n−3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases1–3

Philip C Calder

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) E2; 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-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 diseases.

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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.

**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 PGE2 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, PGE2 possesses both pro- and antiinflammatory actions (Table 1).

**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.

**FIGURE 2.** Diagrammatic representation of the movement of leukocytes through the endothelium and the subsequent generation of inflammatory mediators.
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 PGE2 (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 PGE2 and LTB4 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 E2 (PGE2) and leukotriene B4 (LTB4)

<table>
<thead>
<tr>
<th>PGE2</th>
<th>Proinflammatory</th>
<th>Antiinflammatory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Induces fever</td>
<td>Inhibits production of TNF and IL-1</td>
</tr>
<tr>
<td></td>
<td>Increases vascular permeability</td>
<td>Inhibits 5-LOX (decreases 4-series LT production)</td>
</tr>
<tr>
<td></td>
<td>Causes pain</td>
<td>Induces 15-LOX (increases lipoxin production)</td>
</tr>
<tr>
<td></td>
<td>Enhances pain caused by other agents</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increases production of IL-6</td>
<td></td>
</tr>
<tr>
<td>LTB4</td>
<td>Proinflammatory</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increases vascular permeability</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enhances local blood flow</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chemotactic agent for leukocytes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Induces release of lysosomal enzymes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Induces release of reactive oxygen species by granulocytes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increases production of TNF, IL-1, and IL-6</td>
<td></td>
</tr>
</tbody>
</table>

1 IL, interleukin; LOX, lipoxygenase; TNF, tumor necrosis factor. Modified from reference 4 with permission from the American Oil Chemists’ Society.
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 PGE2 (16, 19, 21, 22), thromboxane B2 (19), LTB4 (15, 17), 5-hydroxyeicosatetraenoic acid (15, 17), and LTE4 (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 PGE2 (by 60%) and LTB4 (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 LTB4, LTE4, and 5-hydroxyeicosapentaenoic acid by inflammatory cells (15, 17, 23), although generation of PGE3 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, LTB4 is 10- to 100-fold less potent as a neutrophil chemotactic agent than LTB4 (26, 27). Recent studies have compared the effects of PGE2 and PGE3 on production of cytokines by cell lines and by human cells. Bagga et al (5) reported that PGE3 was a less potent inducer of COX-2 gene expression in fibroblasts and of IL-6 production by macrophages. However, PGE2 and PGE3 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).

**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 PGE2 (16, 19, 21, 22), thromboxane B2 (19), LTB4 (15, 17), 5-hydroxyeicosatetraenoic acid (15, 17), and LTE4 (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 PGE2 (by 60%) and LTB4 (by 75%) by endotoxin-stimulated mononuclear cells.

**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.

**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.
Long-chain n-3 PUFAs AND INFLAMMATION

The high dose of long-chain n-3 fatty acids, primarily through their derivative eicosanoids, has 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 LTB4, 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. Supplemeting the diet of healthy humans with fish oil providing \( \approx 1.5 \) 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* |

<table>
<thead>
<tr>
<th>Antiinflammatory effect</th>
<th>Mechanism likely to be involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased content of EPA and DHA in cell membrane phospholipids</td>
<td>Decreased content of EPA and DHA in cell membrane phospholipids</td>
</tr>
<tr>
<td>Decreased arachidonic acid–derived eicosanoids</td>
<td>Increased content of EPA and DHA in cell membrane phospholipids</td>
</tr>
<tr>
<td>Decreased generation of arachidonic acid–derived eicosanoids (many with inflammatory actions)</td>
<td>Decreased arachidonic acid in cell membrane phospholipids; inhibition of arachidonic acid metabolism; decreased induction of COX-2, 5-LOX, and 5-LOX activating protein</td>
</tr>
<tr>
<td>Increased expression of adhesion molecules</td>
<td>Decreased generation of arachidonic acid–derived eicosanoids</td>
</tr>
<tr>
<td>Decreased leukocyte chemotaxis</td>
<td>Decreased activation of NF( \kappa B ) (via decreased phosphorylation of I( \kappa B )); altered activity of other transcription factors; differential effects of arachidonic acid– vs EPA-derived eicosanoids</td>
</tr>
<tr>
<td>Decreased generation of reactive oxygen species</td>
<td>Decreased generation of reactive oxygen species</td>
</tr>
<tr>
<td>Decreased generation of inflammatory cytokines (TNF-( \alpha ), IL-1( \beta ), IL-6, and IL-8)</td>
<td>Decreased activation of NF( \kappa B ) (via decreased phosphorylation of I( \kappa B )); altered activity of other transcription factors</td>
</tr>
<tr>
<td>Decreased generation of eicosanoids (many with inflammatory actions)</td>
<td>Not clear; perhaps decreased expression of receptors for some chemoattractants</td>
</tr>
<tr>
<td>Decreased generation of arachidonic acid</td>
<td>Not clear; perhaps decreased expression of receptors for some chemoattractants</td>
</tr>
</tbody>
</table>

* 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.
Both EPA and DHA resulted in decreased plasma TNF-α production. This is confirmed by a study in which persons both EPA (19) and DHA (24) can decrease inflammatory cytokine production. Thus, although most studies have used fish oil, it appears that those who are more likely and those who are less likely to experience specific antiinflammatory effects of fish oil.

Long-chain n–3 PUFAs and inflammatory cytokine production

Cell culture studies show that EPA and DHA can inhibit the production of IL-1β and TNF-α 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-α, IL-1β, 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-α and IL-1β 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-α (by 20%) and IL-1β (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-α 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-α and the +252 TNF-β 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 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.
Several studies have reported antiinflammatory effects of fish oil in patients with rheumatoid arthritis, such as decreased LTB₄ production by neutrophils (101–104) and monocytes (103, 105), decreased IL-1 production by monocytes (106), decreased plasma IL-1β 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 PUFA s used in these trials was between 1.6 and 7.1 g/d and averaged ≈ 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 PUFA s 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 PUFA s 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 A₂ and LTB₄ 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 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 PUFA s by ultrasound,
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 4

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

<table>
<thead>
<tr>
<th>Reference</th>
<th>Dose of EPA + DHA (g/d wk)</th>
<th>Duration</th>
<th>Placebo</th>
<th>Clinical outcomes improved with long-chain n-3 PUFAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>(101)</td>
<td>1.8 + 1.2</td>
<td>12</td>
<td>Paraffin oil</td>
<td>Number of tender joints; duration of morning stiffness</td>
</tr>
<tr>
<td>(102)</td>
<td>2.7 + 1.8</td>
<td>14</td>
<td>Olive oil</td>
<td>Number of tender joints; number of swollen joints; time to fatigue; physician’s global assessment</td>
</tr>
<tr>
<td>(103)</td>
<td>3.2 + 2.0</td>
<td>12</td>
<td>Olive oil</td>
<td>Number of tender joints; grip strength</td>
</tr>
<tr>
<td>(104)</td>
<td>2.0 + 1.3</td>
<td>12</td>
<td>Coconut oil</td>
<td>Number of swollen joints; duration of morning stiffness</td>
</tr>
<tr>
<td>(105)</td>
<td>2.0 + 1.3</td>
<td>12</td>
<td>Coconut oil</td>
<td>Number of swollen joints; joint pain index</td>
</tr>
<tr>
<td>(109)</td>
<td>1.8 + 1.2</td>
<td>24</td>
<td>Mixed oils</td>
<td>Number and severity of tender joints; physician’s global assessment; use of NSAIDs</td>
</tr>
<tr>
<td>(110)</td>
<td>2.0 + 1.2</td>
<td>12</td>
<td>Mixed oils</td>
<td>Number and severity of tender joints</td>
</tr>
<tr>
<td>(111)</td>
<td>3.8 + 2.0</td>
<td>16</td>
<td>Corn oil</td>
<td>Number and severity of tender joints; duration of morning stiffness</td>
</tr>
<tr>
<td>(112)</td>
<td>1.7 + 1.1</td>
<td>52</td>
<td>Air</td>
<td>Use of NSAIDs</td>
</tr>
<tr>
<td>(113)</td>
<td>1.7 + 0.4</td>
<td>52</td>
<td>Olive oil</td>
<td>Physician’s pain assessment; patient’s global assessment; use of NSAIDs or DMARDs</td>
</tr>
<tr>
<td>(114)</td>
<td>4.6 + 2.5</td>
<td>26–30</td>
<td>Corn oil</td>
<td>Number of tender joints; duration of morning stiffness; physician’s assessment of pain; physician’s global assessment; patient’s global assessment</td>
</tr>
<tr>
<td>(115)</td>
<td>Total 40 mg/kg (≈2.2–3.0)</td>
<td>15</td>
<td>Mixed oils</td>
<td>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</td>
</tr>
<tr>
<td>(116)</td>
<td>≈2.4 + 1.8</td>
<td>12</td>
<td>Corn oil</td>
<td>Number of swollen joints; number of tender joints; patient’s global assessment; physician’s global assessment; patient’s assessment of pain</td>
</tr>
<tr>
<td>(117)</td>
<td>1.4 + 0.2 (+ 0.5 γ-linolenic acid)</td>
<td>16</td>
<td>Liquid supplement without added PUFA</td>
<td>None</td>
</tr>
<tr>
<td>(118)</td>
<td>Total 3.0</td>
<td>24</td>
<td>Soybean oil</td>
<td>Duration of morning stiffness; joint pain; time to onset of fatigue; Ritchie’s articular index; grip strength, patient’s global assessment</td>
</tr>
</tbody>
</table>

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

2 Published too late to be considered.
The pathophysiology of inflammatory bowel diseases suggests
the established role of arachidonic acid–derived eicosanoids in
of PGE2 by blood mononuclear cells (138). Small open-label or
production by colonic mucosa (133), and decreased production
of inflammatory cytokines and eicosanoids such as LTB4 (128).

Overview of placebo-controlled studies using long-chain n−3 fatty acids (fish oil) in patients with inflammatory bowel diseases

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

1 AHRQ, Agency for Healthcare Research and Quality; CD, Crohn Disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; UC, ulcerative colitis.
2 Published in abstract form only.
3 Published too late to be considered.

of inflammatory cytokines and eicosanoids such as LTB₄ (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 PUFAs 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 LTB₄ production by neutrophils (134–136) and colonic mucosa (136, 137), decreased PGE₂ and thromboxane B₂ production by colonic mucosa (133), and decreased production of PGE₂ 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, sigmoidoscopic 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),
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 n-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$_2$, LTC$_4$, LTD$_4$, and LTE$_4$ 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$_2$ 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 bronchial 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 α-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 α-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-α, IL-1β, 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 α-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 α-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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