Review

Omega-3 Fatty Acids in Inflammation and Autoimmune Diseases

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Among the fatty acids, it is the omega-3 polyunsaturated fatty acids (PUFA) which possess the most potent immunomodulatory activities, and among the omega-3 PUFA, those from fish oil—eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—are more biologically potent than α-linolenic acid (ALA). Some of the effects of omega-3 PUFA are brought about by modulation of the amount and types of eicosanoids made, and other effects are elicited by eicosanoid-independent mechanisms, including actions upon intracellular signaling pathways, transcription factor activity and gene expression. Animal experiments and clinical intervention studies indicate that omega-3 fatty acids have anti-inflammatory properties and, therefore, might be useful in the management of inflammatory and autoimmune diseases. Coronary heart disease, major depression, aging and cancer are characterized by an increased level of interleukin 1 (IL-1), a proinflammatory cytokine. Similarly, arthritis, Crohn's disease, ulcerative colitis and lupus erythematosis are autoimmune diseases characterized by a high level of IL-1 and the proinflammatory leukotriene LTB_4 produced by omega-6 fatty acids. There have been a number of clinical trials assessing the benefits of dietary supplementation with fish oils in several inflammatory and autoimmune diseases in humans, including rheumatoid arthritis, Crohn's disease, ulcerative colitis, psoriasis, lupus erythematosus, multiple sclerosis and migraine headaches. Many of the placebo-controlled trials of fish oil in chronic inflammatory diseases reveal significant benefit, including decreased disease activity and a lowered use of anti-inflammatory drugs.

Key teaching points:

- In Western diets, omega-6 fatty acids are the predominant polyunsaturated fats. The omega-6 and omega-3 fatty acids are metabolically distinct and have opposing physiologic functions.
- Eicosapentaenoic acid (EPA) is released to compete with arachidonic acid (AA) for enzymatic metabolism inducing the production of less inflammatory and chemotactic derivatives.
- Animal and human studies support the hypothesis that omega-3 PUFA suppress cell mediated immune responses.
- In experimental animals and humans, serum PUFA levels predict the response of proinflammatory cytokines to psychologic stress. Imbalance in the omega-6/omega-3 PUFA ratio in major depression may be related to the increased production of proinflammatory cytokines and eicosanoids in that illness.
- The increased omega-6/omega-3 ratio in Western diets most likely contributes to an increased incidence of cardiovascular disease and inflammatory disorders.
- Patients with autoimmune diseases, such as rheumatoid arthritis, inflammatory bowel disease and asthma, usually respond to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) supplementation by decreasing the elevated levels of cytokines.

Introduction

The first evidence of the important role of dietary intake of omega-3 polyunsaturated fatty acids (PUFAs) in inflammation was derived from epidemiological observations of the low incidence of autoimmune and inflammatory disorders, such as psoriasis, asthma and type-1 diabetes, as well as the complete absence of multiple sclerosis, in a population of Greenland

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Eskimos compared with gender- and age-matched groups living in Denmark [1]. Most of these diseases are characterized by inappropriate activation of T cells resulting on and ultimately destruction of host tissues.

In the 1980's several independent lines of evidence suggested that changes in the natural history of hypertensive, atherosclerotic and chronic inflammatory disorders may be achieved by altering availability of eicosanoid precursors. Native Greenland Eskimos [2] and Japanese [3] have a high dietary intake of long chain omega-3 PUFA from seafood and a low incidence of myocardial infarction and chronic inflammatory or autoimmune disorders, even when compared to their Westernized ethnic counterparts. Diets containing omega-3 PUFA have also been found to reduce the severity of experimental cerebral [4] and myocardial [5] infarction, to retard autoimmune nephritis and prolong survival of NZB × NZW F_1 mice [6,7] and reduce the incidence of breast tumors in rats [8].

The 1980s were a period of expansion in our knowledge about PUFAs in general and omega-3 fatty acids in particular. Today we know that omega-3 fatty acids are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, hypertension, arthritis, other inflammatory and autoimmune disorders and cancer [9]. Research has been carried out in animal models, tissue cultures and human beings. The original observational studies have given way to controlled clinical trials.

In this paper, I review the anti-inflammatory aspects of omega-3 fatty acids relative to prostaglandins and cytokines and their clinical effects in inflammatory and autoimmune diseases, such as cardiovascular disease, major depression, arthritis, inflammatory bowel disease, asthma and psoriasis.

Omega-6 and Omega-3 Fatty Acids and Prostaglandin Metabolism

Omega-6 fatty acids account for the majority of polyunsaturated fatty acids (PUFA) in the food supply. They are the predominant PUFA in all diets, especially Western diets. When diets are supplemented with omega-3 fatty acids, the latter partially replace the omega-6 fatty acids in the membranes of practically all cells (i.e., erythrocytes, platelets, endothelial cells, monocytes, lymphocytes, granulocytes, neuronal cells, fibroblasts, retinal cells, hepatic cells and neuroblastoma cells).

Competition between the omega-6 and omega-3 fatty acids occurs in prostaglandin formation. Eicosapentaenoic acid (EPA), an omega-3 fatty acid, competes with arachidonic acid (AA), an omega-6 fatty acid, for prostaglandin and leukotriene synthesis at the cyclooxygenase and lipoxygenase level (Fig. 1). When humans ingest fish or fish oil, the EPA and docosahexaenoic acid (DHA) from fish or fish oil lead to (1) a decreased production of prostaglandin E_2 (PGE_2) metabolites, (2) a decrease in thromboxane A_2, a potent platelet aggregator and vasoconstrictor (3) a decrease in leukotriene B_4 formation,

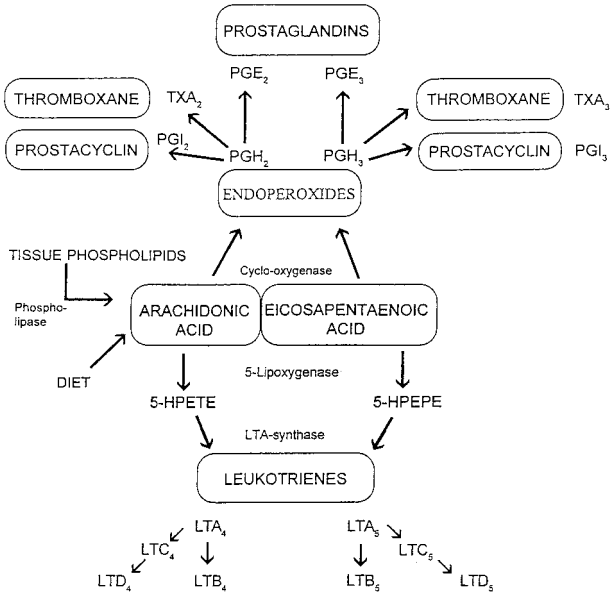

Fig. 1. Oxidative metabolism of arachidonic acid and eicosapentaenoic acid by the cyclooxygenase and 5-lipoxygenase pathways. 5-HPETE denotes 5-hydroperoxyeicosatetranoic acid and 5-HPEPE denotes 5-hydroxyeicosapentaenoic acid.

an inducer of inflammation and a powerful inducer of leukocyte chemotaxis and adherence, (4) an increase in thromboxane A_3, a weak platelet aggregator and a weak vasoconstrictor, (5) an increase in prostacyclin PGI_3, leading to an overall increase in total prostacyclin by increasing PGI_3 without a decrease in PGI_2 (both PGI_2 and PGI_3 are active vasodilators and inhibitors of platelet aggregation) and (6) an increase in leukotriene B_5, a weak inducer of inflammation and a weak chemotactic agent [10,11]. Omega-3 fatty acids modulate prostaglandin metabolism and decrease triglycerides and, in high doses, lower cholesterol and have antithrombotic and anti-inflammatory properties. These studies were extensively reviewed and reported [12–17].

Many factors contribute to the complex course of inflammatory reactions. Microbiological, immunological and toxic agents can initiate the inflammatory response by activating a variety of humoral and cellular mediators. In the early phase of inflammation, excessive amounts of interleukins and lipid mediators are released and play a crucial role. Pro-inflammatory eicosanoids of AA metabolism are released from membrane phospholipids in the course of inflammatory activation. EPA is released to compete with AA for enzymatic metabolism inducing the production of less inflammatory and chemotactic derivatives.

A variety of substances that inhibit the COX pathway have been investigated, including non-steroidal anti-inflammatory drugs (NSAIDs) used for the treatment of inflammation, pain and fever. Although NSAIDs inhibit COX and are efficacious anti-inflammatory agents, serious adverse effects limit their use. Two forms of COX have been identified, a constitutively

expressed COX-1 and a cytokine inducible COX-2. It has been suggested that NSAID toxicity is due to inhibition of COX-1, whereas therapeutic properties are derived from COX-2 inhibition at the site of inflammation [18,19]. In addition, there is evidence that COX-2 inhibition can suppress the growth of colorectal cancer [20].

A new arena for omega-3 fatty acids has emerged as adjuvants to drug treatment leading to synergism (potentiating the effects of drugs) or to decreasing their toxicity (Table 1) [21–32].

Similarly, increasing the intake of omega-3 fatty acids while decreasing the omega-6 fatty acids in the diet has led to improvements and a decrease of non-steroidal anti-inflammatory agents in patients with rheumatoid arthritis [33,34] and asthma [35].

Dietary fish oils, rich in omega-3 PUFA, are rapidly incorporated into the membrane phospholipids of circulating human (monocyte) cells, suggesting that they are likely to have an effect on several aspects of cell function. Moderate dietary supplementation with omega-3 PUFA significantly increases their level in monocytes within two weeks [36]. The levels of EPA reached a maximum accumulation after six weeks' supplementation and DHA reached a peak at 18 weeks [37]. EPA returned rapidly to pretreatment levels in monocytes (although plasma levels remained significantly elevated from baseline after 24 weeks of washout) whereas DHA levels declined more slowly [37].

Omega-3 Fatty Acids, Interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF)

The interactions between immune and inflammatory cells are mediated in large part by proteins, termed interleukins (IL), that are able to promote cell growth, differentiation and functional activation. TNF-α and IL-1 and IL-6 are the most important cytokines produced by monocytes and macrophages. Production of appropriate amounts of TNF, IL-1 and IL-6 is beneficial in response to infection, but in inappropriate amounts or overproduction can be dangerous and these cytokines, especially TNF, are implicated in causing some of the pathological responses that occur in inflammatory conditions. They induce fever and the synthesis of acute phase proteins by the liver, activate T and B lymphocytes and endothelial cells and are involved in many other aspects of the acute phase response.

In addition to their anti-inflammatory effects by suppressing LTB_4, omega-3 supplementation to healthy volunteers suppresses the capacity of monocytes to synthesize interleukin-1 (IL-1) and tumor necrosis factor (TNF) (Table 2) [38]. Omega-3 fatty acids suppress IL-1 mRNA [40,41]. These observations led to studies in patients with inflammatory and autoimmune diseases. The suppression of cytokine synthesis could also be achieved by dietary alteration without fish oil supplementation [34]. The cytokine suppression is probably achieved at the level of transcription, since IL-1 mRNA was decreased. This effect may account for the beneficial effects of omega-3 fatty acids in models of chronic inflammatory disease. IL-1 and TNF influence a wide array of biological functions [42]. Many of the biological functions of IL-1 are shared by TNF [43]. IL-1 potentiates procoagulent activity, increases production of plasminogen activator inhibitor and endothelin and the formation of eicosanoids. Furthermore, it increases leukocyte adhesion by inducing the expression of adhesion molecules and it promotes endothelial protein permeability.

Pharmacologic agents known to reduce the synthesis of IL-1 and TNF are corticosteroids and cyclosporin. Since IL-1 and TNF are principal mediators of inflammation, reduced production of these cytokines contributes to the amelioration of inflammatory symptoms in patients taking omega-3 fatty acid supplements. Studies in normal volunteers indicate that omega-3 fatty acid supplementation reduced the ability of monocytes to produce IL-1β upon stimulation with endotoxin. The effect was most pronounced 10 weeks after stopping the supplementation and suggests prolonged incorporation of omega-3 fatty acids into a pool of circulating monocytes [44]. The capacity of the monocytes from these donors to synthesize IL-1β returned to the pre-supplement level 20 weeks after ending supplementation. Similar results were observed for IL-1α and TNF. These findings have led to trials with omega-3 fatty acids since the above effects (suppression of such magnitude) have been observed and can only be achieved pharmacologically by administration of glucocorticoids or cyclosporin A, which have well-known adverse side effects, particularly during long-term administration. In a one-year intervention trial with dietary fish oil, 66 patients, after renal transplantation and on cyclosporin, randomized, double-blind study, 6 gm of fish oil daily (3 gm of omega-3 fatty acids), had a beneficial effect on renal hemodynamics and on blood pressure. Furthermore, the fish-oil group had significantly fewer rejection episodes than the control group, and there was a trend to increased graft survival [45]. In patients with IgA nephropathy, treatment with fish oil for two years retards the rate at which renal function is lost [46]. The omega-3 fatty acids in fish oil affect eicosanoid metabolism and cytokine production, two important classes of inflammatory modulators, and therefore have the potential to alter renal hemodynamics and inflammation. IgA nephropathy is the most common glomerular disease in the world. Omega-3 fatty acids lower plasma triglycerides and improve red cell flexibility in patients with lupus nephritis [47,48].

Caughey *et al.* [49] demonstrated that a diet enriched with

Table 1. Conditions in which Omega-3 Fatty Acids Have Been Shown to Have Synergistic Effects with Drugs

Human Studies	Reference	Animal Studies	Reference
Hypertension	[21]	Autoimmune Disorders	[27]
Arthritis	[22,23]		
Psoriasis	[24]		
Ulcerative Colitis	[25]		
Restenosis	[26]		

Table 2. Effects of Omega-3 Fatty Acids on Factors Involved in the Pathophysiology of Inflammation

Factor	Function	Effect of ω3 Fatty Acid
Arachidonic acid	Eicosanoid precursor, aggregates platelets, stimulates white blood cells	↓
Thromboxane	Platelet aggregation, vasoconstriction, increase of intracellular Ca^{++}	↓
Prostacyclin ($PGI_{2/3}$)	Prevent platelet aggregation, vasodilation, increase cAMP	↑
Leukotriene (LTB_4)	Neutrophil chemoattractant, increase of intracellular Ca^{++}	↓
Fibrinogen	A member of the acute phase response and a blood clotting factor	↓
Tissue plasminogen activator	Increase endogenous fibrinolysis	↑
Platelet activating factor (PAF)	Activates platelets and white blood cells	↓
Platelet-derived growth factor (PDGF)	Chemoattractant and mitogen for smooth muscles and macrophages	↓
Oxygen free radicals	Cellular damage, enhance LDL uptake via scavenger pathway, stimulate arachidonic acid metabolism	↓
Lipid hydroperoxides	Stimulate eicosanoid formation	↓
Interleukin 1 and tumor necrosis factor	Stimulate neutrophil O_2 free radical formation, stimulate lymphocyte proliferation, stimulate PAF, express intercellular adhesion molecule-1 on endothelial cells, inhibit plasminogen activator, thus, procoagulants	↓
Interleukin-6 [39]	Stimulates the synthesis of all acute phase proteins involved in the inflammatory response: C-reative protein, serum amyloid A, fibrinogen, α_1-chymotrypsin and haptoglobin	↓

Adapted and modified from [38].

flaxseed oil can inhibit the *ex vivo* production of these cytokines by 30% in four weeks, whereas nine grams of fish oil for another four weeks inhibited IL-1β by 80% and TNFα by 74%. Flaxseed increased EPA but not DHA levels in monocytes. Thromboxane A_2 is a facilitator of cytokine synthesis in human monocytes [49]. Results of animal and human studies support the hypothesis that omega-3 PUFA suppress cell mediated immune responses, in part at least by inhibiting antigen presenting-cell function, increase membrane fluidity and alter the expression of membrane proteins, possibly by influencing the vertical displacement of the proteins within the membrane. Most of the human studies have shown that omega-3 fatty acids inhibit proinflammatory cytokines TNF and IL-1. Several studies performed in mice show that omega-3 fatty acids have a stimulatory effect on TNF and IL-1 [50–54]. This species-specific effect may be due to differences in the cell population affected by the PUFAs between the various species [55].

Omega-3 fatty acids suppress platelet activating factor (PAF). PAF is a potent platelet aggregator and leukocyte activator, and it strongly promotes AA metabolism (Table 2). It has been proposed that PAF, a phospholipase A_2 (PLA_2) dependent phospholipid, plays a crucial role in the pathogenesis of rheumatoid arthritis, asthma, endotoxin shock and acute renal transplant rejection.

Other Inflammatory Markers, Interleukin-6 (IL-6) and Cardiovascular Disease

Atherosclerosis and inflammation share similar basic mechanisms involving the adhesion of leukocytes to vascular endothelium in their early phases. There is a strong association between systemic inflammation and coronary artery disease. This association is thought to be causal, i.e. inflammation increases the risk of the disease, rather than simply marking the presence of atherosclerosis, which is an inflammatory process [56,57]. The relationship between infection and cardiovascular disease is likely to have several mediators (including possibly an autoimmune response against protein on the arterial endothelial cell wall) [58]. Cigarette smoking is a well established cardiovascular disease risk factor [59–64], as is high body mass index (BMI) [60], both providing a link between increased inflammation and increased risk. Although regular exercise reduces the risk of cardiovascular events, severe exercise has been shown to be associated with a systemic inflammatory response [65] and increased risk of myocardial infarction [66,67]. In contrast to the risks of severe exercise, moderate exercise and physical fitness are associated with lower baseline levels of inflammatory mediators [62,65,68–70].

Inflammatory markers such as C-reactive protein and fibrinogen are raised in affected people in both chronic coronary artery disease [60,62,63] and peripheral vascular disease compared with unaffected people [71]. The degree of inflammation correlates with disease severity [71–73].

Interleukin-6 (IL-6) is produced and released into the systemic circulation from subcutaneous adipose tissue as well as from cells of the immune system [74] (Table 2). The levels correlate with BMI and percent body fat. A recent theory is that increased IL-6 may be the link between obesity and insulin resistance [75]. Adipose tissue secretes IL-6 whose levels and those of C-reactive protein also correlate with obesity and insulin resistance. There is strong evidence supporting the central role of IL-6 in the inflammatory response. IL-6 is a 26 kDa cytokine, produced by many different cells in the body, including lymphocytes, monocytes, fibroblasts and endothelial cells. Various cytokines are involved in acute phase protein synthesis, including TNFα and IL-1β. However, IL-6 is the only cytokine that can stimulate the synthesis of all the acute

phase proteins involved in the inflammatory response: C-reactive protein, serum amyloid A, fibrinogen, α_1-chymotrypsin and haptoglobin [76]. There is evidence that phospholipase A_2 and cyclooxygenase pathways of AA metabolism are involved in the action of IL-6 in platelets (aggregation). Khalfoun *et al.* [77] examined the effects of PUFA on the production of IL-6 by human unstimulated endothelial cells and stimulated endothelial cells with TNFα, IL-4, LPS (lipopolysaccharide) or PBL (allogeneic peripheral blood lymphocytes). The addition of EPA and DHA significantly reduced the production of IL-6 whereas AA was ineffective even at highest concentrations. EPA was more potent than DHA.

Interleukin-6 occupies a central place in the inflammatory response. Woods *et al.* [39] suggest a link between IL-6 and cardiovascular disease and the pathways involved (Fig. 2). The discovery of genetic polymorphisms involving a change of a single base, from guanine to cytosine, at position—174 in the 5′ flanking region of the interleukin-6 gene is of great importance because the G allele is associated with higher IL-6 production than the C allele. It is quite possible that genetic variation could account for the different responses to omega-3 fatty acids, both in terms of suppression of IL-6 and the inflammatory response [78]. *In vivo* studies found basal IL-6 levels to be twice as high in volunteers with the GG allele than in those with the CC allele. Therefore, the understanding of the genetic mechanisms controlling the IL-6 levels as well as knowing the frequency of GG alleles in the population would provide further evidence that the higher levels of inflammation seen in patients with cardiovascular disease are primary rather than secondary in the development of cardiovascular disease.

Fatty Acids, Cytokines, and Major Depression

Psychologic stress in humans induces the production of proinflammatory cytokines such as interferon gamma (IFNγ), TNFα, IL-6 and IL-10. An imbalance of omega-6 and omega-3 PUFA in the peripheral blood causes an overproduction of proinflammatory cytokines. There is evidence that changes in fatty acid composition are involved in the pathophysiology of major depression. Changes in serotonin (5-HT) receptor number and function caused by changes in PUFA provide the theoretical rationale connecting fatty acids with the current receptor and neurotransmitter theories of depression [79–81].

The involvement of changes in fatty acid composition in the pathophysiology of major depression also revolves around its role in immune function and production of cytokines. There is now evidence that major depression is accompanied by an acute phase response, increased secretion of eicosanoids, such as prostaglandins; cytokines, i.e. the monocyte cytokines, IL-1β and IL-6, as well as the Th-1-like cytokines, IL-2 and IFNγ. IL-1, IL-2 and TNFα activate the hypothalamic adrenal (HPA) axis where proinflammatory cytokines can induce resistance to the effects of glucocorticoid hormones by influencing glucocorticoid receptor expression. In experimental animals and humans (students facing an academic examination), external stressors increase the production of inflammatory cytokines, such as IL-6, TNFα and IFNγ [82], and serum PUFA levels predict the response of proinflammatory cytokines to psychologic stress [83]. The increased C20:4ω6/C20:5ω3 ratio and the imbalance in the omega-6/omega-3 PUFA ratio in major depression may be related to the increased production of

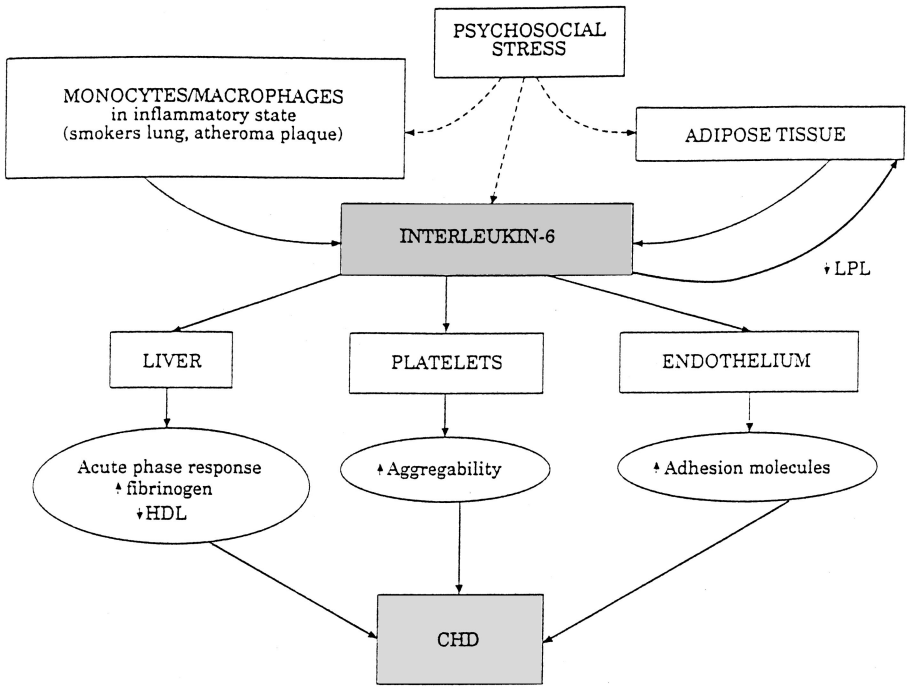

Fig. 2. The central role of interleukin-6 and its effect on the development of coronary artery disease [39].

proinflammatory cytokines and eicosanoids in that illness [79]. The increased omega-6/omega-3 ratio in Western diets most likely contributes to an increased incidence of cardiovascular disease and inflammatory disorders. Lower serum HDL cholesterol and an increased C20:4ω6/C20:5ω3 ratio are related both to depression and to a higher risk of cardiovascular disease, which shows a strong comorbidity with depression [79,80]. There are a number of studies evaluating the therapeutic effect of EPA and DHA in major depression. Stoll and colleagues have shown that EPA and DHA prolong remission, that is, reduce the risk of relapse in patients with bipolar disorder [84,85].

Rheumatoid Arthritis

Interest in the use of omega-3 fatty acids EPA and DHA in rheumatoid arthritis began in the mid-eighties, following the demonstration in several autoimmune strains of mice [NZB (NZB × NZW) F_1, MRL/1pr, and BxSB/Mpj] that omega-3 fatty acids reduced the severity of diffuse proliferative glomerulonephritis. Dose-response studies demonstrated that DHA is more effective than EPA, and that diets with combinations of these two omega-3 fatty acids are synergistic [6,86–88]. Kremer in 1985 [89] carried out a pilot study in 17 patients with rheumatoid arthritis who consumed 1.8 g EPA and 0.9 g DHA. This was a double-blind, controlled, randomized trial of 12 weeks' duration with a follow up evaluation one to two months after the diets and supplements were discontinued. Standard clinical measures of arthritis activity were performed at baseline and after 4, 8 and 12 weeks and at follow up. The results showed a significant difference in morning stiffness between the two groups at the time of the 12-week evaluation, which represented a worsening in the control group, while the fish oil supplemented group remained unchanged. In a subsequent trial, Kremer [90] measured neutrophil LTB_4 production, which was decreased in the patients receiving fish oil. The prolonged suppression of LTB_4 beyond the period of supplementation with fish oils most likely accounted for the continued clinical benefits observed after the period of discontinuation of fish oil. Prolonged effects on the immune system were subsequently reported in normal volunteers ingesting fish oil. Therefore, a crossover format is not appropriate to study the clinical or immune effects of fish oil in patients with inflammatory disease [44]. In a study [91] which examined potential mechanisms of EPA + DHA supplementation in patients with rheumatoid arthritis, 12 patients with active disease consumed 3.6 g EPA and 2.4 g DHA daily for a period of six weeks. After six weeks of fish oil ingestion, LTB_4 was decreased by 33%, and there was a 37% decrease in the quantity of platelet activation factor (PAF). Analyses of fatty acid composition of neutrophil membranes after six weeks' ingestion of fish oils revealed a decline of 33% in AA with a simultaneous twentyfold rise in EPA content from the pre-diet period. DHA was not detected. In the study by Endres [44], IL-1β, IL-1α and TNF were suppressed by 42% at six weeks, but a further decrease was observed 10 weeks after discontinuation. It is therefore possible that fish-oil induced suppression of IL-1 contributes to the amelioration of clinical signs and symptoms of disease activity in patients with rheumatoid arthritis to a greater extent than does inhibition of leukotriene metabolism.

There are at least 13 randomized controlled clinical trials that show benefit from fish oil supplements in patients with rheumatoid arthritis [92]. A common feature of the studies has been a reduction in symptoms and in the number of tender joints. There was a reduction in the dose of analgesic anti-inflammatory drugs. In a subsequent meta-analysis, morning stiffness was decreased, as well as the number of tender joints [93]. Cleland and James have attempted to develop a standard laboratory index of omega-3 nutritional status. They have explored the feasibility of using an assay to guide prevention and therapeutic treatments with omega-3 fatty acids. They have established that there is little diurnal variation in levels of plasma phospholipid EPA, no relationship with meals and a close correlation with cellular EPA levels. Plasma phospholipid EPA correlated very closely with peripheral blood mononuclear cell EPA levels ($r = 0.97$). Thus, they measure non-fasting EPA level, mononuclear cell EPA level and the degree of inhibition in the synthesis of the inflammatory cytokines IL-1β and TNF *ex vivo* in human volunteers given diets fortified with omega-3 fatty acids [49]. They noted substantial inhibition of IL-1β and TNF when the mononuclear cell level of EPA was equal to or greater than 1.5% of total cell phospholipid fatty acids and correlated with a plasma phospholipid EPA level equal to or greater than 3.2%. In their clinic, patients achieving the target EPA level tended to have higher discontinuation rates of NSAIDs [92]. This non-fasting plasma phospholipid EPA may prove to be a useful assay to support the use of dietary omega-3 fatty acids in the treatment of autoimmune diseases and possibly in their prevention. Relative to prevention of rheumatoid arthritis, changes in the diet are recommended in patients with family history who are at special risk for the disease because they carry the HLA-DRβ susceptibility alleles. Population studies suggest that omega-3 fatty acids may have a preventive effect in rheumatoid arthritis. Therefore, persons at a higher risk because of genetic susceptibility are good subjects to carry out preventive measures through dietary change by decreasing the omega-6 fatty acid and increasing the omega-3 fatty acid intake.

Inflammatory Bowel Disease

Crohn's disease and ulcerative colitis, collectively known as inflammatory bowel disease, are related but distinct complex disorders with immunologic, environmental and genetic components [94]. The recent approach to the management of ulcerative colitis has centered on soluble mediators of inflammation. The mediators that have been studied most extensively are

the AA metabolites, prostaglandins, leukotrienes and cytokines. Many others probably remain to be identified, as do all of the cells of origin. Ongoing studies are attempting to define the effects of inflammatory mediators on the functional capabilities of various relevant cell types. The exact point in the pathway of inflammation at which the available therapeutic agents have their greatest effect has not yet been defined. Patients with ulcerative colitis have increased amounts of LTB_4, the end product of AA via the 5-lipoxygenase pathway (Fig. 1), and IL-1β (Table 2). Stenson *et al.* [25] in a double-blind crossover comparison with placebo showed that fish oil supplementation, which increases production of B_5 and decreases production of B_4, did indeed reduce the contents of rectal dialysates of leukotrienes. The clinical improvement was modest. However, the steroid dose could be reduced, and histologic improvement was impressive, even if the gross sigmoidoscopic improvement was not. Encouraging results have been reported by others [95,96]. Belluzzi *et al.* [97] showed a reduced rate of relapse in patients with Crohn's disease in remission by supplementation of 2.7 g of omega-3 enteric-coated fish oil preparation. Endres *et al.* [98] reviewed the evidence of the therapeutic effect of omega-3 fatty acids in patients with inflammatory bowel disease and concluded that some studies have shown a significant improvement in clinical activity and a steroid-sparing effect while others have shown only a trend towards improvement. This variation in response may be due to the heterogeneity of inflammatory bowel disease.

Asthma

Asthma is a mediator driven inflammatory process in the lungs and the most common chronic condition in childhood. The leukotrienes and prostaglandins are implicated in the inflammatory cascade that occurs in asthmatic airways. There is evidence of airway inflammation even in newly diagnosed asthma patients within two to twelve months after their first symptoms [99]. Among the cells involved in asthma are mast cells, macrophages, eosinophils and lymphocytes. The inflammatory mediators include cytokines and growth factors (peptide mediators) as well as the eicosanoids, which are the products of AA metabolism, which are important mediators in the underlying inflammatory mechanisms of asthma (Fig. 1, Table 2). Leukotrienes and prostaglandins appear to have the greatest relevance to the pathogenesis of asthma. The leukotrienes are potent inducers of bronchospasm, airway edema, mucus secretion and inflammatory cell migration, all of which are important to the asthmatic symptomatology. Broughton *et al.* [35] studied the effect of omega-3 fatty acids at a ratio of omega-6:omega-3 of 10:1 to 5:1 in an asthmatic population in ameliorating methacholine-induced respiratory distress. With low omega-3 ingestion, methacholine-induced respiratory distress increased. With high omega-3 fatty acid ingestion, alterations in urinary 5-series leukotriene excretion predicted treatment efficacy and a dose change in >40% of the test subjects (responders) whereas the non-responders had a further loss in respiratory capacity. A urinary ratio of 4-series to 5-series of <1 induced by omega-3 fatty acid ingestion may predict respiratory benefit.

Psoriasis

The recognition that AA metabolism is altered in psoriasis prompted attempts to inhibit the generation of proinflammatory lipoxygenase products, LTB_4 and 12-hydroxyeicosatetraenoic acid (12-HETE), which are markedly elevated in the psoriatic lesions [100]. The addition of MaxEPA® to the standard treatment produced further improvement and a decrease in LTB_4 and 12-HETE (Fig. 1) [11]. In other studies fish oil was successfully used in combination with etretinate to reduce the hyperlipidemia caused by that drug. In patients treated with ultraviolet B (UVB), omega-3 fatty acids prolong the beneficial effects of a course of phototherapy [24]. Fish oil in combination with cyclosporin reduces nephrotoxicity, which is the major side effect of that drug [24].

Conclusions

The anti-inflammatory properties of ω3 fatty acids, especially EPA, are due to competition with arachidonic acid (AA) as a substrate for cyclooxygenases and 5-lipoxygenase. The eicosanoids from the ω6 and ω3 fatty acids have opposing properties. The eicosanoids are considered a link between PUFA, inflammation and immunity. In addition to their effects on prostaglandins, thromboxanes and leukotrienes, ω3 fatty acids suppress the production on interleukin 1 (IL-1β) by suppressing the IL-1β mRNA, as well as the expression of Cox2 (cytooxygenase) mRNA that is induced by IL-1β. Cox2 is overexpressed in colon cancer cells. Both ALA, and EPA and DHA are involved in immune function. The precise effect of ALA depends on the level of linoleic acid (LA) and total PUFA content of the diet. A high dose of ALA (about 15 g/day) will suppress human IL-1 and TNF (tumor necrosis factor). It is unclear whether ALA itself exerts these effects or whether they are the result of its conversion to EPA. Excessive intake of ω6 fatty acids characteristic of Western diets produces an imbalance of ω6 to ω3 PUFAs which leads to an overproduction of the proinflammatory prostaglandins of the ω6 series and cytokines. Supplements of LA rich vegetable oils increase IL-1 and TNFα. Humans given ω3-rich flax seed oil or fish oil supplements have sharply reduced stimulated production of IL-1, IL-2 and TNFα, as well as suppressed mononuclear cell proliferation and expression of IL-2 receptors. Thus, in humans, LA increases proinflammatory cytokine secretion, whereas fish oil reduces proinflammatory cytokine secretion.

Experimental studies have provided evidence that incorporation of omega-3 fatty acids modifies inflammatory and immune reactions, making omega-3 fatty acids potential therapeutic agents for inflammatory and autoimmune diseases. Their effects are brought about by modulation of the type and amount

of eicosanoids and cytokines and by altering gene expression. A number of studies have been carried out in patients with coronary heart disease, cancer, obesity, arthritis, inflammatory bowel disease, psoriasis, asthma, lupus erythematosus, multiple sclerosis, major depression and bipolar depression. Clinical studies indicate that omega-3 fatty acids improve the clinical condition and biochemical factors of patients with arthritis, but the clinical intervention studies in other autoimmune conditions have given conflicting results, most likely due to lack of an adequate number of subjects in some and not taking into consideration the background diet or genetic variation. There is a clear need for more carefully designed and controlled clinical trials in the therapeutic application of omega-3 fatty acids to human autoimmune and inflammatory conditions. Nutritional supplementation with omega-3 fatty acids either as an alternative or adjunct therapy is potentially important, especially since current therapies with drugs have many side effects and the diseases are heterogeneous. In designing clinical interventions, genetic variation should be taken into consideration, since the level of cytokines is to a great extent genetically determined and the dose or amount of omega-3 fatty acids to suppress the proinflammatory state may vary.

The importance of omega-3 essential fatty acids in the diet is now evident, as well as the need to return to a more physiologic omega-6/omega-3 ratio of about 1-4/1 rather than the ratio of 20-16/1 provided by current Western diets. In order to improve the ratio of omega-6/omega-3 essential fatty acids, it will be necessary to decrease the intake of omega-6 fatty acids from vegetable oils and to increase the intake of omega-3 fatty acids by using oils rich in omega-3 fatty acids and increase the intake of fish to two to three times per week or take supplements. Omega-3 fatty acids have been part of our diet since the beginning of time. It is only for the past 150 years that omega-3 fatty acids have been decreased in Western diets due to agribusiness and food processing. The need to return the omega-3 fatty acids into the food supply has been recognized by industry, which is already producing omega-3 enriched products.

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