Dietary polyunsaturated fatty acids and inflammatory mediator production[1,2]

Michael J James, Robert A Gibson, and Leslie G Cleland

ABSTRACT Many antiinflammatory pharmaceutical products inhibit the production of certain eicosanoids and cytokines and it is here that possibilities exist for therapies that incorporate $n-3$ and $n-9$ dietary fatty acids. The proinflammatory eicosanoids prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) are derived from the $n-6$ fatty acid arachidonic acid (AA), which is maintained at high cellular concentrations by the high $n-6$ and low $n-3$ polyunsaturated fatty acid content of the modern Western diet. Flaxseed oil contains the 18-carbon $n-3$ fatty acid α-linolenic acid, which can be converted after ingestion to the 20-carbon $n-3$ fatty acid eicosapentaenoic acid (EPA). Fish oils contain both 20- and 22-carbon $n-3$ fatty acids, EPA and docosahexaenoic acid. EPA can act as a competitive inhibitor of AA conversion to PGE_2 and LTB_4, and decreased synthesis of one or both of these eicosanoids has been observed after inclusion of flaxseed oil or fish oil in the diet. Analogous to the effect of $n-3$ fatty acids, inclusion of the 20-carbon $n-9$ fatty acid eicosatrienoic acid in the diet also results in decreased synthesis of LTB_4. Regarding the proinflammatory cytokines, tumor necrosis factor α and interleukin 1β, studies of healthy volunteers and rheumatoid arthritis patients have shown $\leq 90\%$ inhibition of cytokine production after dietary supplementation with fish oil. Use of flaxseed oil in domestic food preparation also reduced production of these cytokines. Novel antiinflammatory therapies can be developed that take advantage of positive interactions between the dietary fats and existing or newly developed pharmaceutical products. *Am J Clin Nutr* 2000;71(suppl):343S–8S.

KEY WORDS Inflammation, fish oil, flaxseed, eicosapentaenoic acid, cytokine, prostaglandin, eicosanoid, $n-3$ fatty acid, polyunsaturated fatty acid

INTRODUCTION

Inflammation is characterized symptomatically by pain, redness, and swelling; disordered or excessive inflammation also entails loss of function. This clinical pathology results from the release of inflammatory mediators, predominantly from activated leukocytes that migrate into the target area. Among the key inflammatory mediators are the $n-6$ eicosanoids, prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4), which are derived from the $n-6$ polyunsaturated fatty acid (PUFA) arachidonic acid (AA; 20:4$n-6$) (1). Also important are the cytokines, interleukin

1β (IL-1β) and tumor necrosis factor α (TNF-α), and there is strong evidence for the involvement of TNF-α in the joint pathology of rheumatoid arthritis (2, 3).

Many antiinflammatory pharmacotherapies are directed at inhibiting the production of these inflammatory mediators and thus possibilities exist for therapies that incorporate $n-3$ and $n-9$ dietary fatty acids. However, there are competitive interactions between dietary PUFAs; thus, in any examination of the effects of dietary $n-3$ or $n-9$ PUFAs, it is important to consider also the background dietary $n-6$ PUFAs.

POLYUNSATURATED FATTY ACIDS IN THE DIET

In the typical Western diet, 20–25-fold more $n-6$ fats than $n-3$ fats are consumed (4). This predominance of $n-6$ fat is due to the abundance in the diet of linoleic acid (LA; 18:2$n-6$), which is present in high concentrations in soy, corn, safflower, and sunflower oils. By contrast, there is a low intake of the $n-3$ homologue of LA, α-linolenic acid (ALA; 18:3$n-3$), which is present in leafy green vegetables and in flaxseed and canola oils. Once ingested, the 18-carbon fatty acids are desaturated and elongated to 20-carbon $n-6$ fatty acids. LA is converted to AA and ALA is converted to eicosapentaenoic acid (EPA; 20:5$n-3$). Compared with LA, there is little dietary intake of AA and EPA, which are present in meat and fish, respectively.

LA and ALA are necessary for complete health and cannot be synthesized in vertebrates; therefore, they are essential fatty acids. As a consequence, the relative dietary amounts of $n-6$ and $n-3$ fatty acids are determinants of the relative cellular amounts of LA and ALA (**Figure 1**).

Unlike the 18-carbon $n-3$ fatty acid ALA, oleic acid (18:1$n-9$), is consumed in substantial amounts in the typical Western diet and is not an essential fatty acid. There is little eicosatrienoic acid (ETA; 20:3$n-9$) in cell membranes, however, probably because of the overwhelming competition from dietary LA for the relevant desaturase and elongase enzymes (5) (Figure 1).

Fatty acid designation	n−9	n−6	n−3
18-Carbon fatty acids	Oleic acid	Linoleic acid	α-Linolenic acid
		(these are dietary essential fatty acids)	
Dietary sources	Olive oil, Sunola oil, meat	Soy, corn, sunflower, safflower oils	Flaxseed, canola, soy oils
Dietary intake	Large intake (8−15% dietary energy)	Large intake (7−8% dietary energy)	Minor intake (0.3−0.4% dietary energy)
	↓	↓	↓
20-Carbon fatty acids	Eicosatrienoic acid	Arachidonic acid	Eicosapentaenoic acid
Sources	Potentially synthesized from oleic acid	Mainly synthesized from ingested linoleic acid, relatively small amounts in diet (meat, offal)	Synthesized from ingested α-linolenic acid or ingested as fish or fish oil
Leucocyte content (% of total fatty acids)	*0.1%*	*10−16%*	*0.1−0.3%*

FIGURE 1. Dietary fatty acids and their metabolism after ingestion. Sunola oil; Meadow Lea Foods Ltd, Sydney, Australia.

EFFECT OF DIETARY n−3 AND n−9 FATS ON LIPID (EICOSANOID) INFLAMMATORY MEDIATORS

PGE_2 and LTB_4 have proinflammatory biological actions. PGE_2 can cause pain and vasodilation and LTB_4 is a chemoattractant and activator of neutrophils; together they can cause vascular leakage and extravasation of fluid (1). Thus, the lipid mediators may be involved in the pain, redness, and swelling that occurs in acute inflammation.

AA is the progenitor of both PGE_2 and LTB_4 via the cyclooxygenase and 5-lipoxygenase enzymatic pathways, respectively.

EPA, the n−3 homologue of AA, can inhibit AA metabolism competitively via these enzymatic pathways and, thus, can suppress production of the n−6 eicosanoid inflammatory mediators. EPA is a potential cyclooxygenase substrate for the synthesis of PGE_3, which also has inflammatory activity, although PGE_3 synthesis occurs with low efficiency or not at all (6, 7). EPA is also a 5-lipoxygenase substrate and can lead to the formation of LTB_5, but LTB_5 has little inflammatory activity compared with LTB_4 (8, 9). Thus, increasing dietary n−3 fats can shift the balance of the eicosanoids produced to a less inflammatory mixture (**Figure 2**).
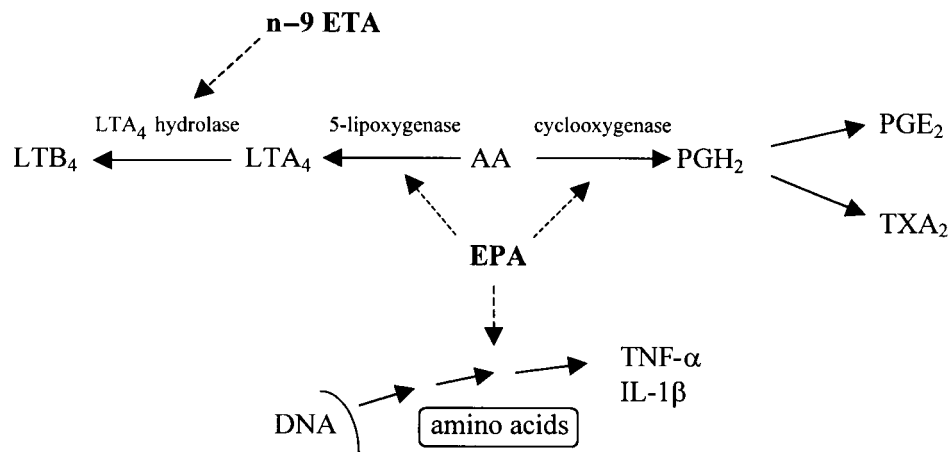
FIGURE 2. Effect of n−3 and n−9 fatty acids on the production of lipid and peptide inflammatory mediators. The solid arrows indicate synthesis and the dashed arrows indicate inhibition. ETA, eicosatrienoic acid, LT, leukotriene; AA, arachidonic acid; PG, prostaglandin; TX, thromboxane; EPA, eicosapentaenoic acid; TNF-α, tumor necrosis factor α; IL-1β, interleukin 1β.

There is potential also for use of dietary ETA for antagonism of AA conversion to LTB_4. ETA is a substrate of 5-lipoxygenase and although LTA_3 formation occurs, LTA_3 is a poor substrate but a good inhibitor of leukotriene-A_4 hydrolase, the enzyme necessary for LTB_4 synthesis in addition to arachidonate 5-lipoxygenase (10, 11). Inhibition of LTB_4 synthesis via inhibition of leukotriene-A_4 hydrolase has been observed in rats (12) and humans (13) during severe LA restriction, leading to elevated ETA concentrations. Furthermore, when ETA was included in the diet of (LA-sufficient) rats, it was incorporated into cell membranes and inhibited LTB_4 synthesis via inhibition of leukotriene-A_4 hydrolase (14–16). Thus, in partial analogy to the situation with EPA, increasing dietary ETA intake can also alter the balance of eicosanoids produced by leukocytes toward a potentially less inflammatory mixture (Figure 2). The effect of ETA on prostaglandin-endoperoxide synthase (cyclooxygenase) is less clear than that with 5-lipoxygenase. ETA lacks the n−6 double bond necessary for prostanoid formation, but may inhibit PGE_2 production because inhibition of endothelial PGI_2 production has been attributed to ETA (17).

EFFECT OF DIETARY n−3 FATS ON PEPTIDE (CYTOKINE) INFLAMMATORY MEDIATORS

The cytokines IL-1β and TNF-α have proinflammatory cellular actions that include stimulating the production of collagenases (2, 18) and increasing the expression of adhesion molecules necessary for leukocyte extravasation (19). More direct biological evidence for the importance of these cytokines in inflammatory joint disease includes the observations that intraarticular IL-1β causes arthritis in rabbits (20), that mice transgenic for a modified human TNF gene that is constitutively expressed develop polyarthritis that is prevented by anti-TNF monoclonal antibody (21), and that intravenous administration of anti-TNF-α monoclonal antibody to patients with rheumatoid arthritis suppresses joint inflammation (3). Whereas the eicosanoids may mediate much of the early pathology of inflammatory joint disease, such as swelling, pain, and leukocyte infiltration, cytokines have been implicated in the late, destructive phase of the disease, which includes cartilage loss, bone resorption, and, ultimately, joint failure (2).

Inclusion of n−3 fats in the diet can suppress the production of both TNF-α and IL-1β. Fish oil is rich in the 20-carbon and 22-carbon n−3 fatty acids, EPA and docosahexaenoic acid (DHA; 22:6n−3) and it has been shown that dietary supplementation with encapsulated fish oil results in decreased monocyte synthesis of TNF-α, IL-1β, or both in healthy subjects (22–24) and in patients with rheumatoid arthritis (25). Results of these

studies are summarized in **Table 1**. Although it is known that fish-oil ingestion effectively elevates cellular concentrations of EPA and DHA, it is not known whether EPA, DHA, or both are involved in the suppression of cytokine production.

An alternate approach to elevating cellular EPA concentrations is to increase intake of ALA, the progenitor of EPA. When flaxseed oil, which contains ≈56% ALA, was used by healthy male volunteers in their domestic food preparation, leukocyte EPA concentrations were increased and both IL-1β and TNF-α production were suppressed by ≈30% after 4 wk (**Figure 3**) (24).

The successful application of any of these dietary approaches to suppressing excessive cytokine production in inflammatory disease rests in our knowledge of the dose-response relations and the mechanisms of action of n−3 fatty acids in the suppression of cytokine production. Regarding the first of these issues, there is an inverse exponential relation between mononuclear cell EPA content and cytokine production. Whether the cellular source of EPA is the diet or endogenous synthesis from dietary ALA, cytokine synthesis decreases as cellular EPA concentrations increase to ≈1% of total membrane fatty acids. Further increases in EPA content, however, do not result in further measurable decreases in cytokine production (**Figure 4**) (24).

The mechanisms responsible for the suppression of cytokine production by n−3 fatty acids remain unknown, although suppression of eicosanoid production by n−3 fatty acids may be involved. Ingestion of n−3 fatty acids leads to suppression of thromboxane A_2 (TXA_2) synthesis by platelets and by gradient preparations of blood mononuclear cells (24). In the mononuclear cell preparations, the TXA_2 is derived from contaminating platelets but also from the monocytes, where it is a major eicosanoid product (26). When TXA_2, but not PGE_2, synthesis was inhibited in purified human monocytes, TNF-α and IL-1β synthesis were inhibited and the inhibition was overcome by the addition of an active TXA_2 analogue. Also, cytokine synthesis was inhibited by TXA-receptor antagonists. Collectively, these results provide evidence of a role for TXA_2 as an autocrine or paracrine facilitator of cytokine synthesis (26). However, other results suggest that the relation between monocyte eicosanoid and cytokine production probably reflects a balance between the opposing effects of TXA_2 and PGE_2 (26). Because n−3 fatty acids inhibit PGE_2 synthesis also, inhibition of TXA_2 synthesis may provide only one element of a complex mechanism responsible for inhibition of cytokine synthesis by n−3 fatty acids.

DIETARY CONSIDERATIONS

When fortifying diets with either EPA, ETA, or both with therapeutic or health-enhancing intent, the background n−6 PUFA

TABLE 1
Studies reporting inhibition of tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) production after dietary fish oil supplementation[1]

Subjects and reference	n	Dietary advice or intervention	Amount of ingested n−3 PUFAs	Inhibition of TNF-α	Inhibition of IL-1β
				%	*%*
Healthy males (22)	9	None	2.7 g EPA, 1.8 g DHA	40	61
Rheumatoid arthritis patients (25)	17	None	3.5 g EPA[2], 2.3 g DHA[2]	Not measured	54
Healthy women (23)	12	None	1.7 g EPA, 0.7 g DHA	58–70	48–90
Healthy men (24)	15	High LA, low ALA	1.6 g EPA, 1.1 g DHA	70	78
Healthy men (24)	13	High LA, low ALA	1.6 g EPA, 1.1 g DHA	77	81

[1] PUFA, polyunsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; ALA, α-linolenic acid.

[2] Amounts of n−3 PUFAs ingested are based on a 65-kg subject.
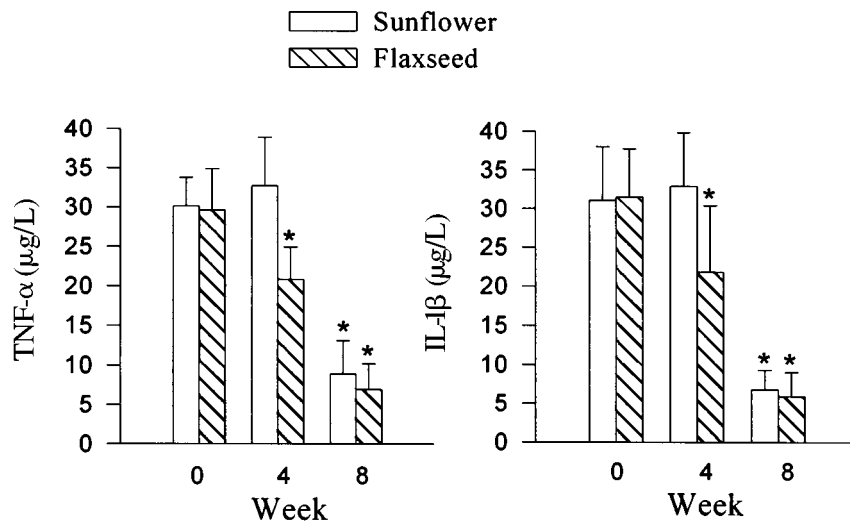
FIGURE 3. Effect on cytokine production by human peripheral blood mononuclear cells of dietary α-linolenic acid from flaxseed oil and dietary eicosapentaenoic acid from fish oil: 0 wk, baseline; 4 wk, after 4 wk of the flaxseed or sunflower oil diets; 8 wk, after an additional 4 wk of the same diets plus fish-oil supplementation (9 g/d). TNF-α, tumor necrosis factor α; IL-1β, interleukin 1β. $\bar{x} \pm$ SD; $n = 15$ for all groups. *Significantly different from baseline value at week 0, $P < 0.05$. Data are from reference 24.

content of the diet is a key issue. The competitive interactions between n−6 and n−3 dietary fatty acids that determine the cellular content of the long-chain, highly unsaturated n−6 and n−3 fatty acids such as AA and EPA have been studied and well described mathematically (5, 27). When competition from the major dietary PUFA, LA, was specifically examined, it was observed that higher tissue concentrations of EPA and ETA occurred when these fatty acids were ingested within diets of lower LA content (16, 28). In at least the United States and Australia, the widespread dietary use of LA-rich oils such as soybean, corn, sunflower, and safflower oils results in intakes of LA at ≈7–8% of dietary energy (29, 30). Whether n−3 or n−9 fats are considered for use as components of foodstuffs or as dietary supplements, the LA content of the modern Western diet merits attention because of its negative effect on tissue concentrations of n−3 and n−9 fatty acids.

FUTURE ROLE OF DIETARY POLYUNSATURATED FATTY ACIDS IN THERAPIES FOR INFLAMMATORY DISORDERS

A beneficial clinical effect of dietary supplementation with fish oil on rheumatoid arthritis was observed in at least 11 double-blind, placebo-controlled studies, and in the studies in which drug use was examined, there was partial sparing of nonsteroidal antiinflammatory drug use [summarized in references 31 and 32 and discussed in detail by Kremer in this supplement (33)]. Common features of the clinical studies that may have moderated the size of the effects observed were that *1*) all studies were conducted against unmodified Western diets, ie, diets high in LA, and *2*) the usual antiinflammatory and antirheumatic medications were used in addition to fish oil in amounts likely to confer maximum suppression of their common molecular and cellular targets.
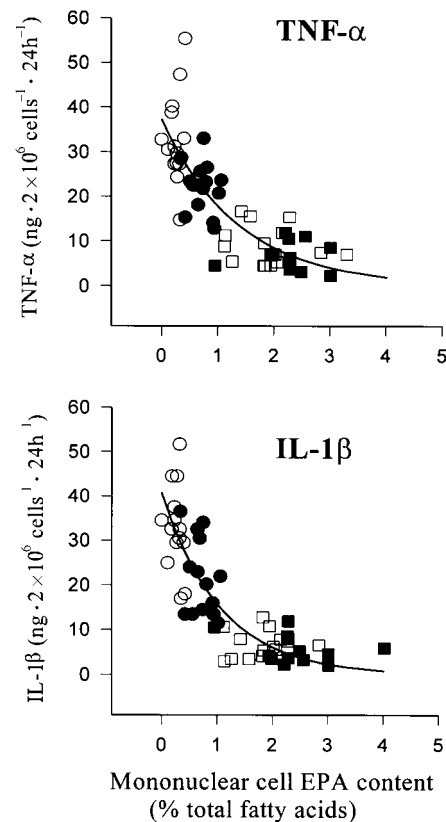

FIGURE 4. Relation between mononuclear cell eicosapentaenoic acid (EPA) content and cytokine production. Open symbols, sunflower oil diet; closed symbols, flaxseed oil diet; circles, 4 wk of vegetable oil diets; squares, additional 4 wk of the same diets plus fish oil; TNF-α, tumor necrosis factor α; IL-1β, interleukin 1β. Reproduced with permission from reference 24.
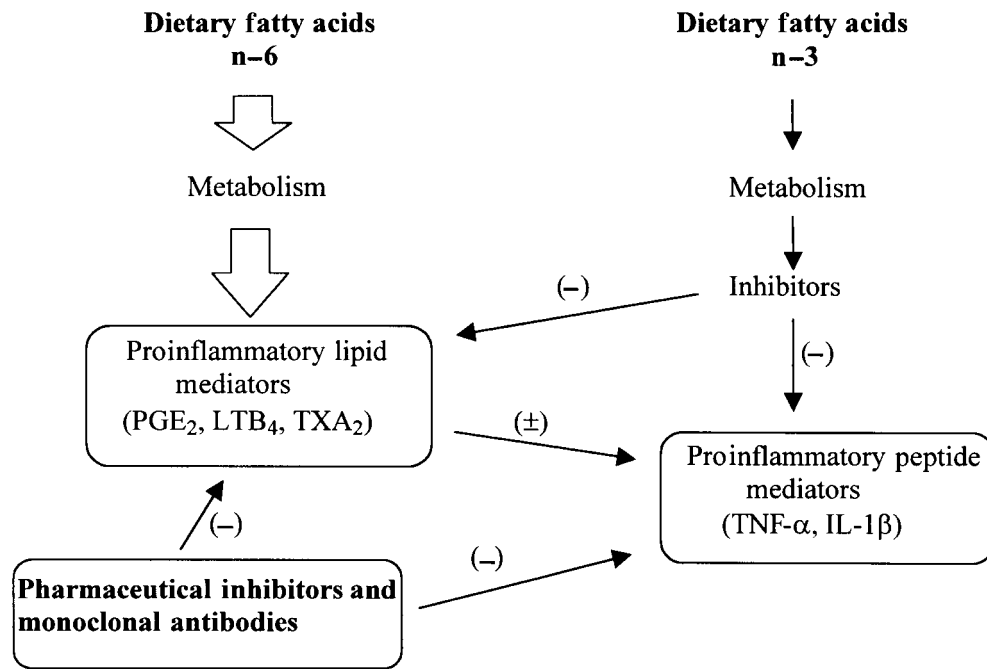
**Dietary fatty acids
n−6**

**Dietary fatty acids
n−3**

Metabolism

Metabolism

Inhibitors

(−)

Proinflammatory lipid
mediators
(PGE$_2$, LTB$_4$, TXA$_2$)

(−)

(±)

Proinflammatory peptide
mediators
(TNF-α, IL-1β)

(−)

(−)

**Pharmaceutical inhibitors and
monoclonal antibodies**

FIGURE 5. Scheme depicting common molecular targets for antiinflammatory therapies that use dietary n−3 fatty acids and pharmaceuticals. PGE$_2$, prostaglandin E$_2$; LTB$_4$, leukotriene B$_4$; TXA$_2$, thromboxane A$_2$; TNF-α, tumor necrosis factor α; IL-1β, interleukin 1β.

The first issue could be addressed by substituting vegetable oils rich in monounsaturated fatty acids for the current oils rich in LA and, preferably, by also using oils with substantial ALA content. The second issue could be addressed by optimizing the possible additive effects of drug-diet combinations. Antiinflammatory drug use could be decreased in some patients with rheumatoid arthritis in concert with increased fish-oil ingestion if both the drug and fish oil are exerting their therapeutic effects through the same molecular actions, eg, inhibition of PGE$_2$ and TXA$_2$ production. This might also apply to new drugs or new treatment modalities that aim to suppress cytokine concentrations, ie, there may be an opportunity for beneficial additive effects with fish-oil supplementation or any other dietary approach to increasing intake of n−3 fats. Similar arguments apply to use of ETA with drugs that target LTB$_4$ synthesis. Thus, the possibility exists for drug-diet interactions that confer greater antiinflammatory benefits than either agent alone or similar antiinflammatory effects with less toxicity (**Figure 5**). Investigation of potentially beneficial interactions will require greater knowledge of the dose-response effects for both the drug (including biological agent therapies) and the dietary intake of n−3 PUFAs.

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