n-3 Fatty acids and the inflammatory response — biological background

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Inflammation can be defined as the reaction of a living vascularized tissue to a localized damage, and plays a role in both normal repair reactions and in the pathogenesis of disease. Inflammatory phenomena are at the basis of a number of disease processes in virtually any systemic or organ-specific disease, ranging from classical rheumatic diseases to bronchial airway hyper-responsiveness, inflammatory bowel disease, kidney diseases, psoriasis and atopic eczema. Modulation of long-chain polyunsaturated fatty acid intake, mostly by increasing the relative proportions of n-3 versus n-6 fatty acids, is the clearest example of how diet may modulate the inflammatory process. It is possible that many of the environmentally attributable epidemiological differences in the incidence of inflammatory diseases among different populations can be tracked back to different nutritional intake of selected, quantitatively minor nutritional components such as omega-3 fatty acids. The increase in dietary intake of these compounds, or their pharmacological supplementation, leads to a moderate quenching of the inflammatory reaction which may prove useful in selected clinical conditions. The clarification of the mechanisms of the biological action of these, as well as of other dietary components, and a better documentation of the spectrum of clinical possibilities offered by dietary manipulation in the intake of such compounds, linking together classical nutritional science, molecular biology, epidemiology and clinical medicine, are a frontier for nutritional research in the years to come. This nutritional approach promises to gain a place in the therapy of some inflammatory disorders.

The inflammatory reaction and points of attack for its modulation

Inflammation can be defined as the reaction of a living vascularized tissue to localized damage[1], and plays a role in both normal repair reactions and in the pathogenesis of disease. The inflammatory reactions are usually defined as acute or chronic on the basis of both their temporal duration and the prevailing phenomena. Acute inflammation, lasting minutes to hours, has its main features in fluid and plasma protein exudation (oedema) and leukocyte (mainly neutrophil) migration. Chronic inflammation lasts longer, is less stereotyped, and is associated histologically with the presence of lymphocytes and macrophages, as well as with the proliferation of small blood vessels and of connective tissue. Inflammatory phenomena are at the basis of a number of disease processes in virtually any systemic or organ-specific disease, ranging from classical rheumatic diseases to bronchial airway hyper-responsiveness, inflammatory bowel disease, kidney diseases, psoriasis and atopic eczema. A scheme of the tissue phenomena occurring in inflammation is given in Table 1. All phases of the inflammatory reaction are sustained by the local production of mediators[2,3], each of which may be a theoretical target for drugs or therapeutic interventions. It has recently been appreciated that many of these phases may be modulated by diet. Dietary modulation of the inflammatory reaction is thus now achievable as a therapeutic option in the treatment of a variety of human diseases. Selected dietary components can also be supplemented in amounts not easily achieved by diet, thus configuring truly ‘pharmacological’ modalities based on dietary components. This paper will review the main options presently available for these interventions, their proposed rationale and mechanism of action. This paper is not intended to review the clinical results obtained in clinical settings.

Omega-3 polyunsaturated fatty acids and inflammation

Present mainly in seafood, and therefore better known as ‘fish oils’, highly unsaturated fatty acids (FAs) of the n-3 series (omega-3 FAs) are probably the best example of how diet may affect inflammation. These compounds exert a remarkable variety of biological effects[4–6].

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because of this, they are currently being tested in a variety of clinical situations as heterogeneous as coronary artery disease, hypertension, hyperlipidaemia, cancer, diabetes, renal diseases, and a number of inflammatory states. The reader is referred to the quoted recent reviews covering their use in these conditions, while this section will focus on their use in inflammatory states.

Biological properties and effects of n-3 fatty acids and their potential relevance to inflammation

Current medical interest in n-3 fatty acids stems from observations of the different prevalence of some chronic diseases in the Greenland (Eskimo) population relative to Western populations. Diseases with lower prevalence in Eskimos compared with control Danes include myocardial infarction, from which the main source of interest for these compounds as preventive agents in coronary artery disease has derived, but also conditions such as psoriasis, bronchial asthma, diabetes mellitus and thyrotoxicosis, which share a background of inflammation or derangement in immunity. Increased nutritional intake of marine fish and mammals, providing increased supply of n-3 FAs, was pointed out as the main factor responsible for these differences. Mammals in general cannot synthesize FAs with double bonds distal to the C-9 (starting counts from the methyl end of the carbon chain), although they are able, to some extent, to elongate (increase carbon-chain length) and further desaturate (increase the number of double bonds) the aliphatic chain. Two main families of long-chain polyunsaturated fatty acids exist, biologically derived from the shortest non-synthesizable precursors linoleic acid (C18:2n-6) and alpha-linolenic acid (C18:3) (Fig. 1). Linoleic acid is abundant in oils from most vegetable seeds such as corn and safflower. Alpha-linolenic acid is found in the chloroplasts of green leafy vegetables. Humans can desaturate and elongate alpha-linolenic acid to eicosapentaenoic acid (EPA) and, further, to docosahexaenoic acid (DHA). However, the elongation and desaturation processes are likely to be slow and possibly further limited with ageing and disease conditions.

n-3 FAs exert a remarkable variety of biological effects, many of which may affect inflammation and clinical conditions related to its presence. The most important of these are now discussed in greater detail.

Production of eicosanoids and related lipid mediators

Until recently, the prevailing hypothesis to explain the proteiform variety of effects of n-3 FAs was that their action could be related to the different profile of activities of neo-synthesized soluble lipid mediators derived from these acids.
from EPA, compared with those derived from the normally more abundant arachidonic acid (AA) (Fig. 3).

Both AA and EPA are fatty acids with twenty (in Greek ‘eicosa’) carbon atoms, and the presence of four or five cis double bonds, each inducing a bending of the otherwise linear aliphatic chain. These bendings allow the occurrence of a ‘hairpin’ configuration and the subsequent enzymatic transformation of the fatty acid precursor in a variety of compounds commonly designated ‘eicosanoids’. This term now encompasses a number of classes of related compounds, named prostaglandins (PGs), thromboxanes (TXs), leukotrienes (LTs), hydroxy- and epoxy-FAs, lipoxins (LXs) and isoprostanes. The initial step in biosynthesis of these compounds is thought to be a receptor- or physical perturbation-mediated influx of Ca\(^{2+}\) ions causing translocation of a cytoplasmic phospholipase A\(_2\) to the cell membrane\[^{19, 20}\]. The enzyme then catalyses the hydrolysis of the esterified AA in the sn-2 position\[^{21, 22}\]. A variety of phospholipases A\(_2\) have now been identified, differing in molecular weight, calcium sensitivity and in the specificity for AA\[^{21, 22}\]. The activity of these enzymes appears to be increased by a phospholipase A\(_2\)-activating protein, which is activated by cytokines such as interleukin(IL)-1 and tumour necrosis factor (TNF)\[^{23}\]. A secretory phospholipase A\(_2\) present on the surface of mast cells and other cells may also be involved in the liberation of AA\[^{24}\].

Physical- or agonist-induced activation of cytoplasmic phospholipase A\(_2\) leads to a liberation of free AA. When EPA partially replaces AA as the polyunsaturated fatty acid in the sn-2 position of glycerophospholipids, free EPA is produced. AA or EPA then become available for a variety of enzymes able to drive their further metabolism in directions depending on the cell type where such activation processes occur (Fig. 3).

In platelets and a few other tissues (including the kidney), AA is further metabolized to TX, a powerful vasoconstrictor and inducer of platelet activation. The replacement of EPA leads to the production of a much weaker TXA\(_3\).

In contrast, in endothelia, the products of AA and EPA are the almost equally-active PGI\(_2\) and PGI\(_3\), both vasodilators and inhibitors of platelet activation. In leukocytes, which are pivotal cells in inflammation, the

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**Figure 1** Metabolism and nomenclature of the main polyunsaturated FAs of the linoleic series (left) and the alpha-linolenic series (right). The two metabolic pathways, although largely using the same enzymes without appreciable substrate specificity, are entirely distinct and not interconvertible in animals and humans. Regulation of elongase and desaturates is largely unknown. Both pathways use the same enzymes for chain elongation and desaturation. Recent findings, however, have indicated that formation of DHA from the 22:5n-3 acid occurs through an initial chain elongation to 24:5n-3 acid (in either mitochondria or peroxisomes), which is in turn desaturated in microsomes at position 6 to yield the 24:6n-3 acid. The chain is then shortened via beta-oxidation to yield DHA. This novel biosynthetic pathways is commonly referred to as the ‘Sprecher’s shunt’\[^{25}\].

Dihomo-gamma linolenic acid (GLA) is the precursor of prostaglandins of the 1 series. Arachidonic acid (AA) is the most common eicosanoid precursor; eicosapentaenoic acid (EPA) is the most common precursor of the prostaglandins of the 3 series and of leukotrienes of the 5 series, and the most abundant polyunsaturated FA present in fish oil concentrates docosahexaenoic acid (DHA) is the most abundant n-3 FA accumulated in tissues (especially the central nervous system) and in fish, and can exert its effects partially by retroconversion to EPA and, partially, by itself. See text for further details (modified with permission\[^{13}\]).

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Main metabolism of AA is towards the production of leukotrienes, endowed with chemotactic properties (LTB₄) or vaso-, broncho-constrictory and endothelial-permeabilizing properties (LTC₄, D₄, and E₄) and other lipoxygenase metabolites. Leukocyte production of less active 5-series leukotrienes and other lipoxygenase metabolites contribute to cellular recruitment and activation. EPA also acts as a poor substrate for AA-metabolizing enzymes, leading to a decreased net production of derived compounds (reviewed in References 4–6, 25). Lipoxygenase products of EPA are the weaker corresponding leukotrienes of the 5-series (LTB₅, C₅, D₅, E₅) (Fig. 3), although the most relevant property of n-3 FA incorporation in membrane phospholipids in this respect appears to be the reduced production of such mediators [26]. As a result, a shift in the relative abundance of AA and EPA leads to a new balance of eicosanoids, favouring vasodilating, antiplatelet and less pro-inflammatory compounds. Elevated TXA₂ (by assays of metabolites of its hydrolytic product TXB₂) has been found in patients with systemic lupus erythematosus [27] and in a variety of renal diseases including chronic glomerular disease [28], diabetic nephropathy [29], renal damage caused by cyclosporine [30], renal transplant rejection [31] and proteinuric syndromes [32–35]. Substitution of EPA for AA reduces platelet [8,36–39] as well as renal production of TXs [35]. These changes may be a partial explanation for some of the anti-inflammatory, antihypertensive and renal effects of n-3 FAs.

**Modulation of cell activation and cytokine production**

In addition to changes in eicosanoid metabolism, increased attention is being now paid to n-3 FAs as possible modulators of cytokine production. When administered to healthy volunteers, n-3 FAs decrease bacterial lipopolysaccharide-induced production of the pro-inflammatory cytokines IL-1 and TNF from peripheral blood lymphomonocytes [40,41]. In a different setting — cultured human endothelial cells — the membrane enrichment of n-3 FAs by supplementing culture medium with DHA reduces the ability of endothelial cells to respond to stimulation with bacterial lipopolysaccharide (LPS), IL-1, IL-4 or TNF in terms of surface expression of the leukocyte adhesion molecules vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin, as well as soluble inflammatory cell recruitment and production of inflammatory mediators.

Figure 2 Biological effects of n-3 FAs and the rationale for their anti-inflammatory use. TX, thromboxane; PG, prostaglandin; LT, leukotriene; PAF, platelet-activating factor; PDGF, platelet-derived growth factor. See text for details and references.
cytes expression of tissue factor by stimulated mono-
ties of n-3 FAs, including the ability to modulate the
vides a basis for a reduced responsiveness of cells to
flammatory stimuli, probably due to the ability of n-3
FAs to modulate the activation of transcription factors
of the immune response
acute inflammation, atherosclerosis and the modulation
the concerted activation of a variety of genes involved in
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Preliminary results support this hypothesis[53], thus
led to speculation that this may be another way
by which omega-3 FAs may decrease the production of
pro-inflammatory prostanooids, independent from
competition with AA.

Other biological properties of n-3 FAs
related to modulation of inflammation

These include: reductions of monocyte and neutrophil
chemotaxis[54-57] and leukocyte inflammatory potential[58],
whose mechanism have not yet been investigated in
detail (possibly cytokine and chemokine production);
a reduction of total blood viscosity[59-61], most prob-
ably through a combined effect on red blood cell
 deformability[62], and plasma viscosity, the main deter-
minant of which, concentration of fibrinogen, is favour-
able reduced by these compounds[63-65]; an increase
endothelium-dependent vasodilatation[66,67]; and a
decrease in vasoconstrictive responses to angiotensin
II[68,69]. At least some of these effects may be due to a
modification of intracellular signal transduction path-
ways, in part due to the function of FAs as intracellular
mediators of ‘endothelial activation’, such as IL-6 and
IL-8, able to provide positive feed-backs for the ampli-
fication of the inflammatory response[42-44]. This pro-
vides a basis for a reduced responsiveness of cells to
flammatory stimuli, probably due to the ability of n-3
FAs to modulate the activation of transcription factors
(nuclear factor-κB, NF-κB)[42-45], which can coordinate
the concerted activation of a variety of genes involved in
acute inflammation, atherosclerosis and the modulation
of the immune response[42,46-49]. Other reported properties
of n-3 FAs, including the ability to modulate the ex-
pression of tissue factor by stimulated monocytes[49,50],
or of platelet-derived growth factor-like prote-
in endothelial cells[51] or monocytes[52], could be
due to the same underlying mechanism of action. We
recently addressed the hypothesis that the cytokine-
induced cyclooxygenase-2 (COX-2), expressed in endo-
thelial cells, macrophages and other cell types, which
is another gene controlled by NF-κB, could be regulated
in its expression by DHA at the transcriptional level.
Preliminary results support this hypothesis[53], thus
leading to speculation that this may be another way
by which omega-3 FAs may decrease the production of
pro-inflammatory prostanooids, independent from
competition with AA.

Figure 3  An updated scheme for the origin of the main eicosanoids deriving from
the linoleic series (metabolites of AA) and of the alpha-linolenic series (metabolites
of EPA) relevant to inflammation physiology and pathophysiology. The best char-
acterized metabolic pathway, catalyzed by the enzyme PGH synthase (cyclo-
oxigenase, COX, of which a constitutive and an inducible form are now known),
leads to the formation of prostanooids [prostaglandins (PG) and thromboxanes (TX)]
of the 2 series from AA, and of the 3 series from EPA. Arachidonic acid and EPA
can also be metabolized, in leukocytes and some connective tissue cells, via the
enzyme 5-lipoxygenase (5-LO) to leukotrienes (LT) A4 and A5, respectively.
These labile intermediates can be converted to the more stable LTB (endowed with potent
chemoattractant properties) or, by the addition of a peptide residue, to the sulfido-peptide
LTs (LTC, LTD, LTE), which are powerful vasoconstrictors and able to increase
vascular permeability. The scheme also outlines the possible complex metabolization
of both AA and EPA towards lipoxins (IX) which are also endowed with vasoactive
properties. Lipoxins arise through the combined action of 5-LO and other lipoxy-
genases (15-LO and 12-LO), 15-HETE=15-hydroxyeicosatetraenoic acid; 15-HEPE=15-
hydroxyeicosapentaenoic acid. Cell–cell interactions, including exchanges of substrates
and of intermediate metabolites, are thought to be particularly relevant to the
reduction in many cellular responses occurring when n-3 FAs are added to the diet.
In particular, EPA is a worse substrate for the metabolizing enzymes than AA,
leading to a net absolute reduction in the amount of metabolites generated. The
scheme also outlines the bidirectional relationship of EPA and DHA, by which this
last compound may serve as a storage compartment for EPA. The asterisk (*)
denotes other potential metabolization of AA and EPA to bioactive compounds,
which have been recently appreciated in particular organ systems. These include
the generation of isoprostanes, omega-3 hydroxylation, epioxygenase and cytochrome
P-450/allylic oxidation products.
second messengers themselves in cell activation\[^{70}\](Fig. 2). In general, n-3 FAs have been found to reduce the increase in intracellular calcium. In particular, the enrichment of cellular phospholipids with DHA inhibits calcium transients\[^{81}-^{83}\]. In cardiac myocytes this may occur through a modulation of the L-type calcium channel\[^{71}\]. Alternatively, changes in agonist-induced increase in intracellular calcium may occur through an alteration of the agonist-receptor affinity\[^{83}\] or cell membrane physico-chemical characteristics\[^{76,77}\]. Post-receptor signalling pathways and the formation of second messengers involved in the mobilization of intracellular calcium may be inhibited by reductions of the production of inositol trisphosphate\[^{78,79}\] or by conversion of FAs to cytochrome P450 epoxygenase metabolites\[^{80,81}\].

**Conclusions**

Modulation of long-chain polyunsaturated FA intake, mostly by increasing the relative proportions of n-3 versus n-6 FA is the clearest example of how diet may modulate the inflammatory process. It is possible that many of the environmentally attributable epidemiological differences in the incidence of inflammatory diseases among different populations can be tracked back to different nutritional intake of selected, quantitatively minor nutritional components such as omega-3 FAs. The increase in dietary intake of these compounds, or their pharmacological supplementation, leads to a moderate quenching of the inflammatory reaction, which may prove useful in selected clinical conditions. The clarification of the mechanisms of the biological action, of these as well as of other dietary components, and a better documentation of the spectrum of clinical possibilities offered by dietary manipulation in the intake of such compounds, linking together classical nutritional science, molecular biology, epidemiology and clinical medicine, are a frontier for nutritional research in the years to come. The nutritional approach with n-3 FAs promises to gain a place in the therapy of some inflammatory disorders.

**References**


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