n-3 Polyunsaturated Fatty Acids and Inflammation: From Molecular Biology to the Clinic

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ABSTRACT: The immune system is involved in host defense against infectious agents, tumor cells, and environmental insults. Inflammation is an important component of the early immunologic response. Inappropriate or dysfunctional immune responses underlie acute and chronic inflammatory diseases. The n-6 PUFA arachidonic acid (AA) is the precursor of prostaglandins, leukotrienes, and related compounds that have important roles in inflammation and in the regulation of immunity. Feeding fish oil results in partial replacement of AA in cell membranes by EPA. This leads to decreased production of AA-derived mediators, through several mechanisms, including decreased availability of AA, competition for cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, and decreased expression of COX-2 and 5-LOX. This alone is a potentially beneficial anti-inflammatory effect of n-3 FA. However, n-3 FA have a number of other effects that might occur downstream of altered eicosanoid production or might be independent of this effect. For example, dietary fish oil results in suppressed production of proinflammatory cytokines and can modulate adhesion molecule expression. These effects occur at the level of altered gene expression. Fish oil feeding has been shown to ameliorate the symptoms of some animal models of autoimmune disease and to protect against the effects of endotoxin. Clinical studies have reported that oral fish oil supplementation has beneficial effects in rheumatoid arthritis and among some asthmatics, supporting the idea that the n-3 FA in fish oil are anti-inflammatory. There are indications that the inclusion of fish oil in enteral and parenteral formulae is beneficial to patients.

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This article will briefly describe the nature of the inflammatory process, the role of n-6 PUFA- derived eicosanoids as mediators and regulators of inflammation, the effects of n-3 PUFA on eicosanoid and inflammatory cytokine production and on adhesion molecule expression, recent developments regarding n-3 PUFA and the expression of inflammatory genes, and the evidence that supports the use of n-3 PUFA in a range of inflammatory conditions.

INFLAMMATION IN HEALTH AND DISEASE

Inflammation is the body's immediate response to infection or injury. Its role is to begin the immunological process of elimination of invading pathogens and toxins and to repair damaged tissue. These responses must be ordered and controlled. The movement of cells into the inflammatory/infected site is induced by the upregulation of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selection on the surface of endothelial cells, allowing leukocyte binding and subsequent diapedesis. The activity of leukocytes is induced by certain triggers. One important exogenous trigger is bacterial endotoxin (also known as lipopolysaccharide or LPS), a component of the cell wall of gram-negative bacteria. LPS can directly activate monocyte/macrophages, inducing them to form cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6 and IL-8; eicosanoids, such as prostaglandin (PG)E_2; nitric oxide; matrix metalloproteinases (MMP); and other mediators. LPS also induces adhesion molecule expression on the surface of endothelial cells and leukocytes. The cytokines produced by monocyte/macrophages also serve to regulate the whole-body response to infection and injury (see Fig. 3 of Ref. 1). Thus, inflammation and the inflammatory response are part of the normal, innate immune response; inflammatory mediators also provide a link between the innate and acquired immune responses (see Fig. 3 of Ref. 1). However, when inflammation occurs in an uncontrolled manner, disease ensues. High levels of TNF-α, IL-1β, and IL-6 are particularly destructive. Chronic overproduction of TNF-α and IL-1 may cause muscle wasting and loss of bone mass. TNF-α, IL-1, and IL-6 are implicated in causing some of the pathologic responses that occur in endotoxic shock, in adult respiratory distress syndrome, and in chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease.

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Abbreviations used: AA, arachidonic acid; COX, cyclooxygenase; FLAP, 5-lipoxygenase activating protein; GM-CSF, granulocyte macrophage colony stimulating factor; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IκB, inhibitory subunit of NFκB; IκK, IκB kinase; IL, interleukin; LOX, lipoxygenase; LPS, lipopolysaccharide; LT, leukotriene; MMP, matrix metalloproteinase; NFκB, nuclear factor κB; PG, prostaglandin; PPAR, peroxisome proliferator-activated receptor; SIRS, systemic inflammatory response syndrome; TIMP, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor; TX, thromboxane; VCAM-1, vascular cell adhesion molecule-1.

FA AND EICOSANOIDS

The FA composition of inflammatory cells. The link between FA and inflammation relates to the composition of inflammatory cell membrane phospholipids. This is because the FA composition of membrane phospholipids can influence various membrane activities, which can, in turn, influence cellular responses (Fig. 1). For example, the physical nature of the membrane, often referred to as fluidity, is regulated in part by the FA composition of its constituent phospholipids (2). Membrane fluidity affects the activity of membrane-bound proteins including receptors, transporters, and enzymes (3); thus, it can alter the responsiveness of inflammatory cells to a stimulus (for a review, see Ref. 4). Intracellular signals, such as DAG, inositol phosphates, and ceramide, are produced from membrane phospholipids in response to a suitable cell stimulus, and there is evidence that the ability of the phospholipase enzymes to generate these signaling molecules can be altered by the FA composition of the substrate phospholipids (for references, see Ref. 5). Another group of mediators, the eicosanoids, are generated from FA liberated from membrane phospholipids; the ability to produce these mediators is therefore strongly influenced by the FA composition of membrane phospholipids (i.e., substrate availability) (see below). Thus, the FA composition of inflammatory cells is important in terms of regulating the functional responses of those cells.

Inflammatory cells typically contain a high proportion of the n-6 PUFA arachidonic acid (AA; 20:4n-6) and low proportions of n-3 PUFA, especially EPA (20:5n-3). The exact proportion of AA in human inflammatory cells varies according to cell type and the lipid fraction examined (see, for example, Refs. 6,7). Increased consumption of fish oil, which is rich in long-chain n-3 PUFA such as EPA and DHA (22:6n-3), results in increased proportions of those FA in inflammatory cell phospholipids, partly at the expense of AA (see, for example, Refs. 6,7).

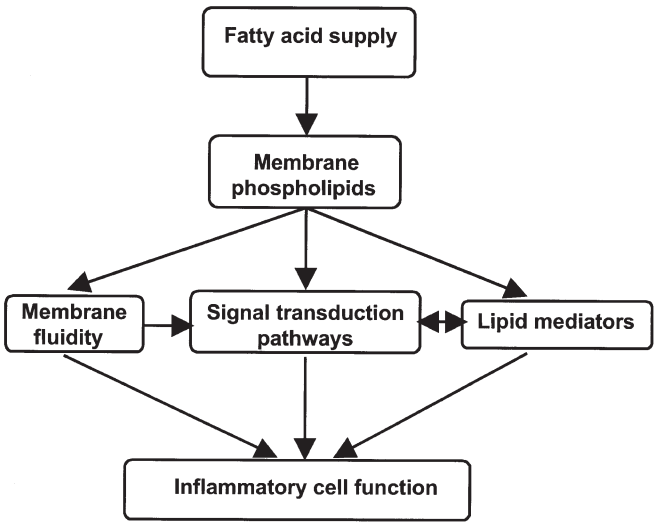

FIG. 1. Mechanisms by which altered FA supply could affect inflammatory cell function.

AA as an eicosanoid precursor. The principal functional role for AA is as a substrate for synthesis of the eicosanoid family of bioactive mediators [e.g., PG, thromboxanes (TX), leukotrienes (LT), or hydroxyeicosatetraenoic acids (HETE); see Fig. 4 of Ref. 1]. AA in cell membranes can be mobilized by various phospholipase enzymes, especially phospholipase A_2, and the free AA can subsequently act as a substrate for the enzymes that synthesize eicosanoids (Fig. 2). Metabolism of AA by cyclooxygenase enzymes (COX) gives rise to the 2-series PG and TX (Fig. 2). There are two isoforms of COX: COX-1 is a constitutive enzyme and COX-2 is induced in inflammatory cells as a result of stimulation and is responsible for the markedly elevated production of PG that occurs upon cellular activation. PG are formed in a cell-specific manner. For example, upon activation monocytes and macrophages produce large amounts of PGE_2 and $PGF_{2\alpha}$, neutrophils produce moderate amounts of PGE_2, and mast cells produce PGD_2. Metabolism of AA by the 5-lipoxygenase (5-LOX) pathway gives rise to hydroxy and hydroperoxy derivatives [5-HETE and 5-hydroperoxyeicosatetraenoic acid (5-HPETE), respectively], and the 4-series LT, LTA_4, B_4, C_4, D_4, and E_4 (Fig. 2). 5-LOX is found in mast cells, monocytes, macrophages, and granulocytes.

AA-derived eicosanoids and inflammation. Eicosanoids are involved in modulating the intensity and duration of inflammatory responses (for reviews, see Refs. 8–10). For example, PGE_2 has a number of proinflammatory effects including inducing fever, increasing vascular permeability and vasodilation, and enhancing pain and edema caused by other agents such as bradykinin and histamine. PGE_2 also promotes IgE production by B lymphocytes; IgE is a mediator of allergic inflammation. PGE_2 suppresses production of TNF-α, IL-1, and IL-6, which, in these respects, is an anti-inflammatory action. LTB_4 increases vascular permeability, is a vasoconstrictor, enhances local blood flow, is a potent chemotactic agent for leukocytes, induces release of lysosomal enzymes, enhances the generation of reactive oxygen species, and enhances production of TNF-α, IL-1, and IL-6. Thus, LTB_4 is proinflammatory in nature. In inflammatory conditions, increased rates of production of AA-derived eicosanoids occur, and elevated levels of these eicosanoids are observed in blood and tissues from patients with trauma, burns, and a variety of inflammatory disorders (8,10). Interestingly, recent studies have shown that PGE_2 inhibits 5-LOX, thereby preventing the generation of the inflammatory 4-series LT (11). Recent studies have also reported novel anti-inflammatory effects of certain lipoxins generated from AA by 15-LOX (12,13). PGE_2 was found to induce generation of one of these, lipoxin A_4 (11), indicating that PGE_2 is involved in mediating the resolution of inflammation through effects on the generation of other eicosanoids.

n-3 PUFA and eicosanoid production. Because significantly increased consumption of long-chain n-3 PUFA results in a decrease in the amount of AA in the membranes of inflammatory cells, there will be less substrate available for synthesis of eicosanoids from AA. However, it is now apparent that the ability of long-chain n-3 PUFA to influence pro-

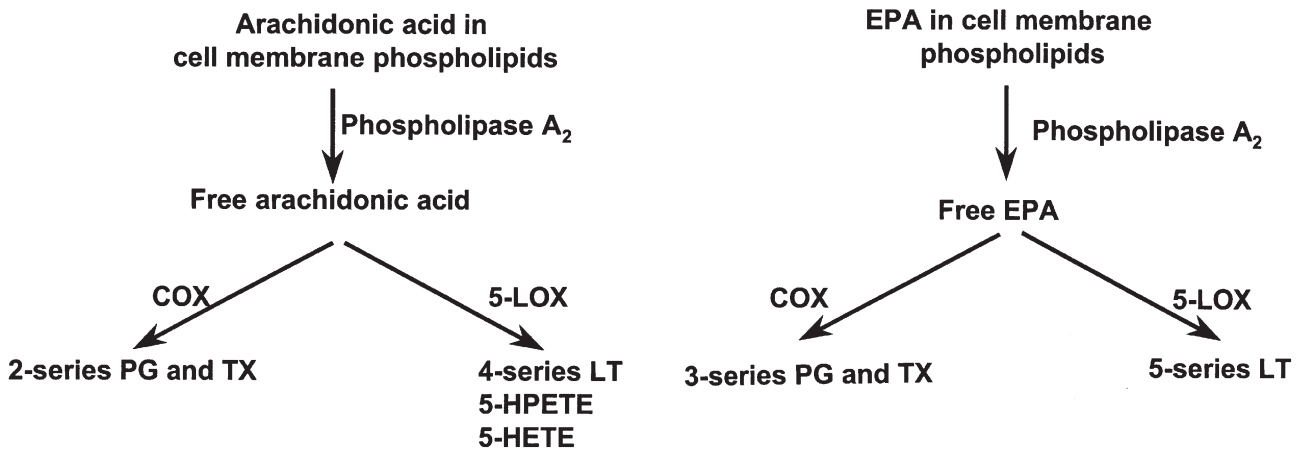

FIG. 2. Synthesis of eicosanoids from arachidonic acid and EPA. COX, cyclooxygenase; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; TX, thromboxane.

duction of eicosanoids extends beyond simply decreasing substrate availability. For example, EPA competitively inhibits the oxygenation of AA by COX (14). Recent cell culture studies have demonstrated that n-3 PUFA suppress cytokine-induction of COX-2 and 5-LOX gene expression (15,16). Owing to these various actions, fish oil feeding results in a decreased capacity of inflammatory cells to synthesize COX- and 5-LOX-derived eicosanoids from AA (see, for example, Refs. 7,17–20). The reduction in the generation of AA-derived mediators that accompanies fish oil consumption has led to the idea that fish oil is anti-inflammatory.

In addition to inhibiting the metabolism of AA, EPA is able to act as a substrate for both COX and 5-LOX (Fig. 2), giving rise to derivatives that differ in structure from those produced from AA (i.e., 3-series PG and TX, and 5-series LT). Thus, the EPA-induced suppression in the production of AA-derived eicosanoids may be accompanied by an elevation in the production of EPA-derived eicosanoids. This is most evident for the 5-LOX products of EPA metabolism (7,20). The eicosanoids produced from EPA are considered to be less biologically potent than the analogs synthesized from AA, although the full range of biological activities of these compounds has not been investigated. Additionally, n-3 PUFA have been shown to give rise to novel anti-inflammatory eicosanoids generated *via* COX-2 (21).

n-3 PUFA AND INFLAMMATORY CYTOKINE PRODUCTION

EPA and DHA can inhibit the production of IL-1β and TNF-α by cultured monocytes (see Ref. 22), and the production of IL-6 and IL-8 by cultured venous endothelial cells (23,24). More recent studies have extended such findings to include suppression of tissue factor production by n-3 PUFA (25). Consistent with cell culture studies involving n-3 PUFA, fish oil feeding decreased *ex vivo* production of TNF-α, IL-1β, and IL-6 by rodent macrophages (26–28) and decreased circulating TNF-α, IL-1β, and IL-6 concentrations in mice injected with LPS (29). Supplementation of the diet of healthy human volunteers with fish oil providing more than 2.4 g/d EPA plus DHA decreased production of TNF-α, IL-1, and IL-6 by mononuclear cells (17,19,30,31). These inhibitory effects of n-3 PUFA on inflammatory cytokine production are not those that would be predicted on the basis of antagonism of PGE_2 production, suggesting some other mechanism of action. This might be *via* effects on production of eicosanoids other than PGE_2 or *via* eicosanoid-independent effects.

n-3 PUFA AND ADHESION MOLECULE EXPRESSION

Culture of murine macrophages with n-3 PUFA decreased their ability to bind to various surfaces (32); how this effect occurred was not investigated. Later studies showed that culture of human venous endothelial cells with EPA or DHA decreased cytokine- or LPS-induced surface expression of E-selectin, ICAM-1, and VCAM-1 (23,33), and diminished the adhesion of ligand-bearing monocytes (33,34). In another cell culture study, EPA decreased surface expression of ICAM-1 on monocytes stimulated with interferon (IFN)-γ (35). Dietary fish oil decreased expression of ICAM-1 on the surface of murine macrophages (36). Supplementing the diet of healthy humans with fish oil providing ~1.5 g/d EPA plus DHA resulted in a lower level of expression of ICAM-1 on the surface of blood monocytes stimulated *ex vivo* with IFN-γ (37). More recently, dietary fish was found to decrease circulating levels of soluble VCAM-1 in elderly subjects (38), but it is not clear whether this represents decreased surface expression of VCAM-1. If so, then this effect might be indicative of decreased endothelial inflammation *in vivo*.

n-3 PUFA AND INFLAMMATORY GENE EXPRESSION

de Caterina *et al.* (23) demonstrated that the downregulation of VCAM-1 expression on the surface of endothelial cells caused by DHA was exerted at the level of VCAM-1 gene expression, and that this effect was independent of any effects on eicosanoid production and on antioxidant status. This was among the first demonstrations of an effect of n-3 PUFA on the expression of inflammatory genes. More recently, it was

shown that culturing bovine chondrocytes with α-linolenic acid, EPA, or DHA dramatically decreased cytokine-mediated induction of expression of the COX-2 (but not COX-1), TNF-α, IL-1α, and aggrecanase-1 and -2 genes (15). Recently, this study was extended by a study using cultured explants of human osteoarthritic cartilage (16). Including α-linolenic acid, EPA, or DHA in the culture medium markedly decreased the cytokine-induced upregulation of expression of the COX-2, IL-1α, IL-1β, TNF-α, 5-LOX, 5-LOX activating protein (FLAP), MMP-3, MMP-13, and aggrecanase-1 genes in these cells. The n-3 PUFA did not affect expression of the COX-1, 12-LOX, or 15-LOX genes, which were not induced by cytokines (16). Also, there was little effect of n-3 PUFA on the expression of genes for the tissue inhibitor of metalloproteinase (TIMP)-1, -2, or -3, which again were not cytokine inducible (16). These studies indicate a marked capacity of n-3 PUFA to suppress the expression of inflammatory genes, with little effect on the expression of housekeeping (e.g., COX-1) or anti-inflammatory (TIMP) genes. They also indicate that an important, hitherto unrecognized contributor to the reduction in the generation of AA-derived eicosanoids after fish oil feeding may be decreased expression of the enzymes and proteins responsible (e.g., COX-2, 5-LOX, FLAP). These observations provide exciting new understanding of the anti-inflammatory effects of n-3 PUFA.

A limited number of feeding studies have demonstrated an effect of dietary fish oil on inflammatory gene expression. Dietary fish oil completely abolished mRNA for TNF-α, IL-1β, and IL-6 in the kidneys of autoimmune disease-prone mice (39). Feeding mice a fish oil-rich diet significantly decreased the level of IL-1β mRNA in LPS-stimulated spleen lymphocytes (40). This study further demonstrated that the lower IL-1β mRNA level was not due to accelerated mRNA degradation but to impaired mRNA synthesis (40). Fish oil lowered LPS-stimulated TNF-α mRNA levels in murine peritoneal macrophages (27). ICAM-1 mRNA levels were lower in peritoneal macrophages from mice fed fish oil (36). Thus, significant evidence is emerging of an effect of dietary fish oil on inflammatory gene expression.

Because eicosanoids derived from AA regulate inflammatory gene expression, the effects of n-3 PUFA might come about through antagonism of the effects of AA-derived mediators. However, at least some of these effects have been demonstrated to occur in an eicosanoid-independent manner (see, for example Ref. 23). Recent studies indicate that n-3 PUFA exert some effects through direct actions on the intracellular signaling pathways that lead to activation of one or more transcription factors.

Nuclear factor κB (NFκB) is a transcription factor involved in the induction of numerous inflammatory genes including COX-2, ICAM-1, VCAM-1, E-selectin, TNF-α, IL-1β, IL-6, nitric oxide synthase, acute phase proteins, and MMP in response to inflammatory stimuli (41–43) (Fig. 3). NFκB exists as an inactive heterotrimer in the cytosol of resting inflammatory cells; one of the subunits is called the inhibitory subunit of NFκB (IκB). Upon stimulation, a signal-

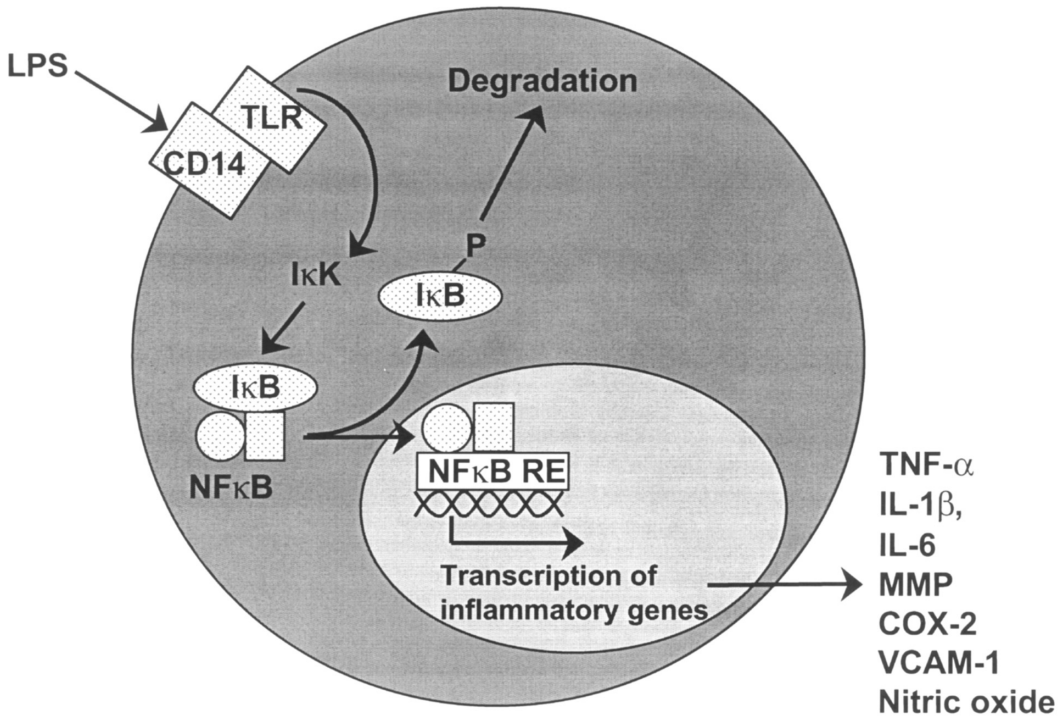

FIG. 3. Outline of the pathway of upregulation of inflammatory gene expression *via* nuclear factor κB. CD14, cluster of differentiation 14 (the LPS receptor); COX, cyclooxygenase; IκB, inhibitory subunit of NFκB; IκK, IκB kinase; IL, interleukin; LPS, lipopolysaccharide; MMP, matrix metalloproteinases; NFκB, nuclear factor κB; RE, response element; TLR, toll-like receptor; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule. Figure previously published in Reference 43.

ing cascade activates a protein complex known as IκB kinase (IκK). Activated IκK phosphorylates IκB, causing its dissociation from the rest of the inactive NFκB trimer (44,45). The phosphorylated IκB is degraded. The remaining NFκB heterodimer is rapidly translocated to the nucleus where it binds to response elements in target genes, thus regulating their transcription. Recent studies suggest that one aspect of the anti-inflammatory action of fish oil is decreased activation of NFκB. For example, dietary fish oil resulted in a lower level of NFκB in the nucleus (i.e., activated NFκB) of LPS-stimulated murine spleen lymphocytes compared with feeding corn oil (46). How n-3 PUFA decreases the activation of NFκB is not clear. However, incubating human monocytes with EPA decreased LPS-induced activation of NFκB, and this was associated with decreased phosphorylation of IκB (47). This suggests an effect of n-3 PUFA on the signaling process leading to activation of IκK. Incubation of a pancreatic cell line with TNF-α markedly upregulated degradation of IκB, and this could be totally abolished by prior incubation of the cells with EPA, but not with AA (48). This effect could be due to inhibition of phosphorylation of IκB, thereby preventing it from being targeted for degradation, or to inhibition of the degradation process itself.

A second group of transcription factors currently eliciting much interest because of their potential role in inflammation is the peroxisome proliferator-activated receptors (PPAR). Although PPARα and -γ play important roles in liver and adipose tissue, respectively (49), they are also found in inflammatory cells (50,51). PPAR act through dimerization with the retinoid-X-receptor and subsequent regulation of gene expression. PPAR can bind, and appear to be regulated by PUFA and eicosanoids (52,53). Mice deficient in PPARα have a prolonged response to inflammatory stimuli (53), leading to the suggestion that PPARα activation might be "anti-inflammatory." Activators of both PPARα and -γ have been shown to inhibit the induction of a range of inflammatory genes including TNF-α, IL-1β, IL-6, IL-8, COX-2, VCAM-1, nitric oxide synthase, MMP, and acute phase proteins (51,54–60). Two mechanisms for the anti-inflammatory actions of PPAR have been proposed (for reviews, see Refs. 61,62). The first is that PPAR stimulate the breakdown of inflammatory eicosanoids through induction of peroxisomal β-oxidation. The second is that PPAR might interfere with or antagonize the activation of other transcription factors, including NFκB. Expression of PPARα and -γ in liver and adipose tissue, respectively, is increased by feeding mice fish oil (63). The effect of fish oil on PPAR expression in inflammatory cells has not been reported. However, it is possible that n-3 PUFA might act by increasing the level of these anti-inflammatory transcription factors in such cells.

A number of other transcription factors are activated by inflammatory signals and play a role in the expression of inflammatory genes (for a review, see Ref. 64). Possibly n-3 PUFA might affect the activation of these factors, but this has not been studied in detail. However, effects of n-3 PUFA on signaling processes that lead to activation of various transcription factors have been reported. For example, incubation of murine macrophages with EPA was found to decrease LPS-induced phosphorylation and activation of mitogen-activated protein kinase (65).

CLINICAL APPLICATIONS OF THE ANTI-INFLAMMATORY EFFECTS OF n-3 PUFA

Chronic inflammatory diseases. Chronic inflammatory diseases are characterized by a dysregulated T-cell response that drives an ongoing immune response to normally benign, often host, antigens. There is a genetic predisposition to such diseases. The response has a strong inflammatory component, and inflammatory mediators are responsible for damage to host tissues and for the metabolic changes seen at the whole-body level. Rheumatoid arthritis is an example of such a disease. It is characterized by a dysregulated T helper 1-type response that promotes the production of inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-8 (66,67). High levels of TNF-α, IL-1β, IL-6, IL-8, and granulocyte/macrophage-colony stimulating factor (GM-CSF) are present in synovial biopsies from patients with rheumatoid arthritis (68). Furthermore, cultured synovial cells produce TNF-α, IL-1β, IL-6, IL-8, and GM-CSF without any additional stimulus (68), suggesting chronic stimulation. COX-2 expression is increased in the synovium of rheumatoid arthritis patients, and in the joint tissues in rat models of arthritis (69). PGE_2, LTB_4, 5-HETE, and platelet activating factor are found in the synovial fluid of patients with active rheumatoid arthritis (70). The efficacy of nonsteroidal anti-inflammatory drugs in rheumatoid arthritis indicates the importance of proinflammatory COX pathway products in the pathophysiology of the disease. Increased expression of E-selection, VCAM-1, and ICAM-1 is found in patients with arthritis, and blocking ICAM-1 or VCAM-1 with antibodies reduces leukocyte infiltration into the synovium and synovial inflammation in animal models of the disease (for references, see Ref. 71).

The effects of fish oil on inflammatory eicosanoid and cytokine production and on adhesion molecule expression suggest that it might have a role in prevention and therapy of rheumatoid arthritis (and other chronic inflammatory diseases). Certainly, dietary fish oil has been shown to have beneficial effects in animal models of arthritis. For example, dietary fish oil delayed the onset and reduced the incidence and severity of type II collagen-induced arthritis in mice (72). It was recently reported that both EPA and DHA suppress streptococcal cell wall-induced arthritis in rats, but that EPA was more effective (73).

Numerous (at least 14) randomized, placebo-controlled, double-blind studies of fish oil in rheumatoid arthritis have been reported. These trials were reviewed in some detail elsewhere (74,75) and are summarized briefly here. The trials used between 1 and 7.1 g/d EPA plus DHA (average dose was 3.3 g/d) with a duration of 12–52 wk. Various improvements in clinical outcomes were reported. These included reduced duration of morning stiffness, reduced number of tender or swollen joints, reduced joint pain, reduced time to fatigue, increased grip strength, and decreased use of nonsteroidal anti-inflammatory drugs. In

an editorial commentary discussing the use of fish oil in rheumatoid arthritis, it was concluded that "the findings of benefit from fish oil in rheumatoid arthritis are robust," "that dietary fish oil supplements in rheumatoid arthritis have treatment efficacy," and that "dietary fish oil supplements should now be regarded as part of the standard therapy for rheumatoid arthritis" (76).

The efficacy of dietary fish oil has been examined in other chronic inflammatory diseases including ulcerative colitis, Crohn's disease, lupus, and multiple sclerosis (reviewed in Ref. 22). Although there are examples of trials reporting clinical improvement in each of these conditions, in general, these studies show little benefit (22). This may be because the dose of n-3 PUFA used was too low, the duration of the studies was too short, the studies were insufficiently powered to detect a significant effect, or a number of other reasons.

Atopic disease. There is currently considerable interest in the relative effects of n-3 and n-6 PUFA in asthma (and other atopic diseases). The discussions center on the roles of various eicosanoids produced from AA in mediating allergic inflammation and in programming T lymphocytes to a phenotype that predisposes to such inflammation. AA-derived eicosanoids such as PGD_2, LTC_4, D_4, and E_4 are produced by the cells that mediate pulmonary inflammation in asthma (mast cells) and are believed to be the major mediators of asthmatic bronchoconstriction. Thus, provision of n-3 PUFA to asthmatics might be beneficial because of the resulting decrease in production of 4-series LT (7,20) and other AA-derived mediators. However, the situation is complicated by the fact that different eicosanoids have different effects, some antagonizing others. For example, the observations that PGE_2 inhibits 5-LOX (11) and promotes the generation of lipoxins that act as inflammation "stop signals" (11–13) indicate that PGE_2 could, in fact, be protective in active asthma. Thus, interventions that aim to suppress PGE_2 production could be counterproductive, at least in some asthmatics. Nevertheless, numerous trials of fish oil in asthma and related atopic diseases have been performed (for reviews, see Refs. 77,78). Most of these studies reveal limited clinical effect, despite significant biochemical changes (e.g., reduced 4-series LT production), although some have shown some significant clinical improvements at least in some patient groups (78). However, a study performed by Broughton *et al.* (79) suggests that fish oil should be used cautiously in asthmatics. These researchers found that although n-3 PUFA ingestion resulted in markedly improved lung function in >40% of adult asthmatic subjects, some patients did not respond favorably to the high n-3 PUFA intake (79). This study suggests that there are patients who respond positively to fish oil intervention and patients who are nonresponders.

Another area of current interest relates to the putative predisposing role of PGE_2 toward atopic disease, particularly in childhood. There has been a rapid increase in the prevalence of childhood atopic disease in developed countries over the last 30 years (80,81), and this coincides with the period of increase in the intake of n-6 PUFA. Because PGE_2 regulates T-lymphocyte differentiation, promoting the development of the T helper 2-type phenotype that underlies sensitization to environmental allergens (82), it is suggested that the pattern of change in dietary FA intake over the last 40 yr is responsible for the increase in childhood atopic disease (83–85). Certainly there are biochemical measurements suggesting an inverse relationship between n-3 FA status and atopic disease. The proportions of EPA, docosapentaenoic acid, and DHA were higher in umbilical cord serum phospholipids from nonallergic compared with allergic mothers (86). Higher n-3 PUFA in breast milk were associated with a decreased likelihood of atopy in the infants (87). The proportions of DHA and of total n-3 PUFA were higher in the serum phospholipids from 12- to 15-yr-old nonatopic controls than in those from children with asthma and/or atopic dermatitis (88). There is also epidemiologic evidence to support a protective role of long-chain n-3 PUFA in atopic disease. Data from the first and second National Health and Nutrition Surveys in the United States found that dietary fish intake was positively associated with lung function (89) and protected against wheezing (90), respectively. Australian schoolchildren who included oily fish in their diet had a much lower likelihood of having asthma than children who did not consume oily fish (91). Schoolchildren who went on to develop atopy had previously consumed more margarine and less butter (and thus more n-6 PUFA) than those who did not develop atopy (92). Despite a biologically plausible mechanism and the supportive biochemical and epidemiologic data, the key to demonstrating a protective effect of increased long-chain n-3 PUFA consumption toward atopic disease must come from well-designed, placebo-controlled intervention studies. It is now recognized that sensitization to allergens occurs early in life (93); thus, the characteristics of the maternal diet may be very important in determining predisposition to atopy, and studies addressing this question should be performed in pregnant women. Several such studies are currently under way, and their findings are eagerly anticipated.

Systemic inflammatory response to surgery and injury. It is now recognized that a hyperinflammatory response, characterized by overproduction of TNF-α, IL-1β, IL-6, and IL-8, is important in the progression of very ill patients toward sepsis, i.e., markedly elevated circulating concentrations of these mediators are seen in sepsis (see, for example, Refs. 94,95); Vervloet *et al.* (96) stated that "these mediators are largely, if not completely, responsible for the clinical signs and symptoms of the septic response to a bacterial infection." Enhanced production of AA-derived eicosanoids such as PGE_2 is also associated with such a pathophysiology (97,98). The inflammatory effects of infection can be mimicked by administration of LPS, which causes an elevation of circulating concentrations of inflammatory cytokines and an upregulation of adhesion molecule expression (for references, see Ref. 43). Laboratory animals can be protected against bacterial- and LPS-induced shock by neutralizing these cytokines or by blocking or gene deletion of VCAM-1 or ICAM-1 (for references, see Ref. 43). The ability of n-3 PUFA to decrease production of inflammatory cytokines and eicosanoids and to decrease adhesion molecule expression suggests that fish oil

might be a useful agent to aid the control of endotoxemia and the so-called systemic inflammatory response syndrome (SIRS). Fish oil feeding or infusions enhanced the survival of guinea pigs after LPS challenge and decreased the accompanying metabolic perturbations in guinea pigs and rats (for references, see Ref. 43). Mice fed fish oil and then injected with LPS had lower plasma TNF-α, IL-1β, and IL-6 concentrations than mice fed safflower oil (29), whereas fish oil-containing parenteral nutrition decreased serum TNF-α, IL-6, and IL-8 concentrations in burned rats (99,100). Postsurgery patients administered parenteral fish oil after major abdominal surgery had lower serum concentrations of TNF-α and IL-6 than those given a standard lipid mix (101). In another study in postsurgical patients, parenteral fish oil decreased TNF-α production by LPS-stimulated whole blood and decreased serum IL-6 concentration compared with the control group that was given standard parenteral nutrition (102). Postoperative stay in the intensive care unit and in hospital tended to be shorter in the fish oil group (102). These studies indicate the potential for significant modification of the inflammatory changes induced by major surgery by infusion of n-3 PUFA in the form of fish oil (101,102). However, larger studies are required to evaluate the effects on complication rates, hospital stay, and mortality.

Numerous clinical trials (at least 20) have been performed in intensive care or surgical patients using enteral formulae containing n-3 PUFA. The majority of these trials used formulae that also contained other nutrients proposed to be beneficial such as arginine and nucleotides. Many of these trials report beneficial outcomes, including decreased numbers of infections and infectious or wound complications, decreased severity of infection, decreased need for mechanical ventilation, decreased progression to SIRS, and decreased length of intensive care unit and/or total hospital stay (for a meta-analysis, see Ref. 103). A number of these studies also measured circulating inflammatory cytokine levels or *ex vivo* cytokine production. These studies reported lower plasma concentrations of inflammatory cytokines (especially IL-6) in patients given n-3 PUFA-containing enteral formula pre- or postsurgery than in those administered standard enteral nutrition (104–107). Although this is in agreement with the effects of n-3 PUFA reported in other settings, and could be used as evidence of their efficacy in the trauma and postsurgery settings, the complex nature of the formulae prevents such a clear interpretation. The effects could be due to any one of the specified nutrients (i.e., arginine, nucleotides, n-3 PUFA) or to the combination of these nutrients.

A trial performed in patients with moderate and severe acute respiratory distress syndrome used an enteral formula claimed to differ only in lipid source from the control (32% canola oil + 25% medium-chain TAG + 20% borage oil + 20% fish oil + 3% soy lecithin vs. 97% corn oil + 3% soy lecithin) (108). However, in addition to the difference in FA composition between the formulae, the n-3 PUFA-rich formula contained more vitamin C and E than the control and contained β-carotene, taurine, and carnitine, whereas the control did not. The study included a number of patients with surgical trauma, sepsis, and pneumonia; all patients had respiratory failure and about one third had failure of at least one other organ system. Patients were given ~7 g EPA, 3 g DHA, 6 g γ-linolenic acid, 1.1 g vitamin C, 400 IU vitamin E, and 6.6 mg β-carotene daily for up to 7 d. By day 4, the numbers of leukocytes and neutrophils in the alveolar fluid had significantly declined in the treatment group and were lower than in the control group. Furthermore, arterial oxygenation and gas exchange were improved in the treatment group. Patients in the treatment group had decreased requirement for supplemental oxygen, reduced time on ventilation support, and shorter length of intensive care unit stay. Total length of hospital stay also tended to be shorter. Fewer patients in the treatment group developed new organ failure. Mortality was 19% in the control group and 12% in the treatment group, but this was not a significant difference. Nevertheless, this study suggests the efficacy of n-3 PUFA (in combination with γ-linolenic acid, medium-chain TAG, antioxidant vitamins, taurine, and carnitine) in this group of patients.

CONCLUDING STATEMENT

Inflammation is a component of a range of acute and chronic human diseases, and is characterized by the production of inflammatory cytokines, AA-derived eicosanoids, other inflammatory mediators (e.g., platelet-activating factor), and adhesion molecules. The n-3 PUFA decrease the production of inflammatory mediators and the expression of adhesion molecules. They act both directly (e.g., by replacing AA as an eicosanoid substrate and inhibiting AA metabolism) and indirectly (e.g., by altering the expression of inflammatory genes through effects on transcription factor activation). Thus, n-3 PUFA are potentially potent anti-inflammatory agents. As such, they may be of therapeutic use in a variety of acute and chronic inflammatory settings. Evidence of their clinical efficacy is stronger in some settings (e.g., in rheumatoid arthritis) than others (e.g., in asthma, in trauma patients).

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