Polyunsaturated fatty acids and inflammation

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Abstract
The n-6 polyunsaturated fatty acid, arachidonic acid, is a precursor of prostaglandins, leukotrienes and related compounds that have important roles as mediators and regulators of inflammation. Consuming increased amounts of long chain n-3 polyunsaturated fatty acids (found in oily fish and fish oils) results in a partial replacement of the arachidonic acid in cell membranes by eicosapentaenoic and docosahexaenoic acids. This leads to decreased production of arachidonic acid-derived mediators. This alone is a potentially beneficial anti-inflammatory effect of n-3 fatty acids. However, n-3 fatty acids have a number of other effects that might occur downstream of altered eicosanoid production or are independent of this. For example, they result in suppressed production of pro-inflammatory cytokines and can modulate adhesion molecule expression. These effects occur at the level of altered gene expression.

PUFA (polyunsaturated fatty acid) structure, nomenclature, sources and intake
The general structure of a fatty acid is a hydrocarbon chain with a carboxy group at one end and a methyl group at the other. The most abundant fatty acids have straight chains of an even number of carbon atoms. Fatty acid chain lengths vary from 2 to 30 or more and the chain may contain double bonds. Fatty acids containing double bonds in the acyl chain are referred to as unsaturated fatty acids; a fatty acid containing two or more double bonds is called a PUFA. Unsaturated fatty acids are named by identifying the number of double bonds and the position of the first double bond counted from the methyl terminus (with the methyl, or ω, carbon as number 1) of the acyl chain. Therefore an 18-carbon fatty acid with two double bonds in the acyl chain and with the first double bond on carbon number six from the methyl terminus is notated as 18:2ω6, often shown as 18:2n-6 (Figure 1). The common name of this fatty acid is linoleic acid and it is the simplest member of the ω-6 or n-6 family of fatty acids. Linoleic acid can be further desaturated by insertion of a double bond between carbons 3 and 4 (counted from the methyl carbon) to yield α-linolenic acid (18:3ω3), the simplest member of the ω-3 or n-3 family of fatty acids. Mammals, but not plants, lack the desaturase enzymes necessary to synthesize linoleic and α-linolenic acids.

Although mammalian cells cannot synthesize linoleic and α-linolenic acids, they can metabolize them by the introduction of further double bonds (desaturation) and by lengthening the acyl chain (elongation). Thus linoleic acid can be converted into γ-linolenic acid (18:3n-6), and dihomogamma-linolenic acid (20:3n-6) into arachidonic acid (20:4n-6) (Figure 1). Using the same series of enzymes as those used to metabolize n-6 PUFA, α-linolenic acid is converted into EPA (eicosapentaenoic acid; 20:5n-3) (Figure 1). Further conversion of EPA into DHA (docosahexaenoic acid; 22:6n-3) involves the addition of two carbons to form docosapentaenoic acid (22:5n-3), the addition of two more carbons to produce 24:5n-3, desaturation to form 24:6n-3 and removal of two carbons by limited β-oxidation to yield DHA [1]. Arachidonic acid can also be metabolized by the same series of enzymes. In mammals, the pathway of desaturation and elongation occurs mainly in the liver.

It is evident from the pathway shown in Figure 1 that there is competition between the n-6 and n-3 fatty acid families for metabolism. Although the preferred substrate for Δ6-desaturase is α-linolenic acid, because linoleic acid is much more prevalent in most human diets when compared with α-linolenic acid, the metabolism of n-6 fatty acids is quantitatively more important.

Plant seed oils are frequently rich in PUFA. For example, corn, sunflower, safflower and soya-bean oils are rich in linoleic acid, which may comprise as much as 75% of the fatty acids present. Thus these oils and foods made from them (e.g. margarines) are important dietary sources of linoleic acid. Some plant oils (e.g. soya-bean oil) also contain α-linolenic acid in smaller amounts; green plant tissues are also a source of this fatty acid. The main PUFA in the Western diet is usually linoleic acid followed by α-linolenic acid. Typical intakes of these two fatty acids in the U.K. are 10–15 and 0.75–1.5 g/day respectively [2]. As to longer chain PUFA, they are consumed in smaller amounts than linoleic and α-linolenic acids. Estimates of the intake of arachidonic acid in Western populations vary between 50 and 300 mg/day for adults. Fish, especially oily fish (salmon, herring, tuna and mackerel) and fish oils are a rich source of EPA and DHA. In the absence of oily fish or fish oil consumption, α-linolenic acid is by far the principal dietary n-3 PUFA. Average intake

Key words: arachidonic acid, cytokine, eicosanoid, fish oil, inflammation, polyunsaturated fatty acid.
Abbreviations used: COX, cyclooxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HETE, hydroxyeicosatriaenoic acid; ICAM-1, intercellular cell-adhesion molecule 1; IL, interleukin; LLOX, lipoxygenase; LT, leukotriene; NF-κB, nuclear factor κB; PG, prostaglandin; PUFA, polyunsaturated fatty acid; TNF, tumour necrosis factor; VCAM-1, vascular cell adhesion molecule 1.
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of the long-chain n–3 PUFAs in the U.K. is estimated at <250 mg/day [2].

**Arachidonic acid and eicosanoids**

The key link between fatty acids and inflammation relates to the fact that the family of inflammatory mediators termed eicosanoids is generated from 20-carbon PUFAs liberated from cell-membrane phospholipids. Inflammatory cells typically contain a high proportion of the n–6 PUFA arachidonic acid and low proportions of n–3 PUFAs, especially EPA (see [3]). Thus arachidonic acid is typically the dominant substrate for eicosanoid synthesis. Eicosanoids include PGs (prostaglandins), TXs (thromboxanes), LTs (leukotrienes), HETEs (hydroxyeicosatetraenoic acids) etc. Arachidonic acid in cell membranes can be mobilized by various phospholipase enzymes, most notably phospholipase A2, and the free acid can subsequently act as a substrate for the enzymes that synthesize eicosanoids (Figure 2). Metabolism by COX (cyclo-oxygenase) enzymes gives rise to the 2-series PG and TX (Figure 2). There are two isoforms of COX: COX-1 is a constitutive enzyme and COX-2 is induced in inflammatory cells as a result of stimulation and is responsible for the markedly increased production of PG that occurs on cellular activation. PGs are formed in a cell-specific manner. For example, monocytes and macrophages produce large amounts of PGE2 and PGF2α, neutrophils produce moderate amounts of PGE2 and mast cells produce PGD2. Metabolism of arachidonic acid by the 5-LOX (5-lipoxygenase) pathway gives rise to hydroxy and hydroperoxy derivatives (5-HETE and 5-hydroperoxyeicosatetraenoic acid respectively), and the 4-series LTs, LTA4, LTB4, LTC4, LTD4 and LTE4 (Figure 2). Neutrophils, monocytes and macrophages produce LTB4, whereas LTC4, LTD4 and LTE4 tend to be produced by mast cells, basophils and eosinophils.

PGE2 has a number of pro-inflammatory effects including inducing fever, increasing the vascular permeability and vasodilation and enhancing pain and oedema caused by other agents such as bradykinin and histamine. Recent studies have demonstrated that PGE2 induces COX-2 in cultured fibroblasts and so up-regulates its own production and induces the production of IL-6 (interleukin-6) by macrophages [4]. LTB4 increases vascular permeability, is a potent chemotactic agent for leucocytes, induces the release of lysosomal enzymes, and enhances generation of reactive oxygen species and production of inflammatory cytokines like TNFα (tumour necrosis factor α), IL-1 and IL-6. LTC4, LTD4 and LTE4 are bronchoconstrictors and they increase vascular permeability and promote hypersensitivity. Under inflammatory conditions, increased rates of production of arachidonic acid-derived eicosanoids occur and increased levels of these eicosanoids are observed in blood and tissues from patients with acute and chronic inflammatory conditions. Despite the on-going emphasis on the pro-inflammatory effects of arachidonic acid-derived eicosanoids, previous studies have shown that PGE2 inhibits 5-LOX and so decreases the production of 4-series LTs [5] and induces 15-LOX, thus promoting the formation of lipoxins [5,6] that have been...
found to have anti-inflammatory effects [7,8]. Thus PGE\(_2\) possesses both pro- and anti-inflammatory actions.

**Long-chain n-3 PUFAs and eicosanoid production**

Increased consumption of fish oil, which is rich in the long-chain n-3 PUFAs EPA and DHA, results in increased proportions of those fatty acids in inflammatory cell phospholipids, partly at the expense of arachidonic acid (see [3,9]). Thus, since there is less substrate available for the synthesis of eicosanoids from arachidonic acid, fish oil supplementation of the human diet has been shown to result in decreased production of PGE\(_2\) [10–13], TXB\(_2\) [12], LTB\(_4\) and 5-HETE [14,15] and LTE\(_4\) [16] by inflammatory cells. EPA also acts as a substrate for COX and LOX enzymes [16,17], thus giving rise to a different family of eicosanoids: the 3-series PGs and TXs, the 5-series LTs and the hydroxy-EPAs. Thus fish oil supplementation of the human diet has been shown to result in increased production of LTB\(_3\), LTE\(_3\) and 5-hydroxy-EPA by inflammatory cells [14–16], although generation of PGE\(_3\) has been more difficult to demonstrate [18]. The functional significance of this is that the mediators formed from EPA are believed to be less potent compared with those formed from arachidonic acid. For example, LTB\(_3\) is 10–100-fold less potent as a neutrophil chemotactic agent than LTB\(_4\) [19,20]. Recent studies have compared the effects of PGE\(_3\) and PGE\(_2\) on the production of cytokines by cell lines and by human cells. Bagga et al. [4] reported that PGE\(_3\) was a less potent inducer of COX-2 gene expression in fibroblasts and of IL-6 production by macrophages, although PGE\(_2\) and PGE\(_3\) had equivalent inhibitory effects on the production of TNF\(_\alpha\) [21,22] and IL-1\(\beta\) [22] by human mononuclear cells stimulated with endotoxin.

In addition to long-chain n-3 PUFAs modulating the generation of eicosanoids from arachidonic acid and to EPA acting as substrate for the generation of alternative eicosanoids, previous studies have identified a novel group of mediators, termed E-series resolvins, formed from EPA by COX-2, that appear to exert anti-inflammatory actions [23–25]. In addition, DHA-derived mediators termed D-series resolvins, docosatrienes and neuroprotectins, also produced by COX-2 under some conditions, have been identified and these too appear to be anti-inflammatory [26–28]. 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.

**Anti-inflammatory effects of n-3 PUFA other than altered eicosanoid production**

Although their action in antagonizing arachidonic acid metabolism is a key anti-inflammatory effect of n-3 PUFAs, these fatty acids have a number of other anti-inflammatory effects that might occur downstream of altered eicosanoid production or might be independent of this.

Cell culture studies demonstrate that EPA and DHA can inhibit the production of IL-1\(\beta\) and TNF\(_\alpha\) by monocytes [29] and the production of IL-6 and IL-8 by venous endothelial cells [30,31]. Fish oil feeding decreased ex vivo production of...
Figure 3 | The current view of anti-inflammatory actions of long-chain n-3 PUFAs

![Diagram of anti-inflammatory actions of long-chain n-3 PUFAs]

n-3 PUFA and inflammatory gene expression

Many of the anti-inflammatory effects of n-3 PUFAs appear to be exerted at the level of altered gene expression. However, these effects have been demonstrated only a limited number of times and often in artificial in vitro settings, and thus the extent of these effects in vivo is not yet clear. Nevertheless, cell-culture and animal feeding studies indicate potentially very potent effects of n-3 PUFA on the expression of a range of inflammatory genes.

Culturing bovine chondrocytes with EPA or DHA markedly decreased the cytokine-mediated induction of expression of the COX-2 (but not COX-1), TNFα, IL-1α, IL-1β, and IL-6 genes [45]. Including EPA or DHA in the culture medium of human osteoarthritic cartilage explants markedly decreased the cytokine-induced up-regulation of expression of the COX-2, IL-1α, IL-1β, TNFα, 5-LOX, 5-LOX activating protein and matrix metalloproteinase genes in these cells [46]. In an earlier study, De Caterina et al. [30] had demonstrated that the down-regulation of VCAM-1 expression on endothelial cells caused by DHA was exerted at the level of VCAM-1 gene expression and that this effect was independent of the effects on eicosanoid production and on antioxidant status. A limited number of feeding studies have demonstrated an effect of dietary fish oil on inflammatory gene expression. Inclusion of fish oil in the diet completely abolished mRNA for TNFα, IL-1β and IL-6 in the kidneys of autoimmune disease-prone mice [47]. Feeding mice with a fish-oil-rich diet significantly decreased the level of IL-1β mRNA in endotoxin- or phorbol ester-stimulated spleen lymphocytes [48]; the lower IL-1β mRNA level was not due to accelerated degradation but due to impaired synthesis. Fish oil feeding to mice lowered basal and endotoxin-stimulated TNFα mRNA levels in peritoneal macrophages [33]. ICAM-1 mRNA levels were lower in fresh peritoneal macrophages from mice fed with fish oil [43].

It is now emerging that n-3 PUFAs might exert their effects on inflammatory gene expression through direct actions on the intracellular signalling pathways that lead to activation of one or more transcription factors such as NF-κB (nuclear factor κB). Previous studies have shown that n-3 PUFAs can down-regulate the activity of the nuclear transcription factor NF-κB. EPA prevented NF-κB activation by TNFα in cultured pancreatic cells, an effect that involved decreased degradation of the inhibitory subunit of NF-κB (IκB), perhaps through decreased phosphorylation [49]. Similarly, EPA or fish oil decreased endotoxin-induced activation of NF-κB in human monocytes [50–52] and this was associated with decreased IκB phosphorylation [51,52], perhaps due to decreased activation of mitogen-activated protein kinases.
[53]. These observations suggest direct effects of long-chain n–3 fatty acids on inflammatory gene expression through the inhibition of activation of NF-κB.

**Conclusion**

Long-chain n–3 PUFAs from oily fish and fish oils decrease the production of inflammatory eicosanoids and cytokines. They act both directly, by replacing arachidonic acid as an eicosanoid substrate and by inhibiting arachidonic acid metabolism, and indirectly, by altering the expression of inflammatory genes through effects on transcription factor activation (Figure 3). Thus long-chain n–3 PUFAs are potentially useful anti-inflammatory agents and would benefit patients at risk in a variety of acute and chronic inflammatory settings (see [3,9,54–57]).

**References**