n−3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases[1–3]

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ABSTRACT

Inflammation is part of the normal host response to infection and injury. However, excessive or inappropriate inflammation contributes to a range of acute and chronic human diseases and is characterized by the production of inflammatory cytokines, arachidonic acid–derived eicosanoids (prostaglandins, thromboxanes, leukotrienes, and other oxidized derivatives), other inflammatory agents (eg, reactive oxygen species), and adhesion molecules. At sufficiently high intakes, long-chain n−3 polyunsaturated fatty acids (PUFAs), as found in oily fish and fish oils, decrease the production of inflammatory eicosanoids, cytokines, and reactive oxygen species and the expression of adhesion molecules. Long-chain n−3 PUFAs act both directly (eg, by replacing arachidonic acid as an eicosanoid substrate and inhibiting arachidonic acid metabolism) and indirectly (eg, by altering the expression of inflammatory genes through effects on transcription factor activation). Long-chain n−3 PUFAs also give rise to a family of antiinflammatory mediators termed resolvins. Thus, n−3 PUFAs are potentially potent antiinflammatory agents. As such, they may be of therapeutic use in a variety of acute and chronic inflammatory settings. Evidence of their clinical efficacy is reasonably strong in some settings (eg, in rheumatoid arthritis) but is weak in others (eg, in inflammatory bowel diseases and asthma). More, better designed, and larger trials are required to assess the therapeutic potential of long-chain n−3 PUFAs in inflammatory diseases. The precursor n−3 PUFA α-linolenic acid does not appear to exert antiinflammatory effects at achievable intakes. *Am J Clin Nutr* 2006;83(suppl):1505S–19S.

KEY WORDS Inflammation, monocyte, macrophage, eicosanoid, cytokine, inflammatory disease

INFLAMMATION IN HEALTH AND DISEASE

Inflammation is part of the body's immediate response to infection or injury. It is typified by redness, swelling, heat, and pain. These occur as a result of increased blood flow; increased permeability across blood capillaries, which permits large molecules (eg, complement, antibodies, and cytokines) to leave the bloodstream and cross the endothelial wall; and increased movement of leukocytes from the bloodstream into the surrounding tissue. Inflammation functions to begin the immunologic 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 or infected site is induced by the up-regulation of adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin on the surface of endothelial cells, which allows leukocyte binding and subsequent diapedesis. The earliest cells to appear at inflamed sites are granulocytes, with monocytes, macrophages, and lymphocytes appearing later. Granulocytes, monocytes, and macrophages are involved in pathogen killing, in clearing up cellular and tissue debris, and in tissue repair. The activity of these cells is induced by certain triggers. One important exogenous trigger is bacterial endotoxin (also known as lipopolysaccharide), a component of the cell wall of Gram-negative bacteria, which can directly activate monocytes and macrophages, inducing them to form cytokines, such as tumor necrosis factor α (TNF-α); interleukin 1 (IL-1), IL-6, and IL-8; eicosanoids, such as prostaglandin (PG) E_2; nitric oxide; matrix metalloproteinases; and other mediators. Endotoxin also induces adhesion molecule expression on the surface of endothelial cells and leukocytes.

The cytokines produced by monocytes and macrophages also serve to regulate the whole-body response to infection and injury (**Figure 1**). Thus, inflammation and the inflammatory response are part of the normal, innate immune response. Inflammatory mediators also provide a link between innate and acquired immune responses (Figure 1). The actions of inflammatory cytokines, which initiate a cascade of inflammatory mediators, thus amplifying the initial inflammatory signal, are opposed by antiinflammatory cytokines such as IL-10 and by receptor antagonists such as IL-1 receptor antagonist.

Although inflammation is a normal response, when it occurs in an uncontrolled or inappropriate manner, excessive damage to host tissues and disease can ensue. Such uncontrolled or inappropriate inflammatory responses are characterized by hyperexpression of endothelial and leukocyte adhesion molecules, appearance of soluble forms of adhesion molecules in the circulation, sequestration of leukocytes to sites where they are not usually found, production of inflammatory mediators, and damage to host tissues (**Figure 2**). High concentrations of TNF-α, IL-1β, and IL-6 are particularly destructive and are implicated in some of the pathologic responses that occur in endotoxic shock, in acute respiratory distress syndrome, and in chronic inflammatory diseases such as rheumatoid arthritis and inflammatory

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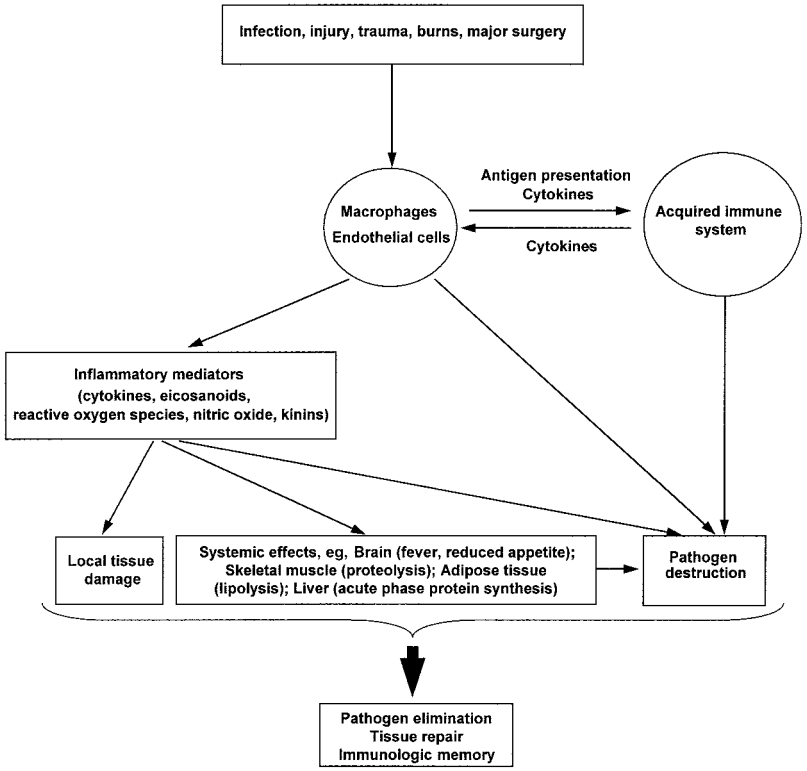

FIGURE 1. The role of inflammatory cells and mediators in regulating the whole-body metabolic and immunologic responses to infection and injury. Modified from reference 1 with permission from the American Oil Chemists' Society.

bowel disease. Chronic overproduction of TNF-α and IL-1 can cause adipose tissue and muscle wasting and loss of bone mass and may account for alterations in body composition and tissue loss seen in inflammatory diseases and in cancer cachexia. As well as its clear and obvious association with classic inflammatory diseases, inflammation is now recognized to play an important role in the pathology of other diseases, such as cardiovascular disease and neurodegenerative diseases of aging. Additionally, the realization that adipose tissue is a source of inflammatory cytokines has given rise to the notion that obesity, the metabolic syndrome, and type 2 diabetes have an inflammatory component.

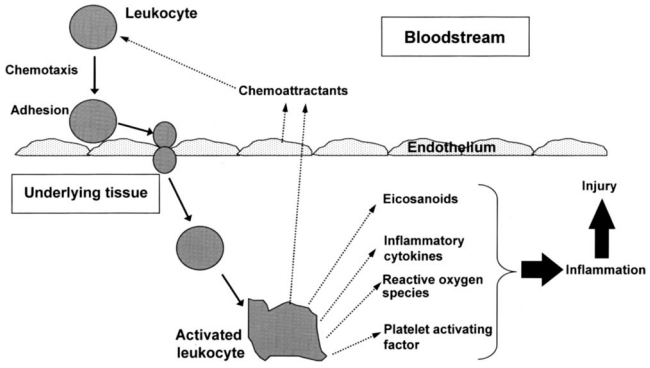

FIGURE 2. Diagrammatic representation of the movement of leukocytes through the endothelium and the subsequent generation of inflammatory mediators.

ARACHIDONIC ACID–DERIVED EICOSANOIDS AND INFLAMMATION

The key link between polyunsaturated fatty acids (PUFAs) and inflammation is that eicosanoids, which are among the mediators and regulators of inflammation, are generated from 20-carbon PUFAs. Because inflammatory cells typically contain a high proportion of the n−6 PUFA arachidonic acid (20:4n−6) and low proportions of other 20-carbon PUFAs, arachidonic acid is usually the major substrate for eicosanoid synthesis. Eicosanoids, which include PGs, thromboxanes, leukotrienes (LTs), and other oxidized derivatives, are generated from arachidonic acid by the metabolic processes summarized in **Figure 3**. Eicosanoids are involved in modulating the intensity and duration of inflammatory responses (*see* references 2 and 3 for reviews), have cell- and stimulus-specific sources, and frequently have opposing effects (**Table 1**). Thus, the overall physiologic (or pathophysiologic) outcome will depend on the cells present, the nature of the stimulus, the timing of eicosanoid generation, the concentrations of different eicosanoids generated, and the sensitivity of the target cells and tissues to the eicosanoids generated. Recent studies have shown that PGE_2 induces cyclooxygenase 2 (COX-2) in fibroblasts cells and so up-regulates its own production (5), induces the production of IL-6 by macrophages (5), inhibits 5-lipoxygenase (5-LOX) and so decreases production of the 4-series LTs (6), and induces 15-LOX and so promotes the formation of lipoxins (6, 7), which have been found to have antiinflammatory effects (8, 9). Thus, PGE_2 possesses both pro- and antiinflammatory actions (Table 1).

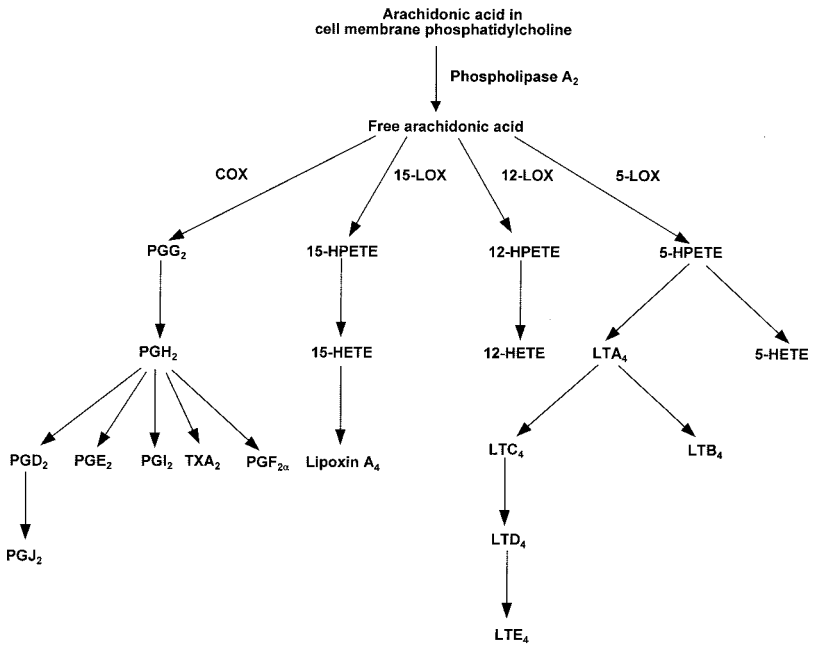

FIGURE 3. Generalized pathway for the conversion of arachidonic acid to eicosanoids. COX, cyclooxygenase; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; TX, thromboxane.

ARACHIDONIC ACID AND INFLAMMATORY MEDIATOR PRODUCTION

Animal feeding studies have shown a strong positive relation between the amount of arachidonic acid in inflammatory cells and the ability of those cells to produce eicosanoids such as PGE_2 (10). In turn, the amount of arachidonic acid in inflammatory cells can be increased by including arachidonic acid in the diet of rats (10) or by increasing the amount of it in the diet of humans (11). The amount of arachidonic acid in inflammatory cells may also be influenced by dietary intake of its precursor, linoleic acid (18:2n−6), although the range of linoleic acid intake over which this relation occurs has not been defined for humans. Increasing linoleic acid intake by 6.5 g/d in humans who habitually consume 10–15 g/d did not alter the arachidonic acid content of blood mononuclear cells (12). Nevertheless, the role of arachidonic acid as a precursor for the synthesis of eicosanoids indicates the potential for dietary n−6 PUFAs (linoleic or arachidonic acid) to influence inflammatory processes. This has been little investigated in humans. Supplementation of the diet of healthy young men with 1.5 g arachidonic acid/d for 7 wk resulted in a marked increase in production of PGE_2 and LTB_4 by endotoxin-stimulated mononuclear cells (13). However, production of TNF-α, IL-1β, and IL-6 by these cells was not significantly altered (13). Thus, increased arachidonic acid intake may result in changes indicative of selectively increased inflammation or inflammatory responses in humans. Supplementation of the diet of healthy elderly subjects with arachidonic acid [0.7 g/d in addition to a habitual intake of ≈0.15 g/d (11)] for 12 wk did not affect endotoxin-stimulated production of TNF-α, IL-1β, or IL-6 by mononuclear cells; did not alter reactive oxygen species (superoxide) production by neutrophils or monocytes; and did not alter plasma soluble VCAM-1, ICAM-1, or E-selectin concentrations (14). This lack of effect was despite incorporation of arachidonic acid into target cells (11). Taken together, these studies suggest that modestly increased intake of arachidonic acid results in incorporation of arachidonic acid into cells involved in inflammatory responses (11), but that this does not affect the production of inflammatory cytokines (13, 14), the generation of superoxide (14), or the shedding of adhesion molecules (14), although production of inflammatory eicosanoids is increased (13).

TABLE 1
Pro- and antiinflammatory effects of prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4)[1]

PGE_2
Proinflammatory
Induces fever
Increases vascular permeability
Increases vasodilatation
Causes pain
Enhances pain caused by other agents
Increases production of IL-6
Antiinflammatory
Inhibits production of TNF and IL-1
Inhibits 5-LOX (decreases 4-series LT production)
Induces 15-LOX (increases lipoxin production)
LTB_4
Proinflammatory
Increases vascular permeability
Enhances local blood flow
Chemotactic agent for leukocytes
Induces release of lysosomal enzymes
Induces release of reactive oxygen species by granulocytes
Increases production of TNF, IL-1, and IL-6

[1] IL, interleukin; LOX, lipoxygenase; TNF, tumor necrosis factor. Modified from reference 4 with permission from the American Oil Chemists' Society.

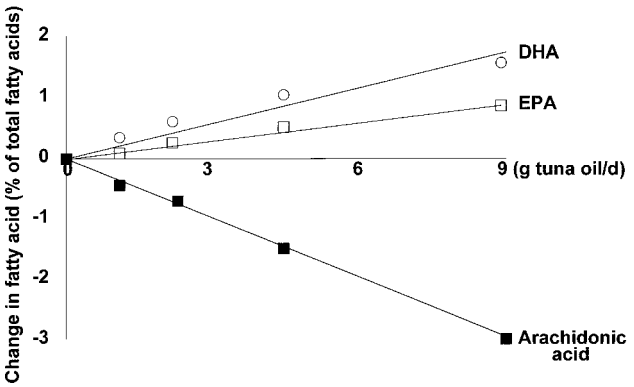

FIGURE 4. Relation between tuna oil consumption and the fatty acid content of human neutrophils. Healthy male volunteers consumed differing amounts of tuna oil in capsules for 12 wk. Neutrophils were isolated before and at the end of the intervention period, and the fatty acid composition of their phospholipids determined. The mean changes in the proportions of arachidonic acid, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) were linearly related to the increase in tuna oil consumption (g/d). Data are from reference 20.

LONG-CHAIN n−3 PUFAs AND INFLAMMATORY EICOSANOID PRODUCTION

Increased consumption of long-chain n−3 PUFAs, such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), results in increased proportions of those fatty acids in inflammatory cell phospholipids (12, 15–20). The incorporation of EPA and DHA into human inflammatory cells occurs in a dose-response fashion and is partly at the expense of arachidonic acid (**Figure 4**). Because less substrate is available for synthesis of eicosanoids from arachidonic acid, fish oil supplementation of the human diet has been shown to result in decreased production of PGE_2 (16, 19, 21, 22), thromboxane B_2 (19), LTB_4 (15, 17), 5-hydroxyeicosatetraenoic acid (15, 17), and LTE_4 (23) by inflammatory cells. Although these studies used fish oil, Kelley et al (24) showed that 6 g DHA/d resulted in decreased production of PGE_2 (by 60%) and LTB_4 (by 75%) by endotoxin-stimulated mononuclear cells.

EPA can also act as a substrate for both COX and 5-LOX, giving rise to eicosanoids with a slightly different structure from those formed from arachidonic acid (**Figure 5**). Thus, fish oil supplementation of the human diet has been shown to result in increased production of LTB_5, LTE_5, and 5-hydroxyeicosapentaenoic acid by inflammatory cells (15, 17, 23), although generation of PGE_3 has been more difficult to demonstrate (25). The functional significance of this is that the mediators formed from EPA are believed to be less potent than those formed from arachidonic acid. For example, LTB_5 is 10- to 100-fold less potent as a neutrophil chemotactic agent than LTB_4 (26, 27). Recent studies have compared the effects of PGE_2 and PGE_3 on production of cytokines by cell lines and by human cells. Bagga et al (5) reported that PGE_3 was a less potent inducer of COX-2 gene expression in fibroblasts and of IL-6 production by macrophages. However, PGE_2 and PGE_3 had equivalent inhibitory effects on the production of TNF-α (28, 29) and IL-1β (29) by human mononuclear cells stimulated with endotoxin. The reduction in generation of arachidonic acid–derived mediators that accompanies fish oil consumption has led to the idea that fish oil is antiinflammatory (**Figure 6**).

In addition to long-chain n−3 PUFAs modulating the generation of eicosanoids from arachidonic acid and to EPA acting as a substrate for the generation of alternative eicosanoids, recent studies have identified a novel group of mediators, termed E-series resolvins, formed from EPA by COX-2 that appear to exert antiinflammatory actions (30–32). In addition, DHA-derived mediators termed D-series resolvins, docosatrienes and neuroprotectins, also produced by COX-2, have been identified and also appear to be antiinflammatory (33–35). This is an exciting new area of n−3 fatty acids and inflammatory mediators and the implications for a variety of conditions may be of great importance. This area was recently reviewed (36, 37).

ANTIINFLAMMATORY EFFECTS OF LONG-CHAIN n−3 PUFAs OTHER THAN ALTERED EICOSANOID PRODUCTION

Although their action in antagonizing arachidonic acid metabolism is a key antiinflammatory effect of n−3 PUFAs, these fatty

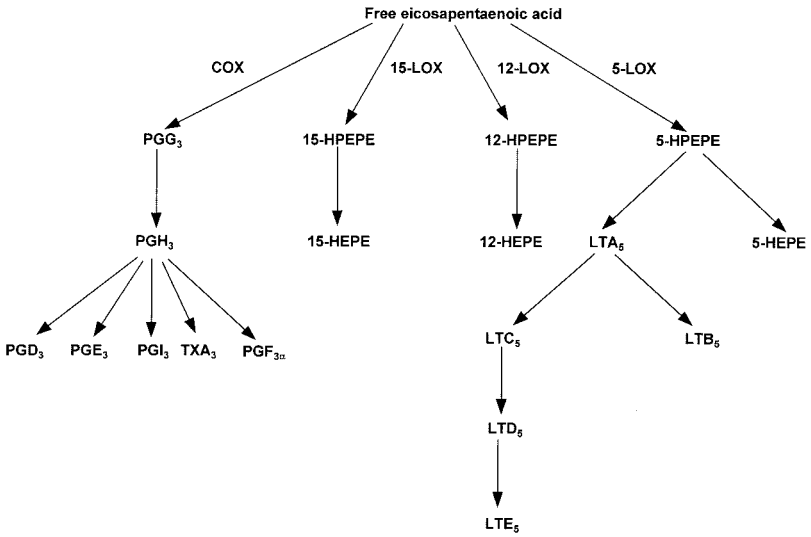

FIGURE 5. Generalized pathway for the conversion of eicosapentaenoic acid to eicosanoids. COX, cyclooxygenase; HEPE, hydroxyeicosapentaenoic acid; HPEPE, hydroperoxyeicosapentaenoic acid; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; TX, thromboxane.

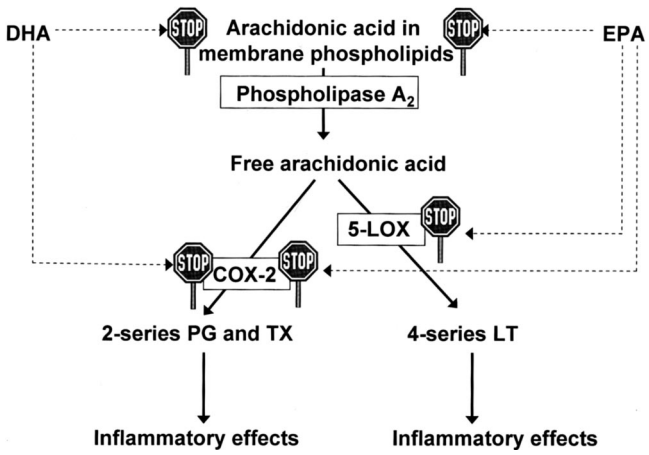

FIGURE 6. Classic mechanism of the antiinflammatory action of long-chain n−3 fatty acids. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) decrease the amounts of arachidonic acid available as a substrate for eicosanoid synthesis and also inhibit the metabolism of arachidonic acid. COX, cyclooxygenase; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; TX, thromboxane.

acids have several other antiinflammatory effects that might result from altered eicosanoid production or might be independent of this. For example, studies have shown that, when consumed in sufficient quantities, dietary fish oil results in decreased leukocyte chemotaxis, decreased production of reactive oxygen species and proinflammatory cytokines, and decreased adhesion molecule expression (**Table 2**).

Long-chain n−3 PUFAs and leukocyte chemotaxis

Several dietary supplementation studies that used between 3.1 and 14.4 g EPA+DHA/d have shown a time-dependent decrease in chemotaxis of human neutrophils and monocytes toward various chemoattractants, including LTB_4, bacterial peptides, and human serum (15–17, 38–40). Both the distance of cell migration and the number of cells migrating were decreased. Despite the high dose of long-chain n−3 PUFAs used in these studies, a dose-response study by Schmidt et al (41) suggests that near-maximum inhibition of chemotaxis occurs at an intake of 1.3 g EPA+DHA/d. A lower intake (0.55 g EPA+DHA/d) did not affect monocyte chemotaxis (42). However, Healy et al (20) did not find an effect of several doses of fish oil providing up to 2.25 g EPA+DHA/d on neutrophil chemotaxis. The apparently divergent reports of Schmidt et al (42) and Healy et al (20) could be explained by the fact that the latter study used a low-EPA, high-DHA fish oil such that the highest dose provided 0.58 g EPA/d, which is less than the amount of EPA provided by the lowest dose of fish oil used by Schmidt et al. If this is so, then the antichemotactic effects of fish oil might be due to EPA rather than DHA. No studies have attempted to discriminate the effects of EPA and DHA on chemotaxis.

Long-chain n−3 PUFAs and adhesion molecule expression

Cell culture (43–46) and animal feeding studies (47) report decreased expression of some adhesion molecules on the surface of monocytes (46), macrophages (47), or endothelial cells (43–45) after exposure to long-chain n−3 PUFAs. Supplementing the diet of healthy humans with fish oil providing ≈1.5 g EPA+DHA/d results in a lower level of expression of ICAM-1 on the surface of blood monocytes stimulated ex vivo with interferon-γ (48). Dietary fish oil providing 1.1 g EPA+DHA/d was found to decrease circulating concentrations of soluble VCAM-1 in elderly subjects (49), but it is not clear whether this represents decreased surface expression of VCAM-1.

Long-chain n−3 PUFAs and reactive oxygen species production

Supplementation studies providing 3.1–8.4 g EPA+DHA/d have reported 30–55% decreases in the production of reactive oxygen species (superoxide or hydrogen peroxide) by stimulated human neutrophils (50–52). Supplementation with 6 g EPA+DHA/d was shown to decrease hydrogen peroxide production by human monocytes (53). Studies using lower doses of long-chain n−3 PUFAs (0.55–2.3 g/d) failed to demonstrate

TABLE 2
Summary of the antiinflammatory effects of long-chain n−3 fatty acids[1]

Antiinflammatory effect	Mechanism likely to be involved
Decreased generation of arachidonic acid–derived eicosanoids (many with inflammatory actions)	Decreased arachidonic acid in cell membrane phospholipids; inhibition of arachidonic acid metabolism; decreased induction of COX-2, 5-LOX, and 5-LOX activating protein
Increased generation of EPA-derived eicosanoids (many with less inflammatory actions than those produced from arachidonic acid)	Increased content of EPA in cell membrane phospholipids
Increased generation of EPA and DHA-derived resolvins (with antiinflammatory actions)	Increased content of EPA and DHA in cell membrane phospholipids
Decreased generation of inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8)	Decreased activation of NFκB (via decreased phosphorylation of IκB); activation of PPARγ; altered activity of other transcription factors; differential effects of arachidonic acid– vs EPA-derived eicosanoids
Decreased expression of adhesion molecules	Decreased activation of NFκB (via decreased phosphorylation of IκB); altered activity of other transcription factors
Decreased leukocyte chemotaxis	Not clear; perhaps decreased expression of receptors for some chemoattractants
Decreased generation of reactive oxygen species	Not clear; perhaps altered membrane composition affecting signaling processes

[1] COX, cyclooxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IκB, inhibitory subunit of NFκB; IL, interleukin; LOX, lipoxygenase; NFκB, nuclear factor κB; PPAR, peroxisome proliferator-activated receptor; TNF, tumor necrosis factor. Modified from reference 4 with permission from the American Oil Chemists' Society.

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