Marine omega-3 fatty acids and inflammation

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Abstract

Marine n-3 polyunsaturated fatty acids (PUFAs) may influence inflammation through a variety of mechanisms; many of these are mediated by, or at least associated with, changes in fatty acid composition of inflammatory cell membranes. Changes in fatty acid composition can modify membrane fluidity, cell signaling mechanisms leading to altered gene expression, and the pattern of lipid mediator production. Human inflammatory cells are typically rich in the n-6 fatty acid arachidonic acid, but the contents of arachidonic acid and of the n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can be altered through oral administration of EPA and DHA. Eicosanoids produced from arachidonic acid (e.g. prostaglandin E\textsubscript{2}) have roles in inflammation. EPA also gives rise to eicosanoids and these may have differing properties from those of arachidonic acid-derived eicosanoids, often being less potent. EPA and DHA give rise to resolvins which are anti-inflammatory and inflammation resolving. Increased membrane content of EPA and DHA (and decreased arachidonic acid content) results in a changed pattern of production of eicosanoids and probably also of resolvins. Changing the fatty acid composition of inflammatory cells also affects the production of peptide mediators of inflammation (cytokines, adhesion molecules etc.). Thus, the fatty acid composition of human inflammatory cells influences their function; the contents of arachidonic acid, EPA and DHA appear to be especially important. The anti-inflammatory actions of marine n-3 PUFAs suggest that they may be of therapeutic use in diseases involving chronic inflammation.

Short title: Fatty acids and inflammation

Key words: Arachidonic acid, Eicosapentaenoic acid; Docosahexaenoic acid; Eicosanoid, Cytokine
Introduction

Inflammation is a normal defense mechanism that protects the host from infection and other insults; it initiates pathogen killing as well as tissue repair processes and helps to restore homeostasis at infected or damaged sites. It is typified by redness, swelling, heat, pain and loss of function, and involves interactions amongst many cell types and the production of, and responses to, a number of chemical mediators. Where an inflammatory response does occur, it is normally well regulated in order that it does not cause excessive damage to the host, is self-limiting and resolves rapidly. This self-regulation involves the activation of negative feedback mechanisms such as the secretion of anti-inflammatory mediators, inhibition of pro-inflammatory signaling cascades, shedding of receptors for inflammatory mediators, and activation of regulatory cells. As such, when controlled properly, regulated inflammatory responses are essential to remain healthy and maintain homeostasis. Pathological inflammation involves a loss of tolerance and/or of regulatory processes. Where this becomes excessive, irreparable damage to host tissues and disease can occur. Irrespective of the cause of the inflammation, the response involves four major events:

- An increased blood supply to the site of inflammation;
- Increased capillary permeability caused by retraction of endothelial cells. This permits larger molecules, not normally capable of traversing the endothelium, to do so and thus delivers soluble mediators to the site of inflammation;
- Leukocyte migration from the capillaries into the surrounding tissue. This is promoted by release of chemoattractants from the site of inflammation and by the upregulation of adhesion molecules on the endothelium. Once in the tissue the leukocytes move to the site of inflammation;
- Release of mediators from leukocytes at the site of inflammation. These may include lipid mediators (e.g. prostaglandins (PGs), leukotrienes (LTs)), peptide mediators (e.g. cytokines), reactive oxygen species (e.g. superoxide), amino acid derivatives (e.g. histamine), and enzymes (e.g. matrix proteases) depending upon the cell type involved, the nature of the inflammatory stimulus, the anatomical site involved, and the stage during the inflammatory response. These mediators normally would play a role in host defense, but when produced inappropriately or in an unregulated fashion they can cause damage to host tissues, leading to disease. Several of these mediators may act to amplify the inflammatory process acting, for example, as chemoattractants. Some of the inflammatory mediators may escape the inflammatory site into the circulation and from there they can exert systemic effects. For example, the cytokine interleukin (IL)-6 induces hepatic synthesis of the acute phase protein C-reactive
protein (CRP), while the cytokine tumour necrosis factor (TNF)-α elicits metabolic effects within skeletal muscle, adipose tissue and bone.

Fatty acid composition of human inflammatory cells and its modification by marine n-3 fatty acids

Polyunsaturated fatty acids (PUFAs) are important constituents of the phospholipids of all cell membranes. The bulk phospholipids of inflammatory cells (e.g. neutrophils, lymphocytes, monocytes) from the blood of humans consuming typical Western diets contain about 10 to 20% of fatty acids as arachidonic acid (20:4n-6), with about 0.5 to 1% eicosapentaenoic acid (20:5n-3; EPA) and about 2 to 4% docosahexaenoic acid (22:6n-3; DHA) 1-12, although there are differences between the different phospholipid classes in terms of the content of these fatty acids 3). The fatty acid composition of these cells can be modified by increasing intake of marine n-3 fatty acids 1-8,10-12. This occurs in a dose response fashion (Figure 1) and over a period of days to weeks, with a new steady-state composition reached within about 4 weeks (Figure 2). Typically the increase in content of n-3 PUFAs occurs at the expense of n-6 PUFAs, especially arachidonic

Figure 1. Dose-dependent incorporation of EPA into human blood mononuclear cells. Healthy young males supplemented their diet with differing amounts of an EPA-rich oil for a period of 12 weeks. Plasma and blood mononuclear cell phospholipids were isolated and their fatty acid composition determined by gas chromatography. Data are mean ± SEM from 23 or 24 subjects per group and are expressed as change in EPA from week 0 (study entry). Data are from Rees et al. 12).

Figure 2. Time course of incorporation of EPA and DHA into human blood mononuclear cells. Healthy subjects supplemented their diet with fish oil capsules providing 2.1 g EPA plus 1.1 g DHA per day for a period of 12 weeks. Blood mononuclear cell phospholipids were isolated at 0, 4, 8 and 12 weeks and their fatty acid composition determined by gas chromatography. Data are mean ± SEM from 8 subjects and are from Yaqoob et al. 6).
Changing membrane phospholipid fatty acids can impact on inflammatory cell function

PUFAs play roles assuring the correct environment for membrane protein function, maintaining membrane fluidity and regulating cell signaling, gene expression and cellular function. In addition, some PUFAs, particularly arachidonic acid, act as substrates for synthesis of eicosanoids, which are involved in regulation of many cell and tissue responses, including aspects of inflammation and immunity. Thus, changes in membrane phospholipid fatty acid composition might be expected to influence immune cell function in a variety of ways:

- alterations in the physical properties of the membrane such as membrane order (“fluidity”) and raft structure;
- effects on cell signaling pathways, either through modifying the expression, activity or avidity of membrane receptors or modifying intracellular signal transduction mechanisms. As a result of these effects, transcription factor activation is altered and gene expression modified;
- alterations in the pattern of lipid mediators produced. The different mediators have different biological activities and potencies.

Lipid mediators: biosynthesis, roles in inflammation, and the impact of marine n-3 fatty acids

Eicosanoids generated from arachidonic acid

Eicosanoids are key mediators and regulators of inflammation and immunity and are generated from 20 carbon PUFAs. Eicosanoids, which include PGs, thromboxanes, LTs and other oxidised derivatives, are generated from arachidonic acid by the metabolic processes summarized in Figure 3. Eicosanoids are involved in modulating the intensity and duration of inflammatory responses, have cell- and stimulus-specific sources and frequently have opposing effects. Thus, the overall physiological (or pathophysiological) outcome will depend upon the cells present, the nature of the stimulus, the timing of eicosanoid generation, the concentrations of different eicosanoids generated and the sensitivity of target cells and tissues to the eicosanoids generated. Because of the relatively high amount of arachidonic acid in inflammatory cell membrane phospholipids, this fatty acid is typically the major precursor for eicosanoid mediators, which are produced in greatly increased amounts upon cellular stimulation. Thus, amongst the mix of eicosanoids produced, those synthesized from arachidonic acid (e.g. PGE2 and LTB4) predominate although the exact eicosanoid profile depends upon the cell type concerned (e.g. neutrophils and mast cells...
produce a lot of PGD₂ whereas monocytes produce a lot of PGE₂) and the nature of the stimulus; the profile will also change over time as the nature of the response to the stimulus alters. In general arachidonic acid-derived eicosanoids act in a pro-inflammatory way, although this is an over-simplification since it is now recognised that PGE₂, for example, has both pro- and anti-inflammatory effects, and that another eicosanoid lipoxin A₄ is anti-inflammatory¹⁶⁻¹⁸).

**Fatty acid modification of eicosanoid profiles**

Animal studies have shown a direct relationship between arachidonic acid content of inflammatory cell phospholipids and ability of those cells to produce PGE₂¹⁹), such that PGE₂ production is increased by arachidonic acid feeding¹⁹) and decreased by EPA or DHA feeding¹⁹⁻²¹). It is well documented that PGE₂ and 4 series-LT production by human inflammatory cells can be significantly decreased by fish oil supplementation of the diet for a period of weeks to months¹⁻³,⁵,²²,²³). EPA is also a substrate for the cyclooxygenase and lipoxygenase enzymes that produce eicosanoids, but the mediators produced have a different structure from the arachidonic
acid-derived mediators, and this influences their potency. Increased generation of 5-series LTs has been demonstrated using macrophages from fish oil-fed mice and neutrophils from humans supplemented with oral fish oil for several weeks. The functional significance of the generation of eicosanoids from EPA is that EPA-derived mediators are often much less biologically active than those produced from arachidonic acid (Figure 4) or may even antagonise the action of those produced from arachidonic acid, although this is not always the case and the full range of possible actions has not been compared.

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

EPA and DHA also give rise to resolvins through pathways involving cyclooxygenase and lipoxygenase enzymes. These mediators have been demonstrated in cell culture and animal feeding studies to be anti-inflammatory and inflammation resolving (Figure 4). The latter effect of resolvins and related compounds may be very important because resolution of inflammation is important in shutting off the ongoing inflammatory process and in limiting tissue damage.

![Figure 4. General overview of synthesis and actions of lipid mediators produced from arachidonic acid, EPA and DHA. COX, cyclooxygenase; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin.](image)

**Influence of marine n-3 fatty acids on leukocyte chemotaxis**

A number of dietary supplementation studies with fish oil have demonstrated a time-dependent decrease in chemotaxis of human neutrophils and monocytes towards various chemoattractants including LTB₄, bacterial peptides and human serum. Both the distance of cell migration and the number of cells migrating were decreased. Despite the high dose of marine n-3 PUFAs used in many of these studies, a dose response study by Schmidt et al. suggests that near-maximum inhibition of chemotaxis occurs at an intake of 1.3 g EPA+DHA/day. The mechanism by which n-3 PUFAs inhibit chemotaxis is not clear but may relate to reduced expression or antagonism of receptors for chemoattractants.
Influence of marine n-3 PUFAs on adhesion molecules and adhesive interactions

Cell culture 31-34) and animal feeding studies 35) report decreased expression of some adhesion molecules on the surface of monocytes 34), macrophages 35) or endothelial cells 31-33) following exposure to marine n-3 PUFAs. In some cases this was shown to result in decreased adhesion between leukocytes and endothelial cells. Supplementing the diet of healthy humans with fish oil providing about 1.5 g EPA+DHA/day resulted in a lower level of expression of ICAM-1 on the surface of blood monocytes stimulated \textit{ex vivo} with interferon-\(\gamma\) 36). Dietary fish oil providing 1.1 g EPA+DHA/day was found to decrease circulating levels of soluble VCAM-1 in elderly subjects 37), but it is not clear if this represents decreased surface expression of VCAM-1.

Influence of marine n-3 fatty acids on inflammatory cytokines

**Transcription factors involved in regulating inflammatory gene expression**

In addition to effects on inflammation mediated by changes in the pattern of eicosanoids and other lipid mediators produced, marine n-3 PUFAs have also been shown to alter the production of inflammatory proteins including chemokines, cytokines, growth factors and matrix proteases. This effect may be mediated by altered activation of key transcription factors involved in regulating inflammatory gene expression. Two transcription factors that are likely to play a role in inflammation are nuclear factor \(\kappa\) B (NF\(\kappa\)B) and peroxisome proliferator activated receptor (PPAR)-\(\gamma\). NF\(\kappa\)B is the principal transcription factor involved in upregulation of inflammatory cytokine, adhesion molecule and cyclooxygenase-2 genes 38,39). NF\(\kappa\)B is activated as a result of a signalling cascade triggered by extracellular inflammatory stimuli and involving phosphorylation of an inhibitory subunit (inhibitory subunit of NF\(\kappa\)B (I\(\kappa\)B)) which then allows translocation of the remaining NF\(\kappa\)B dimer to the nucleus 40). The second transcription factor, PPAR-\(\gamma\), is believed to act in an anti-inflammatory manner. While PPAR-\(\gamma\) directly regulates inflammatory gene expression, it also interferes with the activation of NF\(\kappa\)B creating an intriguing interaction between these two transcription factors 41). Both NF\(\kappa\)B and PPAR-\(\gamma\) may be regulated by n-3 PUFAs.

**Fatty acid modulation of inflammatory cytokine production and of transcription factor activation**

EPA and DHA inhibited endotoxin-stimulated production of IL-6 and IL-8 by cultured human endothelial cells and EPA or fish oil inhibited endotoxin-induced TNF-\(\alpha\) production by cultured monocytes 29). EPA or fish oil decreased endotoxin-induced activation of NF\(\kappa\)B in human monocytes 29) and this was associated with decreased I\(\kappa\)B phosphorylation, perhaps due to decreased activation of mitogen-activated protein kinases. These
observations suggest effects of marine n-3 PUFAs on inflammatory gene expression via inhibition of activation of the transcription factor NFκB.

Animal feeding studies with fish oil support the observations made in cell culture with respect to the effects of marine n-3 PUFAs on NFκB activation and inflammatory cytokine production. Several studies in healthy human volunteers involving supplementation of the diet with fish oil have demonstrated decreased production of TNF-α, IL-1β, IL-6 and various growth factors by endotoxin-stimulated monocytes or mononuclear cells (a mixture of lymphocytes and monocytes), although not all studies confirm this effect.

Conclusions

Marine n-3 PUFAs may influence inflammation through a variety of mechanisms; many of these are mediated by, or at least associated with, changes in fatty acid composition of inflammatory cell membranes. Changes in these compositions can modify membrane fluidity, phospholipid-based cell signaling leading to altered gene expression, and the pattern of lipid mediator production. Human inflammatory cells are typically rich in the n-6 fatty acid arachidonic acid, but the contents of arachidonic acid and of the n-3 fatty acids EPA and DHA can be altered through oral administration of EPA and DHA. Eicosanoids produced from arachidonic acid have roles in inflammation. EPA also gives rise to eicosanoids and these may have differing properties from those of arachidonic acid-derived eicosanoids. EPA and DHA give rise to newly discovered resolvins which are anti-inflammatory and inflammation resolving. Increased membrane content of EPA and DHA (and decreased arachidonic acid content) results in a changed pattern of production of eicosanoids and probably also of resolvins, although the latter are not well examined in the human context. Changing the fatty acid composition of inflammatory cells also affects the production of peptide mediators of inflammation (cytokines, adhesion molecules etc.). Thus, the fatty acid composition of human inflammatory cells influences their function; the contents of arachidonic acid, EPA and DHA appear to be especially important. The anti-inflammatory actions of marine n-3 PUFAs suggest that they may be of therapeutic use in diseases involving chronic inflammation.

REFERENCES


2) Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, van der Meer JM, Cannon JG, Rogers TS, Klemppner MS, Weber PC, Schaeffer EJ, Wolff SM and


5) Caughey GE, Mantzioris E, Gibson RA, Cleland LG and James MJ. The effect on human tumor necrosis factor \( \alpha \) and interleukin 1\( \beta \) production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. Am J Clin Nutr, 63: 116-122, 1996.


