Dietary fatty acids and inflammation

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Abstract
Introduction: It is commonly believed that inflammation can be reduced by lowering the dietary ratio of n-6 (linoleic acid) to n-3 polyunsaturated fatty acids as a means of lowering arachidonic acid levels in cell membranes. This review will examine this proposition.

Results: Although many pro-inflammatory molecules can be produced from arachidonic acid and this long-chain n-6 fatty acid can be produced from linoleic acid, changing dietary linoleic acid intake over a wide range does not have any significant effect on arachidonic acid levels in cell membranes, inflammation or immune functions. There are no data that show lowering dietary n-6 polyunsaturated fats reduces inflammation. In contrast, arachidonic acid levels in cell membranes and inflammatory parameters are lowered by increasing intakes of long-chain n-3 polyunsaturated fatty acids. Resolvins and protectins, both derivatives of long-chain n-3 fats, also have potential anti-inflammatory activity. In high doses, long-chain n-3 fats may have modest, beneficial effects on inflammation in rheumatoid arthritis. However, many studies have shown no effects of fish oil on inflammation parameters.

Conclusions: The dietary n-6/n-3 ratio is not a useful measure of the inflammatory nature of a diet, though the absolute amount of dietary long-chain n-3 polyunsaturated fatty acids may be a guide.

Key words: linoleic acid, arachidonic acid, inflammation mediators, eicosanoids, prostaglandins

INTRODUCTION

Inflammation describes the body’s response to pathogens, allergens, dead and damaged cells and chemical and physical irritants. It is primarily a response mediated via the vascular system, in concert with the immune system in the case of pathogens and allergens, to bring more blood-borne chemicals and cells to the site of invasion or damage. Vasodilation, which increases blood flow, is one of the key primary events and in the skin leads to redness, hence the name from the Latin ‘inflammatio’—to set on fire. A second primary event is swelling as interstitial fluid increases as capillaries and venules become more leaky. This allows easy access of larger protein mediators and accumulation of white cells at the focus of damage or invasion—in initially neutrophils in acute inflammation and later lymphocytes and monocytes as the inflammation persists and becomes chronic. During the final resolution stage of inflammation, fibrosis might occur and impair tissue function. Significant damage can occur as a result of the inflammatory response and the resolution phase, which might be much greater than the original insult, so the process has many control check points, some of which clearly fail in chronic inflammatory disorders. Chronic inflammation typically occurs in auto-immune diseases, like rheumatoid arthritis where the stimulating auto-antigen is always present, but some bacterial infections, such as tuberculosis, become chronic as the invading organism is never eliminated. Both acute and chronic inflammations are part of the pathogenesis of atherosclerosis, with acute inflammation being particularly important in plaque rupture through dissolution of extracellular matrix by metalloproteinases.

The role of fatty acids in inflammation

Fatty acids can be saturated or unsaturated with the first double bonds in the 3, 6 or 9 position from the methyl end. Only longer-chain (>18 carbons) essential n-3 and n-6 fatty acids have a role in producing inflammatory mediators. Endogenously produced N9 fatty acids (e.g. oleic acid) have no inflammatory role. The most important fatty acid is the 20-carbon n-6 fatty acid arachidonic acid (AA), which is the basis for a whole family of inflammatory mediators the 20-carbon eicosanoids. AA can be derived directly from the diet, especially from meat, but is also derived from another important n-6 fatty acid linoleic acid (LA). AA produces both inflammatory molecules, such as prostaglandins D, E and F and thromboxanes and leukotrienes, and anti-inflammatory molecules, including prostacyclin and lipoxins, and the balance between pro- and anti-inflammatory compounds varies enormously depending on the clinical condition. Compounds derived from n-3 polyunsaturated fatty acids are also important in promoting and inhibiting inflammation.
and compete at all points with n-6 fats for elongation and desaturation (Figure 1), incorporation into membranes, release from membranes and conversion into prostaglandins and leukotrienes from eicosapentaenoic acid (EPA). Anti-inflammatory resolvins and protectins are derived from EPA and docosahexaenoic acid (DHA). Aspirin and non-steroidal anti-inflammatory drugs are important drugs that block production of all prostaglandins, but usually the pro-inflammatory components are affected more than anti-inflammatory compounds, although with specific cyclooxygenase 2 inhibitors this might not be true.

Higher cell membrane levels of AA would be expected to be pro-inflammatory, and lower levels anti-inflammatory, but what is the role of dietary fats in determining AA levels in cell membranes? AA is also found in plasma as a free fatty acid and also in plasma lipoproteins in triglycerides and phospholipids, but only exerts its inflammatory effects when membrane-bound and is freed intracellularly by a phospholipase.

**Effects of dietary linoleic acid on AA levels in cell membranes**

Although the pathway in Figure 1 shows that LA might be converted to AA, a direct relationship between dietary LA and cell membrane levels of AA only exists at very low intakes of LA, that is, essential fatty acid deficiency. Above this level of LA intake, the level of AA in membranes is relatively constant and controlled, and increasing dietary LA has no effect on cell membrane AA levels or inflammatory mediators derived from AA. These points are detailed in the studies below.

Lands et al. showed that in rats AA levels in cell membranes reached their maximum with dietary LA at 0.33% of energy; whereas in humans 2–3% of dietary energy as LA is required to avoid essential fatty acid deficiency. Thus, it would appear that the only way of lowering tissue AA levels through manipulating LA intakes is to lower LA intakes to deficient levels.

James et al. and Mantziouis et al. showed that there was no relationship between dietary LA and the tissue level of its major metabolite AA in white cells derived from humans on either free-living or controlled diets with high (17.5% of energy) and low (2.5% of energy) LA contents for four weeks.

Following dietary supplementation with long-chain n-3 fatty acids, no differences in neutrophil membrane AA levels or leukotriene B4 production were observed between the high and low LA diets. Thus, widely divergent levels of dietary LA had no effect on at least one major inflammatory molecule.

Other key inflammatory molecules appear to be little influenced by dietary LA. Blair et al. fed seven healthy women diets containing 3.0% or 8.3% energy from LA for 40 days on each diet. Urinary excretion of prostaglandin E2 (PGE2), a major inflammatory prostaglandin derived from AA, did not change significantly. Systemic thromboxane B2 production was lower on the low-LA diet, and renal and systemic prostacyclin and renal thromboxane A2 were not altered. Adam et al. used formula diets in six healthy women and found no effect on PGE2 of changing from 0 to 8% of energy as LA, but did find a 50% increase in PGE2 at 20% of energy as LA. It should be noted that these authors used a radioimmunoassay that was not selective for PGE2. Overall, very large changes in LA status are probably required to see changes in prostaglandin production.

Rallidis et al. found that feeding 15 mL/day of LA-rich safflower oil for 12 weeks had no effect on the inflammatory molecules serum amyloid A protein, interleukin-6 (IL-6) or C reactive protein (CRP), although alpha linolenic acid (ALA) was effective. Similarly, Liou et al. found no effect of decreasing LA from 10% to 3.8% of energy on CRP, IL-6 and platelet aggregation.

Although dietary LA has no effect on AA levels in cell membranes, EPA levels are affected. Cleland et al. found that higher levels of dietary LA reduced EPA incorporation in neutrophil phospholipids by 25%, associated with a 30% increase in membrane LA levels. Dietary LA also somewhat inhibits the elongation of ALA to EPA. Liou et al. found that changing from a high-LA intake of 10.5% of energy to a low intake of 3.8% with ALA stable at 1% increased plasma phospholipid EPA levels from 0.6% to 0.8%. So within the normal range of LA intakes, the effect on EPA levels is small and not sufficient to exert an anti-inflammatory effect.

In summary, there is essentially no effect on AA or pro-inflammatory molecules of large changes in dietary LA intake.

**Effects of n-3 polyunsaturated fatty acids on AA and inflammatory markers**

Long-chain n-3 polyunsaturated fatty acids clearly have the potential to be anti-inflammatory. Feeding EPA and DHA to animals and humans and supplying the fatty acids to cells reduce the level of AA in the membrane of all cells examined and inhibits the release of AA from it. EPA also com-

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**Figure 1** Elongation and desaturation of essential fatty acids.
petes with AA for the active sites of the enzymes that produce prostaglandins and leukotrienes, and thus reduces the AA-derived end products. Some, but not all, of the eicosanoids produced from EPA are less potent than the AA derivatives but compete for the same receptor, and thus antagonise their action.9 Lee et al. demonstrated the potency of high doses of fish oil on a variety of inflammatory pathways.10 Seven normal subjects were fed 3.2 g of EPA and 2.2 g of DHA, increasing the EPA content in neutrophils and monocytes more than sevenfold. When the neutrophils were activated, the release of labelled AA and its metabolites was reduced on average by 37%, and the maximum generation of leukotrienes was reduced by more than 48%. So in these ex vivo tests, long-chain n-3 polyunsaturated fats have quite a potent effect, but these are not necessarily translated into a similar potency in vivo, especially in inflammatory conditions where there are many activators and redundancy is in-built. For example, Yaqoob et al. fed 3.3 g/day of encapsulated fish oil to eight volunteers for 12 weeks and observed no immunological changes or changes in the production of a variety of cytokines, despite a 12-fold change in the AA/EPA ratio compared with placebo.11

Long-chain n-3 polyunsaturated fatty acids can inhibit the production of IL-1 beta and tumour necrosis factor alpha (TNF alpha)—two very important cytokines in inflammation. Maximal production of these molecules in cells stimulated in vitro is dramatically reduced when the cells are taken from humans fed high doses of long-chain n-3 fat. Fish oil (9 g/day) fed to humans for four weeks inhibits ex vivo monocyte production of IL1 and TNF alpha by 74–80%.12 This is similar to what Endres et al.13 found with a much higher dose of long-chain n-3 fat (18 g/day). IL2 production and the proliferation of cells in response to stimulation were also suppressed by the same dose.14 However, contrary evidence is available. For example, Blok et al.15 fed 58 monks up to 3 g/day of fish oil for a year and found no effects at all on immunological parameters, whereas Trebble et al.16 found inhibition at a low dose (1 g) on TNF alpha and IL-6 production with a loss of this inhibition at higher doses (2 g/day).

Adhesion molecules contribute to the inflammatory response by binding white cells to the endothelium and allowing them to cross it. This process can be inhibited by DHA, but not EPA. The adhesion molecules inhibited by DHA include vascular cell adhesion molecule-1, E-selectin and intercellular adhesion molecule-1, IL-6 and IL-8.17,18 A progressive increase in inhibitory activity was observed with dietary intake of fatty acids with the same chain length but increasing double bonds, that is, from monounsaturated to n-6 fatty acids and, further, to n-3 fatty acids.19

Long-chain n-3 polyunsaturated fatty acids also give rise to resolvins (resolution phase interaction products) and protectins, which possess potent anti-inflammatory, neuroprotective20 and inflammation-resolving properties. Aspirin causes biosynthesis of these compounds and a related series of variants that have potent anti-inflammatory actions.21,22

In summary, in vitro studies indicate that long-chain n-3 polyunsaturated fatty acids are anti-inflammatory as they displace AA from cell membranes and give rise less inflammatory derivatives that compete with those of AA.

Clinical effects of long-chain n-3 polyunsaturated fatty acids

All of the above studies on long-chain n-3 fats have examined the potential impact on inflammation, but the ultimate proof lies in treatment of human inflammatory conditions. A recent meta-analysis of 17 randomised controlled studies of the effect of long-chain n-3 polyunsaturated fatty acids for joint pain in rheumatoid arthritis showed that supplementation for three to four months was effective in lowering: (i) patient-assessed pain; (ii) duration of morning stiffness; (iii) number of painful and/or tender joints; and (iv) non-selective non-steroidal anti-inflammatory drug consumption. However, physician-assessed pain and the Ritchie articular index (a graded assessment of 26-joint regions to assess tenderness plus a 44-joint count to assess swelling) were not altered.23 In relation to inflammatory bowel disease, there is insufficient evidence to make a statement about induction of remission in ulcerative colitis,24 but the data that have addressed the question of maintenance of remission suggest that long-chain n-3s are not effective,25 especially in Crohn’s disease.26 A Cochrane review has concluded that fish oil has no effects in asthma,27 although if fed to normal pregnant women it might reduce the risk of asthma in the offspring after long-term (16 years) follow up.28

Although not related to anti-inflammatory effects, fish oil also lowers plasma triglycerides and inhibits ventricular fibrillation, thus contributing to a lower death rate in patients after a myocardial infarction.29 Parenteral fish oil in surgical patients shortens hospital stay by 21%,30 and reduces intensive care stay and mortality in doses of 0.1–0.2 g/kg.31 It also reduces parenteral nutrition-associated liver disease in children with short bowel syndrome.32

Is the dietary n-6/n-3 ratio helpful?

The hypothesis that the dietary n-6/n-3 ratio is indicative of the inflammatory nature of the diet is based on the assumptions that increasing intakes of n-6 polyunsaturated fatty acids increase cell membrane levels of AA and is thereby pro-inflammatory, and that increasing intakes of long-chain n-3 polyunsaturated fatty acids is anti-inflammatory. It follows that ‘improving’ the ratio can be achieved by either lowering intake of n-6 fats or increasing intake of n-3 fats and that both dietary manoeuvres would be similarly effective in lowering inflammation. Based on the above discussion, it is apparent that dietary n-6 polyunsaturated fatty acids have negligible effect on AA levels in cell membranes and markers of inflammation. In contrast, long-chain n-3 polyunsaturated fatty acids lower AA levels in cell membranes and lower levels of several markers of inflammation. Therefore, employing the n-6/n-3 ratio is inappropriate as
only the denominator has the potential to affect inflammation. Similar ratios achieved by manipulating either the numerator or the denominator will have very different implications for inflammation; for example, lowering the ratio by reducing the amount of n-6 would be expected to have no effect on inflammation, whereas lowering the ratio by the same amount by increasing n-3 intake would be expected to be anti-inflammatory. In both cases, the effect on inflammation is almost entirely driven by total intake of long-chain n-3 polyunsaturated fatty acids. Thus, use of the ratio as a marker of the inflammatory nature of the diet is misleading and there are no published data to support its use. One of the few epidemiological studies to investigate the effects of n-3 and n-6 fats on inflammatory markers in humans provided no support to the ratio concept, the lowest levels of inflammatory markers being associated with the highest intakes of both n-6 and n-3 fats.35

Recommended intakes for the general population for both n-6 and n-3 polyunsaturated fatty acids are best expressed individually as in Nutrient Reference Values.34 Adequate intakes for LA are 8–13 g/day, ALA 0.8–1.3 g/day and long-chain n-3 fats 90–160 mg/day for men and women, respectively. There are no recommendations for people with chronic inflammatory conditions, nor is there sufficient evidence to recommend an overall ideal range of LA intakes, although the National Heart Foundation recommends that LA should be 10% of calories for optimal low-density lipoprotein cholesterol lowering. For people at risk of or with pre-existing heart disease, it is recommended by the American Heart Association that 0.8–1.5 g/day of long-chain n-3 fatty acids be consumed per day.35 The current intake of LA is about 10–11 g/day based on the Melbourne Collaborative Cancer Study 1990–1994.36 mean 11.3 ± 1.5 g/day (n = 3737) with 0.95 ± 0.33 g/day of linoleic acid and about 0.28 g/day of EPA and DHA, and the National Nutrition Survey 199537 (n = 10 851) where average daily intakes of LA, AA, ALA, EPA, DPA and DHA were 10.8, 0.052, 1.17, 0.056, 0.026 and 0.106 g, respectively, with long-chain n-3 polyunsaturated fatty acids (addition of EPA, DPA and DHA) totalling 0.189 g; median intakes were considerably lower (9.0 g LA, 0.024 g AA, 0.95 g LNA, 0.008 g EPA, 0.006 g DPA, 0.015 g DHA and 0.029 g long-chain n-3 polyunsaturated fatty acids). In relation to coronary risk, Van Schacky and Harris have proposed an omega-3 index based on the % of EPA and DHA of total red blood cell fatty acids.36 A cardioprotective level of 8% or greater and a level associated with increased risk of cardiovascular events was <4%. This was based on a literature review and has not yet been tested prospectively, nor has it been examined in elation to inflammatory disease.

CONCLUSIONS AND FUTURE TRENDS

At present, there is little evidence that the LA currently being consumed is enhancing inflammatory conditions. Apart from inflammatory arthritis, there is little evidence yet that fish oils are particularly helpful, but use in parenteral nutrition is a new and emerging field in which the benefit of fish oil is appearing. Larger clinical trials are required.

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REFERENCES

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