



# n-3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases<sup>1-3</sup>

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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  $\alpha$ -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  $\alpha$  (TNF- $\alpha$ ); interleukin 1 (IL-1), IL-6, and IL-8; eicosanoids, such as prostaglandin (PG) E<sub>2</sub>; nitric oxide; matrix metalloproteinases; and other mediators. Endotoxin also induces adhesion molecule expression on the surface of endothelial cells and leukocytes.

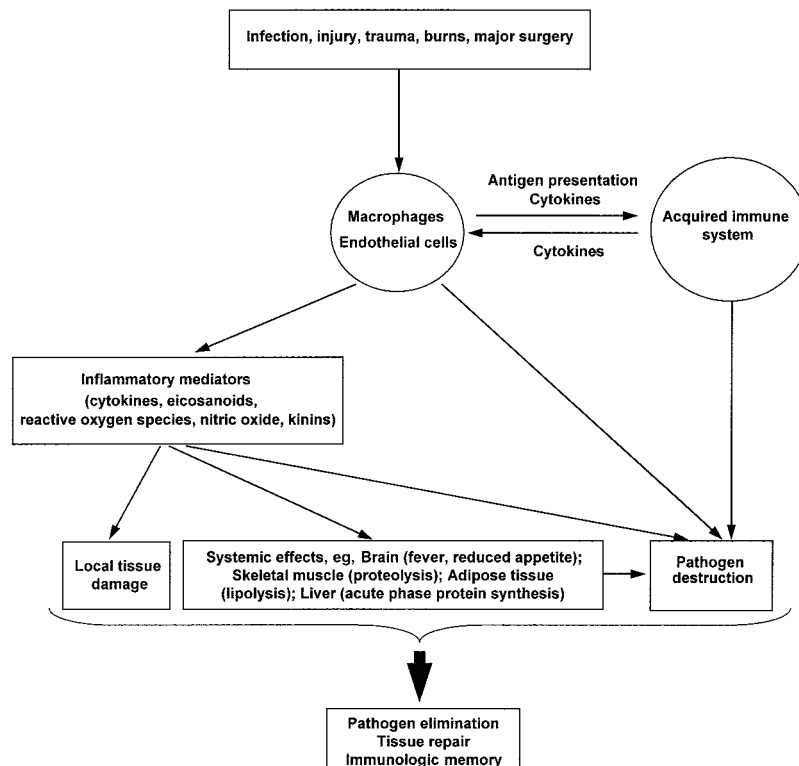
The cytokines produced by monocytes and macrophages also serve to regulate the whole-body response to infection and injury (**Figure 1**). Thus, inflammation and the inflammatory response are part of the normal, innate immune response. Inflammatory mediators also provide a link between innate and acquired 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 anti-inflammatory 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- $\alpha$ , IL-1 $\beta$ , 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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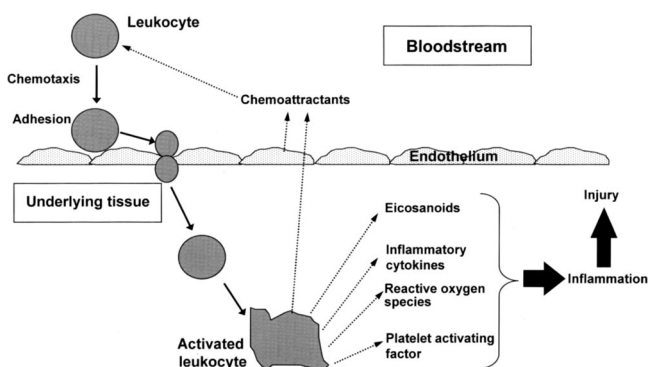


**FIGURE 1.** The role of inflammatory cells and mediators in regulating the whole-body metabolic and immunologic responses to infection and injury. Modified from reference 1 with permission from the American Oil Chemists' Society.

bowel disease. Chronic overproduction of TNF- $\alpha$  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.

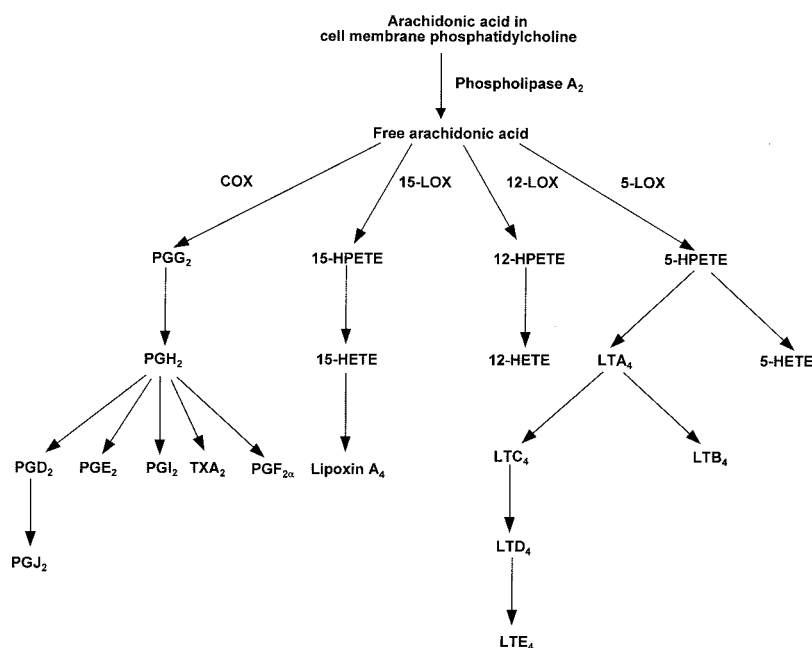
## ARACHIDONIC ACID-DERIVED EICOSANOIDS AND INFLAMMATION

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<sub>2</sub> 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<sub>2</sub> possesses both pro- and antiinflammatory actions (**Table 1**).



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





**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<sub>2</sub> (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

**TABLE 1**

Pro- and antiinflammatory effects of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and leukotriene B<sub>4</sub> (LTB<sub>4</sub>)<sup>1</sup>

#### PGE<sub>2</sub>

##### 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)

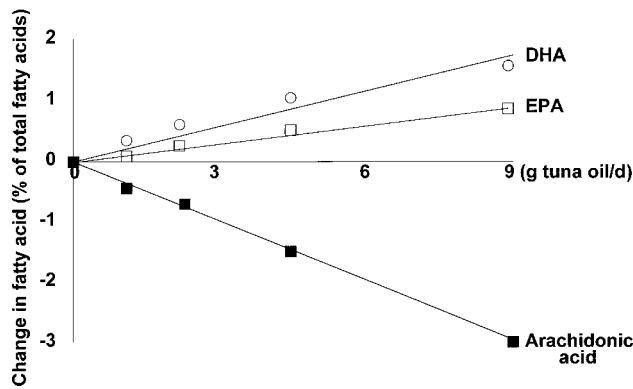
#### LTB<sub>4</sub>

##### 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

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

(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<sub>2</sub> and LTB<sub>4</sub> 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).



**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

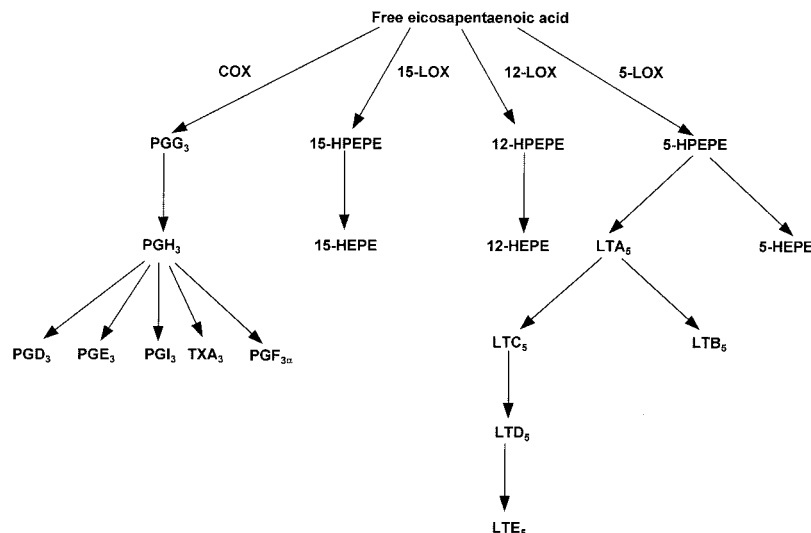
Increased consumption of long-chain n-3 PUFAs, such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), results in increased proportions of those fatty acids in inflammatory cell phospholipids (12, 15–20). The incorporation of EPA and DHA into human inflammatory cells occurs 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<sub>2</sub> (16, 19, 21, 22), thromboxane B<sub>2</sub> (19), LTB<sub>4</sub> (15, 17), 5-hydroxyeicosatetraenoic acid (15, 17), and LTE<sub>4</sub> (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<sub>2</sub> (by 60%) and LTB<sub>4</sub> (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<sub>5</sub>, LTE<sub>5</sub>, and 5-hydroxyeicosapentaenoic acid by inflammatory cells (15, 17, 23), although generation of PGE<sub>3</sub> 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<sub>5</sub> is 10- to 100-fold less potent as a neutrophil chemotactic agent than LTB<sub>4</sub> (26, 27). Recent studies have compared the effects of PGE<sub>2</sub> and PGE<sub>3</sub> on production of cytokines by cell lines and by human cells. Bagga et al (5) reported that PGE<sub>3</sub> was a less potent inducer of COX-2 gene expression in fibroblasts and of IL-6 production by macrophages. However, PGE<sub>2</sub> and PGE<sub>3</sub> had equivalent inhibitory effects on the production of TNF- $\alpha$  (28, 29) and IL-1 $\beta$  (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).

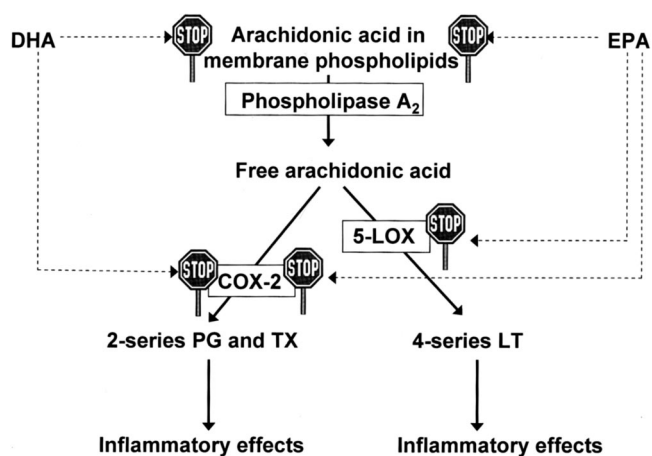
In addition to long-chain n-3 PUFAs modulating the generation of eicosanoids from arachidonic acid and to EPA acting as a substrate for the generation of alternative eicosanoids, recent studies have identified a novel group of mediators, termed E-series resolvins, formed from EPA by COX-2 that appear 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<sub>4</sub>, 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 anti-chemotactic 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- $\gamma$  (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<sup>1</sup>

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- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8)	Decreased activation of NF $\kappa$ B (via decreased phosphorylation of I $\kappa$ B); activation of PPAR $\gamma$ ; altered activity of other transcription factors; differential effects of arachidonic acid– vs EPA-derived eicosanoids
Decreased expression of adhesion molecules	Decreased activation of NF $\kappa$ B (via decreased phosphorylation of I $\kappa$ B); 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

<sup>1</sup> COX, cyclooxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; I $\kappa$ B, inhibitory subunit of NF $\kappa$ B; IL, interleukin; LOX, lipoxygenase; NF $\kappa$ B, nuclear factor  $\kappa$ B; PPAR, peroxisome proliferator-activated receptor; TNF, tumor necrosis factor. Modified from reference 4 with permission from the American Oil Chemists' Society.

effects on reactive oxygen species production by either neutrophils (14, 20, 54, 55) or monocytes (14, 42, 54, 55). A study by Halvorsen et al (56) reported that 3.8 g of either EPA or DHA per day did not affect production of hydrogen peroxide by human monocytes. This lack of effect might relate either to the different stimulus used in this study (*Escherichia coli*) compared with the other "high" dose study with monocytes (latex beads) (53) or to the fact that 3.8 g long-chain n-3 PUFAs/d is below and 6 g/d is above the threshold that affects hydrogen peroxide production by monocytes.

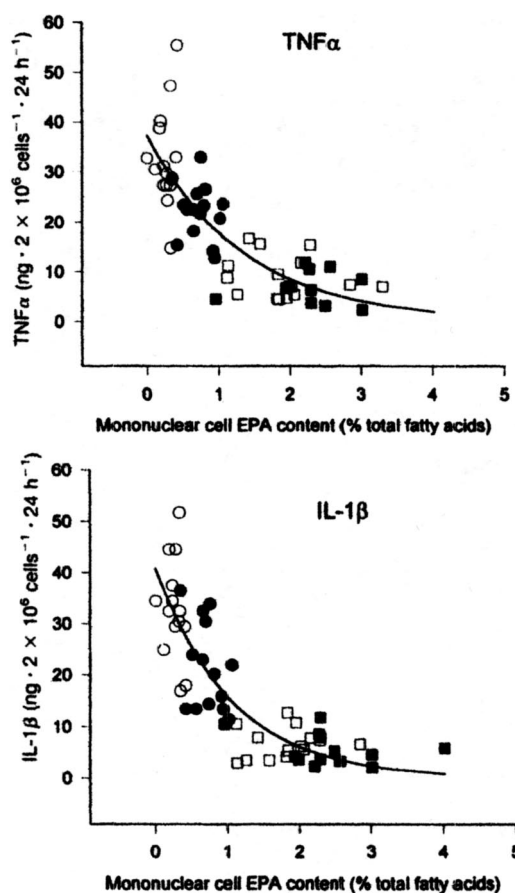
### Long-chain n-3 PUFAs and inflammatory cytokine production

Cell culture studies show that EPA and DHA can inhibit the production of IL-1 $\beta$  and TNF- $\alpha$  by monocytes (57-60) and the production of IL-6 and IL-8 by venous endothelial cells (43, 61). Fish oil feeding decreases the ex vivo production of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 by rodent macrophages (62-64). Supplementation of the diet of healthy humans with fish oil providing >2 g EPA+DHA/d was shown to decrease the production of TNF or IL-1 or IL-6 by mononuclear cells in some studies (16, 19, 21, 65-67). Caughey et al (19) reported a significant inverse correlation between the EPA content of mononuclear cells and the ability of those cells to produce TNF- $\alpha$  and IL-1 $\beta$  in response to endotoxin (Figure 7). Kelley et al (24) showed that 6 g DHA/d for 12 wk resulted in decreased production of TNF- $\alpha$  (by 20%) and IL-1 $\beta$  (by 35%) by endotoxin-stimulated mononuclear cells. Thus, although most studies have used fish oil, it appears that both EPA (19) and DHA (24) can decrease inflammatory cytokine production. This is confirmed by a study in which persons with type 2 diabetes were given 4 g EPA or DHA/d for 6 wk (68). Both EPA and DHA resulted in decreased plasma TNF- $\alpha$  concentrations, although DHA was more potent (35% reduction compared with 20% for EPA). Note, however, that several other studies failed to show effects of dietary long-chain n-3 PUFAs on the production of inflammatory cytokines in humans. Some of these studies provided <2 g EPA+DHA/d (14, 42, 54, 69, 70), although others provided higher doses (12, 55, 71-74). It is not clear what the reason for these discrepancies in the literature is, but technical factors are likely to contribute (75). The relative contributions of EPA and DHA might also be important in determining the effect of fish oil. One other factor that was recently identified is polymorphisms in genes affecting cytokine production (76). It was found that the effect of dietary fish oil on cytokine production by human mononuclear cells was dependent on the nature of the -308 TNF- $\alpha$  and the +252 TNF- $\beta$  polymorphisms. This study raises the possibility of being able to identify those who are more likely and those who are less likely to experience specific antiinflammatory effects of fish oil.

## CLINICAL APPLICATIONS OF THE ANTIINFLAMMATORY EFFECTS OF LONG-CHAIN n-3 PUFAs

### Introductory comments

Inflammation is an overt or covert component of numerous human conditions and diseases. Although the inflammation may afflict different body compartments, one common characteristic of these conditions and diseases is excessive or inappropriate production of inflammatory mediators, including eicosanoids



**FIGURE 7.** Inverse relation between the eicosapentaenoic acid (EPA) content of human mononuclear cells and the production of tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and interleukin 1 $\beta$  (IL-1 $\beta$ ). Healthy male volunteers consumed various combinations of sunflower oil, flaxseed oil, and fish oil resulting in various EPA levels in blood mononuclear cells. The mononuclear cells were isolated and stimulated ex vivo with endotoxin for 24 h. The total intra- and extracellular accumulation of TNF- $\alpha$  and IL-1 $\beta$  was measured by specific enzyme-linked immunosorbent assays. The concentration of each inflammatory cytokine was inversely related to the EPA content of the mononuclear cells. Reproduced from reference 19 with permission.

and cytokines. The roles of n-6 and n-3 PUFAs in shaping and regulating inflammatory processes and responses suggest that the balance of these fatty acids might be important in determining the development and severity of inflammatory diseases. For example, a high intake of n-6 PUFAs, especially arachidonic acid, could contribute to inflammatory processes and so could predispose to or exacerbate inflammatory diseases. Conversely, the recognition that the long-chain n-3 PUFAs have antiinflammatory actions suggests that increasing their intake by patients with inflammatory diseases, for example, through dietary supplementation, may be of clinical benefit. Possible therapeutic targets for long-chain n-3 PUFAs are listed in Table 3. Supplementation trials have been conducted for most of these diseases. Those trials dealing with rheumatoid arthritis, inflammatory bowel diseases (Crohn disease and ulcerative colitis), and asthma will be reviewed in some detail here. This is because a larger number of trials have been conducted for these diseases or because the evidence of benefit is strongest in these diseases.

**TABLE 3**

Diseases and conditions with an inflammatory component in which long-chain n-3 fatty acids might be of benefit<sup>1</sup>

Disease	Reference to the role of inflammation
Rheumatoid arthritis	(77)
Crohn disease	(78)
Ulcerative colitis	(78)
Lupus	(79)
Type 1 diabetes	(80)
Type 2 diabetes	(80, 81)
Cystic fibrosis	(82)
Childhood asthma	(83)
Adult asthma	(84)
Allergic disease	(85)
Psoriasis	(86)
Multiple sclerosis	(87)
Neurodegenerative disease of aging	(87, 88)
Atherosclerosis	(89, 90)
Acute cardiovascular events	(90, 91)
Obesity	(92)
Systemic inflammatory response to surgery, trauma, and critical illness	(93)
Acute respiratory distress syndrome	(94)
Cancer cachexia	(95)

<sup>1</sup> Note: the list is not exhaustive.

### Rheumatoid arthritis

Rheumatoid arthritis is a chronic inflammatory disease characterized by joint inflammation that manifests as swelling, pain, functional impairment, morning stiffness, osteoporosis, and muscle wasting. Joint lesions are characterized by infiltration of activated macrophages, T lymphocytes, and plasma cells into the synovium (the tissue lining the joints) and by proliferation of synovial cells called synoviocytes. Synovial biopsies from patients with rheumatoid arthritis contain high concentrations of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor (GM-CSF), and synovial cells cultured *ex vivo* produce TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, and GM-CSF for extended periods of time without additional stimulus (77). COX-2 expression is increased in the synovium of rheumatoid arthritis patients, and in the joint tissues in rat models of arthritis (96). PGE<sub>2</sub>, LTB<sub>4</sub>, 5-hydroxyeicosatetraenoic acid, and platelet-activating factor are found in the synovial fluid of patients with active rheumatoid arthritis (97). The efficacy of nonsteroidal antiinflammatory drugs in rheumatoid arthritis indicates the importance of proinflammatory COX pathway products in the pathophysiology of the disease. Increased expression of E-selectin, 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 (*see* 98 for references).

Dietary fish oil has been shown to have beneficial effects in animal models of arthritis. For example, compared with feeding vegetable oil, feeding mice fish oil delayed the onset (mean: 34 d compared with 25 d) and reduced the incidence (69% compared with 93%) and severity (mean peak severity score: 6.7 compared with 9.8) of type II collagen-induced arthritis (99). Both EPA and DHA suppressed streptococcal cell wall-induced arthritis in rats, but EPA was more effective (100).

Several studies have reported antiinflammatory effects of fish oil in patients with rheumatoid arthritis, such as decreased LTB<sub>4</sub> production by neutrophils (101–104) and monocytes (103, 105), decreased IL-1 production by monocytes (106), decreased plasma IL-1 $\beta$  concentrations (107), decreased serum C-reactive protein concentrations (101), and normalization of the neutrophil chemotactic response (108). Several randomized, placebo-controlled, double-blind studies of fish oil in rheumatoid arthritis have been reported. The characteristics and findings of these trials are summarized in **Table 4**. The dose of long-chain n-3 PUFAs used in these trials was between 1.6 and 7.1 g/d and averaged  $\approx$ 3.5 g/d. Almost all of these trials showed some benefit of fish oil. Such benefits 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 antiinflammatory drugs (Table 4).

Arachidonic acid may contribute to inflammatory processes by acting as a precursor to eicosanoids known to have a role in rheumatoid arthritis (77, 78). Additionally, long-chain n-3 PUFAs may act as antiinflammatory agents by competing with arachidonic acid for incorporation into inflammatory cell membranes and for metabolism by enzymes of eicosanoid synthesis. Thus, it is possible that greater efficacy of n-3 PUFAs may be achieved in rheumatoid arthritis by simultaneously decreasing n-6 PUFA intake, especially that of arachidonic acid. This was investigated by Adam et al (116). Fish oil, providing 4.2 g EPA + DHA/d, or placebo was given to patients against a background of a typical Western diet, providing 0.1–0.25 g arachidonic acid/d, or of a diet that restricted the intake of arachidonic acid-rich foods (meat, egg yolk, etc) and that provided 0.025–0.09 g arachidonic acid/d; the latter diet was termed an antiinflammatory diet. The fish oil-induced decreases in plasma concentrations of thromboxane A<sub>2</sub> and LTB<sub>4</sub> and the urinary concentration of PG metabolites were greater in patients consuming the antiinflammatory diet than in those consuming the Western diet. The reductions in the number of swollen joints, number of tender joints, patient's global assessment, physician's global assessment, and patient's assessment of pain seen with fish oil supplementation were all also greater for patients consuming the antiinflammatory diet (116). Nonsteroidal antiinflammatory drug use declined in patients receiving fish oil against the background of the antiinflammatory diet but not against the background of the Western diet.

Several reviews of the trials of fish oil in rheumatoid arthritis have been published (121–126), and each concluded that there is benefit from fish oil. 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,” “dietary fish oil supplements in rheumatoid arthritis have treatment efficacy,” and “dietary fish oil supplements should now be regarded as part of the standard therapy for rheumatoid arthritis” (127). A meta-analysis that included data from 9 trials published between 1985 and 1992 inclusive and from one unpublished trial concluded that dietary fish oil supplementation for 3 mo significantly reduces tender joint count (mean difference: -2.9;  $P = 0.001$ ) and morning stiffness (mean difference: -25.9 min;  $P = 0.01$ ) (119). A recent meta-analysis that included data from 10 trials published between 1985 and 2002 was conducted (120), although this included one study of flaxseed oil, one study that did not use a control for fish oil, and one study in which transdermal administration of n-3 PUFAs by ultrasound,

TABLE 4

Overview of placebo-controlled studies using long-chain n-3 fatty acids (fish oil) in patients with rheumatoid arthritis<sup>1</sup>

Reference	Dose of EPA + DHA	Duration	Placebo	Clinical outcomes improved with long-chain n-3 PUFAs	Included in Fortin et al meta-analysis (119)	Included in AHRQ meta-analysis (120)
(101)	<i>g/d</i> 1.8 + 1.2	<i>wk</i> 12	Paraffin oil	Number of tender joints; duration of morning stiffness	Y	Y
(102)	2.7 + 1.8	14	Olive oil	Number of tender joints; number of swollen joints; time to fatigue; physician's global assessment	Y	N
(103)	3.2 + 2.0	12	Olive oil	Number of tender joints; grip strength	Y	Y
(104)	2.0 + 1.3	12	Coconut oil	Number of swollen joints; duration of morning stiffness	Y	N
(106)	1.7 + 1.2	24	Olive oil	Number of tender joints; number of swollen joints; grip strength; physician's global assessment	Y	Y
(106)	3.5 + 2.4	24	Olive oil	Number of tender joints; number of swollen joints; grip strength; physician's global assessment; duration of morning stiffness	Y	Y
(105)	2.0 + 1.3	12	Coconut oil	Number of swollen joints; joint pain index	Y	Y
(109)	1.8 + 1.2	24	Mixed oils	Number and severity of tender joints; physician's global assessment; use of NSAIDs	N	Y
(107)	2.0 + 1.2	12	Mixed oils	Number and severity of tender joints	N	N
(110)	2.0 + 1.2	12	Vegetable oil	Number of tender joints; duration of morning stiffness	Y	Y
(111)	3.8 + 2.0	16	Corn oil	Number and severity of tender joints; duration of morning stiffness	Y	N
(112)	1.7 + 1.1	52	Air	Use of NSAIDs	N <sup>2</sup>	N
(113)	1.7 + 0.4	52	Olive oil	Physician's pain assessment; patient's global assessment; use of NSAIDs or DMARDs	N <sup>2</sup>	Y
(114)	4.6 + 2.5	26-30	Corn oil	Number of tender joints; duration of morning stiffness; physician's assessment of pain; physician's global assessment; patient's global assessment	N <sup>2</sup>	N
(115)	Total 40 mg/kg (≈2.2-3.0)	15	Mixed oils	Number of swollen joints; duration of morning stiffness; patient's assessment of pain; patient's global assessment; physician's global assessment; health assessment by questionnaire	N <sup>2</sup>	N
(116)	≈2.4 + 1.8	12	Corn oil	Number of swollen joints; number of tender joints; patient's global assessment; physician's global assessment; patient's assessment of pain	N <sup>2</sup>	N
(117)	1.4 + 0.2 (+ 0.5 γ-linolenic acid) in a liquid supplement	16	Liquid supplement without added PUFA	None	N <sup>2</sup>	N <sup>2</sup>
(118)	Total 3.0	24	Soybean oil	Duration of morning stiffness; joint pain; time to onset of fatigue; Ritchie's articular index; grip strength, patient's global assessment	N <sup>2</sup>	N <sup>2</sup>

<sup>1</sup> AHRQ, Agency for Healthcare Research and Quality; DHA, docosahexaenoic acid; DMARDs, disease-modifying antirheumatic drugs; EPA, eicosapentaenoic acid; NSAIDs, nonsteroidal antiinflammatory drugs.

<sup>2</sup> Published too late to be considered.

rather than the oral route, was used. This meta-analysis concluded that fish oil supplementation has no effect on "patient report of pain, swollen joint count, disease activity, or patient's global assessment." However it also stated that "in a qualitative analysis of seven studies that assessed the effect of n-3 fatty acids on anti-inflammatory drug or corticosteroid requirement, six demonstrated reduced requirement for these drugs" and concluded that "n-3 fatty acids may reduce requirements for corticosteroids." The effects of long-chain n-3 PUFAs on tender joint count was not assessed in reference 120, which reiterated the findings of the earlier meta-analysis (119) that "n-3 fatty

acids reduce tender joint counts." Thus, reasonably strong evidence suggests that long-chain-3 PUFAs have some clinical benefits in rheumatoid arthritis.

### Inflammatory bowel diseases

Ulcerative colitis and Crohn disease are chronic inflammatory diseases of the alimentary tract. In ulcerative colitis, the mucosa of the colon is mainly affected, whereas in Crohn disease, any part of the alimentary tract from the mouth to the anus can be affected, although it is usually the ileum and colon. In both diseases, the intestinal mucosa contains elevated concentrations



TABLE 5

Overview of placebo-controlled studies using long-chain n-3 fatty acids (fish oil) in patients with inflammatory bowel diseases<sup>1</sup>

Reference	Disease	Dose of EPA + DHA g/d	Duration wk	Placebo	Effect of long chain n-3 PUFAs on clinical outcomes	Considered in AHRQ report (120)
(132)	UC & CD	>1.8 + 1.3	12	Olive oil	Decreased sigmoidoscopy score; decreased disease activity in UC (NS); no effect on disease activity in CD	Y
(140)	UC	2.7 + 1.8	12	Mixed oils	Decreased disease activity; decreased use of corticosteroids; no effect on sigmoidoscopy score; no effect on gut mucosal histology score	Y
(134)	UC	4.5 + 1.1	52	Olive oil	Decreased use of corticosteroids in relapsing patients; induction of remission in relapsing patients; no effect on relapse for patients in remission	Y
(139)	UC	3.2 + 2.2	16	Linoleic acid-rich vegetable oil	Decreased gut mucosal histology score; decreased use of corticosteroids; increased weight gain	Y
(141)	UC	2.2 + 1.5 for 4 wk; then 1.1 + 0.75 for 20 wk	24	Olive oil	No effect on sigmoidoscopy score; no effect on gut mucosal histology score; no effect on relapse; no effect on rectal bleeding	Y
(142)	CD	1.8 + 0.9	52	Short-chain fatty acids	Increased maintenance in remission; decreased relapse	Y
(143)	UC	Total 5.1	104	Corn oil	No effect on gut mucosal histology score; no effect on disease activity; no effect on relapse	Y
(144)	CD	2.8 + 1.5	52	Corn oil	No effect on relapse	Y
(145)	UC	3.2 + 2.1	52	Olive oil	No effect on relapse	Y
(146, 147)	UC	3.2 + 2.4	24	Sunflower oil	Decreased sigmoidoscopy score; decreased gut mucosal histology score; decreased disease activity	Y
(148)	UC	Total 5.6	24	Sunflower oil	Decreased disease activity; decreased sigmoidoscopy score	Y <sup>2</sup>
(149)	CD	1.6 + 1.1	24	Olive oil	No effect on disease activity; no effect on body composition	N <sup>3</sup>

<sup>1</sup> AHRQ, Agency for Healthcare Research and Quality; CD, Crohn Disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; UC, ulcerative colitis.

<sup>2</sup> Published in abstract form only.

<sup>3</sup> Published too late to be considered.

of inflammatory cytokines and eicosanoids such as LTB<sub>4</sub> (128). The established role of arachidonic acid-derived eicosanoids in the pathophysiology of inflammatory bowel diseases suggests that a high dietary intake of n-6 PUFAs may play a part in establishing or perpetuating the disease. Indeed, using multivariate analysis, Shoda et al (129) determined that the increased incidence of Crohn disease in Japan was significantly associated with an increase in the ratio of n-6 to n-3 PUFAs in the diet. They suggested that a diet high in n-6 PUFAs relative to n-3 PUFAs somehow plays a causal role in the disease, and that an increase in n-3 PUFA intake may be of benefit.

Certainly, dietary fish oil has beneficial effects in animal models of colitis (130, 131). Long-chain n-3 PUFAs are incorporated into gut mucosal tissue of patients with inflammatory bowel disease who supplement their diet with fish oil (132-134), and there are reports that this results in antiinflammatory effects, such as decreased LTB<sub>4</sub> production by neutrophils (134-136) and colonic mucosa (136, 137), decreased PGE<sub>2</sub> and thromboxane B<sub>2</sub> production by colonic mucosa (133), and decreased production of PGE<sub>2</sub> by blood mononuclear cells (138). Small open-label or pilot studies reported clinical benefit of fish oil supplementation in ulcerative colitis (135, 139).

Several randomized, placebo-controlled, double-blind studies of fish oil in inflammatory bowel disease have been reported. The characteristics and findings of these trials are summarized in

**Table 5.** The dose of long-chain n-3 PUFAs used in these trials was between 2.7 and 5.6 g/d and averaged ≈4.5 g/d. Some of these trials indicate benefits of fish oil, including improved clinical score, improved gut mucosal histology, improved sigmoidoscopic score, lower rate of relapse, and decreased use of corticosteroids. One study of special note is that of Belluzzi et al (142) in which patients with Crohn disease in remission were randomly assigned to receive placebo or 2.7 g long-chain n-3 PUFAs/d from an enterically coated fish oil preparation for 1 y. The primary outcome was relapse. There was a significant difference in the proportion of patients who relapsed over 12 mo: 11/39 (28%) in the fish oil group compared with 27/39 (69%) in the placebo group ( $P < 0.001$ ). Likewise, there was a significant difference in the proportion of patients who remained in remission at 12 mo: 59% in the fish oil group compared with 26% in the placebo group ( $P = 0.003$ ).

Reviews of trials of fish oil in inflammatory bowel diseases have been published (150-152), and these conclude that there is some benefit from fish oil. A recent meta-analysis identified 13 studies of fish oil supplementation in inflammatory bowel diseases reporting outcomes related to clinical score, sigmoidoscopy score, gut mucosal histology score, induced remission, and relapse (120). However, there were sufficient data to perform the meta-analysis only for relapse and only for ulcerative colitis. Relapse was reported in 5 studies in ulcerative colitis (Table 5),

TABLE 6

Overview of placebo-controlled studies using long-chain n-3 fatty acids (fish oil) in patients with asthma<sup>1</sup>

Reference	Dose of EPA + DHA	Duration	Placebo	Effects of long chain n-3 PUFAs on clinical outcomes	Included in Thien et al meta-analysis (174)	Included in AHRQ systematic review (165)
(163, 166)	3.2 + 2.2 <i>g/d</i>	10 <i>wk</i>	Olive oil	Improved PEF	Y	Y
(164)	4.0 + trace	8	Low n-3 PUFA	None identified	Y	Y
(167)	3.6 + 2.4	10	Olive oil	None identified	Y	Y
(168)	Total 1.0	52	Not specified	Improved FEV <sub>1</sub>	Y	Y
(169)	2.7 + 1.8	10	Olive oil	None identified	Y	Y
(170)	3.2 + 2.2	24	Olive oil	None identified	Y	Y
(171)	Not clear (total ≈3.3) but n-6 to n-3 PUFA ratio of diet + capsules = 2	4	Not clear	Overall none identified, but FEV <sub>1</sub> , FVC, PEF, and FEF <sub>25-75</sub> in response to methacholine challenge improved in 40% of patients	N	Y
(172)	0.72 + 0.48 (+ some α-linolenic acid) <sup>2</sup>	24	Mixed oils	None identified	Y	Y
(173)	17-27 mg/kg + 7- 11.5 mg/kg <sup>2</sup>	40	Olive oil	Decreased asthma symptom scores; decreased bronchial hyperresponsiveness to acetylcholine challenge	Y	Y

<sup>1</sup> AHRQ, Agency for Healthcare Research and Quality; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FEV<sub>1</sub>, forced expiratory volume at 1 s; FEF<sub>25-75</sub>, maximum forced expiratory flow; FVC, forced vital capacity; PEF, peak expiratory flow.

<sup>2</sup> Study in children.

and 3 of these were used for the meta-analysis (134, 143, 145). Two of these studies reported a higher rate of relapse with fish oil than with placebo (134, 143), although this was not significant in either study, whereas one reported no effect (145). The pooled risk of relapse with long-chain n-3 PUFAs relative to placebo was 1.13 (95% CI: 0.91, 1.57). This meta-analysis concluded that “n-3 fatty acids have no effect on relative risk of relapse in ulcerative colitis” and “there was a statistically nonsignificant reduction in requirement for corticosteroids for n-3 fatty acids relative to placebo in two studies” (120). A recent study reported no effect of 2.7 g EPA+DHA/d for 24 wk on disease activity in patients with Crohn disease (149).

Thus, despite several favorable studies, the overall view at the moment must be that only weak evidence exists that long-chain-3 PUFAs have clinical benefits in inflammatory bowel diseases. However, the apparent ability of long-chain n-3 PUFAs to retain Crohn disease patients in remission (142) is a striking finding.

### Asthma

Arachidonic acid-derived eicosanoids such as PGD<sub>2</sub>, LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub> are produced by the cells that mediate pulmonary inflammation in asthma (eg, mast cells) and are believed to be major mediators of asthmatic bronchoconstriction. The 4-series LTs have been detected in the blood, bronchoalveolar lavage fluid, and urine of asthmatics (153). In addition to the role of arachidonic acid-derived eicosanoids as mediators of asthma, PGE<sub>2</sub> is also involved in regulating the development of the T helper type 2 phenotype of T lymphocytes that predisposes to allergic inflammation (154) and promotes the formation of immunoglobulin E by B lymphocytes (155). Thus, a hypothesis has evolved that an increased intake of n-6 PUFAs has played a causal role in increased asthma incidence (156, 157). Epidemiologic data link high n-6 PUFA or low n-3 PUFA consumption with childhood asthma (158, 159). Early exposure to long-chain n-3 PUFAs does appear to alter cytokine production by neonatal T cells (160, 161), although the longer-term clinical impact of

this is not yet clear. Nevertheless, the role of arachidonic acid-derived eicosanoids in asthma has prompted a series of studies attempting to modify the disease with fish oil treatment. Several studies have reported antiinflammatory effects of fish oil in patients with asthma, such as decreased 4-series LT production (162-164) and leukocyte chemotaxis (163, 164). Several uncontrolled or open-label trials of fish oil have shown clinical benefit of fish oil; these are discussed in detail elsewhere (165).


Several randomized, placebo-controlled, double-blind studies of fish oil in asthma have been reported. The characteristics and findings of these trials are summarized in **Table 6** and are discussed in great detail elsewhere (165, 174). Thien et al (174) included 8 studies published between 1988 and 2000 in a systematic review. They identified that there was “no consistent effect on forced expiratory volume at one second, peak flow rate, asthma symptoms, asthma medication use or bronchia hyperreactivity.” They conceded that one study in children showed improved peak flow and reduced asthma medication use. A more recent report covering 26 studies (both randomized placebo-controlled and others) concluded that “no definitive conclusion can yet be drawn regarding the efficacy of n-3 fatty acid supplementation as a treatment for asthma in children and adults” (165). However, the studies of Broughton et al (171) and Nagakura et al (173) indicate that there may be subgroups of asthmatic subjects who benefit greatly from long-chain n-3 PUFAs. Clearly, more needs to be done in this area.

### IS THERE A ROLE FOR α-LINOLENIC ACID IN MODULATING INFLAMMATION?

Relatively few studies have examined the effect of the precursor n-3 PUFA α-linolenic acid on inflammatory outcomes in humans. Caughey et al (19) reported that 13.7 g α-linolenic acid/d for 4 wk resulted in a decrease in production of TNF-α and IL-1β by endotoxin-stimulated mononuclear cells by 27% and 30%, respectively. By comparison, fish oil

providing 2.7 g EPA+DHA/d decreased production of these cytokines by 70% and 78%, respectively (19). Thus, on a g/d-basis, long-chain n-3 PUFAs are about 9 times as potent as  $\alpha$ -linolenic acid with respect to this outcome in healthy subjects. In contrast with the observations of Caughey et al, several studies using lower intakes of  $\alpha$ -linolenic acid (2-9.5 g/d) did not find effects on neutrophil chemotaxis (20); neutrophil respiratory burst (14, 20, 54); monocyte respiratory burst (14, 54); TNF- $\alpha$ , IL-1 $\beta$ , or IL-6 production by endotoxin-stimulated mononuclear cells (14, 54, 70); ICAM-1 expression on monocytes (54); or soluble adhesion molecule concentrations (14). Taken together, these data suggest that increasing  $\alpha$ -linolenic acid intake to >10 g/d is required for antiinflammatory effects to be seen. Even then, the effects will be much more modest than those exerted by long-chain n-3 PUFAs (19).

## CONCLUSIONS

Inflammation is a component of a range of acute and chronic human diseases and is characterized by the production of inflammatory cytokines, arachidonic acid-derived eicosanoids, other inflammatory mediators, and adhesion molecules. Long-chain n-3 PUFAs decrease the production of inflammatory mediators (eicosanoids, cytokines, and reactive oxygen species) and the expression of adhesion molecules. They 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) (1, 4, 175-177). Long-chain n-3 PUFAs also give rise to antiinflammatory mediators (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. However, because information about the relative antiinflammatory potencies of EPA and DHA is lacking, comparisons between these 2 fatty acids in various settings should be made. Evidence of the clinical efficacy of long-chain n-3 PUFAs is 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 in inflammatory diseases to assess the therapeutic potential of long-chain n-3 PUFAs. The precursor n-3 PUFA  $\alpha$ -linolenic acid does not appear to exert antiinflammatory effects at achievable intakes. The antiinflammatory efficacy of n-3 PUFAs may be improved if intakes of n-6 PUFAs, especially arachidonic acid, are decreased. 

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