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.
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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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Table 1   Tissue events in acute and chronic inflammation and mediators involved

Events
  Modifications of blood flow and vessel diameter
  Modifications of vascular permeability
  Leukocyte exudation and phagocytosis
    Margination
    Adhesion
    Migration
    Diapedesis
    Phagocytosis
    Extracellular release of leukocyte products
  Remodelling of extracellular matrix
  Cell proliferation (fibroblasts, lymphocytes, monocyte-macrophages)

Mediators
  Vasoactive amines (histamine, serotonin)
  Plasma proteases (complement components, bradykinin, coagulation and fibrinolytic
    components, vascular endothelial growth factor)
  Arachidonic acid metabolites (mainly prostaglandins, thromboxanes and leukotrienes); platelet-
    activating factor; reactive oxygen species; interleukin-1 and 4, chemokines (IL-8, monocyte-
    chemoattractant protein-1), other cytokines and growth factors
  Lysosomal components; cytokines modulating collagen synthesis, matrix metalloproteinases
  Other cytokines and growth factors

because of this, they are currently being tested in a variety of clinical situations as heterogeneous as coronary artery disease[7], hypertension[8,9], hyperlipidaemia[10], cancer[11], diabetes[12], renal diseases[13], 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[14]. 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[14], 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 such differences[15,16]. 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[17] and disease conditions[18]. For these reasons, EPA and DHA are considered, to a large extent, nutritionally essential and nearly exclusively derived from fish. Fish increase their membrane content by eating the phytoplankton rich in either the precursor alpha-linolenic acid or the more elongated compounds EPA and DHA. Fatty fish living in cold seas (e.g. mackerel, salmon and herring) are particularly rich in these compounds, which may give them a selective advantage in preventing low-temperature-related loss in membrane fluidity in cell membranes[17]. Therefore, EPA and DHA may be considered nutritionally essential for most practical purposes and largely derived from fish and marine oils. Concentrated formulations of these compounds are now available from industrial processing of the body fat from fish.

n-3 FAs exert a remarkable variety of biological effects, many of which may affect inflammation and clinical conditions related to its presence (Fig. 2). 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
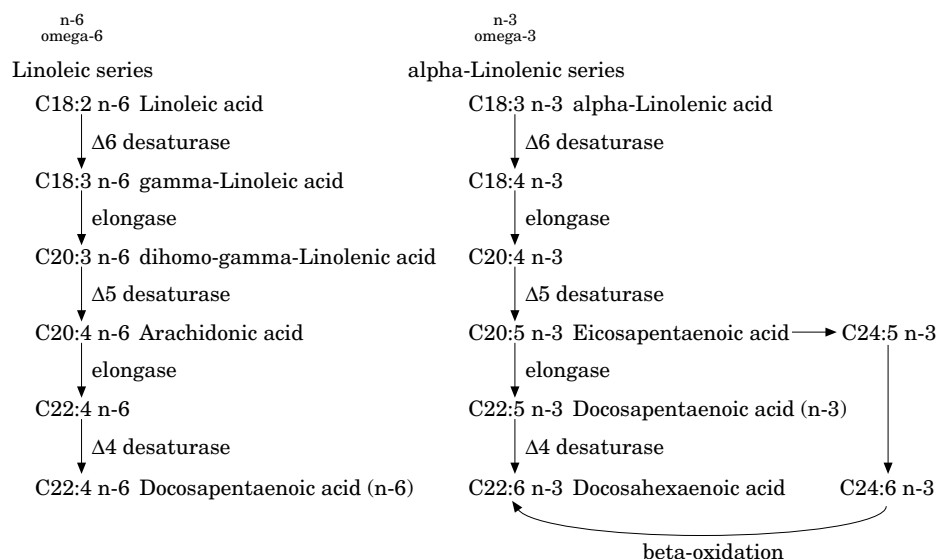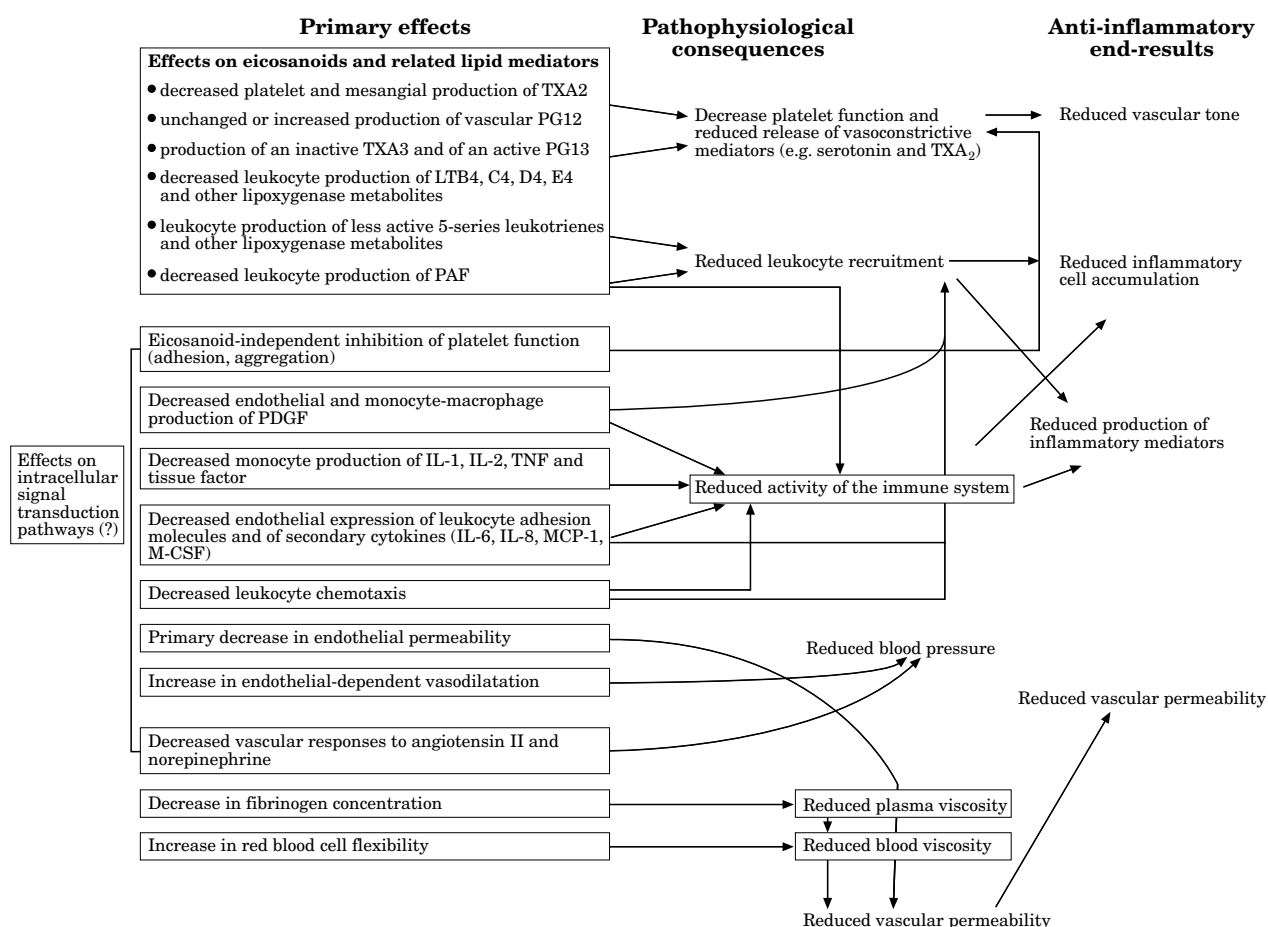
Figure 1 **Metabolism and nomenclature of the main polyunsaturated FAs of the linoleic series (left) and the alpha-linolenic series (right). The two metabolic pathways, although largely using the same enzymes without appreciable substrate specificity, are entirely distinct and not interconvertible in 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'[82]. 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]).**

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). Thus, 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

Primary effects

Pathophysiological consequences

Anti-inflammatory end-results

Effects on eicosanoids and related lipid mediators
- decreased platelet and mesangial production of TXA2
- unchanged or increased production of vascular PG12
- production of an inactive TXA3 and of an active PG13
- decreased leukocyte production of LTB4, C4, D4, E4 and other lipoxygenase metabolites
- leukocyte production of less active 5-series leukotrienes and other lipoxygenase metabolites
- decreased leukocyte production of PAF

Effects on intracellular signal transduction pathways (?)

- Eicosanoid-independent inhibition of platelet function (adhesion, aggregation)
- Decreased endothelial and monocyte-macrophage production of PDGF
- Decreased monocyte production of IL-1, IL-2, TNF and tissue factor
- Decreased endothelial expression of leukocyte adhesion molecules and of secondary cytokines (IL-6, IL-8, MCP-1, M-CSF)
- Decreased leukocyte chemotaxis
- Primary decrease in endothelial permeability
- Increase in endothelial-dependent vasodilatation
- Decreased vascular responses to angiotensin II and norepinephrine
- Decrease in fibrinogen concentration
- Increase in red blood cell flexibility

Decrease platelet function and reduced release of vasoconstrictive mediators (e.g. serotonin and TXA$_2$)

Reduced leukocyte recruitment

Reduced activity of the immune system

Reduced blood pressure

Reduced plasma viscosity

Reduced blood viscosity

Reduced vascular permeability

Reduced vascular tone

Reduced inflammatory cell accumulation

Reduced production of inflammatory mediators
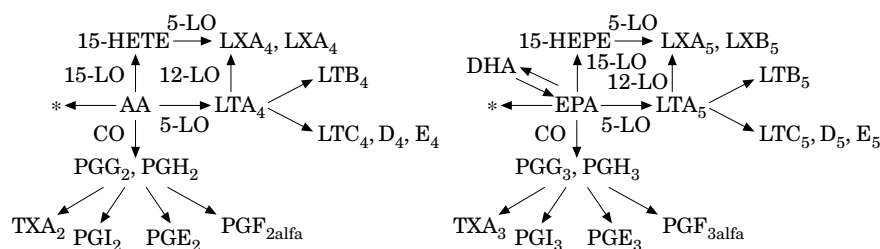
Reduced vascular permeability

Figure 2 **Biological effects of n-3 FAs and the rationale for their antiinflammatory use. TX, thromboxane; PG, prostaglandin; LT, leukotriene; PAF, platelet-activating factor; PDGF, platelet-derived growth factor. See text for details and references.**

main metabolism of AA is towards the production of leukotrienes, endowed with chemotactic properties (LTB$_4$) or vaso-, broncho-constrictory and endothelial-permeabilizing properties (LTC$_4$, D$_4$ and E$_4$). 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 (LTB$_5$, C$_5$, D$_5$, E$_5$) (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 TXA$_2$ (by assays of metabolites of its hydrolytic product TXB$_2$) 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 pro-

duction of TXs[35]. These changes may be a partial explanation for some of the anti-inflammatory, anti-hypertensive 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-I, 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

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\begin{array}{ll}
15\text{-HETE} \xrightarrow{5\text{-LO}} \text{LXA}_4, \text{LXA}_4 & 15\text{-HEPE} \xrightarrow{5\text{-LO}} \text{LXA}_5, \text{LXB}_5 \\
15\text{-LO} \uparrow \quad 12\text{-LO} \uparrow \quad\quad \searrow \text{LTB}_4 & \text{DHA} \searrow \quad 15\text{-LO} \uparrow \quad \searrow \text{LTB}_5 \\
* \leftarrow \text{AA} \longrightarrow \text{LTA}_4 & 12\text{-LO} \\
\text{CO} \downarrow \; 5\text{-LO} \quad\quad \searrow \text{LTC}_4, \text{D}_4, \text{E}_4 & * \leftarrow \text{EPA} \longrightarrow \text{LTA}_5 \\
\text{PGG}_2, \text{PGH}_2 & \text{CO} \downarrow \; 5\text{-LO} \quad \searrow \text{LTC}_5, \text{D}_5, \text{E}_5 \\
& \text{PGG}_3, \text{PGH}_3 \\
\text{TXA}_2 \; \text{PGI}_2 \; \text{PGE}_2 \; \text{PGF}_{2alfa} & \text{TXA}_3 \; \text{PGI}_3 \; \text{PGE}_3 \; \text{PGF}_{3alfa}
\end{array}
$$

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 characterized metabolic pathway, catalyzed by the enzyme PGH synthase (cyclo-oxygenase, COX, of which a constitutive and an inducible form are now known), leads to the formation of prostanoids [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 chemotactic 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-hydroxytetraenoic acid; 15-HEPE = 15-hydroxypentaenoic acid. Cell–cell interactions, including exchanges of substrates and of intermediate metabolites, are thought to be particularly relevant to the generation of LO metabolites. On average, metabolites derived from EPA are less active than the corresponding species derived from AA, potentially explaining 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.**

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 inflammatory 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[42,46–48]. Other reported proper-ties of n-3 FAs, including the ability to modulate the expression of tissue factor by stimulated mono-cytes[49,50], or of platelet-derived growth factor-like pro-teins 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 prostanoids, 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 poten-tial[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-ably reduced by these compounds[63–65]; an increase in 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 modulation of intracellular signal transduction path-ways, in part due to the function of FAs as intracellular

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[71–73]. In cardiac myocytes this may occur through a modulation of the L-type calcium channel[74]. Alternatively, changes in agonist-induced increase in intracellular calcium may occur through an alteration of the agonist-receptor affinity[75] 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.

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