the late allergic response in 13 asthmatic patients supplemented for 2 weeks with n-3 PUFA, and in another study showed that n-3 PUFA supplementation resulted in a significant decline of the late allergic response and reduced drug doses in 27 asthmatic patients (Masuev, 1997b). Provocative tests with

allergen after 10 weeks of either n-3 PUFA or placebo showed a significant decline in the late allergic response and suppression of inflammatory mediators (50% reduction in the capacity of PMN to produce LTB₄) in the treatment group (Arm *et al*, 1989). Broughton *et al* (1997) demonstrated that supplementing the diet with 3.3 g/day of EPA and DHA daily in 27 asthmatic subjects ameliorated methacholine-induced respiratory distress, which may be predicted by LT metabolism. Emelyanov *et al* (2002) recently showed a decrease in daytime wheeze, concentration of exhaled hydrogen peroxide (a marker of airway inflammation), and an increase in morning peak expiratory flow rate in 46 atopic asthmatic patients receiving a lipid extract of New Zealand green-lipped mussel, rich in n-3 PUFA, for 8 weeks compared to placebo (olive oil).

The inconsistency among study results assessing the efficacy of fish oil supplementation in asthma may be attributable to the heterogeneity in definitions of the (1) Settings (eg hospital vs outpatient; countries); (2) Populations (eg age; gender; clinical picture of asthma, including its severity and concomitants, or triggers with the potential to impact asthma control); (3) Interventions and their contrasts with comparators (eg different types and amounts of oil and n-3 PUFA contents; controlled vs uncontrolled dosing); (4) the dosage (1–4 g/day) and duration (3 weeks to 12 months) of fish oil supplementation varies greatly among published studies; (5) Cointerventions (eg asthma medication with varying capacities to control asthma in the short or long term). For example, failing to assure that there is not an uneven distribution of corticosteroid users or doses across study arms/cohorts can restrict the ability to meaningfully attribute a significant or null effect to the actions of n-3 PUFA supplementation. The capacity of asthma medications to improve asthma symptoms can mask the benefits linked to the use of n-3 PUFA supplementation; and (6) the most commonly employed respiratory outcome measure in a large majority of the published studies evaluating the efficacy of n-3 PUFA supplementation in asthma is peak expiratory flow. However, peak expiratory flow may not accurately reflect changes in airway function as assessed by more reliable measurements such as FEV₁ or forced vital capacity (Brusasco, 2003; Miller *et al*, 2003).

Woods *et al* (2003) in The Cochrane Database of Systematic Reviews, assessing the efficacy of fish oil for asthma in adults and children, identified 22 studies for possible inclusion; however, the authors only included nine studies. Reasons for noninclusion were (a) not a randomized controlled trial (four studies), (b) not using marine fatty acids in asthma (three studies), (c) no outcome measures reported (three studies), and (d) an inadequate intervention period (one study). None of the studies reported asthma exacerbations, health status (quality of life), or hospital admissions. These authors stressed that further studies should address these issues. Woods *et al* (2003) concluded that they were unable to determine the effect of fish oil supplementation in asthma or answer the question whether increasing dietary marine n-3

PUFA by increased fish intake results in improved asthma control.

It may be that the absolute and relative quantities of other PUFA and saturated fatty acids are equally important, as suggested by Chang *et al* (1993), and that an excess of other PUFA such as the *n*-6 subset may be influential. Given the small number of studies, which have been conducted to date, and the limited range of clinically important outcomes that have been reported, there is a need for further research in this area. To date, in the studies involving the efficacy of fish oil supplementation in asthma, the total number of asthmatic subjects studied has only been 187, methodologies have been variable, and the outcome measures of asthma exacerbations, hospital admissions, and quality of life scores have been lacking (Woods *et al*, 2003). Future research must address these issues.

Manipulation of dietary omega-3 fatty acids in asthma

In only one study (Hodge *et al*, 1998) was dietary manipulation of *n*-3 PUFA performed as part of the treatment phase. It is noteworthy that this study demonstrated a significant improvement in peak expiratory flow and a reduction in asthma medication use on the *n*-3 PUFA diet (canola oil and canola-based margarines and salad dressings), and a decrement in resting peak expiratory flow and increased medication use was observed on the *n*-6 PUFA diet (sunflower oil and sunflower oil-based margarines and salad dressings).

While consumption of supplements may be the most efficacious way to increase *n*-3 PUFA in the body, a food-based approach that is accepted and tolerated is a preferable way to ensure long-term nutrient intake. As this dietary approach will enhance *n*-3 PUFA intake to a lesser extent than most capsule supplementation methods, changes in mediator generation are likely to come about more slowly (James *et al*, 2000). Dietary approaches that allow an individual to meet their nutrient needs through multiple types of foods generally reduce an individual's risk of exposure to toxins, such as methyl mercury and PCBs, which are common contaminants of fish and other sea foods (Kris-Etherton *et al*, 2002). For those individuals who do not eat fish, have limited access to a variety of fish, or cannot afford to purchase fish, a fish oil supplement may be considered. On the other hand, some individuals may prefer to eat whole fish rather than taking fish oil capsules, since a common complaint from taking encapsulated fish oil are fishy aftertaste, gastrointestinal disturbances, and nausea (Kris-Etherton *et al*, 2002).

Whether the type of *n*-3 PUFA (18-, 20-, or 22-carbon fatty acid) exerts differences on plasma lipids, inflammatory mediator generation, and airway hyper-responsiveness has not been adequately addressed under carefully controlled dietary conditions. Elongation and desaturation of α -linolenic acid to EPA and other *n*-3 PUFA occurs at low levels; this shorter fatty acid does get incorporated into tissues at

the expense of *n*-6 PUFA, and could modestly contribute to changing the inflammatory mediator environment (James *et al*, 2000). It has been suggested that the extent of the conversion of α -linolenic acid to the longer-chain *n*-3 PUFA is modest (Kris-Etherton *et al*, 2002). For example, Emken *et al* (1994) reported a 15% conversion, whereas Pawlosky *et al* (2001) found 0.2% conversion; both reported that the conversion to DHA was much less than that to EPA. However, these studies were performed against a background diet high in *n*-6 PUFA. The desaturation and elongation enzymes that convert α -linolenic acid to EPA also convert linoleic acid (18:2*n*-6) to AA. Hence, these previous studies on EPA concentrations may have been performed under suboptimal conditions for conversion of α -linolenic acid to EPA. Mantzioris *et al* (1994) demonstrated that dietary flaxseed oil, used in domestic food preparations, increased tissue EPA levels comparable with fish oil supplementation against a background diet low in *n*-6 PUFA in healthy male volunteers. In a follow-up study using healthy volunteers with a diet low in *n*-6 PUFA consumption, Caughey *et al* (1996) confirmed that both flaxseed oil and fish oil supplementation resulted in a comparable increase in mononuclear cell EPA content with a concomitant inhibition of TNF- α and IL-1 β synthesis. Flaxseed, but not flaxseed oil, is also rich in lignans, which may have biological properties independent of *n*-3 PUFA. Lignans possess anti-platelet-activating factor activity and are antioxidant in nature. However, their role in inflammatory conditions, such as asthma, is presently undetermined.

Omega-3 fatty acids and EIB

Arm *et al* (1988) evaluated the effect of fish oil (*n*-3 PUFA) supplementation on the airway response to exercise in patients with asthma (Arm *et al*, 1988). After 10 weeks of daily supplementation with 3.2g EPA and 2.2g 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% inhibition of total LT synthesis (LTB₄ and LTB₅), there was no detectable change in the clinical outcome (eg histamine response, exercise response, specific conductance of the airway, or symptom scores).

Recently, Mickleborough *et al* (2003) demonstrated that 3 weeks of fish oil supplementation reduces the severity of EIB and resulted in a significant suppression of several proinflammatory mediators in nonatopic elite athletes who exhibited 'asthma-like symptoms' following exercise (Mickleborough *et al*, 2003). The fish oil supplement had no effect on baseline pulmonary function in EIB (*n* = 10) and control subjects (*n* = 10) or following exercise in control subjects. However, in the group of athletes who had a history of airway narrowing following exercise, the fish oil supplement reduced the fall in FEV₁ at 15 min postexercise by almost 80% (Figure 3) in conjunction with a greater than 20%

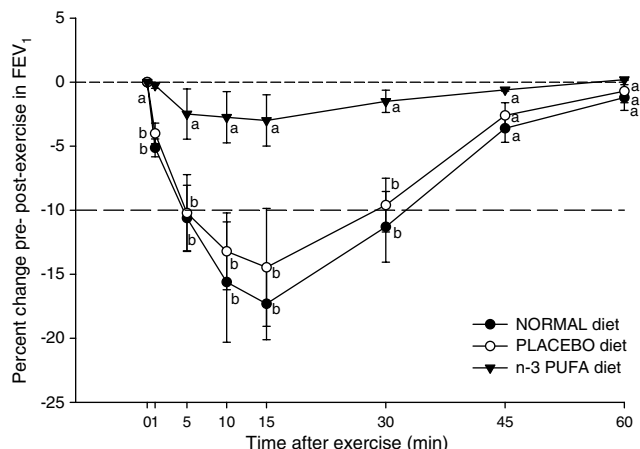


Figure 3 Percent change in FEV₁ pre- to postexercise in elite athletes with exercise-induced bronchoconstriction. A reduction in FEV₁ in excess of 10% represents abnormal pulmonary function and is diagnostic of EIB. Letters (a and b) refer to comparisons by diet within respective time period. Different letters designate significant difference ($P < 0.017$). Adapted from Mickleborough *et al* (2003).

reduction in bronchodilator 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 LTE₄, PGD₂ metabolite 9 α , 11 β -PGF₂, and LTB₄, and pro-inflammatory cytokines IL-1 β and TNF- α .

The divergent findings between the Mickleborough *et al* (2003) study and that of Arm *et al* (1988) are difficult to reconcile, especially since the Arm *et al* study had a longer duration supplementation period with an identical fish oil dosage. The negative findings observed by Arm *et al* (1988) may be due to methodological and statistical limitations of their study. These authors exercised a cohort of mild asthmatics at very low exercise intensity (80% predicted maximal oxygen consumption for 8 min at ambient temperature and humidity). It is generally accepted that inhaling cold-dry air at high ventilation rates initiates EIB. Rundell *et al* (2000) have shown that out of 23 subjects who tested positive for EIB in cold-dry air, 18 (78%) subjects tested negative in ambient conditions (21°C and 50% relative humidity). This suggests that the exercise protocol performed in ambient conditions in the Arm *et al* (1988) study may have been less sensitive to identifying changes in airway hyper-responsiveness following exercise due to inadequate environmental stress. In addition, an assessment of the numbers used in the airway response to exercise of Arm *et al*'s (1988) study (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. The grade of fish oil also differed between the two studies: Mickleborough *et al* (2003) used pharmaceutical-grade fish oil, while the majority of earlier studies, including the study by Arm *et al* (1988), used a

lower-grade fish oil supplement. Pharmaceutical-grade fish oil has only recently become available and enables the experimental evaluation of the specific mechanism of n-3 PUFA action without the confounding variables of impurity. In addition, pharmaceutical-grade fish oil has a higher percentage of total long-chain n-3 PUFA than lower-grade fish oil.

In a follow-up study, Mickleborough and colleagues (unpublished observations) recently examined the effect of fish oil supplementation in asthmatic patients who experienced EIB. This pilot study was conducted as a randomized, double-blind crossover trial over 8 weeks in a manner similar to our previous work (Mickleborough *et al*, 2003) and used a similar pharmaceutical-grade fish oil (3.2 g EPA and 2.0 g DHA) and placebo supplementation dosage and duration (3 weeks). In all, 16 mild atopic asthmatic subjects with documented EIB volunteered for the study. There were no significant differences in baseline pulmonary function following exercise across diet. However, the n-3 PUFA diet reduced the postexercise fall in FEV₁ by approximately 64%. In addition, there was a significant improvement in asthma symptoms scores and a reduction in bronchodilator use (total number of doses/puffs) on the n-3 PUFA diet. As per our previous study (Mickleborough *et al*, 2003), proinflammatory mediator concentration was significantly suppressed on the n-3 PUFA diet. Using the relatively noninvasive technique of sputum induction, we demonstrated that a diet supplemented with fish oil reduced airway inflammation in mild atopic asthmatics with EIB. Specifically, we found that sputum differential eosinophil, neutrophil, lymphocyte, and macrophage cell counts, and sputum supernatant concentrations of proinflammatory eicosanoids LTC₄-E₄, LTB₄, PGD₂, and cytokines IL-1 β and TNF- α were significantly reduced on the fish oil diet. These results strongly suggest that dietary supplementation with n-3 PUFA could decrease exercise-induced airway narrowing in asthmatics.

Conclusions

In view of the clinical consequences, these findings point towards prophylactic and acute therapeutic effects of fish oil supplementation in inflammatory diseases such as asthma, which seem to be attainable by simple rearrangement of nutritional components. It is possible that anti-inflammatory drug use could be decreased in some patients with asthma and EIB in concert with increased fish oil ingestion if both the drug and n-3 PUFA are exerting their therapeutic effects through the same molecular actions. There may be an opportunity for beneficial additive effects with fish oil supplementation or other dietary approaches to increasing intake of n-3 PUFA. Thus, the possibility exists for drug-diet interactions that confer greater anti-inflammatory benefits than either intervention alone or at least similar anti-inflammatory effects with less toxicity.

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