Fatty acids
Long-chain fatty acids and inflammation

Philip C. Calder
Institute of Human Nutrition and Human Development and Health Academic Unit, Faculty of Medicine, University of Southampton, Tremona Road, Southampton SO16 6YD, UK

Inflammation plays a key role in many common conditions and diseases. Fatty acids can influence inflammation through a variety of mechanisms acting from the membrane to the nucleus. They act through cell surface and intracellular receptors that control inflammatory cell signalling and gene expression patterns. Modifications of inflammatory cell membrane fatty acid composition can modify membrane fluidity, lipid raft formation and cell signalling leading to altered gene expression and can alter the pattern of lipid and peptide mediator production. Cells involved in the inflammatory response usually contain a relatively high proportion of the n-6 fatty acid arachidonic acid in their membrane phospholipids. Eicosanoids produced from arachidonic acid have well-recognised roles in inflammation. Oral administration of the marine n-3 fatty acids EPA and DHA increases the contents of EPA and DHA in the membranes of cells involved in inflammation. This is accompanied by a decrease in the amount of arachidonic acid present. EPA is a substrate for eicosanoid synthesis and these are often less potent than those produced from arachidonic acid. EPA gives rise to E-series resolvins and DHA gives rise to D-series resolvins and protectins. Resolvins and protectins are anti-inflammatory and inflammation resolving. Thus, the exposure of inflammatory cells to different types of fatty acids can influence their function and so has the potential to modify inflammatory processes.

Abbreviations: COX, cyclooxygenase; IκB, inhibitory subunit of NF-κB; LPS, lipopolysaccharide; TLR, Toll-like receptor.

Corresponding author: Professor Philip C. Calder, fax +44 2380 795255, email pcc@soton.ac.uk

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and functional roles and biological activities. Indeed, several fatty acids have roles and activities that oppose one another, indicating that the overall biological outcome will be the result of interactions among several fatty acids. With regard to inflammation it is often considered that it is the PUFA of the n-6 and n-3 families that are most important, these often acting to oppose one another’s actions. However, it is now recognised that other fatty acids and fatty acid families are likely also involved in inflammation, with fatty acid exposures affecting inflammatory cell function, and presumably also inflammatory processes. This article will deal with three of these mechanisms:

(i) Action directly via surface or intracellular fatty acid receptors.
(ii) Incorporation into the phospholipids of inflammatory cell membranes, where the fatty acids play important roles assuring the correct environment for membrane protein function, maintaining membrane order (‘fluidity’), influencing lipid raft formation and modifying membrane-generated intracellular signalling cascades.
(iii) Acting as precursors of extracellular signalling molecules such as PG.

Fatty acids and NF-κB-induced inflammatory gene expression

NF-κB is a key transcription factor involved in upregulation of inflammatory cytokine, adhesion molecule and cyclooxygenase (COX)-2 genes. Inactive NF-κB is a trimmer localised within the cytosol; it is activated via a signalling cascade triggered by extracellular inflammatory stimuli, and which involves phosphorylation of an inhibitory subunit (inhibitory subunit of NF-κB (IkB)), which then dissociates allowing translocation of the remaining NF-κB dimer to the nucleus. Bacterial lipopolysaccharide (LPS), which is also known as endotoxin, induces inflammation by activating NF-κB, as do some inflammatory cytokines and UV irradiation. Cell culture studies with the n-3 PUFA EPA and DHA show inhibition of LPS-induced production of COX-2, inducible NO synthase, TNFα, IL-1, IL-6, IL-8 and IL-12 in endothelial cells, macrophages, and dendritic cells. Animal feeding studies with fish oil, a source of EPA and DHA, support the observations made in vitro with respect to the effects of n-3 PUFA on inflammatory cytokine production. For example, dietary fish oil decreased production of TNFα, IL-1β and IL-6 by LPS-stimulated macrophages. Some studies in healthy human subjects have demonstrated that oral fish oil supplements can decrease production of TNFα, IL-1β, IL-6 and various growth factors by LPS-stimulated monocytes or mononuclear cells, although not all studies confirm this effect. The effects of n-3 PUFA have been shown to involve inhibition of LPS-induced activation of NF-κB associated with decreased IkB phosphorylation. In contrast, SFA, especially lauric acid, enhanced NF-κB activation in macrophages and dendritic cells and so promoted inflammatory gene expression. Lee et al. found that EPA and DHA, as well as other unsaturated fatty acids (arachidonic, linoleic and oleic acids), were able to prevent the pro-inflammatory effect of lauric acid in macrophages. It has not been clear how fatty acids can influence activation of NF-κB although their effects might be as far upstream as the plasma membrane. Consistent with this, Lee et al. showed that the activation of NF-κB and induction of COX-2 expression by lauric acid did not occur in macrophages expressing a dominant-negative mutant of the cell surface LPS receptor. Toll-like receptor (TLR)-4, suggesting that lauric acid somehow interacts with TLR-4 (Fig. 1). Myeloid differentiation primary response gene 88 is a cell membrane-associated adapter protein used by TLR-4 to activate NF-κB. DHA inhibited COX-2 expression in macrophages bearing constitutively active TLR-4 but not in those bearing constitutively active myeloid differentiation primary response gene 88 suggesting that the effects of DHA are at the level of TLR-4. More recently, Wong et al. demonstrated that exposure of macrophages to lauric acid induced association of TLR-4, myeloid differentiation primary response gene 88 and other signalling proteins into organised signalling platforms within the plasma membrane termed membrane rafts in much the same way as LPS acts. Furthermore they showed that DHA inhibited the ability of both LPS and lauric acid to promote recruitment of these signalling proteins into rafts. Thus, the differential effects of fatty acids on inflammatory signalling initiated through TLR-4 and impacting on NF-κB appear to relate to their ability to promote or disrupt membrane raft formation.

Actions of fatty acids on inflammation via fatty acid receptors

Fatty acids, PPARγ and inflammation

PPARγ is a transcription factor that acts in an anti-inflammatory manner. It is able to directly regulate...
inflammatory gene expression, but it also interferes with the activation of the prototypical pro-inflammatory transcription NF-κB(26). PUFA and their derivatives are endogenous ligands for PPARγ. The n-3 PUFA DHA induced PPARγ in dendritic cells and this was associated with reduced production of the pro-inflammatory cytokines TNFα and IL-6 following endotoxin stimulation(14). In addition, DHA induced a number of known PPARγ target genes in dendritic cells, suggesting this as an important anti-inflammatory mechanism of action(27).

**Fatty acids, GPR120 and inflammation**

The cell surface G-protein coupled receptor termed GPR120 is highly expressed on inflammatory macrophages, and a GPR120 agonist GW9508 inhibited responsiveness of macrophages to LPS(28). This involved reduced phosphorylation of the IkB and its maintenance in the cytosol (phosphorylated IkB is degraded) and reduced TNFα and IL-6 production. These observations suggest that GPR120 is anti-inflammatory. DHA and another n-3 PUFA, EPA, but not arachidonic, palmitic or myristic acids, promoted GPR120-mediated gene activation, although they were much less potent than GW9508. The effects of DHA were further explored(28). Its inhibitory effects on LPS-induced IkB phosphorylation, IkB degradation and TNFα, IL-6 and also on monocyte chemotactic protein-1 production did not occur in GPR120 knockdown cells. These observations suggest that the inhibitory effect of DHA (and probably also those of EPA) on responsiveness to LPS occur via GPR120 (Fig. 1).

**Modification of inflammatory cell membrane fatty acid composition and consequent alteration of lipid mediator profiles**

**Modification of inflammatory cell membrane fatty acid composition**

PUFA are important constituents of the phospholipids of the membranes of inflammatory cells. Typically these contain a relatively high proportion of the n-6 PUFA, arachidonic acid; this is seen in both laboratory animals(29–38) and human subjects(18,21,39–48). Increased oral supply of the n-3 PUFA EPA and DHA results in an increase in the amount of those fatty acids in inflammatory cells, seen in both laboratory animals(29,30,32–38) and human subjects(18,21,39–44,46–48). The increase in content of EPA and DHA occurs over the course of days(49) to weeks(42), occurs in a dose–response manner(48) and is accompanied by a decrease in content of arachidonic acid.

**Fatty acid modification of eicosanoid profiles**

Eicosanoids, which include PG, thromboxanes and leukotrienes, are long-recognised mediators and regulators of inflammation. They are formed from C20 PUFA, typically arachidonic acid, by the COX and lipoxygenase enzymes. In general, arachidonic acid-derived eicosanoids act in a pro-inflammatory way, although this is an oversimplification since it is now recognised that PGE2, for example, has both pro- and anti-inflammatory effects(3), and that another eicosanoid derived from arachidonic acid, lipoxin A4, is anti-inflammatory(50–53).

The decrease in arachidonic acid content of inflammatory cell membranes that occurs with incorporation of the n-3 PUFA reduces the availability of the usual eicosanoid substrate and so the production of the major 2-series PG and 4-series leukotrienes is decreased(17–19,21,39,40,54–56). EPA is also a substrate for the COX and lipoxygenase, but the mediators produced have a different structure from the arachidonic acid-derived mediators, and this often influences their potency(57). For example EPA-derived leukotriene B5 is ten- to 100-fold less potent as a neutrophil chemoattractant compared with leukotriene B4(58,59). Furthermore, EPA-derived eicosanoids may antagonise the action of those produced from arachidonic acid, as was recently demonstrated for PGD3 v. PGD2(60).

**Novel anti-inflammatory and inflammation resolving mediators produced from EPA and DHA: resolvins and protectins**

EPA and DHA are substrates for synthesis of fairly recently discovered lipid mediators that are potent anti-inflammatory and inflammation resolving agents. These include resolvins and protectins, which are produced through pathways involving COX and lipoxygenase enzymes(61–63). Examples of the activities are these compounds include the inhibition of transendothelial migration of neutrophils by resolin E1, resolin D1 and protectin D1, and inhibition of TNFα and IL-1β production by protectin D1(63).

**Therapeutic benefits of the anti-inflammatory actions of n-3 fatty acids**

A number of human conditions and diseases have an inflammatory component, and it seems that, irrespective of the body compartment(s) involved, these conditions and...
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Summary and conclusions

Fatty acids can influence inflammation through a variety of mechanisms, including acting via cell surface and intracellular receptors/sensors that control inflammatory cell signalling and gene expression patterns. Some effects of fatty acids on inflammatory processes involve lipid mediators generated from the fatty acids themselves. Often these fatty acids will be released from cell membrane phospholipids prior to their conversion to the bioactive mediators. Cells involved in the inflammatory response are typically rich in the n-6 fatty acid arachidonic acid which is a precursor to inflammatory eicosanoids. The membrane contents of arachidonic acid and of the n-3 fatty acids EPA and DHA can be altered through oral administration of EPA and DHA. EPA also gives rise to eicosanoids and these often have differing properties from those of the arachidonic acid-derived analogues, typically being less potent. EPA and DHA give rise to resolvins, and DHA to protectins. In these ways, n-3 PUFA act to oppose the pro-inflammatory actions of SFA and of n-6 PUFA. The roles of n-3 PUFA in shaping and regulating inflammatory processes and responses suggest that the level of exposure to these fatty acids might be important in determining the development and severity of inflammatory diseases. The recognition that n-3 PUFA have anti-inflammatory actions has led to numerous studies supplementing the diet of patients with inflammatory diseases to evaluate clinical benefit. Studies in patients with rheumatoid arthritis have been the most successful among those in patients with an overt inflammatory disease, with a number of trials reporting clinical benefits(65), these benefits being supported by meta-analyses(66,67). Studies in patients with inflammatory bowel diseases (Crohn’s disease and ulcerative colitis) provide equivocal findings with some showing some benefits and others not(68). Similarly studies conducted in patients with asthma do not provide a clear picture with most studies conducted in adults not showing a clinical benefit, although there are indications of benefits of n-3 PUFA in children and adolescents(69). In most other inflammatory diseases and conditions there are too few studies to draw a clear conclusion of the possible efficacy of n-3 PUFA. One reason for these discrepancies may be that the dose of n-3 PUFA required to treat different inflammatory conditions is not known, although it is evident that the anti-inflammatory effects of these fatty acids are dose-dependent(48).

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