Therapeutic potential of n-3 polyunsaturated fatty acids in disease

JAMES W. FETTERMAN JR. AND MARTIN M. ZDANOWICZ

Purpose. The potential therapeutic benefits of supplementation with n-3 polyunsaturated fatty acids (PUFAs) in various diseases are reviewed, and the antiinflammatory actions, activity, and potential drug interactions and adverse effects of n-3 PUFAs are discussed.

Summary. Fish oils are an excellent source of long-chain n-3 PUFAs, such as eicosapentaenoic acid and docosahexaenoic acid. After consumption, n-3 PUFAs can be incorporated into cell membranes and reduce the amount of arachidonic acid available for the synthesis of proinflammatory eicosanoids (e.g., prostaglandins, leukotrienes). Likewise, n-3 PUFAs can also reduce the production of inflammatory cytokines, such as tumor necrosis factor α, interleukin-1, and interleukin-6. Considerable research has been conducted to evaluate the potential therapeutic effects of fish oils in numerous conditions, including arthritis, coronary artery disease, inflammatory bowel disease, asthma, and sepsis, all of which have inflammation as a key component of their pathology. Additional investigations into the use of supplementation with fish oils in patients with neural injury, cancer, ocular diseases, and critical illness have recently been conducted. The most commonly reported adverse effects of fish oil supplements are a fishy aftertaste and gastrointestinal upset. When recommending an n-3 PUFA, clinicians should be aware of any possible adverse effect or drug interaction that, although not necessarily clinically significant, may occur, especially for patients who may be susceptible to increased bleeding (e.g., patients taking warfarin).

Conclusion. The n-3 PUFAs have been shown to be efficacious in treating and preventing various diseases. The wide variation in dosages and formulations used in studies makes it difficult to recommend dosages for specific treatment goals.

Index terms: Docosahexaenoic acid; Drug interactions; Eicosapentaenoic acid; Fish oils; Mechanism of action; Polyunsaturated fatty acids; Toxicity

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Polyunsaturated fatty acids (PUFAs) are important dietary components involved in a number of diverse physiological processes. In addition to their role as structural components of cellular lipids, PUFAs also serve as substrates for the synthesis of several physiological mediators, such as arachidonic acid and the eicosanoids. Key PUFAs, such as linoleic and linolenic acids, cannot be synthesized by human cells and must be obtained from the diet; these substances are termed "essential." There are two major classes of essential PUFAs: n-6 (e.g., linoleic acid) and n-3 (e.g., linolenic acid). The classification refers to the number of carbons from the methyl terminus (with the methyl carbon counted as *1*) to the double bond closest to that terminus (Figure 1). In the older biochemistry literature, n-3 and n-6 PUFAs were referred to as ω-3 and ω-6 PUFAs, respectively.

The predominant n-6 PUFA derived from the diet is linoleic acid, found in high concentrations in grains, many seeds, and meats. In human cells, linoleic acid serves as a substrate for the synthesis of arachidonic acid and other eicosanoids. The predominant dietary n-3 PUFA, linolenic acid, is found in high concentrations in plants, seeds, leafy vegetables, legumes, and nuts. Although a minor component of human tissue lipids, linolenic

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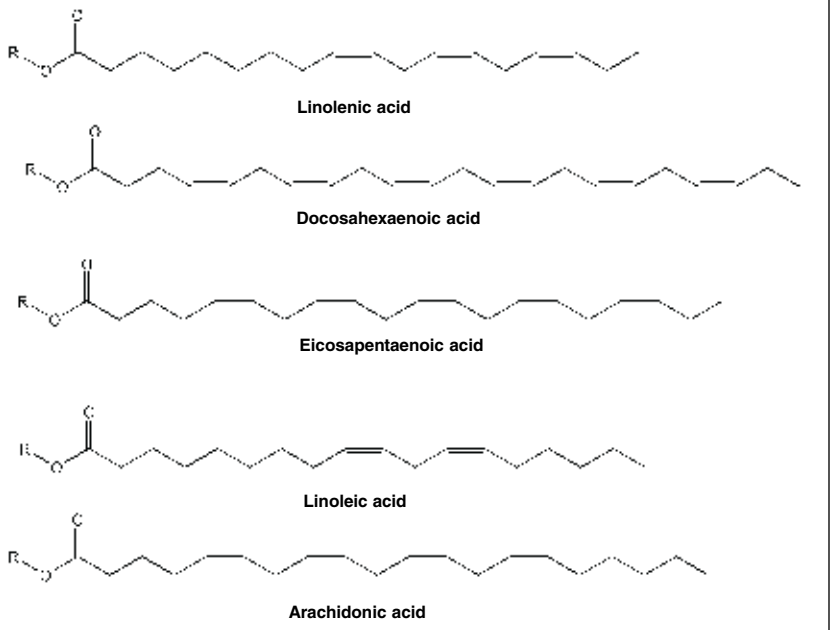
Figure 1. Structures of n-3 and n-6 polyunsaturated fatty acids (PUFAs). The n-3 or n-6 classification refers to the number of carbons from the methyl terminus (right, with the methyl carbon counted as *1*) to the double bond closest to that terminus. Linolenic acid, docosahexaenoic acid, and eicosapentaenoic acid are n-3 PUFAs, whereas linoleic acid and arachidonic acid are n-6 PUFAs.



acid serves as an important substrate for other important n-3 fatty acids, such as eicosapentaenoic acid (EPA), docosapentaenoic acid, and docosahexaenoic acid (DHA). EPA is an important component of nerve cell lipids and serves as a precursor for the synthesis of the PG_3 series of prostaglandins. All three of these fatty acids are found in significant concentrations in oils derived from cold-water fish. Both EPA and DHA have been the focus of considerable research as a result of their reported antiinflammatory and tissue-protective effects. This article provides a comprehensive, updated review of the therapeutic potential of the n-3 PUFAs in various diseases and summarizes their proposed mechanisms of benefit in these conditions. Although the n-6 PUFAs served as placebos in a number of studies reviewed, they are not the focus of this article.

Antiinflammatory actions of n-3 PUFAs

Arachidonic acid is an important component of mammalian cell membranes. During the early stages of inflammation, arachidonic acid is released from the cell membrane through the activation of phospholipase A_2 and serves as a substrate for the synthesis of bioactive eicosanoids (e.g., prostaglandins, leukotrienes, thromboxane) (Figure 2).[1]

There are two distinct arachidonic acid pathways. The first involves cyclooxygenase, which converts arachidonic acid into thromboxane A_2 (TXA_2) and various prostaglandins. There are two distinct cyclooxygenase isoenzymes: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). COX-1 is expressed constitutively in most cells; COX-2 is inducible in many cells, and its expression is increased by various stimuli. Prostaglandins, such as prostaglandin E_2 (PGE_2), are potent mediators of inflammation and also contribute to pain, fever, and increased vascular permeability. The second arachidonic acid pathway involves the enzyme 5-lipoxygenase and yields various leukotrienes. Leukotriene B_4 (LTB_4), leukotriene C_4, and leukotriene D_4 are potent proinflammatory agents that increase vascular permeability, enhance the activity of immune cells, and stimulate the release of inflammatory cytokines. TXA_2 is a key stimulator of platelet aggregation. Both human and animal studies have reported that diets high in DHA and EPA increase the proportion of these PUFAs in the membranes of inflammatory cells, white blood cells, and lymphocytes and reduce levels of arachidonic acid.[2,3]

Since EPA is also a substrate for cyclooxygenase and 5-lipoxygenase, n-3 PUFA in cell membranes may compete with arachidonic acid for conversion by cyclooxygenase. EPA is a relatively poor substrate for cyclooxygenase and has been found to inhibit the conversion of arachidonic acid by cyclooxygenase in vitro.[4] Human and animal studies have found that dietary supplementation with EPA can reduce the formation of PGE_2, TXA_2, and LTB_4 and maintain levels of prostaglandin I_2 (prostacyclin), an inhibitor of platelet aggregation.[5,6]

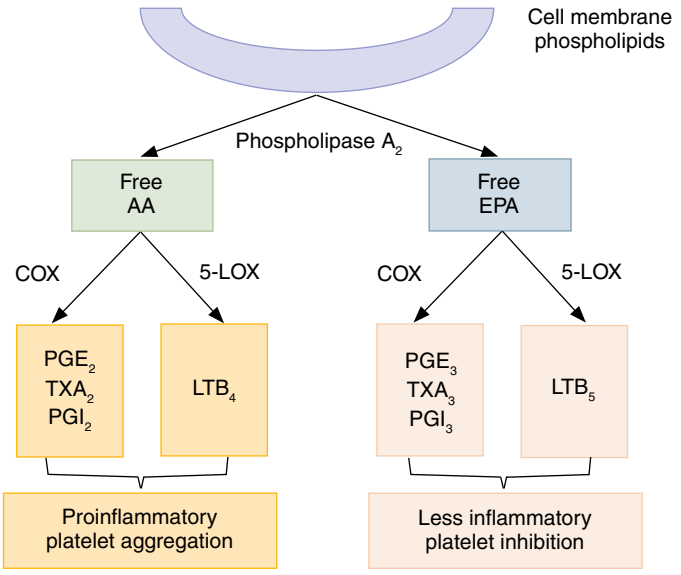
While the products of arachidonic acid conversion (PGE_2, TXA_2, and LTB_4) are proinflammatory, the products of EPA conversion (thromboxane A_3, prostaglandins I_3 and E_3, and leukotriene B_5) are significantly less potent at stimulating inflammation, vasoconstriction, and platelet aggregation and may be antagonistic toward the classic proinflammatory eicosanoids.

Supplementation with n-3 PUFA also appears able to reduce production of proinflammatory cytokines, such as interleukin-1, interleukin-6, and interleukin-8, and tumor necrosis factor-α (TNF-α) that are released

from activated immune cells.[7] While these cytokines are potent activators of immune function, excess activity of these substances contributes to the pathological inflammation seen in conditions such as inflammatory bowel disease (IBD) and rheumatoid arthritis (RA). TNF-α also plays a key role in the development of cachexia in patients with cancer. Numerous studies have shown that dietary supplementation with n-3 PUFA can reduce the production of inflammatory cytokines from activated immune cells (e.g., monocytes, macrophages).[8-10]

The production of these inflammatory cytokines is regulated by the availability of arachidonic-acid-derived eicosanoids, which can be modulated by n-3 PUFA supplementation. Suppression of inflammatory cytokine production by n-3 PUFA may also occur during gene transcription, since the expression of a number of inflammatory genes, such as cytokines and cellular adhesion molecules, is reduced in response to n-3 PUFA exposure.[2,11,12] Evidence also suggests that n-3 PUFA may directly affect intracellular signaling pathways associated with the activation of transcription factors, such as nuclear factor κ B and peroxisome proliferator-activated receptors, that regulate a number of inflammatory genes.[12,13] Inflammation is a key feature in a number of clinical conditions, such as IBD, RA, and asthma. The potential antiinflammatory actions of the n-3 PUFAs make them candidates for use in these conditions.

Figure 2. Overview of eicosanoid pathways and the conversion of eicosapentaenoic acid (EPA). In response to inflammation, both arachidonic acid (AA) and EPA are released from cell membranes and act as substrates for cyclooxygenase (COX) and 5-lipooxygenase (5-LOX) in the synthesis of eicosanoids. Free AA is a substrate for the synthesis of eicosanoids such as prostaglandin (PG) E_2, thromboxane (TX) A_2, and leukotriene (LT) B_4, which are potent inflammatory and platelet-aggregating substances. Supplementation with n-3 polyunsaturated fatty acids may increase the amount of EPA present in cell membranes. Free EPA may compete with AA as a substrate and lead to the production of eicosanoids PGE_3, TXA_3, and LTB_5, which are substantially less potent inflammatory and platelet-aggregating substances.



IBD

The etiology of IBD (Crohn's disease and ulcerative colitis) is complex and poorly understood. Several epidemiologic studies have suggested that diets high in n-6 PUFA may contribute to the development of IBD.[14,15] Conversely, several studies have found that patients with IBD have low serum levels of total PUFAs and n-3 PUFAs, which may contribute to a worsening of these patients' pathologies.[16,17] Inflammatory cytokines (e.g., interleukins, tumor necrosis factor) play major roles in the pathological inflammation associated with IBD and are the targets of drug therapy. While current pharmacologic interventions are effective for reducing relapses in patients with IBD, these interventions may cause significant adverse effects with long-term use. The documented effects of n-3 PUFAs on reducing inflammatory cytokines make n-3 PUFAs potentially important agents to consider when treating IBD.

Studies of IBD in animals have shown that n-3 PUFAs can substantially reduce the production of PGE, TNF-α, LTB_4, and TXA_2.[18,19] Rats with induced ulcerative colitis that were fed n-3 PUFA from fish oils showed reduced levels of inflammatory mediators and improvement in several histological and biochemical markers of cellular injury.[20] Supplementation with olive oil did not provide the same benefits in these rats.[15,20,21] While the results of some studies of various n-3 PUFA preparations have shown reduced rates of relapse, improved symptoms, and reduced oxidative stress in the intestines of human patients with IBD, definitive studies on the efficacy of n-3 PUFA use for IBD are still lacking.[22]

Patients with IBD have low total serum PUFA levels. Patients with Crohn's disease have a specific deficiency of n-3 PUFA.[23] Many studies have examined clinical, histological, or endoscopic scores as they relate to the effects of n-3 PUFA in patients

with IBD. While these studies did not have sufficient data to render a conclusive opinion on the benefits of n-3 PUFA use in patients with IBD, the studies did demonstrate that patients receiving n-3 PUFA and corticosteroids were, in some cases, able to decrease their corticosteroid dosage or discontinue the drugs completely.[16]

In one double-blind, placebo-controlled study (n = 78), Beluzzi et al.[22,23] administered 2.7 g of an enteric-coated fish oil preparation to patients with Crohn's disease and found that there was a decrease in the relapse of Crohn's disease. After one year, 59% (n = 46) of the patients in the fish oil group remained in remission compared to 26% (n = 20) in the placebo group. The results of this study were promising; however, in comparative studies, the delivery method, dose, and purity of the fish oil products used should be considered because not all fish oil products are extracted and manufactured to the requirements of an over-the-counter product.

RA

RA is a complex autoimmune disorder characterized by progressive destruction of joint tissues. Marked tissue inflammation and joint inflammation are hallmarks of RA. Considerable evidence suggests that cytokines released from activated T cells play a major role in the inflammatory processes associated with RA. Treatment of RA currently centers on antiinflammatory drugs and immune-suppressing agents—classes of drugs that can have significant adverse effects with long-term use. Cytokine antibodies are used but can have serious adverse effects. Investigations into the potential role of n-3 PUFA in RA has been ongoing since the 1980s. Results of human and animal studies have shown that n-3 PUFA supplementation from fish oils can substantially reduce (42% in the study by Endres et al.[7]) serum levels of interleukin-1, interleukin-2, interleukin-6, and interleukin-8, as well as TNF-α and LTB_4.[6,24,25] Reduced levels of these inflammatory mediators were associated with improved symptoms, such as reduced joint tenderness and stiffness.[24,26] Several studies found that patients with RA who took fish oil supplements reduced their use of nonsteroidal antiinflammatory drugs (NSAIDs) and other antiinflammatory medications.[26-28]

James and Cleland[29] reviewed 12 double-blind, placebo-controlled studies that incorporated n-3 fatty acids from fish oils into the daily regimen of patients with RA. The studies ranged from 12 to 52 weeks and included n-3 fatty acid dosages of 1–7.1 g daily. Most studies reviewed measured similar clinical outcomes to determine the significance of the response to the supplements given. The outcomes were patient or physician global assessment of disease activity, the number of tender joints, the number of swollen joints, the duration of morning stiffness, and grip strength. In 11 studies related to the effects of fish oil in patients with RA, patients showed improvement in at least two clinical outcomes, while patients showed improvement in all of the measured clinical outcomes in 4 studies. The most commonly reported improved outcome was a decrease in joint tenderness. Some studies continued the use of NSAIDs after a certain time interval. The results of the studies that continued NSAID use showed that the NSAID dosage could be reduced in patients taking fish oils, resulting in fewer adverse drug effects. One confounding factor was that some studies that examined the effects of n-3 fatty acids on RA used formulations and placebos with a high content of n-6 fatty acids, which are the precursors to proinflammatory cytokines. James and Cleland suggested that if the products that were used did not contain n-6 fatty acids, there may have been greater antiinflammatory effects of the n-3 fatty acid supplements, as well as a greater NSAID-sparing effect.

Bronchial asthma

Two key features of bronchial asthma are airway hyperreactivity and inflammation. Eicosanoids, interleukin-4, interleukin-5, and tumor necrosis factor are important mediators of the bronchoconstriction and inflammation that occur in patients with asthma. Studies of animals with induced lung inflammation or acute injury revealed benefits with n-3 PUFA administration.[30-32] The administration of n-3 PUFA reduced the production of thromboxane B_2 that occurred with acute lung injury in pigs, while decreasing the amount of edema observed in rabbit lungs during acute inflammation.

Results of recent studies in athletes with exercise-induced asthma also showed substantial reductions in the release of inflammatory mediators and improved pulmonary function before and after intense exercise.[33-35] However, other clinical studies examining the potential benefits of fish oil supplementation in patients with asthma have yielded conflicting results.[36,37] Although fish oil supplementation reduces the production of inflammatory mediators in patients with asthma, these reduced levels do not necessarily translate into direct clinical benefit. Perhaps the strongest data supporting fish oil use in patients with asthma have been collected from studies examining individuals with exercise-induced bronchoconstriction.

Clinical data supporting the use of fish oil supplementation in patients with asthma are limited. Part of the variability observed in these studies may be due to individual differences in asthma etiology, the types and purity of n-3 PUFA used, and the dramatic differences in doses used throughout the studies. The beneficial effect of fish oil supplementation in patients with exercise-induced

asthma also raises the possibility that the underlying etiology of this condition might differ from that of typical bronchial asthma.

Clinical studies examining the antiinflammatory effects of fish oil use have generally found considerable variability in its efficacy. In addition to the varying sources and dosages of n-3 PUFA used, variability may partly be explained by polymorphisms in the genes for cytokine production. In 2002, Grimble et al.[38] found that the ability of fish oils to suppress TNF-α in human volunteers was influenced by the presence of certain polymorphisms in genes for this particular cytokine.

Cardiovascular disease

The first evidence of the potential cardioprotective effects of n-3 PUFA came from studies that reported very low levels of heart disease in Greenland Eskimos despite their consumption of foods high in fats from seals and fish.[39] Subsequent studies in similar populations revealed very low levels of other inflammatory diseases as well.[14] Atherosclerosis is a long-term process that is multifactorial in origin. Two key components of atherosclerosis are hyperlipidemia and inflammation. The lipid-lowering effects of n-3 PUFA have been well documented in human and animal studies.[40-44] Fish oils have been shown to lower plasma cholesterol and triglyceride levels through the inhibition of very-low-density lipoprotein and triglyceride synthesis by the liver without reducing production of beneficial high-density lipoprotein (HDL) molecules.[40,44-46] Data from in vitro studies support these findings and suggest that fish oils can shift cellular synthesis of fats toward membrane phospholipids.[47] In contrast, vegetable oils, which are rich in n-6 fatty acids, can cause substantial increases in plasma triglycerides while lowering plasma HDL levels.[40,44,46,47]

The beneficial effect of n-3 PUFA on cardiovascular disease has been documented in human and animal studies.[48-57] Studies have suggested that DHA may be a more potent cardioprotectant than EPA.[48-50] Fish oils reduce the occurrence of atherosclerotic lesions, decrease the frequency of cardiac arrest, and reduce overall mortality in patients at risk for cardiovascular disease.[50-53] Small but important decreases in blood pressure have also been documented with n-3 PUFA.[49,54] While the exact mechanism of the protective effect of n-3 PUFA is unknown, it likely involves an improved lipid profile, reduced vascular inflammation, and decreased platelet aggregation. The n-3 PUFAs also appear to exert a number of potentially beneficial effects on vascular smooth muscle, including inhibition of calcium currents, decreased proliferation of smooth muscle cells (through inhibition of growth factors), and increased production of nitric oxide.[50]

In addition to its effects on hyperlipidemia and atherosclerosis, n-3 PUFA may exert antiarrhythmic effects.[50,51,55] The antiarrhythmic actions of these substances may be related to their ability to inhibit L-type calcium channels in cardiac cells, which in turn prolongs the refractory period and makes the myocardium less susceptible to potentially dangerous arrhythmias. Shimojo et al.[56] used cultured cardiac myocytes and found that EPA may have protective effects against cardiac hypertrophy by inhibiting the molecular effects of endothelin-1, a vascular hormone believed to be involved in the process of myocyte hypertrophy. Fish oils have been shown to accumulate rapidly in human cardiac cells and might be of particular value when used in patients after myocardial infarction.[57]

Clinical studies have shown a decreased rate of cardiovascular disease in patients who consumed fish at least once weekly. It was postulated that the mechanisms of this beneficial effect were secondary to a decrease in serum triglycerides, decreased blood pressure, reduced platelet aggregation, and a lower frequency of arrhythmia.[42,50,53]

Wang et al.[53] conducted an extensive review of more than 800 articles and several thousand patients from several countries. The investigators examined the beneficial effects of n-3 PUFA in both primary and secondary prevention studies and concluded that patients who ingested n-3 PUFA from fish or supplements derived from fish had reduced all-cause mortality, decreased cardiac death, decreased sudden death, and possibly reduced frequency of stroke. Wang et al. also concluded that these same protective effects may or may not be seen with other forms of n-3 fatty acids, such as those derived from α-linolenic acid (ALA). ALA, an n-3 PUFA derived from plants, is converted in the body to EPA and DHA; however, less than 5% of ALA is converted to EPA and DHA in humans.[58]

A study by Mozaffarian et al.[52] examined whether the type of fish consumed altered the potentially beneficial cardiac effects of fish oils. The investigators found a significant decrease in the total risk of ischemic heart disease in patients age 65 years or older who ingested fish high in oils (e.g., tuna). The method of fish preparation also seemed to be a factor. Patients who had a higher intake of broiled or baked tuna had a decreased risk of ischemic cardiac events, but those who consumed fried tuna had a greater risk of death due to an ischemic heart event.

Neuroprotective effects

Data have recently begun to emerge regarding the possible role of n-3 PUFAs as neuroprotective agents. In animal and in vitro studies, n-3 PUFA protected nerve cells against injury induced by ischemia and neurotransmitter excitotoxicity.[59-61] In animal models of diabetes, n-3 PUFA (specifically DHA) supplementation

was effective in preventing the functional and anatomical changes in nerve cells that are commonly associated with diabetic neuropathy.[62,63] Supplementation with n-3 PUFA also reduced oxidative damage and prevented learning disability (based on the Morris water maze) in rats with traumatic brain injury.[64] Recently, considerable research has focused on the potential protective effects of n-3 PUFA in animal models of Alzheimer's disease, since patients with the disease have low serum and brain cell membrane levels of DHA. Rats fed diets low in DHA have demonstrated impaired learning and cognitive function, effects that are reversed with DHA supplementation.[65,66] In a mouse model of Alz-heimer's disease, diets enriched with n-3 PUFA reduced the accumulation of β-amyloid by over 70%.[65,66]

The neuroprotective mechanisms of the n-3 PUFAs are likely multifactorial and may be related to a number of cellular and molecular effects in the central nervous system (CNS). In vitro, n-3 PUFAs have been shown to prevent neuronal accumulations of calcium, which can trigger a destructive cellular cascade of events that leads to neuronal injury and death. Evidence also suggests that n-3 PUFAs can directly alter glutaminergic transport and neurotransmission, a key amino acid neurotransmitter involved in excitotoxicity.[66] The n-3 PUFAs can also affect voltage-gated potassium channels in the CNS and protect rats against NMDA (*N*-methyl-D-aspartate)-receptor-mediated excitotoxicity.[60] The ability of n-3 PUFA to reduce oxidative stress may be an important component of its overall neuroprotective actions. Numerous animal and in vitro studies have found that n-3 PUFA can affect the expression of many genes in the CNS, a number of which represent transcription factors.[67] The protective effects of n-3 PUFA in patients with diabetic neuropathy may be attributable to several effects, including maintenance of nerve blood flow, preservation of membrane sodium–potassium adenosinetriphosphatase activity, alteration of nerve cell lipid composition, and altered metabolism of nerve cell lipids.[62,63]

Cancer

One area of great interest for the potential use of n-3 PUFA is that of cancer and cancer-related cachexia. Studies in mice and cultured cells have found that diets containing EPA or DHA retarded the growth and metastasis of primary tumors and implanted human breast carcinoma cells.[68,69] Dietary fish oil concentrates also increased the efficacy of chemotherapeutic agents such as doxorubicin in human breast cancer xenografts without a concomitant increase in drug toxicity.[70] Supplementation with n-3 PUFA induced apoptosis and cell differentiation while reducing cell proliferation in cancer cell cultures.[71-73] In a rat model of colon cancer, n-3 PUFA blocked drug-induced tumor formation by increasing cell differentiation and apoptosis during the early stages of tumor formation.[74] Davidson et al.[75] found that n-3 PUFA directly altered gene expression related to tumorigenesis and apoptosis and reduced DNA damage in rat colon cancer cells. These agents also modulated key intracellular signaling molecules and nuclear receptors (peroxisome proliferator-activated receptor, retinoid X receptor) that appear to play a role in regulating growth and differentiation of cancer cells.[76-78] Inhibition of prostaglandin production by n-3 PUFA may also help modulate tumor cell growth and apoptosis.[77,78] A study by Siezen et al.[79] found that certain polymorphisms in genes involved with the arachidonic acid pathways might increase an individual's risk for colorectal adenoma. Consumption of fish by these at-risk individuals modified some of these risk associations.

Cachexia, a complex syndrome of anorexia, tissue wasting, and weight loss, is seen in many patients with cancer or acquired immune deficiency syndrome. The presence of cachexia is a poor prognostic indicator that complicates drug therapy and increases drug toxicities. A number of pharmacologic and nonpharmacologic therapies have been studied for use in the treatment of cachexia, including n-3 PUFA. The effectiveness of fish oil supplementation on cachexia in patients with cancer remains unresolved. In a number of studies, fish oil supplementation improved appetite, moderated weight loss, increased lean body mass, and improved quality of life.[80-82] The reduction of proinflammatory cytokines by n-3 PUFA would clearly benefit patients with cancer-related cachexia since these substances are associated with the anorexia, weight loss, and hypermetabolism that accompany this condition.[83] However, other human studies with fish oils and cancer-related cachexia have not found a significant benefit with supplementation of these agents.[84]

Critical illness

One of the body's main responses to injury and infection is activation of the immune and inflammatory pathways. The release of inflammatory cytokines in critically ill patients activates important immune cells, such as T and B cells, but also stimulates fever and localized tissue inflammation, which can lead to cellular injury. Eicosanoids derived from arachidonic acid likewise contribute to the systemic inflammatory reactions observed in patients with sepsis and critical injury. Parenteral use of fish oils in critically ill patients makes sense, given the potential ability of n-3 PUFA to modulate production of both inflammatory eicosanoids and cytokines. Interestingly, many of the parenteral nutrition formulations given to critically ill patients are rich in n-6 PUFA, which may

heighten inflammation and worsen immunosuppression.

A number of studies have been published in recent years on the beneficial effects of fish oil supplementation in patients with critical illness. In patients recovering from major abdominal surgery, fish oil supplementation decreased overall mortality and reduced the number of patients requiring mechanical ventilation.[85] In this study, the greatest benefit occurred when patients were pretreated with fish oils before surgery. Presumably, this pretreatment allowed the n-3 PUFAs sufficient time to incorporate into various tissues and cell membranes. Unfortunately, pretreatment is only possible with elective types of surgery. In a study of 663 critically ill patients from 82 German hospitals, supplementation with fish oils reduced overall mortality, decreased antibiotic use, and decreased the length of overall hospitalization time.[86] In several other studies of patients with critical illness or sepsis, fish oil infusion suppressed the production of proinflammatory cytokines while reducing the frequency of septic events and infectious complications.[87,88]

In a study of patients with severe acute pancreatitis, Wang et al.[89] found a decrease in proinflammatory factors and an increase in plasma EPA levels after infusion with n-3 PUFA. In that double-blind, randomized controlled study of 40 patients started on parenteral nutrition, the study group (n = 20) received a soybean oil–fish oil mixture for the fatty acid component of the parenteral nutrition, and the control group (n = 20) received a standard soybean fatty acid emulsion. The results showed not only a reduction in inflammatory mediators among fish oil recipients but also a decrease in the harmful effects normally seen in the kidneys and lungs of such patients. Decreased morbidity, mortality, and intensive care unit and hospital stays were also observed. Excellent data continue to emerge regarding the clinical benefits of fish oil supplementation in patients after surgery or with sepsis.[3,88]

Diseases of the eyes

A number of studies have been published in recent years examining the potential protective effects of n-3 PUFA supplementation in ocular disease. Several studies focused on risk factors for the development of age-related maculopathy (ARM), such as a history of smoking and cardiovascular diseases.[90,91] These studies found that patients with a high dietary intake of saturated fat and cholesterol had a greater risk of developing ARM. Because the retina and macula have high levels of n-3 PUFAs, especially DHA, they may play a role in maintaining normal functioning of the eye. Smith et al.[90] examined the association between diets high in fish or saturated fat and the development of ARM. The study (3654 patients age 49 years or older) revealed that patients with a higher intake of fish (at least once per week) had a decreased risk of developing late ARM. These authors also reported that lower levels of fish intake might be more protective against ARM than higher levels of fish intake in older patients. In elderly patients, higher fish intake has been reported to compromise the activity of vitamin E, which is needed by the retina.

Cangemi[92] examined the effects of dietary supplements on visual acuity in 73 patients with intermediate dry age-related macular degeneration (AMD). Patients were given a nutritional supplement rich in n-3 PUFA (EPA and DHA). The formulation also contained taurine, antioxidants, and lutein. Of the 37 patients receiving the formulation, over three quarters showed a stabilization or improvement of visual acuity at six months. This study demonstrated both statistical and clinical significance of visual acuity improvement or stability in those patients with intermediate AMD and treated with the nutritional supplement.

Sources and supplements of n-3 PUFA

Sources rich in n-3 PUFAs include walnuts, flaxseed oil, canola oil, and fatty fish or fish oils (Table 1). Although plant sources are a more palatable source of n-3 PUFAs, the shorter-chain α-linolenic acid found in plants must be converted by the body to longer-chain DHA and EPA. Fish oil, on the other hand, does not have to undergo this conversion.[93]

There has been concern about the presence of methylmercury and other environmental contaminants

Table 1.
Dietary Sources of Polyunsaturated Fatty Acids (PUFAs)[a]

PUFA	Source
n-3	
α-Linolenic acid	Walnuts, flaxseed oil, canola oil
Eicosapentaenoic acid	Fatty fish, fish oils
Docosahexaenoic acid	Fatty fish, fish oils
n-6	
Linoleic acid	Corn oil, safflower oil, soybean oil, cottonseed oil, sunflower oil
γ-Linolenic acid	Evening primrose oil, borage oil, black current seed oil
Arachidonic acid	Meat, poultry, eggs

[a]Adapted from the International Food Information Council Foundation. www.ific.org/publications/factsheets/omega3fs.cfm (accessed 2008 Oct 8).

in the fish supply, especially in larger predatory fish.[42] Fish oil is very susceptible to oxidation and can become rancid and even toxic if not processed correctly.[94] Proper processing of the fish, such as molecular distillation, leading to the purification and deodorizing of all fish used in the production of fish oil will greatly reduce the presence of toxins and non-n-3 PUFAs while decreasing the chances of oxidization and potential toxicities. Appropriate processing also decreases the "fishy" odor of supplements and increases their shelf life. The use of the smaller "feeder fish" (e.g., anchovies, sardines) and other small fish will also decrease the presence of environmental toxins, because these fish are closer to the bottom of the food chain and have not been as extensively exposed to pollutants as larger predatory fish.

Manufacturers of dietary supplements, including most fish oil products, are responsible for ensuring that their products are safe before marketed. FDA may take action against any unsafe dietary supplement after it is marketed. Generally, manufacturers are not required to register their products with FDA or obtain FDA approval before producing or selling dietary supplements.

The United States Pharmacopeia (USP) awards those manufacturers who meet the established criteria the "USP Verified Dietary Supplement Mark," indicating that the finished product passed a comprehensive verification process.[95] According to USP, in order to receive the "Verified Mark," the manufacturer must (1) meet established quality, purity, and potency standards, (2) allow its facilities, practices, records, and quality-control processes to be examined by USP, (3) perform routine tests from samples already distributed to the retail setting, and (4) ensure that labels are reviewed to confirm that the proper information is on the label for the consumer. USP is expected to release a monograph for n-3 PUFAs as a dietary supplement in 2009. The "Verified Mark" ensures the integrity, purity, dissolution, and safe manufacturing of the supplement.

There are many brands of n-3 PUFAs available on the market today. They are all currently nonprescription, with the exception of the prescription-only omega-3-acid ethyl esters (Lovaza, GlaxoSmithKline), which is indicated for the treatment of hypertriglyceridemia. The nonprescription products can be found in nutritional specialty stores, pharmacies, and grocery stores and online. Nonprescription products are held to specified nutritional standards; however, a very small percentage of these products are processed and manufactured to the stricter European Pharmacopoeia (and USP) standards.[96]

Even with the purer products, the problem of a fishy odor may remain. One method to decrease the odor is to store the product in the refrigerator or freezer; this will greatly enhance the palatability of the product and help with compliance.

Potential adverse effects of n-3 PUFAs

The most commonly reported adverse effect of n-3 fish oil supplements is a fishy aftertaste, which can be troublesome at times. Another commonly reported adverse effect is gastrointestinal upset, which appears to be dosage dependent.[97]

Even though FDA has ruled that up to 3 g of fish oils daily is "generally recognized as safe for inclusion in the diet," there are some possible adverse effects and drug interactions at higher doses that could potentially be of concern (Table 2).[42,97] The n-3 PUFAs may have the potential to increase bleeding time, especially at dosages exceeding 3 g daily; however, this effect is generally not considered to be clinically significant. High dosages of fish oil have also been associated with an increase in glucose levels, as well as an increase in the production of low-density-lipoprotein cholesterol. However, these effects are not clinically significant.[98]

Table 2.
Influence of Fish Oil on Therapy with Certain Drugs[26,42]

Drug(s)	Potential Effect of Fish Oil: Beneficial	Potential Effect of Fish Oil: Adverse
NSAIDs[a]	Reduction in dosage needed for analgesia	None known
Cyclosporine	Reduction in hypertensive and nephrotoxic effects	None known
Blockers of TNF	Inhibition of TNF and IL-1 synthesis	None known
Methotrexate	Reduction in gastrointestinal toxicity	None known
Aspirin, warfarin	None known	Enhanced bleeding
HMG-CoA reductase inhibitors	Enhancement of pharmacologic effects	None known

[a]NSAIDs = nonsteroidal antiinflammatory drugs, TNF = tumor necrosis factor, IL-1 = interleukin-1, HMG-CoA = hydroxymethylglutaryl–coenzyme A.

Discussion

Currently, there are many n-3 products available. When choosing an n-3 PUFA, several factors must be considered, two of the most important being the quality and the purity of the product. USP has a list of manufacturers on its website (www.usp.org/USPVerified/DietarySupplements/companies.html) that follow good manufacturing practices and quality testing for their raw and finished products. It is also important to look for products that use the smaller feeder fish as the source of n-3 PUFAs

and review the process by which the oils are extracted.

When recommending an n-3 PUFA, clinicians should be aware of any possible adverse effect or drug interaction that may occur, especially for the patient who may be susceptible to increased bleeding (from taking warfarin, for example). Therefore, the patient should be instructed in how and what to monitor and when to bring any potential problems or questions to the attention of the physician or pharmacist.

Several of the clinical studies we reviewed did not report statistically significant results regarding the effects of n-3 PUFA in certain diseases (e.g., asthma). A potentially confounding factor in these studies may have been the use of fish oil formulations and placebos that contained relatively high levels of n-6 fatty acids (precursors to proinflammatory cytokines). It is possible that if these studies were repeated with different formulations not containing n-6 PUFA, statistical and clinical significance may be demonstrated. However, with the evidence available to date, the use of n-3 PUFAs appears beneficial for improving or preventing certain diseases. There is also sufficient evidence to conclude that n-3 PUFAs can potentiate the effects of certain drugs, thereby allowing a reduction of their required dose (e.g., NSAIDs, corticosteroids).

Conclusion

The n-3 PUFAs have been shown to be efficacious in treating and preventing various diseases. The wide variation in dosages and formulations used in studies makes it difficult to recommend dosages for specific treatment goals.

References

1. Devlin TM. Textbook of biochemistry with clinical correlations. 6th ed. Hoboken, NJ: Wiley-Liss; 2006.
2. Calder PC. Dietary modification of inflammation with lipids. *Proc Nutr Soc.* 2002; 61:345-58.
3. Calder PC. n-3 Polyunsaturated fatty acid, inflammation, and inflammatory disease. *Am J Clin Nutr.* 2006; 83(suppl 6):1505S-19S.
4. Obata T, Nagakura T, Masaki T et al. Eicosapentaenoic acid inhibits prostaglandin D2 generation by inhibiting cyclo-oxygenase-2 in cultured human mast cells. *Clin Exper Allergy.* 1999; 29:1129-35.
5. Trebble TM, Wooten SA, Miles EA et al. Prostaglandin E2 production and T cell function after fish-oil supplementation: response to antioxidant cosupplementation. *Am J Clin Nutr.* 2003; 78:376-82.
6. Simopoulos AP. Omega-3 fatty acids in inflammation and autoimmune disease. *J Am Coll Nutr.* 2002; 21:495-505.
7. Endres S, Ghorbani R, Kelley VE et al. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N Engl J Med.* 1989; 320:265-71.
8. Wanten GJ, Calder PC. Immune modulation by parenteral lipid emulsions. *Am J Clin Nutr.* 2007; 85:1171-84.
9. Calder PC, Yaqoob P, Thies F et al. Fatty acids and lymphocyte functions. *Br J Nutr.* 2002; 87:S31-48.
10. Yang M, Cook ME. Dietary conjugated linoleic acid decreased cachexia, macrophage tumor necrosis factor-alpha production, and modifies splenocyte cytokines production. *Exp Biol Med (Maywood).* 2003; 228:51-8.
11. Khan SA, Vanden Heuvel JP. Role of nuclear receptors in the regulation of gene expression by dietary fatty acids (review). *J Nutr Biochem.* 2003; 14:554-67.
12. Xi S, Cohen D, Barve S et al. Fish oil suppressed cytokines and nuclear factor kappa B induced by murine AIDS virus infection. *Nutr Res.* 2001; 21:865-78.
13. Lapillone A, Clark SD, Heird WC. Polyunsaturated fatty acids and gene expression. Lipid metabolism and therapy. *Curr Opin Clin Nutr Metab Care.* 2004; 7:151-6.
14. Kromann N, Green A. Epidemiological studies Upernavik district, Greenland. Incidence of some chronic diseases 1950–1974. *Acta Med Scand.* 1980; 208:401-6.
15. Shoda R, Matsueda K, Yamato S et al. Epidemiologic analysis of Crohn's disease in Japan: increased dietary intake of n-6 polyunsaturated fatty acids and animal protein relates to the increased incidence of Crohn's disease in Japan. *Am J Clin Nutr.* 1996; 63:741-5.
16. MacLean CH, Mojica WA, Newberry SJ et al. Systematic review of the effects of n-3 fatty acids in inflammatory bowel disease. *Am J Clin Nutr.* 2005; 82:611-9.
17. Kuroki F, Iida M, Matsumoto T et al. Serum n3 polyunsaturated fatty acids are depleted in Crohn's disease. *Dig Dis Sci.* 1997; 42:1137-41.
18. Teitelbaum JE, Walker AW. Review: the role of omega 3 fatty acids in intestinal inflammation. *J Nutr Biochem.* 2001; 12:21-32.
19. Camuesco D, Gálvez J, Nieto A et al. Dietary olive oil supplemented with fish oil, rich in EPA and DHA (n-3) polyunsaturated fatty acids, attenuates colonic inflammation in rats with DSS-induced colitis. *J Nutr.* 2005; 135:687-94.
20. Nieto N, Torres MI, Ríos A et al. Dietary polyunsaturated fatty acids improve histological and biochemical alterations in rats with experimental ulcerative colitis. *J Nutr.* 2002; 132:11-9.
21. Gil A. Polyunsaturated fatty acids and inflammatory diseases. *Biomed Pharmacother.* 2002; 56:388-96.
22. Beluzzi A, Boschi S, Brignola C et al. Polyunsaturated fatty acids and inflammatory bowel disease. *Am J Clin Nutr.* 2000; 71(suppl 1):339S-42S.
23. Beluzzi A, Brignola C, Campieri M et al. Effect of an enteric-coated fish-oil preparation on relapses in Crohn's disease. *N Engl J Med.* 1996; 334:1557-60.
24. Kremer JM. n-3 fatty acid supplements in rheumatoid arthritis. *Am J Clin Nutr.* 2000; 71(suppl 1):349S-51S.
25. Adam O, Beringer C, Kless T et al. Anti-inflammatory effects of a low arachidonic acid diet and fish oil in patients with rheumatoid arthritis. *Rheumatol Int.* 2003; 23:27-36.
26. Cleland LG, James MJ, Proudman SM. The role of fish oils in the treatment of rheumatoid arthritis. *Drugs.* 2003; 63:845-53.
27. Geusens P, Wouters C, Nijs J et al. Long-term effect of omega-3 fatty acid supplementation in active rheumatoid arthritis. A 12 month, double-blind, controlled study. *Arthritis Rheum.* 1994; 37:824-9.
28. Rennie KL, Hughes J, Lang R et al. Nutritional management of rheumatoid arthritis: a review of the evidence. *J Human Nutr Diet.* 2003; 16:97-109.
29. James MJ, Cleland LG. Dietary n-3 fatty acids and therapy for rheumatoid arthritis. *Semin Arthritis Rheum.* 1997; 27:85-97.
30. Murray MJ, Kumar M, Gregory TJ et al. Select dietary fatty acids attenuate cardiopulmonary dysfunction during acute lung injury. *Am J Physiol.* 1995; 269(6 pt 2):H2090-9.
31. Koch T, Duncker HP, Klein A et al. Modulation of pulmonary vascular resistance and edema formation by short-term infusion of a 10% fish oil emulsion. *Infusionther Tranfusionmed.* 1993; 20:291-300.
32. Baybutt RC, Rosales C, Brady H et al. Dietary fish oil protects against lung and liver inflammation and fibrosis in monocrotaline treated rats. *Toxicology.* 2002; 175:1-13.
33. Mickleborough TD, Murray RL, Ionescu AA et al. Fish oil supplementation reduces severity of exercise-induced bronchoconstriction in elite athletes. *Am J Respir Crit Care Med.* 2003; 168:1181-9.
34. Mickleborough TD, Ionescu AA, Rundell KW. Omega-3 fatty acids and airway hyperresponsiveness in asthma. *J Altern Complement Med.* 2004; 10:1067-75.

35. Mickleborough TD, Lindley MR, Ionescu AA et al. Protective effect of fish oil supplementation on exercise-induced bronchoconstriction in asthma. *Chest*. 2006; 129:39-49.
36. Woods RK, Thien FC, Abramson MJ et al. Dietary marine fatty acids (fish oil) for asthma in adults and children. *Cochrane Database Syst Rev.* 2002; 3:CD001283.
37. Schachter H, Reisman J, Tran K et al. Agency for Healthcare Research and Quality. Evidence report/technical assessment. Number 91. Health effects of omega-3 fatty acids on asthma. www.ahrq.gov/downloads/pub/evidence/pdf/o3asthma/o3asthma.pdf (accessed 2009 Mar 24).
38. Grimble RF, Howell WM, O'Reilly G et al. The ability of fish oil to suppress tumor necrosis factor alpha production by peripheral blood mononuclear cells in healthy men is associated with polymorphisms in genes that influence tumor necrosis factor alpha production. *Am J Clin Nutr.* 2002; 76:454-9.
39. Dyerberg J, Bang HO, Stofferson E et al. Eicosapentaenoic acid and prevention of thrombus and atherosclerosis? *Lancet*. 1978; 2:117-9.
40. Philipson BE, Rothrock DW, Connor WE et al. Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oil in patients with hypertriglyceridemia. *N Engl J Med.* 1985; 312:1210-6.
41. Connor WE. Importance of n-3 fatty acids in health and disease. *Am J Clin Nutr.* 2000; 71(suppl 1):171S-5S.
42. Kris-Etherthon PM, Harris WS, Appel LJ et al. Fish consumption, fish oil, omega-3 fatty acids and cardiovascular disease. *Circulation*. 2002; 106:2747-57. [Erratum, *Circulation.* 2003; 107:512.]
43. Zatsick NM, Mayket P. Fish oil: getting to the heart of it. *J Nurse Pract.* 2007; 3:104-9.
44. Balk EM, Lichtenstein AH, Chung M et al. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. *Atherosclerosis.* 2006; 189:19-30.
45. Harris WS, Connor WE, Illingworth DR et al. Effect of fish oil on VLDL triglyceride kinetics in humans. *J Lipid Res.* 1990; 31:1549-58.
46. Harris WS. n-3 fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr.* 1997; 65(suppl 5):1645S-54S.
47. Benner KG, Sasaki A, Gowen DR et al. The differential effect of eicosapentaenoic acid and oleic acid on lipid synthesis and VLDL secretion in rabbit hepatocytes. *Lipids.* 1990; 25:534-40.
48. Mori TA, Watts GF, Burke V et al. Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men. *Circulation*. 2000; 102:1264-9.
49. Mori TA, Bao DQ, Burke V et al. Docosahexaenoic acid but not eicosapentaenoic acid lowers ambulatory blood pressure and heart rate in humans. *Hypertension.* 1999; 34:253-60.
50. Hirafuji M, Machida T, Hamaue N et al. Cardiovascular protective effects of n-3 polyunsaturated fatty acids with special emphasis on docosahexaenoic acid. *J Pharmacol Sci.* 2003; 92:308-16.
51. Leaf A, Kang JX, Xiao YF et al. Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. *Circulation*. 2003; 107:2646-52.
52. Mozaffarian D, Lemaitre RN, Kuller LH et al. Cardiac benefits of fish consumption may depend on the type of fish meal consumed. *Circulation.* 2003; 107:1372-7.
53. Wang C, Harris WS, Chung M et al. n-3 fatty acids from fish or fish oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. *Am J Clin Nutr.* 2006; 84:5-17.
54. Borghi C, Cicero AF. Recent evidence on the role of omega-3 polyunsaturated fatty acids on blood pressure control and hypertension-related complications. *Future Lipidology.* 2006; 1:569-77.
55. Leaf A, Albert CM, Josephson M et al. Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. *Circulation.* 2005; 112:2762-8.
56. Shimojo N, Jesmin S, Zaedi S et al. Eicosapentaenoic acid prevents endothelin-1-induced cardiomyocyte hypertrophy in vitro through the suppression of TGF-beta 1 and phosphorylated JNK. *Am J Physiol Heart Cir.* 2006; 291:H835-45.
57. Metcalf RG, James MJ, Gibson RA et al. Effects of fish oil supplementation on myocardial fatty acid in humans. *Am J Clin Nutr.* 2007; 85:1222-8.
58. Brenna JT. Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man. *Curr Opin Clin Nutr Metab Care.* 2002; 5:127-32.
59. Lauritzen I, Blondeau N, Heurteaux C et al. Polyunsaturated fatty acids are potent neuroprotectors. *EMBO J.* 2000; 19:1784-93.
60. Högyes E, Nyakas C, Kiliaan A et al. Neuroprotective effect of developmental docosahexaenoic acid supplement against excitotoxic brain damage in infant rats. *Neuroscience*. 2003; 119:999-1012.
61. Lonergan PE, Martin DS, Horrobin DF et al. Neuroprotective actions of eicosapentaenoic acid on lipopolysaccharide-induced dysfunction in rat hippocampus. *J Neurochem.* 2004; 91:20-9.
62. Gerbi A, Maixent JM, Ansaldi JL et al. Fish oil supplementation prevents diabetes-induced nerve conduction velocity and neuroanatomical changes in rats. *J Nutr.* 1999; 129:207-13.
63. Coste TC, Gerbi A, Vague P et al. Neuroprotective effects of docosahexaenoic acid-enriched phospholipids in experimental diabetic neuropathy. *Diabetes.* 2003; 52:2578-85.
64. Wu A, Ying Z, Gomez-Pinilla F. Dietary omega-3 fatty acids normalize BDNF levels, reduce oxidative damage, and counteract learning disability after traumatic brain injury in rats. *J Neurotrauma*. 2004; 21:1457-67.
65. Catalan J, Moriguchi T, Slotnick B et al. Cognitive defects in docosahexaenoic acid-deficient rats. *Behav Neurosci.* 2002; 116:1022-31.
66. Lim GP, Calon F, Morihara T et al. A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. *J Neurosci.* 2005; 25:3032-40.
67. Berger A, Mutch DM, German JB et al. Dietary effects of arachidonate-rich fungal oil and fish oil on murine hepatic and hippocampal gene expression. *Lipid Health Dis.* 2002; 1:2.
68. Rose DP, Connolly JM, Rayburn J et al. Influence of diets containing eicosapentaenoic acid on growth and metastasis of breast cancer cells in nude mice. *J Nat Cancer Inst.* 1995; 87:587-92.
69. Gonzalez MJ, Schemmel RA, Dugan L Jr et al. Dietary fish oil inhibits human breast carcinoma growth: a function of increased lipid peroxidation. *Lipids*. 1993; 28:827-32.
70. Hardman WE, Avula CP, Fernandes G et al. Three percent dietary fish oil concentrate increased efficacy of doxorubicin against MDA-MB 231 breast cancer xenographs. *Clin Cancer Res.* 2001; 7: 2041-9.
71. Yam D, Peled A, Schinitzky M. Suppression of tumor growth and metastasis by dietary fish oil combined with vitamins E and C and cisplatin. *Cancer Chemother Pharmacol.* 2001; 47:34-40.
72. Llor X, Pons E, Roca A et al. The effects of fish oil, olive oil, oleic acid and linoleic acid on colorectal neoplastic processes. *Clin Nutr.* 2003; 22:71-9.
73. Wu M, Harvey KA, Ruzmetov N et al. Omega-3 polyunsaturated fatty acids attenuate breast cancer growth through activation of a neutral sphingomyelinase-mediated pathway. *Int J Cancer*. 2005; 117:340-8.
74. Chang WL, Chapkin RS, Lupton JR. Fish oil blocks azoxymethane-induced rat colon tumorigenesis by increasing cell differentiation and apoptosis rather than deceasing cell proliferation. *J Nutr.* 1998; 128:491-7.
75. Davidson LA, Nguyen DV, Hokanson RM et al. Chemoprotective n-3 polyunsaturated fatty acids reprogram genetic signatures during colon cancer initiation and progression in the rat. *Cancer Res.* 2004; 64:6797-804.
76. Gupta RA, Sarraf P, Brockman JA et al. Peroxisome proliferator-activated receptor gamma and transforming growth factor-beta pathways inhibit intestinal epithelial cell growth by regulating levels of TSC-22. *J Biol Chem.* 2003; 278:7431-8.
77. Fan YY, Spencer TE, Wang N et al. Chemopreventive n-3 fatty acids activate RXRalpha in colonocytes. *Carcinogenesis*. 2003; 24:1541-8.

78. Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell.* 1995; 83:493-501.
79. Siezen CL, Van Leeuwen AI, Kram NR et al. Colorectal adenoma risk is modified by the interplay between polymorphisms in arachidonic acid pathway genes and fish consumption. *Carcinogenesis.* 2005; 26:449-57.
80. Barber MD, Ross JA, Voss AC et al. The effect of an oral nutritional supplement enriched with fish oil on weight loss in patients with pancreatic cancer. *Br J Cancer.* 1999; 81:80-6.
81. Fearon KC, Von Meyenfeldt M, Moses AG et al. An energy and protein dense, high n-3 fatty acid oral supplement promotes weight gain in cancer cachexia. *Eur J Cancer.* 2001; 37(suppl 6):S27-8.
82. Fearon KC, Von Meyenfeldt MF, Moses AG et al. Effect of a protein and energy dense n-3 fatty acid enriched oral supplement on loss of weight and lean tissue in cancer cachexia: a randomised double blind trial. *Gut.* 2003; 52:1479-86.
83. Deans C, Wigmore SJ. Systemic inflammation, cachexia and prognosis in patients with cancer. *Curr Opin Clin Nutr Metabol Care.* 2005; 8:265-9.
84. Bruera E, Strasser F, Palmer JL et al. Effect of fish oil on appetite and other symptoms in patients with advanced cancer and anorexia/cachexia: a double-blind placebo-controlled study. *J Clin Oncol.* 2003; 21:129-34.
85. Tsekos E, Reuter C, Stehle P et al. Perioperative administration of parenteral fish oil supplements in a routine clinical setting improves patient outcomes after major abdominal surgery. *Clin Nutr.* 2004; 23:325-30. [Erratum, *Clin Nutr.* 2004; 23:755-6.]
86. Heller AR, Rössler S, Litz RJ et al. Omega-3 fatty acids improve the diagnosis-related clinical outcome. *Crit Care Med.* 2006; 34:972-9.
87. Mayer K, Gokorsch S, Fegbeutel C et al. Parenteral nutrition with fish oil modulates cytokine response in patients with sepsis. *Am J Respir Care.* 2003; 167: 1321-8.
88. Mayer K, Schaefer MB, Seeger W. Fish oil in the critically ill: from experimental to clinical data. *Curr Opin Clin Nutr Metabol Care.* 2006; 9:140-8.
89. Wang X, Li W, Li N et al. Omega-3 fatty acids—supplemented parenteral nutrition decreases hyperinflammatory response and attenuates systemic disease sequelae in severe acute pancreatitis: a randomized and controlled study. *JPEN J Parenter Enteral Nutr.* 2008; 32:236-41.
90. Smith W, Mitchell P, Leeder SR. Dietary fat and fish intake and age-related maculopathy. *Arch Ophthalmol.* 2000; 118:401-4.
91. Seddon JM, George S, Rosner B. Cigarette smoking, fish consumption, omega-3 fatty acid intake, and associations with age-related macular degeneration: the U.S. Twin Study of Age-Related Macular Degeneration. *Arch Ophthalmol.* 2006; 124:995-1001.
92. Cangemi FE. TOZAL study: an open case control study of an oral antioxidant and omega-3 supplement for dry AMD. *BMC Ophthalmol.* 2007; 7:3.
93. Williams CM, Burdge G. Long chain n-3 PUFA: plant v. marine sources. *Proc Nutr Soc.* 2006; 65:42-50.
94. Bays H. Safety considerations with omega-3 fatty acid therapy. *J Cardiol.* 2007; 99:S35-43.
95. U.S. Pharmacopeia. USP verified dietary supplements. www.usp.org/USPVerified/dietarySupplements/ (accessed 2009 Apr 16).
96. Omega-3 triglycerides. *Pharmacopeial Forum.* 2008; 34:662-3.
97. Covington MB. Omega-3 fatty acids. *Am Fam Physician.* 2004; 70:133-40.
98. Substances affirmed as generally recognized as safe: menhaden oil. *Fed Reg.* 1997; 62:30750-7.