Original Article

Fish oil - an example of an anti-inflammatory food

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With regard to anti-inflammatory effects of diet away from the gut, altering the balance of dietary poly-unsaturated fatty acids (PUFA) in favour of *n*-3 PUFA provides the best documented examples of effective dietary intervention. PUFA are essential macronutrients of which there are two non-interchangeable classes, *n*-6 and *n*-3. These fatty acids are metabolized to mediators that regulate cardiovascular homeostasis and inflammation. *n*-6 rich diets tend to be pro-inflammatory and, by comparison diets rich in *n*-3 PUFA are anti-inflammatory. The difference is explained by the action of *n*-3 PUFA as competitive inhibitors of enzymes that metabolize *n*-6 fats and by the lesser biological activities of most *n*-3 mediators, compared with their *n*-6 counterparts. Fish oils are a particularly rich source of desirable long chain *n*-3 PUFA. Fish oil has been used with benefit in the treatment of inflammatory diseases of joints and other organs and tissues. Our long-term studies in rheumatoid arthritis (RA) show that this approach, in conjunction with pharmacotherapy, can be sustained in the long term (>5 years). A potential collateral benefit is reduced risk for adverse cardiovascular events, which are increased in RA. Lack of knowledge amongst physicians of relevant biochemistry, evidence of efficacy, dose response relationships, latency in effect, availability of affordable preparations and tactics for discussing issues efficiently with patients appears to be a barrier to broader clinical use.

Key words: Fish oil, anti-inflammatory, rheumatoid arthritis, inflammatory diseases

Introduction

A number of dietary factors have irritant or immunological effects in the gut, which allow an anti-inflammatory effect from food avoidance in susceptible subjects. There are also factors that can be enriched in the diet to achieve anti-inflammatory effects, of which fish oil provides the best documented example. This paper reviews the evidence for the anti-inflammatory effects of fish oil and also examines its safety, collateral health benefits and barriers to its use.

Mechanistic considerations

Fish oil is rich in the long chain *n*-3 fatty acids eicosapentaenoic acid (EPA;20:5 *n*-3) and docosahexaenoic acid (DHA; 22:5 *n*-3), which can displace arachidonic acid (AA; 20:4 *n*-6) from cell membranes (Fig 1). These *n-3* fatty acids are also released with AA by phospholipases and act as substrate inhibitors of conversion of AA by cyclo-oxygenases (COX) and the terminal synthases to the pro-inflammatory oxygenated inflammatory mediators known as eicosanoids (a reference to the 20 carbons in AA and these products, eicosa = 20 in Greek) (Fig 2). EPA is structurally identical to AA with the exception of its additional *n*-3 double bond and can be converted to eicosanoids that resemble *n*-6 eicosanoids but have the additional *n*-3 double bond. This structural difference is sufficient to confer substantial differences in activity between *n*-6 leukotriene B4, (LTB4), a very potent chemo-toxin and leucocyte agonist and *n*-3 LTB5, which is a weak chemo-toxin and weak agonist. *n*-3 thromboxane A3 (TXA_3) appears to lack the potency of *n*-6 thromboxane TXA_2, which is an aggregator of platelets and vasoconstrictor. *n*-3 prostaglandin E3 (PGE_3) seems to have similar oedomogenic activity to *n*-6 PGE_2, but very little PGE_3 is produced by monocytes either from endogenous or exogenous EPA in vitro. *n*-6 prostacyclin (PGI_2) and *n*-3 PGI_3 are thought to have similar activities as agents for vascular patency (Fig. 2) [see ref (1) for review].

In addition to these effects on inflammatory eicosanoid synthesis, dietary fish oils have been shown to reduce the production of the inflammatory cytokines IL-1β and TNF α by monocytes stimulated in vitro. These cytokines are important effector molecules in inflammatory responses and TNF α blocking agents are now used widely to treat rheumatoid disease that has proven refractory to less expensive therapies. In vitro studies have also shown inhibition of release of the metalloproteinases that are implicated in the tissue damage that is the hallmark of rheumatoid arthritis and other inflammatory diseases.[2-4]

Epidemiologic studies

Epidemiological studies of Greenland Inuits in the late 1970s drew attention to the possibility of an anti-inflammatory effect of dietary long chain *n*-3 fatty acids. The study of Kromann and Green showed both a very low

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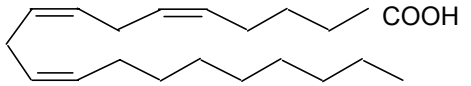
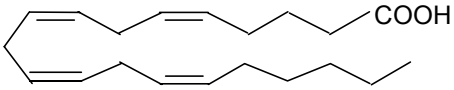
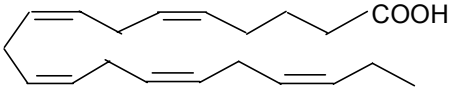


Eicosatrienoic Acid (C20:3 ω-9)



Arachidonic Acid (C20:4 ω-6) [AA]



Eicosapentaenoic Acid (C20:5 ω-3) [EPA]

Figure 1. Structure of arachidonic acid (AA) and eicosapentaenoic acid (EPA)

incidence of coronary heart disease and low frequency of several major categories of inflammatory disease.[5] The Greenland Inuits of this era were unique in consuming a diet based almost entirely on marine mammals that eat fish, fish and sea birds. In this regard, they contrast with continental Inuits, for whom meat from grazing animals, such as caribou, contribute to the diet. Thus, the Greenland Inuits represent an extreme case in which the diet is comprised of foods very rich in long chain *n*-3 fats (7-10g/day) and poor in *n*-6 fats. With the introduction of market foods, the *n*-3 dominance of their aboriginal diet has been diluted by Western products with a higher *n*-6 fatty acid content. The very high *n*-3 content of the Greenland Inuit diet needs to be recognized when extrapolating the putative bleeding tendency of the Inuits to the possible risks of fish oil supplements taken against the background of an *n*-6 abundant Western diet.

Immunogenetic studies of circumpolar Inuits have shown a high frequency of alleles of human leucocyte antigens (HLA) HLA DRB1 0401 and HLA-B27 that in other populations have been associated with increased risk for a variety of arthritides and other inflammatory conditions.[6,7] For example, HLA DR B1 0404 is associated with increased risk for and severity of rheumatoid arthritis. HLA B27 is associated with spondyloarthritis, seronegative arthritis, uveitis and the peripheral arthritis, conjunctivitis and urethritis of Reiter's syndrome. Since these genotypes have been found in a high proportion of Inuits, one can speculate that they may, through strong antigen presentation of certain peptides, provide a selective advantage in relation to defence against particular infections, which may be critical in the context of an anti-inflammatory *n*-3 dominant diet. These same antigen presentation phenotypes may lead to unwanted inflammation and auto-immunity when the diet is rich in pro-inflammatory *n*-6 fats.

Epidemiological studies of the Japanese, whose traditional diet contains about 3G long chain *n*-3 fats, equivalent to an anti-inflammatory dose of fish oil in Western studies, is also revealing. The incidence of RA in Japan is approximately one third that seen in Western countries, in spite of a disproportionately high prevalence of HLA DR4 allele DRB1 0405 that predisposes to RA.[8,9] The Women's Health Study in Seattle involved a case control analysis of fish consumption with regard to disease prevalence. Women eating two or more fish meals per week were found to have less than half the prevalence of seropositive RA compared to women eating less than one fish meal per week.[10] Collectively, the above studies suggest that a diet rich in long chain *n*-3 fatty acids may be protective against RA.

Animal studies

The effects of fish oil feeding has yielded mixed results in animal models of inflammation. Fish oil diets have been shown to have a striking protective effect when used prophylactically in mice genetically predisposed to systemic lupus.[11] A fish oil diet is also effective when introduced after the emergence of signs of murine lupus, but less effective than when used prophylactically.[12] A fish oil diet increased the frequency but not the severity of collagen-induced arthritis in mice[13] and results in rats with adjuvant-induced arthritis were strain specific.[14] This latter model causes universally severe disease in susceptible strains and is resistant to a number of other treatments that are effective in all but the most severe forms of RA in humans. Collectively, the animal studies support an anti-inflammatory effect of dietary long chain *n*-3 fats, but caution that this effect cannot be generalised to all inflammatory diseases.

Dietary fish oil in rheumatoid arthritis

Multiple studies have shown symptomatic benefit in RA with fish oil treatment. The anti-inflammatory dose appears to be at least 2.6g long chain n-3 fatty acids per day.[15] This dose requires 9 standard fish oil capsules per

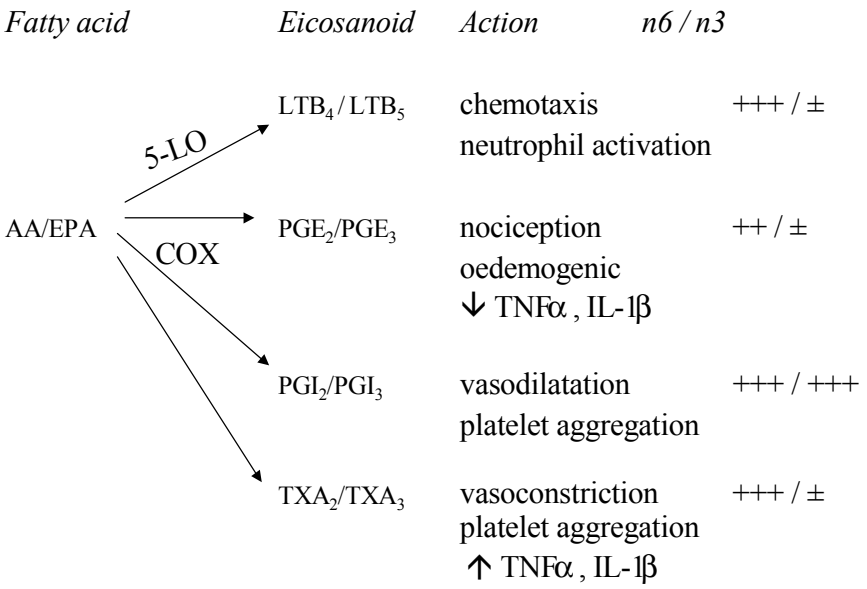


Figure 2. *n6* and *n3* eicosanoids, their precursors and respective biological activities

day. Most studies have used 15 – 20 standard fish oil capsules per day to deliver 4.5 – 6g long chain *n*-3 fatty acids per day. The salient benefits have been reduced joint pain and tenderness. There is usually a latency of six to twelve weeks from the introduction of fish oil to symptomatic response, which appears shorter with higher doses. The need for the analgesic effect of non-steroidal anti-inflammatory drugs (NSAIDs) is reduced by fish oil treatment. Fish oil has been mainly tested as an adjunct to long acting therapies for RA, known as the disease modifying anti-inflammatory drugs (DMARDs) [reviewed in (16)].

The place of fish oil in treatment of recent onset RA

There are no reports of fish oil trials within the context of combination therapy with multiple agents applied early in the course of RA. Since studies in RA published to date have dealt largely with late disease, the role of fish oil in treatment of early RA is not known. This is a matter under investigation in the Rheumatology Unit, Royal Adelaide Hospital. Preliminary observations indicate that fish oil is acceptable to patients as a component of first line treatment for RA. In a protocol that recommends NSAIDs for rescue analgesia only, less than 20% of subjects were using NSAIDs after two years in contrast to an 80% continuation of fish oil. The other components of the treatment protocol include the combination of methotrexate, sulphasalazine and hydroxychloroquine, with addition in sequence of leflunomide, gold sodium thiomalate injections, cyclosporin and etanercept, if required by virtue of failure to meet predefined disease suppression criteria or drug intolerance. The intent has been to determine the practicality, safety, efficacy and influence of fish oil (through a blinded sub study in which fish oil or a comparator oil is taken) of the predefined treatment strategy. The feasibility has been established in a pilot study and a partially completed double blind study in which data for the fish oil-comparator analysis are being gathered. A safety analysis of the pilot phase in which 55 patients have been studied for periods up to 5 years, (37.7 ± 13.7, mean + SD, range 6-63 mths), revealed only one potentially drug related serious adverse event. This involved a woman who developed shortness of breath, during an acute Mycoplasma pneumoniae infection, while taking methotrexate. Methotrexate was withdrawn and the dyspnoea resolved but recurred on re-challenge. However, drug intolerance was common with only 25 of 55 patients remaining on all three starting medications at the time of last observation. No serious unwanted events attributable to fish oil were observed and in particular no abnormal bleeding tendency was noted. Preliminary efficacy analysis has been encouraging with more than 70% of patients achieving a good re-sponse by EULAR disease activity score (DAS) criteria after 6 months of treatment. Remission rates were in the order of 50%. Some patients achieving remission later showed re-emergence of disease activity requiring dose adjustment. These data compare favourably with published studies. While the influence of fish oil on these favourable outcomes is not known, the effectiveness of the regimen of medications cannot necessarily be extrapolated to patient populations not receiving fish oil.

Safety of fish oil with long term use in anti-inflammatory doses

As there is no previously documented experience with long term use of fish oils in anti-inflammatory doses, this question requires special attention. Through our Pilot Early Arthritis Study (open label fish oil), we have an experience with anti-inflammatory doses of fish oil extending over a period of more than 6 years for some patients. In the quest of cost efficiency, we have developed a method for giving bottled fish oil on juice in a way that masks the fishy taste of the oil. Initially, we used

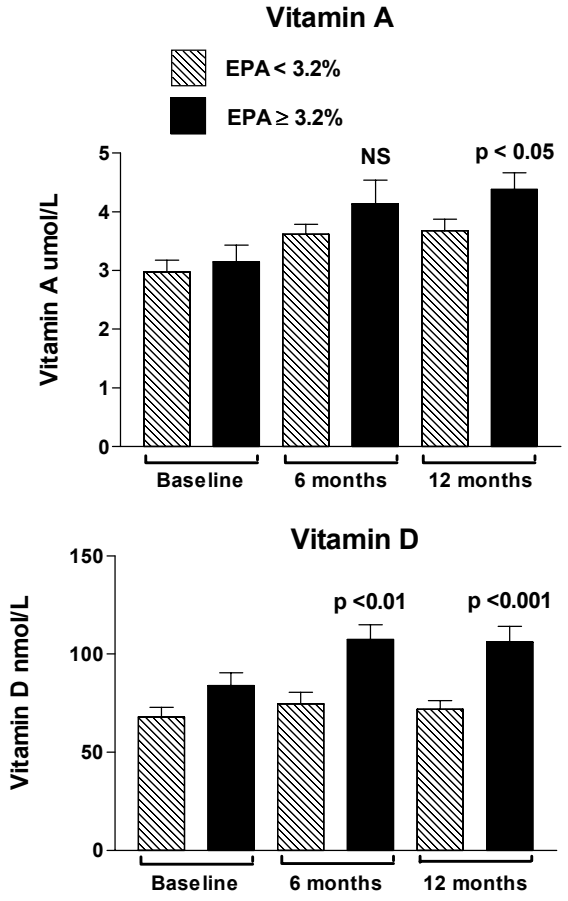


Figure 3. Plasma vitamin A and D (mean± SD) in rheumatoid arthritis patients prescribed 20 ml cod liver oil per day. Results are stratified by plasma phospholipid levels of the omega-3 fatty acid, eicosapentaenoic acid (EPA) (low EPA n=25, high EPA n=23) as a measure of compliance.

a commercially available cod liver oil preparation that contained EPA 100mg, DHA 100mg, vitamin D 80[u], vitamin A 610[u] per mL. The recommended dose was 20mL daily, which delivers 4g long chain *n*-3 fatty acid per day, 1600[u] cholecalciferol and 12,500[u] vitamin A. Compliance with the regimen was reflected in plasma phospholipids EPA.[17] A mean increase in both vitamin D and vitamin A levels was seen at 12 months (Fig 3). Some vitamin D levels approached or slightly exceeded the upper limit of the normal reference range, but all were far below levels at which vitamin D toxicity has been reported.[18] A single patient who displayed hypercalcaemia was found to have primary hyperparathyroidism. In some cases slight elevations of vitamin A above the reference range were also seen. Patients were monitored for bone mineral density and no difference in bone loss was seen relative to RA patients no taking fish oil.

Notwithstanding, because recent reports show higher levels of vitamin A supplementation correlate inversely with bone mineral density,[19] we decided to switch from use of cod liver oil to a fish body oil, since fish body oils contain trace amounts only of fat soluble vitamins. As there was no available retail supply of fish oil other than in capsules, we purchased fish oil in bulk and arranged bottling in our hospital pharmacy. We advised a 15mL dose of this preparation, which delivers 4.5g long chain omega-3 fatty acids daily.

Long term use of fish oil raises concerns regarding the possible ingestion of industrial toxins found in fish. The presence of mercury in the meat of carnivorous fish has attracted considerable attention. Mercuric chloride is not lipophilic and mercury is not present in fish oils. A greater concern is polychlorinated biphenyls (PCBs), which are lipophilic and are present in trace amounts in fish oil. Components of this family are produced as by products of chemical synthetic reactions and are not biodegradable. Processes that generate these compounds have been outlawed but PCBs persist to varying degrees in the environment. Since they are relatively volatile they can be removed using standard fractionation processes such as molecular distillation. Notwithstanding, taking anti-inflammatory doses of fish oils harvested from industrial regions without adequate processing could involve ingestion of PCBs at or above currently recommended intakes, albeit below intakes prior to institution of avoidance measures. The issue of PCBs dictates selection of quality fish oils for long-term therapeutic use.

Effectiveness of fish oil in other inflammatory disease

Fish oil supplements have been shown to reduce relapse in Crohn's disease by more than 60% and to reduce substantially loss of renal function and progression to end

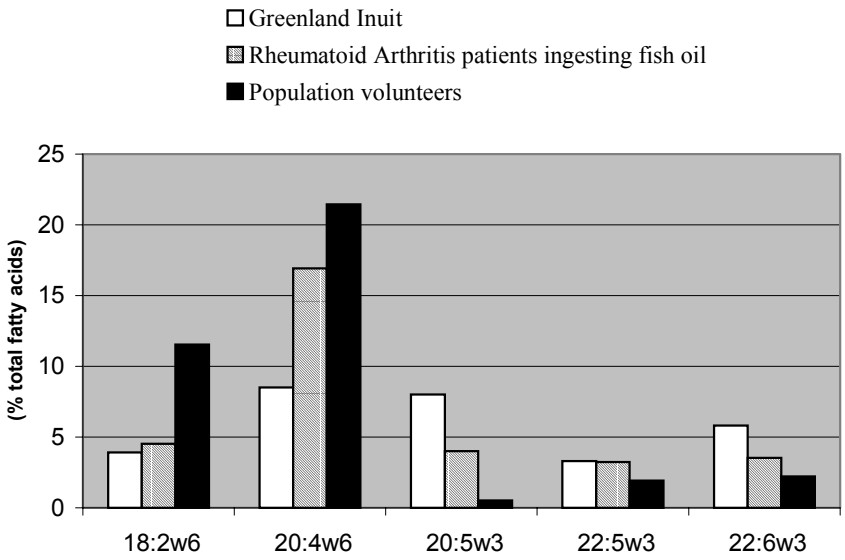


Figure 4. Platelet phospholipid fatty acids of interest in Greenland Inuits[28] (n=21), Rheumatoid Arthritis Patients prescribed 20ml per day fish oil (n=11), and an Australian popu-lation control group not ingesting fish oil (n=30).

stage renal failure in IgA nephropathy.[20,21] Some, but not all, variants of psoriasis have been shown to respond to fish oil treatment [reviewed in (22)]. Control of systemic lupus has been shown to improve with fish oil supplements.[23,24] Dietary fish oil has been shown to improve outcomes in ischaemic heart disease, to which patients with RA are especially prone.[25,26] Cardiovascular benefits of fish oil include a myocardial membrane stabilizing effect, reduced incidence of malignant arrhythmias and sudden death, improved blood pressure control, reduction in raised plasma triglycerides and, in experimental animals, and anti-atherogenic effect.[27]

Bleeding tendency and fish oil supplements

In spite of theoretic concerns that fish oil supplementation may lead to an increased bleeding tendency, this has not been our experience. The concerns centre on an extrapolation from a putatively increased bleeding times in Greenland Inuits[28] and somewhat increased incidence of apoplexy.[5] The latter is likely multifactorial with high dietary salt intake potentially a factor. The bleeding time data from Greenland Inuit studies show a moderate increase in bleeding time toward the upper end of the Danish reference range.[28] Whether this putative effect seen in Inuits with very high dietary long chain *n*-3 intake and low *n* -6 diet will translate to Westerners in whom an *n*-3 rich fish oil supplement is being taken against the background of a Western diet abundant in *n* -6 fatty acids is doubtful. In any case, we have compared competitor AA and EPA in platelets of patients with RA on long term therapy with fish oil (>3years) with those reported for Greenland Inuits (Fig. 4). The AA is far more suppressed and the EPA higher in the Inuits than the fish oil treated patients. Thus, on biochemical grounds a lesser effect on platelet function in patients on fish oil can be expected than seen in Inuits. Also, it has been reported that consumption of 3.4g/day of omega-3 fats in conjunction with 300 mg/day of aspirin had no effect on bleeding time, or episodes of bleeding in patients undergoing coronary artery bypass surgery.[29]

Barriers to implementation

In spite of the many documented health benefits of fish oil, it use in anti-inflammatory doses has not been widely implemented beyond clinical trials. The reasons for this lack of application are manifold but include cost, ineffective marketing, lack of professional detailing, absence of suitable formulations, lack of professional awareness of benefits, ignorance of how to advise, and inaccurate community perceptions as to what constitutes an effective dose.

The issue of suitable preparations centres around the lack of availability of bottled fish oil preparations suited to daily dosing of 15mL, which is equivalent to slightly more than 14 standard capsules. Bottled fish oil on juice is easier to take and a small fraction of the cost of fish oil capsules that deliver the same quantity of *n*-3 fatty acids. Furthermore, taking a large number of 1g capsules can be uncomfortable and is often perceived, understandably, as an unreasonably large dose, whereas taking the same amount of oil on juice is easier and does not seem large.

We purchased bulk fish oil (minimum quantum for sale outside a capsule 200L!) and had arranged bottling in our hospital pharmacy. Within 12 months it became evident that the impact of the bottling on work flows in the hospital pharmacy was not sustainable and arrangements were made for bottling through a company that bottles cod liver oil and organically grown seed oils for the health food industry (Melrose Laboratories, Mitcham, Victoria, Australia 3132).

The product, a 500mL bottle of fish body oil, can be purchased through the RAH Preventive Care Centre for $15 a bottle. This contrasts with a cost of $50 - $200 for similar amounts of fish oil in capsules. The cost for fish oil capsules can dwarf the costs of other treatments, in countries like Australia, where pharmaceutical expenses, but not fish oil, are government subsidised.

Favourable interactions between fish oil and anti-inflammatory drugs

As discussed above, fish oil reduces recourse to NSAIDs for analgesia in RA and thereby reduces risk for upper GI haemorrhage. Fish oil contrasts with the highly selective COX-2 inhibitor rofecoxib, which has been associated with increased serious cardiovascular events, by reducing risk for these events.[30,31] Anti-inflammatory doses of fish oil have been shown to reduce the hypertensive and nephrotoxic effects of cyclosporin.[32]

Oils containing *n* -6 gamma linolenic acid (GLA, 18:3 *n* -6)

Oils rich in GLA may have anti-inflammatory effects. The putative biochemical basis for this effect is relative accumulation of the elongation product of GLA, dihomogamma linolenic acid (DGLA, 20:3 *n* -6) which, like EPA, can compete in metabolic pathway that are usually dominated by AA. The result is fewer AA derived eicosanoids with production of homologous metabolites products of DGLA such as PGE_1 (one less double bond than AA derived PGE_2). GLA rich oils appear to reduce symptoms in RA but available evidence is far less than that for fish oil in RA.[33]

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