Review

Continuing Education Questionnaire, page 170
Meets Learning Need Codes 4000, 5000, 5110, and 9020

Clinical Efficacy of n-3 Fatty Acid Supplementation in Patients with Asthma

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ABSTRACT
The rising prevalence of asthma is an alarming health concern. The morbidity and mortality associated with asthma not only disrupts the quality of life, but it also escalates health care costs. Asthma is a chronic inflammatory disease of the respiratory tract. An exaggerated production of the arachidonic acid-derived eicosanoids, leukotrienes, has been implicated as the chemical trigger for inflammation. n-3 fatty acid supplementation has been shown to suppress the synthesis of the n-6 series of leukotrienes by competing and inhibiting the metabolism of arachidonic acid. The results from epidemiological studies suggested that fish consumption was beneficially associated with lung function and prevalence of asthma. The data generated from clinical trials, however, indicated that n-3 fatty acid supplementation did not consistently improve severity of symptoms, lung functions, airway responsiveness, and medication use in asthmatic patients. Future research should focus on the effects of long-term supplementation using weight-based dosages on specific biochemical markers and clinical outcomes. Leading organizations have not included nutrition as part of the management guidelines for asthma. Meanwhile, regular fish consumption at least three times per week should be highly encouraged as part of a well-balanced diet and to meet the adequate intake levels established for n-3 fatty acids.
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Asthma is a chronic inflammatory disease of the respiratory tract that is characterized by increased airway hyperresponsiveness and mucus production that leads to episodes of wheezing, coughing, and shortness of breath. According to data collected for the Centers for Disease Control and Prevention's National Health Interview Survey, the prevalence of asthma has steadily increased in the past 2 decades. An estimated 30 million people in the United States were diagnosed with this condition in 2001 (1). Asthma disproportionately affects children and African Americans and causes significant work and school absenteeism. In addition to its impact on the quality of life, an estimated cost of $11 billion was attributed to this condition in 1998 (2). The increasing morbidity and mortality associated with asthma have become a major health concern as reflected in its inclusion in the Healthy People 2010 objectives (3). Finding an effective treatment for asthma is, therefore, important.

Although medications and environmental manipulation remain the cornerstones of asthma management, dietary intervention has emerged as a potential therapy. Research efforts on the anti-inflammatory properties of n-3 fatty acids, essential polyunsaturated fatty acids that contain more than one double bond, have gained momentum in recent years. These fatty acids are found in high concentrations mainly in oily fish and include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which can be synthesized from dietary α-linolenic acid. The results from an epidemiologic study suggested that children eating fish more than once per week were three times less likely to develop asthma than those who did not eat fish (4). Other research results have suggested a beneficial association between a high intake of fish and lung function (5), asthma symptoms (6,7), and the prevalence of asthma (8) in children and adults. The purpose of this article is to review data from clinical trials that studied the impact of n-3 fatty acid supplementation on the underlying pathology and clinical outcomes of asthma.

REVIEW OF LITERATURE

Proposed Pathogenesis of Asthma

Individuals with asthma have airways that are chronically and irreversibly inflamed and are hypersensitive to specific allergens in the environment. This hypersensitiv-

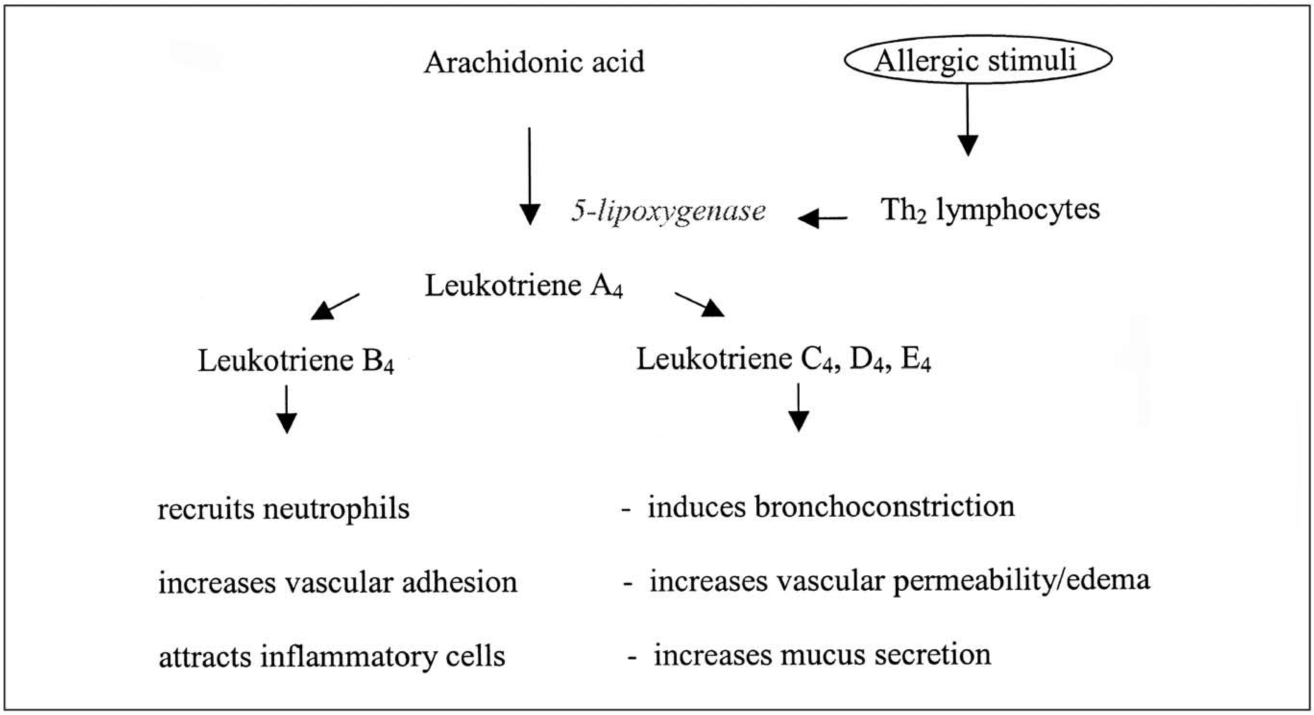

Figure 1. Leukotriene series and their physiologic impact on airways.

ity is believed to be caused by an exaggerated response of T-helper cells. When exposed to stimuli, T-helper lymphocytes orchestrate the release of leukotrienes from activated inflammatory cells through the catalytic action of 5-lipoxygenase (9) (Figure 1). Leukotrienes are one type of eicosanoids derived from arachidonic acid and are the major chemical mediators in the pathogenesis of asthma. Leukotrienes are potent chemoattractants that, on binding to specific target receptors, directly induce bronchoconstriction and mucus production of the respiratory cells. Individuals with asthma were reported to produce significantly greater quantities of leukotrienes than nonasthmatic individuals (10). Controlling the production and action of leukotrienes is therefore pivotal to asthma care, and thus is the pharmacologic goal of several antiasthmatic drugs (eg, montelukast, zafirlukast, and zileuton) (11). The efficacy of these drugs supports the importance of leukotrienes in the pathogenesis of asthma. Hence, n-3 fatty acids serving as anti-inflammatory agents may play a promising role in the intervention for asthma.

Physiologic Effects of n-3 Fatty Acids on the Metabolic Pathways of Eicosanoids

n-3 fatty acids are postulated to have a protective role in the development of inflammatory diseases such as diabetes mellitus, cardiovascular disease, rheumatoid arthritis, and asthma. It is possible that n-3 fatty acids suppress inflammation by exerting an inhibitory and competitive effect on the biosynthetic pathways of leukotrienes and other proinflammatory eicosanoids.

Effects on Arachidonic Acid Metabolism

Polyunsaturated fatty acids are major constituents of the membrane phospholipids of the inflammatory cells. During inflammation, intracellular concentration of Ca^{2+} increases and the ionized calcium binds to the calcium-dependent regulatory protein, calmodulin. When its four binding sites are fully occupied by Ca^{2+}, calmodulin undergoes a conformational change that enables it to phosphorylate an active site residue (Ser^{505}) within the catalytic site of phospholipase A_2 (12). The activated phospholipase A_2 hydrolyzes the second-position ester bond of the phospholipids and releases n-6 fatty acid (linoleic acid 18:2n6), which is then converted into proinflammatory arachidonic acid (ARA)–based eicosanoid.

Depending on the levels of dietary intake, n-3 fatty acids compete with and displace n-6 fatty acids for the acylation sites in the cellular membranes. It has been shown that the saturation of n-3 in cell membranes can significantly increase within 2 weeks' supplementation and the levels of EPA could reach peak accumulation after 6 weeks of supplementation (13). Dietary fat intake may change the membrane composition of fatty acids and modulate the types of eicosanoids produced in the pathway, thus influencing the inflammatory response of the cells.

In addition, the results from an in vivo study suggested that EPA supplementation of 3,000 mg/d suppresses the synthesis of ARA through its inhibitory effect on the 5-desaturase pathway (14). n-6 is metabolized into ARA through a process of desaturation and elongation (Figure 2).

The metabolic pathway of ARA begins with the enzyme

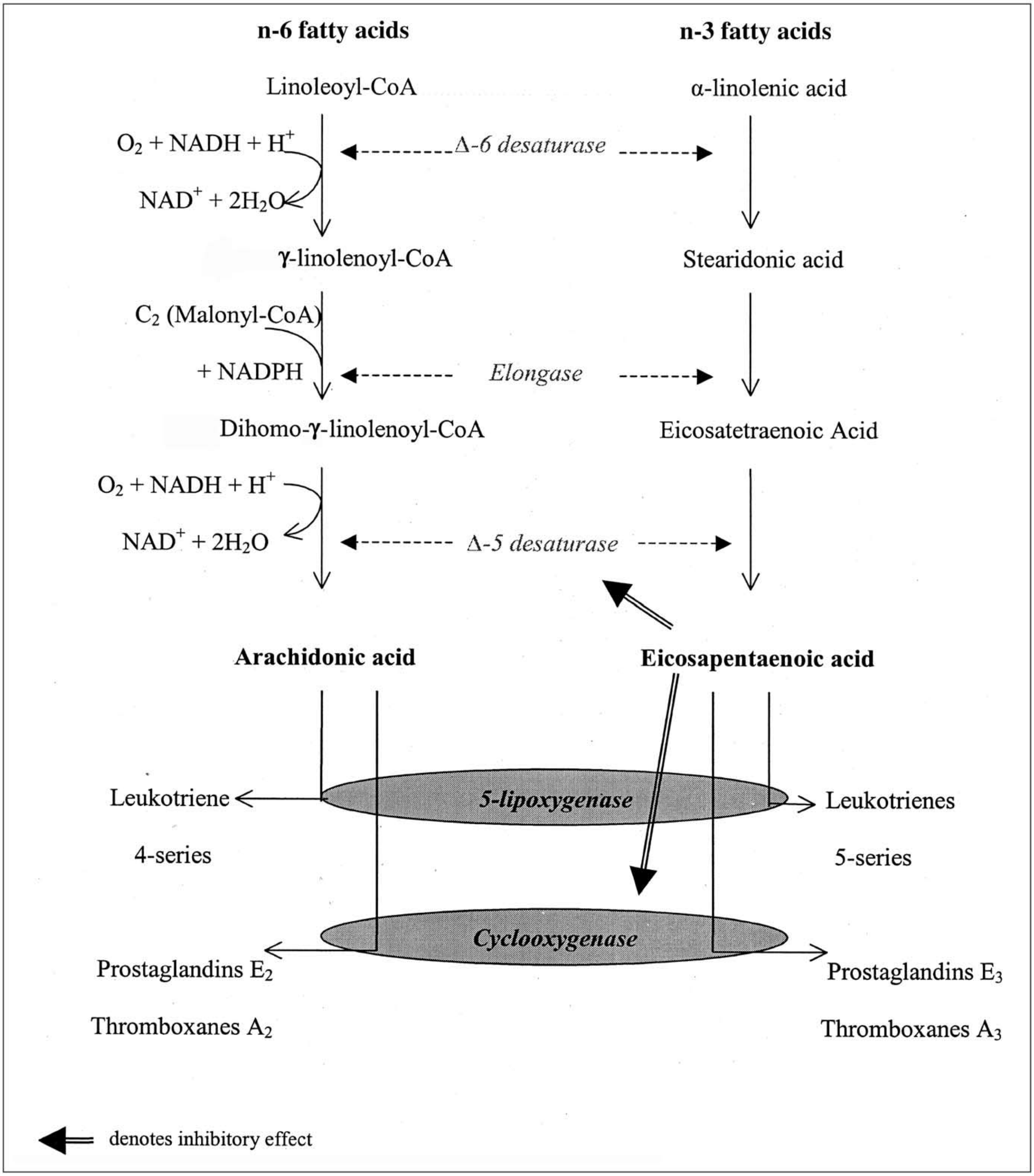

Figure 2. Effect of n-3 fatty acids on arachidonic acid and leukotriene metabolism.

system Δ-6 desaturase, which consists of three proteins, nicotinamide adenine dinucleotide (NADH)-cytochrome b_5 reductase, cytochrome b_5, and a non–heme iron containing desaturase. The NADH donates reducing equivalents for the reduction of cytochrome b_5. The Δ-6 desaturase uses oxygen and electrons from reduced cytochrome

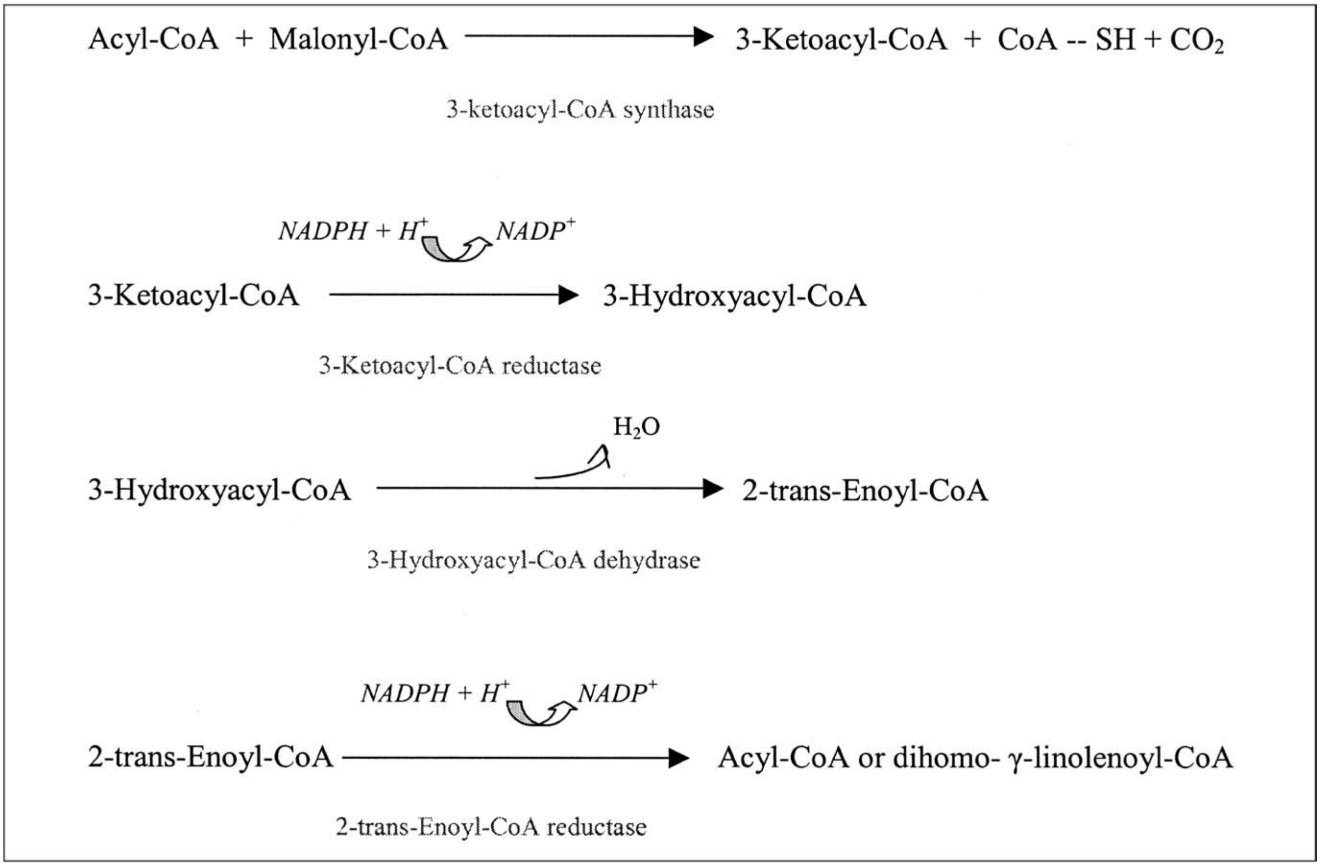

Figure 3. Microsomal elongase system for fatty acid chain elongation.

b_5 to catalyze the insertion of a *cis* double bond between carbons six and seven and yields γ-linolenoyl-coenzyme A (CoA). Then a complex microsomal chain elongation system, elongase, converts the γ-linolenoyl-CoA to dihomo-γ-linolenoyl-CoA. This elongation pathway begins with a condensation reaction between malonyl-CoA and acyl-CoA and is followed by a reduction of the 3-ketoacyl-CoA with nicotinamide adenine dinucleotide phosphate (Figure 3). The elongation of fatty acid is completed after subsequent steps of dehydration and reduction.

The Δ-5 desaturase is an enzyme that catalyzes the last step of the conversion of dihomo-γ-linolenate (Figure 2). The presence of EPA acts as a negative allosteric effector of Δ-5 desaturase, which is the rate-limiting enzyme of ARA metabolism. Hence, EPA limits the direct precursors of the leukotrienes. This physiologic mechanism was evidenced by the results from in-vivo studies showing that EPA supplementation of 3,200 mg/d for 6 to 10 weeks blunted leukotriene generation by neutrophils and monocytes (15,16). The ability of n-3 fatty acids to hinder ARA metabolism parallels the pharmacologic effect of corticosteroids. Corticosteroids are the mainstay of anti-inflammatory drugs that are used to control asthma by inhibiting the release of arachidonic acid from cell membranes. n-3 fatty acids may, therefore, serve as an adjuvant therapy in the care of asthmatic patients.

Effects on the Biosynthesis of Leukotrienes

In addition to inhibiting ARA production, EPA competes with ARA as the substrate for 5-lipoxygenase, which is a key regulatory enzyme in the pathway of leukotriene synthesis (Figure 2). Instead of yielding the chemoattracting four-series of leukotrienes, the metabolism of EPA produces the five-series of leukotrienes, which are weaker chemoattractants and are less inflammatory.

Effects on the Production of Immunoglobulin E

Individuals with asthma have elevated serum levels of immunoglobulin E (IgE), which is responsible for the initiation of an allergic response to antigens. EPA may suppress IgE production through the cyclooxygenase pathway (Figure 2). Cyclooxygenase is a regulatory enzyme that catalyzes the conversion of ARA to thromboxane A_2 and prostaglandin E_2; the latter induces T-lymphocyte response and drives the formation of IgE. Alternatively, cyclooxygenase metabolizes EPA to far less inflammatory mediators, prostaglandin E_3 and thromboxane A_3. EPA is capable of both inhibiting the activity of cyclooxygenase and competing with ARA in this synthetic pathway (9). The results from a randomized, placebo-controlled study with 16 healthy subjects indicated that a 3-month supplementation with fish oil (2,000 mg/d) could lead to a reduction of prostaglandin E_2 levels (17). Overall, n-3 fatty acids seemed to modulate the inflammatory response of asthma by serving as a competitive and inhibitory agent on the biosynthetic pathways of proinflammatory eicosanoids such as their precursor (ARA), leukotrienes (B_4, C_4, D_4, and E_4), and IgE. With the shift toward less inflammatory metabolites, it is speculated that less inflammation would be seen in the lungs, and therefore an increase in the amelioration of asthmatic symptoms would be experienced.

Review of Intervention Trials

The protective roles of n-3 fatty acids against asthma are based on the premises that (a) asthma is primarily an inflammatory disease that is potentiated by an excessive production of proinflammatory leukotrienes, (b) n-3 fatty acid supplementation can reduce leukotriene generation, and (c) there is a correlation between fish intake and a decreased risk of asthma and improved lung function. Several clinical trials have been conducted in the past few years that have investigated the efficacy of n-3 fatty acid supplementation. The results from the interventional studies published so far have yielded conflicting data.

A double-blind, placebo-controlled study was conducted with 46 adults with mild to moderate asthma (18). The subjects were randomized to receive 200 mg/d of an EPA/DHA supplement or 150 mg/d of olive oil for 2 months. Significant reductions in daytime wheezing, decreases in the concentration of exhaled hydrogen peroxide (H_2O_2), and increases in peak expiratory flow were observed in the supplemented group compared with the placebo group. No change was observed in the forced expiratory volume (FEV_1). A reduced H_2O_2 in the exhaled breath might indicate a dampened oxidative stress associated with airway inflammation, which could explain the improvement in asthmatic symptoms and lung function. The results of this study, however, could have been confounded by the presence of carotenoids in the supplement. Carotenoids, acting as antioxidants, also could have reduced the level of oxidative stress and H_2O_2 generation. Therefore, the observed effects could not be isolated to EPA/DHA alone.

In another study, 14 adults were randomized to receive 10 to 20 g/d of Perilla seed oil, which is rich in α-linolenic acid, or corn oil, for 1 month, and their lung function and leukotriene generation were monitored (19). Significant improvements in all measurements of lung function and a reduction in leukotriene synthesis was recorded in the supplemented group, whereas no improvement was observed in the control group. Although the results from this study were encouraging, the internal validity could be contaminated by researchers' bias because they were not blinded to the treatment.

Researchers conducting a small study (N=7) also reported positive findings after using a higher dose for the EPA/DHA supplement (3,000 mg/d) (20). After 1 month of supplementation, a significant decrease in airway responsiveness was observed during the inhalation challenges in comparison with the pretreatment values ($P<.05$). FEV_1, however, remained unchanged, and surprisingly, a decrease in the residual volume was reported posttreatment. The investigators were unable to explain the incongruent results. Also, the validity of this study was challenged because it was not a randomized, comparative trial.

Asthma is an irreversible condition in which inflammation can be long established even before the appearance of the first symptom. It is plausible that any impact on clinical efficacy with n-3 fatty acid supplementation will take a long time to surface in this condition. Thus, a double-blind trial was conducted for 12 months during which 12 asthmatic subjects were randomized to receive 1,000 mg/d of an EPA/DHA supplement (21). FEV_1 reportedly increased by 23% in the treatment group, an improvement that was not seen in the control group ($P<.005$). This improvement, however, did not translate to a reduction in medication use. In addition, the constituents of the placebo used in the study were not identified by the investigators.

Trials with Control for Dietary Variables

All of the studies discussed so far did not account for the dietary intake of the individual subjects. Dietary intake could be a major independent variable by which high n-6 consumption might potentially negate the efficacy of n-3 fatty acid supplementation. Currently, the typical dietary ratio of n-6 to n-3 fatty acid is estimated to be greater than 10:1 in the United States, compared with the suggested ratio of 4:1 as advised by the World Health Organization (22). An epidemiologic study involving 974 children suggested that a high intake of n-6 fatty acids was associated with an increased risk of asthma (23).

Hodge and colleagues (24) partially controlled the dietary intake of n-6 fatty acid by manipulating the subjects' eating patterns, in addition to including the use of an n-3 fatty acid supplement in a double-blind, randomized, placebo-controlled design. About half of the children were randomized to ingest 1,200 mg/d of an EPA/DHA supplement and were instructed to eat fish more than once per month in their canola oil–based diet. The subjects in the control group were assigned to take placebo capsules and were asked to avoid fish in their sunflower oil–based diet. Diet compliance was assured by checking food diaries recorded during baseline and after 3 and 6 months of the intervention. The investigators reported a significant increase in the levels of plasma n-3 fatty acids and EPA along with a decrease in the level of tumor necrosis factor-α and eosinophils in the treatment group after 3 and 6 months of supplementation. Despite the positive data on the biochemical markers, no meaningful changes were observed in the clinical indicators, such as lung functions, asthma severity score, and airway responsiveness.

Executing a tighter control on dietary variables, Broughton and colleagues administered supplements according to an individual's intake level to achieve a specific balance between n-3 and n-6 fatty acids in the diet (25). Nineteen adults with asthma served as their own controls in a crossover design in which they consumed a low-ratio diet (0.1:1) of n-3 to n-6 fatty acids for 1 month. This regimen was immediately switched to a high-ratio diet (0.5:1) for the second month. A high-ratio diet was estimated to contain 3,300 mg/d EPA. Leukotriene synthesis was significantly more reduced with the high-ratio diet than with the low-ratio diet. Furthermore, close to 50% of the subjects showed an improved breathing capacity on an antigen challenge.

To effectively control potential confounding variables such as dietary intake and environmental factors that may influence the outcomes of treatment, it may be best to conduct an in-patient study. Nagakura and colleagues

(26) randomized 29 hospitalized children to receive six to 12 capsules per day of either the EPA/DHA supplement or olive oil. All subjects consumed the same diet and lived in the same environment during the 10 months of the intervention. Under a double-blind monitoring system, the data indicated a significant increase in plasma EPA levels, an improvement in asthmatic symptoms, and a decrease in airway responsiveness on challenge in the supplemented group, although these effects were not observed in the control group. It may be important to note that the subjects in this study received a weight-based dosage (24 to 38 mg/kg/d) of EPA/DHA. This protocol was different from the protocols of previous studies that delivered a fixed daily amount of supplement regardless of subjects' body sizes. The discrepancy between results from studies using different protocols emphasizes the need to complete additional experiments that evaluate the effectiveness of EPA/DHA supplementation that is based on an individual's weight. In addition, the clinical benefits of n-3 fatty acid supplementation may unambiguously unfold when other confounding variables are well-controlled as in an in-patient, stable setting.

Prevention Trial

Because the clinical efficacy of n-3 fatty acids remains uncertain, its use as a preventive tool is challenged. An Australian group designed a large-scale longitudinal study that investigated the effectiveness of modifying the dietary ratio of n-3 to n-6 fatty acids as a primary prevention strategy for infants who were at high risk of developing asthma (27). The subjects (N=554) were randomized before birth into either the treatment group, in which 500 mg/d fish oil was administered and canola oil was used for meal preparation, or the control group, in which sunflower oil and margarine were used. The treatment group was designed to maintain a dietary n-3 to n-6 fatty acid ratio of approximately 1:5, compared with the control group of 1:15 to 1:20. Clinical outcomes and food intake were monitored every 3 months, and the results were first summarized at 18 months of life of the subjects in the 5-year-long study. At 18 months of age, the treatment resulted in a 9.8% reduction in the prevalence of wheeze, although it had no effect on doctors' diagnoses of asthma or serum IgE levels. The results from this well-executed study provided some promising data on the benefits of n-3 fatty acid supplementation in reducing asthmatic symptoms when the diet therapy was used at a very early age. Because the diagnosis of asthma cannot truly be identified until later in life, which is usually around the age of 5 years, the impact of the supplementation on prevention remains to be seen.

FUTURE DIRECTIONS

n-3 fatty acids are expected to improve the clinical severity of asthma based on their anti-inflammatory properties. They reportedly suppress the metabolism of arachidonic acid and the production of leukotrienes and IgE. Indeed, the results from the studies reviewed showed that n-3 fatty acid supplementation could effectively increase plasma concentrations of n-3 and EPA and, in some cases, lead to a decrease in the production of the four-series of leukotrienes and other proinflammatory cytokines. However, these anti-inflammatory effects did not render a consistent improvement in clinical outcomes such as severity of symptoms, measured lung functions, airway responsiveness, and medication use. The limited sample sizes and the aforementioned flaws in some of the study designs could explain the discrepancies and insignificant findings that were reported.

There are two instruments that have been validated in appraising the quality of the study designs and the significance of the results (28). The first instrument is designed with a series of questions that examines whether the research can be applied to different populations and whether the measured outcomes are meaningful and clinically relevant. The second instrument measures the quality of the study designs by questioning the appropriateness of subjects' selection, methods of randomization and blinding, and statistical analysis. Both of these instruments use a numerical rating system to score each question. Based on these evaluation tools, the majority of the selected studies were conducted in an ethical manner and were relevant to current practice. The methodologic quality, however, varied greatly from 0.51 to 0.93; a score of 1.0 would signify methods of maximum quality. Many of the studies did not account for known confounders by study design or by analysis. In addition, a sample size justification was lacking in every study.

Furthermore, only a few studies examined the biochemical markers of inflammation (19,24,25,27). Therefore, it remains equivocal whether the leukotriene-suppressing property of n-3 fatty acids would indeed lead to an improvement in the underlying etiology of asthma. Finally, the differing dosages and durations of interventions adopted in the studies made it difficult to validate the clinical impact of n-3 fatty acid supplementation.

In light of the chronicity of the disease, long-term supplementation may be required to generate an effect on clinical outcomes. Future research should investigate the proper dosage of supplementation, preferably prescribed according to the body weights of the subjects. In addition to supplementation, executing control on the dietary intake of n-3 and n-6 fatty acids would be advisable to account for this potential confounder. A heavy focus should also be placed on the primary preventive trials in children who are at high risk for asthma. This type of study may help to illustrate the preventative roles of n-3 fatty acids on downregulating the inflammatory response before the onset of asthmatic symptoms. Overall, none of the studies reported any harmful side effects with the n-3 fatty acid supplement.

CONCLUSIONS

Nutrition professionals are delivering health care in an era of evidence-based practice. Based on the data generated so far, there is a lack of evidence supporting the use of n-3 fatty acid supplementation or fish oil on improving the clinical outcomes of asthma. This conclusion concurs with the reviewers' findings in a recent publication of The Cochrane Library, which is considered the premier source of high-quality evidence-based data (29). The American Academy of Allergy, Asthma, and Immunology is also in accordance and stated that, in trials, "fish oil therapy does reduce the capacity to make leukotrienes but did not have an impressive beneficial effect in asthma" (30).

The National Asthma Education and Prevention Program, which is organized by The National Heart, Lung, and Blood Institute of The National Institutes of Health, primarily emphasized controlling environmental triggers in its published guidelines for the management of asthma (31). The guidelines did not include nutrition as part of the adjuvant therapy. Understandably, the clinician should not endorse the use of n-3 fatty acid supplementation until more evidence is available. The food sources of n-3 fatty acids, such as fish, should be highly encouraged as part of a well-balanced diet in people with asthma. It has been suggested that a decrease in fish intake is one of the contributing factors to an increased ratio of n-6 to n-3 fatty acids in the diet and is conjecturably accountable for the increasing trend in the incidence of many inflammatory diseases (32). Based on US consumption data, individuals will need to increase fish consumption by about fourfold to approximate the ideal fatty acid ratio (33).

Because n-3 fatty acid deficiency is nonexistent in healthy people, adequate dietary intake levels have been established based on the median intakes in the United States (34). The adequate dietary intake level for men and women is 1.6 and 1.1 g/d, respectively. The level for children from birth to 13 years old is set from 0.5 to 1.2 g/d. An individual would therefore be able to fulfill the recommended intake levels by consuming roughly a 3-oz serving of fish at least twice to three times per week. Fatty fishes such as halibut, mackerel, herring, and salmon are rich sources of EPA and DHA. Individuals who are allergic to fish or who are vegetarians may consider the plant-derived n-3 fatty acids. α-linolenic acid, which is the precursor for EPA and DHA, is found in flaxseed oil, walnuts, and canola oil. As the world is waiting for more clinical evidence endorsing the use of n-3 fatty acid supplementation, a healthful approach to a well-balanced diet is undoubtedly warranted for individuals with asthma.

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