Dietary polyunsaturated fats and inflammation

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Summary

Dietary polyunsaturated fats are classified as n-3 or n-6 according to their double bond chemistry and these chemical differences confer differential biological effects on fatty acids from these two classes. In the modern Australian diet, the intake of n-6 fats exceeds that of n-3 fats by approximately 25-fold. This relative abundance of n-6 fat intake is reflected in the cell membranes where the ratio of n-6:n-3 PUFA is approximately 7:1. While this relative excess of n-6 to n-3 fat has been driven by agricultural and industrial changes as well as dietary changes aimed at lowering blood cholesterol levels, there is considerable evidence that increasing the amount of dietary n-3 fat can suppress inflammatory mediator production and can suppress inflammation. Animal studies using models of inflammatory disease have demonstrated that ingestion of fish oil, rich in n-3 fats, can suppress inflammation. In human studies, at least 11 double-blind, placebo-controlled clinical trials with rheumatoid arthritis patients have demonstrated that dietary supplements of fish oil can provide symptomatic benefits. The mechanisms for these clinical responses lie in the effects which n-3 fats have on the production of inflammatory mediators. Dietary fish oil which contains 20- and 22-carbon n-3 fatty acids and flaxseed oil which contains their 18-carbon n-3 progenitor fatty acid, can inhibit the production of the eicosanoid inflammatory mediators, prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) and the cytokine inflammatory mediators, interleukin-1β (IL-1β) and tumour necrosis factor-α (TNFα). Because n-6 fats can decrease the levels of n-3 fats in cell membranes, it is most likely that the optimum anti-inflammatory effects of n-3 fats will be within the context of diets also containing lower levels of n-6 fats than those in the current Australian diet.

Introduction

There has been a tendency amongst nutritionists to consider polyunsaturated fatty acids as a single group of fatty acids which are unlike saturates in that they do not elevate blood cholesterol levels. However, dietary polyunsaturates can be divided into two biochemical families based on the position of the double bond proximal to the methyl end of the molecule. These families are designated n-6 and n-3, but are also known as omega-6 and omega-3, respectively. These designations have biological significance in that n-3 and n-6 fatty acids frequently have different biological actions. The following presentation will discuss the role of dietary n-6 and n-3 fats as determinants of inflammation and the possible cellular and biochemical mechanisms responsible.

Polyunsaturates in the diet

In the typical Western diet, the n-6 fats are present at levels 20 to 25-fold those of n-3 fats (1). The n-6 fat predominance is due to the abundance of the 18-carbon n-6 fatty acid, linoleic acid (LA; C18:2 n-6), which is present at high levels in sunflower, safflower, and corn oil. By contrast, there is a low intake of its 18-carbon n-3 homologue, α-linolenic acid (α-LNA; C18:3 n-3) which is present in leafy green vegetables and in flaxseed oil and canola oil. Once ingested, the 18-carbon fatty acids are desaturated and elongated to 20-carbon fatty acids. LA is converted to arachidonic acid (AA; C20:4 n-6) and α-LNA is converted to eicosapentaenoic acid (EPA; C20:5 n-3), a 20-carbon n-3 fatty acid. Compared with LA, there is little dietary intake of AA and EPA which are present in meat and fish, repectively.

Because these fatty acids are necessary for complete health and because they cannot be synthesized in vertebrates, they are dietary essential fatty acids. As a consequence, the relative levels of dietary

n-6 and n-3 fatty acids are determinants of their relative cellular levels and these, in turn, can be determinants of cell function (Figure 1).

Fatty acid families	**n-6** (denotes the position of the double bond proximal to the omega terminus)	**n-3**
	↓	↓
18-Carbon fatty acids	**linoleic acid** (these are dietary essential fatty acids)	**α-linolenic acid**
Dietary sources	sunflower, safflower corn oil	flaxseed, canola oil
Dietary intake	Large intake (7-8% dietary energy)	Minor intake (0.3-0.4% dietary energy)
	↓	↓
20-Carbon fatty acids	**arachidonic acid**	**eicosapentaenoic acid (EPA)**
Sources	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
Amount in leucocytes (% of total fatty acids)	10 to 16%	0.1 to 0.3%

Figure 1. Dietary fats and their metabolism after ingestion.

Effect of dietary n-3 fats on lipid (eicosanoid) mediators of inflammation

AA can be converted by cyclooxygenase and 5-lipoxygenase enzymes to the eicosanoids, prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4), respectively. These are both lipid inflammatory mediators with PGE_2 causing pain and vasodilatation, LTB_4 acting as a chemoattractant and activator of neutrophils, and in conjunction, they cause vessel leakage and extravasation of fluid. Together, they have actions which occur in acute inflammation and result in pain, redness, and swelling (2). By contrast, EPA, which is the n-3 homologue of AA, can antagonise AA metabolism and thereby decrease PGE_2 and LTB_4 production (3-5). EPA is a potential substrate for conversion to PGE_3 which has inflammatory activity, but PGE_3 formation occurs with low efficiency or not at all (6,7). EPA is converted to LTB_5 but this has little inflammatory activity compared with LTB_4 (8). Thus, increasing dietary n-3 fats can shift the balance of eicosanoids produced to a less inflammatory mixture (Figure 2).

Effect of dietary n-3 fats on peptide (cytokine) mediators of inflammation

The cytokines, interleukin-1β (IL-1β) and tumour necrosis factor-α (TNFα), have pro-inflammatory actions. They can stimulate collagenase production (9,10), increase expression of adhesion molecules which is necessary for leucocyte extravasation (11), intraarticular IL-1β is arthritogenic in rabbits (12), TNF transgenic mice develop polyarthritis which is prevented by anti-TNF monoclonal antibodies (13), and intravenous anti-TNF monoclonal antibody treatment in RA patients is a potent suppressor of disease (14). Whereas the eicosanoids may mediate many of the early pathogenic events in inflammatory joint disease such as swelling, leucocyte infiltration and

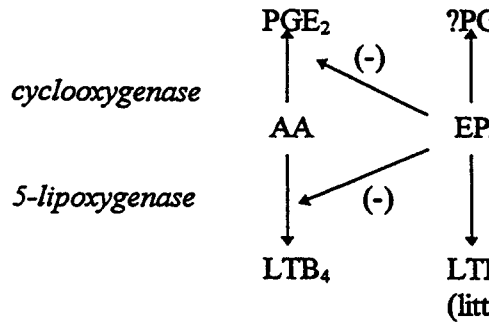

Figure 2. Effect of eicosapentaenoic acid (EPA) on arachidonic acid (AA) metabolism by cyclooxygenase or 5-lipoxygenase

pain, cytokines may mediate the late destructive phase of rheumatoid arthritis which includes cartilage loss and bone resorption resulting in eventual joint failure (15). Inclusion of n-3 fats in the diet can suppress the production of both IL-1β and TNFα.

Fish oil is rich in the 20- and 22-carbon n-3 fatty acids, EPA and docosahexaenoic acid (DHA). Dietary supplements of encapsulated fish oil resulted in decreased monocyte synthesis of TNFα and/or IL-1β in healthy subjects and in patients with rheumatoid arthritis (5,16-18). Results of these studies are summarized in Table 1.

The mechanisms responsible for the effect of fish oil on cytokine synthesis are not clear. While it is not clear whether EPA or DHA, or both are necessary, we observed an inverse exponential relationship between mononuclear cell EPA content and the level of cytokine production. As cellular EPA levels increase to approximately 1% of total fatty acids, cytokine synthesis decreased. Further increases in EPA were not accompanied by a further decline in cytokine sythesis (5).

Table 1. Studies reporting inhibition of TNFα and IL-1β production after dietary fish oil supplementation.

Subjects	No. of subjects	Dietary advice or intervention	Amount of ingested n-3 PUFA	Inhibition of TNFα (%)	Inhibition of IL-1β (%)
healthy males (16)	9	Nil	2.7 g EPA 1.8 g DHA	40%	61%
rheumatoid arthritis patients (17)	17	Nil	3.5 g EPA* 2.3 g DHA*	not done	54%
healthy women (18)	6	Nil	1.7 g EPA 0.7 g DHA	58 - 70%	48 - 90%
healthy males (5)	15	High LA, Low α-LNA	1.6 g EPA 1.1 g DHA	70%	78%
	13	Low LA, High α-LNA	1.6 g EPA 1.1 g DHA	77%	81%

* amounts of n-3 PUFA ingested are based on a 65 kg subject

In addition to demonstrating that fish oil supplementation suppressed cytokine synthesis, we demonstrated that ingestion of α-LNA, the n-3 progenitor of EPA, can lead also to suppression of cytokine synthesis (5). In this study, 30 healthy males were randomized to two groups. One group, the Sunflower group, maintained a normal, high n-6 and low n-3, diet by use of sunflower oil and its derivative products. The other group was supplied with flaxseed oil which is rich in α-LNA, and a flaxseed/butter spread and advised on the avoidance of n-6 fats from other dietary sources. This latter group, the Flaxseed oil group, maintained a low n-6 and high n-3 fat diet. Both groups maintained their diets for eight weeks. After the initial four weeks, both groups

supplemented their respective diets with 9 g fish oil / day for the last four weeks. At zero, four, and eight weeks, peripheral blood mononuclear cells were isolated and stimulated with bacterial lipopolysaccharide (LPS), and the cytokines produced were measured by ELISA (Figure 3).

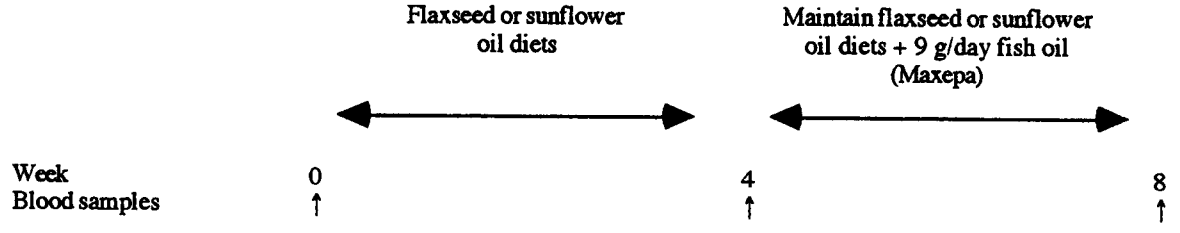

Figure 3. Scheme of experimental plan

Ingestion of flaxseed oil increased cellular EPA levels by more than 2-fold and resulted in approximately 30% inhibition of TNFα and IL-1β production after four weeks; there was no change in the sunflower oil group (Figure 4). After a further four weeks with fish oil ingestion, TNFα and IL-1β production were inhibited by 70 - 80% in both the sunflower and flaxseed oil groups (Figure 4). The synthesis of PGE_2 and thromboxane A_2 (TXA_2) were inhibited also by flaxseed or fish oil ingestion (results not shown).

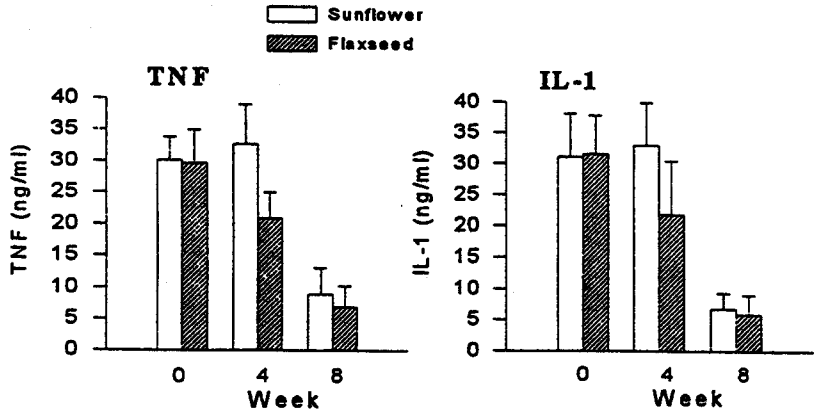

Figure 4. Effect on cytokine production by human peripheral blood mononuclear cells of dietary α-linolenic acid (α-LNA) from flaxseed oil and dietary eicosapentaenoic acid (EPA) from fish oil. 0 weeks, baseline; 4 weeks, after 4 weeks on the flaxseed or sunflower oil diets; 8 weeks, after a further four weeks of fish oil supplementation (9 g / day).

In this study, subjects used the flaxseed oil and spread for their domestic food preparation. Analysis of weighed food records indicated that α-LNA had been substituted for LA and there was no difference in total fat intake between the two groups.

Effect of dietary n-3 fats on rheumatoid arthritis

There have been at least 11 double-blind, placebo-controlled studies which examined the clinical effects in rheumatoid arthritis of increased dietary n-3 fats. All of these studies used fish oil supplementation. The total amount of n-3 fats (EPA + DHA) ingested daily varied between the studies from 1.0 to 7.1 g and the duration of the studies varied from 12 to 52 weeks. The

outcome measures included the number of tender joints, the number of swollen joints, the duration of morning stiffness, grip strength, patient and physician global assessment. In three of the studies, change in anti-inflammatory drug use was used also as an outcome measure.

In all studies, fish oil ingestion resulted in an improvement in at least two clinical measures and in four of the studies, at least four measures indicated symptomatic improvement. In the studies in which it was measured, anti-inflammatory drug use decreased as a result of fish oil ingestion. A more detailed summary of these studies has been published previously (19). Overall, there was a consistent beneficial effect of fish oil ingestion.

This beneficial effect was observable despite the presence of at least two potentially mitigating factors. Firstly, anti-inflammatory and anti-rheumatic drugs were continued at subject entry in all studies. Thus, the beneficial anti-inflammatory effects of fish oil which were observed were additional to those already provided by a full range of anti-inflammatory and anti-rheumatic medication. In clinical trials of new drugs in these classes, existing medication of the same class is ceased as a condition of study entry. In this respect, fish oil was tested under more stringent conditions than those applied in the testing of new anti-inflammatory or anti-rheumatic drugs.

The second potentially mitigating factor is the high n-6 fat content of the background diet. We have reported that, in rats and humans, dietary n-6 fats decrease the incorporation of dietary EPA into cells (4,20). In none of the studies was advice given to lower n-6 fat intake. In only four studies was dietary advice given and this varied from maintaining fat intake to increasing the (n-6) polyunsaturated fat intake (details in ref 19). It is expected that these modern Western diets provide suboptimal backgrounds for the anti-inflammatory effects of n-3 fats to be observed. Thus, when n-3 fats are ingested, the consequent tissue levels will be greater if dietary n-6 fats are restricted. This can be easily achieved by replacement with monounsaturated fats (20).

Conclusions

The biochemical and clinical effects of dietary n-3 fats suggest that they provide symptomatic benefits in rheumatoid arthritis in part, at least, by inhibiting the production of lipid inflammatory

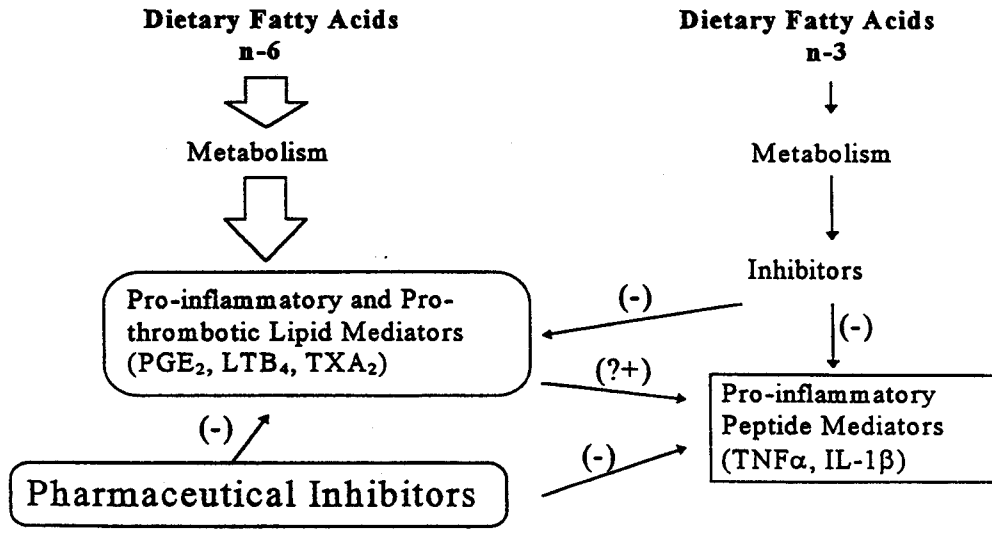

Figure 5. Schematic outlining the possible effects on inflammatory mediator production of anti-inflammatory drugs and dietary polyunsaturated fatty acids.

mediators (eicosanoids) and peptide inflammatory mediators (cytokines). Current pharmacotherapies for rheumatoid arthritis generally seek to inhibit the production of these same inflammatory mediators. However, it is probable that the current diet, high in n-6 and low in n-3

fats, does not provide the most favorable background for beneficial drug effects (Figure 5). While increased intake of n-3 fats can suppress inflammatory mediator production and have a beneficial effect in rheumatoid arthritis, this approach will be most efficient if dietary n-6 fat is decreased from the current levels of approximately 7% of dietary energy. Thus, increasing n-3 and decreasing n-6 fats are complementary changes. They are achievable by using a combination of monounsaturated oils such as olive or ®Sunola (Meadow Lea Foods Inc.) in conjunction with increased intake of n-3 fats from fish or vegetable oils such as flaxseed and canola. These products can provide practical choices within a total diet for increasing the ratio of n-3:n-6 fats ingested.

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