Effects of Omega-3 Fatty Acids on Cytokines and Adhesion Molecules

Raffaele De Caterina, MD, PhD, Rosalinda Madonna, MD, PhD, and Marika Massaro, DBiol, PhD*

Address
*Institute of Cardiology, "G. d'Annunzio" University – Chieti, C/o Ospedale S. Camillo de Lellis, Via Forlanini 50, 66100 Chieti, Italy.
E-mail: rdecater@unich.it

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The dietary intake of omega-3 (n-3) polyunsaturated fatty acids has emerged, over the past 20 years, as an important way to modify cardiovascular risk. This likely occurs through beneficial effects at all stages in the natural history of vascular disease, from the inception of atherosclerotic lesions, to their growth and acute complications (plaque rupture in most instances), up to protection of myocardium from the consequences of ensuing acute myocardial ischemia. This review specifically focuses on the modulating effects of n-3 fatty acids on biologic events involved in early atherogenesis, including important properties of these natural substances on endothelial expression of adhesion molecules and cytokines, processes collectively denoted as "endothelial activation." By decreasing the endothelial responsiveness to proinflammatory and proatherogenic stimuli, n-3 fatty acids act on molecular events not targeted by any other drugs or interventions, and thereby complementary to those of already implemented pharmacologic treatments.

Introduction

The dietary intake of omega-3 (n-3) polyunsaturated fatty acids (FA) has emerged over the past 20 years as an important way to modify cardiovascular risk. This likely occurs through beneficial effects at all stages in the natural history of vascular disease, from the inception of atherosclerotic lesions, to their growth and acute complications (plaque rupture in most instances), up to protection of myocardium from the consequences (including ventricular arrhythmias) of ensuing acute myocardial ischemia. In this review we focus on the modulating effects of n-3 FA on biologic events involved in early atherogenesis, including important properties of these natural substances on the endothelial cell expression of adhesion molecules and cytokines. We first briefly review the biochemistry and metabolic origin of n-3 FA and the epidemiologic evidence for their protective role in atherogenesis. After describing the molecular events featured in early atherogenesis, we then review experimental data showing the modulating effects of n-3 FA on the endothelial expression of adhesion molecules and cytokines and point out the likely molecular targets of these substances.

Omega-3 Fatty Acids and Cardiovascular Disease

Biochemistry

Fatty acids are organic acids with an aliphatic chain and a carboxyl ($^{-}$COOH) group. The aliphatic chain may be completely saturated (*ie*, containing only single bonds between carbon atoms) or unsaturated, with one (monounsaturated FA) or more double bonds (polyunsaturated FA). Numbering of carbon atoms in FA starts from the carboxyl carbon, designated as 1 in the nomenclature of the International Union for Pure and Applied Chemistry (IUPAC). The next carbon atom is designated as 2, as well as α in the traditional nomenclature. The terminal methyl carbon is, therefore, designated as "n" according to the IUPAC nomenclature, and as "ω' according to the traditional nomenclature. The position of a double bond may be thus indicated by counting backwards from this end of the chain. If, for example, a double bond is present between the third and fourth carbon atoms from the methyl end, it is described as n-3 ("n minus 3") or ω-3 ("omega-3"). The structure of FA is thus abbreviated, indicating the number of carbon atoms, followed by the number of double bonds and the position of the most distal unsaturation. For example, eicosapentaenoic acid (EPA) is abbreviated as C20:5 n-3 (or C20:5ω-3); this abbreviation indicates a FA with 20 carbon atoms and 5 double bonds, the last of which is located at the third carbon atom from the distal end of the aliphatic chain. Biologic properties of FA depend on the length of the aliphatic chain, the number of double bonds (degree of unsaturation), and their position. Polyunsaturated FAs belong to two different series: the n-6 family (n-6 FA), deriving from linoleic acid (LA) as metabolic precursor, and the n-3 FA deriving from alpha-linolenic acid (ALA). These FAs are defined as "essential" because humans (and mammals in general) are unable to synthesize them

and, therefore, must introduce them with the diet. LA is widely distributed in nature and is found in most vegetable oils. ALA is abundant in some plant oils (such as canola oil, rapeseed oil, and linseed oil) and in walnuts. All n-3 FAs may be synthesized from simple precursors in the chloroplasts of green leaves, in marine phyto- and zooplankton, and in some worms, which are able to convert n-6 into n-3 FA. This is an enzymatic process missing in higher organisms, including mammals. Mammals, therefore, derive all their intake of n-3 FA from their diet. Furthermore, although longer chain (with 20 or more carbon atoms, also called "highly unsaturated" FA) acids can be synthesized to some extent from shorter 18-carbon atom precursors, this process is very limited in mammals, with the practical consequence that most of EPA and docosahexaenoic acid (DHA) in our body has to be introduced preformed from the diet (usually from fish).

Omega-6 and n-3 FA are fundamental components of phospholipids in cellular membranes, esterified in the sn-2 position of the phospholipids. They can be released through the action mainly of phospholipase A_2 and then metabolized through reactions catalyzed by cyclooxygenase and lipoxygenases to eicosanoids, including prostaglandins (PG), thromboxanes (TX), and leukotrienes (LT), which are important mediators of a vast number of biologic effects. Arachidonic acid (AA) is the precursor of prostanoids of the 2-series (PGI_2 and TXA_2), whereas EPA is the precursor of prostanoids of the 3-series (PGI_3 and TXA_3). Increasing the content of n-3 FAs in the diet causes a partial substitution of the FA of n-6 series, especially decreasing the relative proportions of AA in cell membrane phospholipids. This causes a net decrease in the production of prostanoids (because n-3 FAs are worse substrates for the metabolyzing enzymes) and favors the synthesis of generally weaker prostanoids, especially TXA_3, which contrary to AA-derived TXA_2, has minimal platelet-aggregating and vasoconstricting activity. The results of these changes in eicosanoid production are vasodilatation and inhibition of platelet aggregation. The increase in vascular synthesis of nitric oxide or the reduced expression of cytokines, tissue factor, and growth factors may also play an important role in vasoactive responses induced by dietary n-3 FA.

In leukocytes and monocytes, AA and EPA are substrates of 5-lipoxygenase for the synthesis of LT. LTB_4, derived from AA, has potent chemotactic and other leukocyte-activating properties, whereas sulphido-peptide LT (LTC_4, LTD_4, LTE_4) have a vasoconstrictive effect and can increase vascular permeability. Through 5-lipoxygenase, EPA gives rise to LT of the 5-series, namely LTB_5, LTC_5, LTD_5, and LTE_5, which have weaker proinflammatory and vasoconstrictive activities than those of the 4-series. Introducing n-3 FA into the diet, therefore, may thus decrease inflammatory reactions.

In addition to nutritional sources, intake of n-3 FA may also now derive from fish oil supplements, some of which have been tested clinically.

Epidemiologic evidence for effects of omega-3 fatty acid on atherosclerotic vascular disease in humans

Several studies (summarized by De Caterina and Zampolli [1]) have examined the potentially protective role of n-3 FA against atherosclerosis in experimental animals. The evidence for such a protective effect is not fully persuasive from animal studies. In humans, however, nutritional intake of n-3 FA is highly likely to lead to cardioprotection. Protection from death related to cardiovascular disease has been shown in a 30-year follow-up in men free of overt cardiovascular disease at baseline who consumed up to 35 g/d of fish [2]. Baseline blood values of long-chain n-3 FA have been associated with reduced risk of sudden death in a nested case-study conducted on healthy subjects from the Physicians' Health Study cohort [3]. As a summary for this evidence, the American Heart Association recently recommended that all adults eat fish twice a week as a means of coronary heart disease prevention [4•]. However, because of the multifactorial nature of ischemic heart disease, of which atherosclerosis is one, albeit an important, component, evidence about the occurrence of a true antiatherogenic effect of n-3 FA in humans is not easy to gather. Autopsy studies in Alaskan natives (who consume high amounts of fish-derived products) and non-natives (who mostly consume Western-type diets) provide circumstantial evidence about a lesser extent of atherosclerosis in populations exposed to high nutritional intake of n-3 FA. Middaugh [5] and Newman *et al*. [6] reported decreased percent of intima covering with fatty streaks and raised lesion in Alaskan natives with a high n-3 FA dietary intake compared with non-natives. In the study by Newman *et al*. [6], the magnitude of difference in fatty streak development in natives compared with non-natives appears to be larger in younger age groups, suggesting an effect of diet mainly in the early events leading to fully developed atherosclerotic lesions. Prospective studies in humans are few, but mostly point to the true occurrence of such effects. A study of high-dose n-3 FA supplementation on coronary artery disease regression evaluated by angiography was negative [7], but a subsequent well-controlled study, the Study on prevention of Coronary atherosclerosis by Intervention with Marine Omega-3 fatty acids (SCIMO) [8], showed a slower progression in subjects supplemented with lower doses (1.65 g/d of EPA plus DHA). The same authors have recently reported no effect of the treatment on carotid intima-media thickness evaluated by carotid ultrasound in the very same subjects [9], suggesting some district-specificity for the fish oil effect. It has been speculated that 0.5 to 2.0 g/d of n-3 FA is effective in reducing clinical endpoints [10], whereas higher doses would yield no effect [7]. This contention is, however, based on very few studies examining the effects of these substances on true atherogenesis and not on a mixed endpoint. One study after coronary bypass surgery indicates that n-3 FAs significantly reduce vein graft stenosis [11], a process that may be regarded as an accelerated form of atherosclerosis.

Studies on restenosis after percutaneous coronary angioplasty have been contradictory and, at the end, largely inconclusive [12–23], although issues of study design still leave the door open to the possibility that n-3 FA can have some efficacy on restenosis [24•]. Restenosis after percutaneous interventions is, however, the result of a mechanical injury to an already diseased vessel wall, and its relevance to native atherosclerosis is controversial.

Evidence for an antiatherogenic effect of n-3 FA is more persuasive in human than in animal studies, being now based on at least one placebo-controlled prospective study in native atherosclerosis in the coronary arteries and a placebo-controlled prospective study in coronary bypass surgery grafts. These studies indicate reduced atherogenesis in the coronary arteries and probably the aorta related to increased intake.

Biologic Steps and Mediators in Atherogenesis

Atherosclerosis and inflammation share similar basic mechanisms involving the adhesion of leukocytes to the vascular endothelium in their early phases. Multiple protein families, each with a distinct function, provide "traffic signals" for leukocytes. These include 1) the selectin family of adhesion molecules; 2) chemoattractants, some of which (classic chemoattractants), such as N-formyl peptides, complement components, LTB_4, and platelet-activating factor, act broadly on neutrophils, eosinophils, basophils, and monocytes, whereas more recently described chemokines, such as monocyte chemoattractant protein-1 (MCP-1) and interleukin 8 (IL-8), have selectivity for leukocyte subsets; and 3) the immunoglobulin superfamily members on the endothelium (intercellular adhesion molecule [ICAM]-1, ICAM-2, ICAM-3, and vascular cell adhesion molecule-1 [VCAM-1]), which recognize integrin ligands on the leukocyte surface. For neutrophil and probably lymphocyte adhesion, selectins mediate initial tethering of the circulating leukocyte over the endothelium, allowing it to roll over the endothelium, considerably slowing down its speed and allowing leukocytes to sense the presence of chemotactic gradients. Final firm attachment of leukocytes to the endothelium requires the interaction of integrin ligands on the leukocyte surface with immunoglobulin superfamily members expressed on the endothelium, such as ICAM-1, ICAM-2, and VCAM-1. The multiple molecular choices available for each of these ligand-ligand interactions provide great combinatorial diversity in signals, allowing the selective responses of different leukocyte classes to inflammatory agents, the preferential recirculation patterns of lymphocyte subpopulations, or the selective binding of monocytes to arterial endothelium during early phases of atherogenesis.

Because monocyte recruitment into the intima of large arteries is specific for atherosclerosis as compared with other forms of leukocyte-endothelial interactions, it was hypothesized that these localized monocyte-endothelium interactions reflect specific molecular changes in the adhesive properties of the endothelial surface, leading to surface expression of "athero-endothelium-leukocyte adhesion molecules." The first such protein, originally identified in the rabbit hypercholesterolemic model, is VCAM-1, a member of the immunoglobulin superfamily and expressed on human vascular endothelium at least in two molecular forms. Both forms are able to bind a heterodimeric integrin receptor, very late antigen 4 (VLA4), whose leukocyte selectivity of expression on monocytes and lymphocytes, but not on neutrophils, can explain the selectivity of monocyte recruitment in early atherogenesis [25]. Endothelial cells express VCAM-1 early during cholesterol feeding in the rabbit, before the appearance of macrophages/foam cells in the intima of developing fatty streaks, in a temporal pattern consistent with its pathogenetic role in lesion development [26]. Experiments in mice selectively lacking the VCAM-1 gene have recently proven a true causal role for this molecule in atherosclerosis [27]. Pathophysiologically relevant stimuli for VCAM-1 expression in atherogenesis could include minimally oxidized low-density lipoproteins (LDL) or very low-density lipoproteins (VLDL), the advanced glycosylation end products (AGE) associated with diabetes, lipoprotein(a), or perhaps homocysteine, elevated in homocysteinuria and in subtler forms of congenital or acquired enzyme defects its biosynthetic pathway. In addition to these humoral stimuli, VCAM-1 endothelial gene expression also responds to hemodynamic forces, thus potentially explaining the localization of atherosclerosis in particular points of the arterial vasculature. Subsequent to monocyte adhesion, chemoattraction, as well as monocyte activation and proliferation, ensue. Experiments with targeted gene destruction in mice indicate a role for MCP-1 and LTB_4, as well as for macrophage-colony stimulating factor (M-CSF). Plaque growth and a weakening of the extracellular matrix component with final plaque rupture are likely due to an interplay of action of several endothelial- and macrophage-derived products, including the CD/40-CD40 ligand system, cyclooxygenase-2 (COX-2), and matrix metalloproteinase [28,29,30••].

Control of Cytokine and Adhesion Molecule Expression by Unsaturated Fatty Acids

Decreased expression of cytokine and adhesion molecules upon incubation with omega-3 fatty acids relative to other fatty acids

Much of the knowledge on the control of early atherogenesis by n-3 FA has been due to the availability of in vitro models of such early events. De Caterina *et al.* [31,32] used human adult saphenous vein endothelial cells activated by cytokines in one such model. We first assessed the effects of various fatty acids on the surface expression of endothelial leukocyte adhesion molecules and on monocyte/monocytoid cell adhesion, and subsequently characterized mechanisms and functional relevance of such effects. One n-3 FA, DHA, when added to cultured endothelial cells hours to

days before the stimulation with cytokines (which is early enough to allow a significant incorporation of this fatty acid in cell membrane phospholipids), significantly inhibited events connected with endothelial activation, including the expression of adhesion molecules such as VCAM-1, E-selectin, and, to a lesser extent, ICAM-1 after stimulation with virtually any stimulus able to elicit the coordinated expression of such genes [31,32]. Thus, this inhibition could be demonstrated with IL-1α and β, tumor necrosis factor α (TNF-α), IL-4, and bacterial lipopolysaccharide (LPS) (Fig. 1). Inhibition of adhesion molecule expression occurred in a range of DHA concentrations compatible with nutritional supplementation of this fatty acid to normal Western diet, occurred at any time point after the appearance of cytokine effect (modifying the specific kinetics of surface expression of adhesion molecules) and was strictly related in its magnitude to the extent of incorporation into total cell lipids. Indeed, the extent of VCAM-1 inhibitory effect paralleled the incorporation of DHA and the overall increase in incorporation of n-3 FA and was inversely related to the content of n-6 FA. Experiments following the fate of ^{14}C-labelled DHA into cell phospholipids showed a significant incorporation of DHA into the phosphatidyl ethanolamine pool (*ie*, in a specific and not the most abundant phospholipid pool), likely in the inner plasma membrane, and, therefore, in a possibly strategic position to alter intracellular signal transduction pathways. This effect was not limited to the expression of transmembrane molecules involved in leukocyte recruitment but appeared to occur also for other cytokine-activated products, such as the soluble proteins IL-6 and IL-8, involved in either the amplification of the inflammatory response (IL-6) or in the specific chemoattraction for granulocytes (IL-8), and was accompanied by a functional counterpart (*ie*, a reduced monocyte or monocytoid cell adhesion to cytokine activated endothelium). Compared with DHA, EPA was a weaker inhibitor of the expression of these molecules and of monocyte adhesion, although still more potent than other fatty acids. De Caterina *et al*. [31,32] also showed that DHA's effects on VCAM-1 expression are accompanied by parallel reductions in VCAM-1 mRNA steady state levels, as assessed by Northern analysis. Similar results in experiments with remarkably similar design were later reported by Weber *et al*. [33]. These authors also carried these investigations one step further, demonstrating by electrophoretic mobility shift assay an inhibition by DHA of the activation of the nuclear factor (NF)-κB system of transcription factors [33], which controls the coordinated expression of adhesion molecules and of leukocyte-specific chemoattractants upon cytokine stimulation [34,35]. These findings were recently fully confirmed in endothelial cells transfected with a construct of the n-3 desaturase (fat-1) gene, encoding for the enzyme that catalyzes the transformation of an n-6 to an n-3 FA. By these means, endothelial cells are even more enriched in n-3 FA than upon exposure to exogenous FA. In such conditions, about 50% inhibition on the expression of adhesion molecules and monocyte adhesion were confirmed (Kang, Personal communication) [36••].

The double bond as the minimum necessary and sufficient requirement for the inhibition of endothelial activation by fatty acids

De Caterina *et al*. [37] further analyzed endothelial effects of various FA differing in chain length, number, position (n-3 vs n-6 vs n-9) and *cis*/*trans* configuration of the double bonds. From a large number of such experiments using VCAM-1 surface expression (by enzyme immunoassay or flow cytometry) as a readout, we concluded that: 1) saturated FA is inactive; 2) potency of polyunsaturated FA increases in parallel with the increasing number of unsaturations; 3) potency does not depend on chain length; 4) the single double bond present in the monounsaturated FA oleic acid is indeed sufficient to produce all the effects obtainable with higher unsaturated FA, albeit at higher concentrations; and 5) for such an effect to occur, even the configuration (*cis* vs *trans*) of the double bond does not really matter, because oleic acid (19:1 n-9 cis) and its *trans* stereoisomer elaidic acid are of equal potency [37]. Indeed, inhibition of NF-κB activation could also be reproduced upon incubation of endothelial cells with oleic acid [38].

Possible molecular mechanisms by which unsaturated fatty acids inhibit endothelial activation

In order to ascertain mechanisms for these effects, De Caterina *et al*. [39] demonstrated inhibition of NF-κB activation by DHA (the most potent FA inhibitor of endothelial activation) in parallel with measurements of production of hydrogen peroxide by cultured endothelial cells. This reactive oxygen species (or one of more of its downstream unstable products) appears to be a critical mediator of NF-κB activation because of our previous demonstration that treatment of endothelial cells with polyethylene glycol–complexed superoxide dismutase (a cell membrane–permeable form of this enzyme catalyzing the conversion of superoxide anion to hydrogen peroxide) does not much affect VCAM-1 mRNA production, contrary to a treatment with polyethylene glycol catalase, which acts by accelerating the degradation of hydrogen peroxide [39]. These results suggested that some specific reactive oxygen species (hydrogen peroxide or some downstream products) are involved more directly than others (*eg*, superoxide anion) in the activation of NF-κB. Massaro *et al*. [40•] assessed the production of extracellular hydrogen peroxide by endothelial cells stimulated with the cytokine transforming growth factor β (TGF-β) and, more pertinent to results described previously, the production of intracellular hydrogen peroxide (and/or its downstream products) by measuring the intracellular fluorescence after endothelial cell loading with dichloro-fluoresceine before or after stimulation with IL-1 or TNF. In both these experimental systems, we could document (preliminary results) a decrease in baseline production of hydrogen peroxide (or some of its downstream

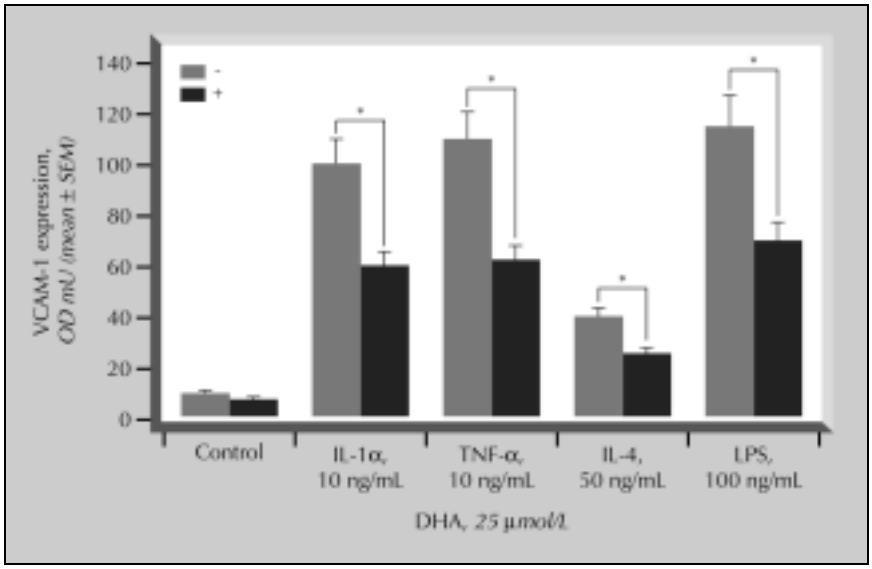

Figure 1. The inhibition of vascular cell adhesion molecule-1 (VCAM-1) expression by docosahexaenoic acid (DHA), occurring with diverse stimuli, including interleukin (IL)-1α, tumor necrosis factor α (TNF-α), IL-4, and bacterial lipopolysaccharide (LPS). *Asterisk* denotes significant differences at $P < 0.01$. (OD—optimal dose; SEM—standard error of mean.)

products) after cell membrane enrichment with DHA, and an even more pronounced dampening of the increase produced by stimulation with cytokines. Saturated FA served as a negative control in these experiments, whereas some effect was also demonstrated with oleic acid [40•]. This suggests that a property related to FA peroxidability (the presence of multiple double bonds) and usually regarded as a detrimental consequence of polyunsaturated FA enrichment of cell membranes, is indeed also directly related to inhibitory properties in the release of some reactive oxygen species crucial for cell responsiveness to cytokines. A tentative model of the site of action of n-3 FA in inhibiting endothelial activation is shown in Figure 2.

These results have led to a reappraisal of how FA may act on endothelial cells in modulating general phenomena such as atherogenesis (mostly investigated by our experimental systems), but also, potentially, inflammation or some immune responses. Because all these effects could be confirmed to occur even in the presence of inhibitors of metabolic conversion of fatty acids to eicosanoids, they provide a novel explanation for the modulating effect of n-3 FA in atherogenesis, distinct from the classic, and now outdated, hypothesis of substrate substitution [41]. The results with oleic acid might also be an explanation for at least some of the beneficial effects of diets rich in olive oil (*ie*, "Mediterranean" diet) on atherogenesis. It is noteworthy in this regard that oleic acid mostly appeared to incorporate at the expense of saturated FA, thus disclosing the possibility of additive effects with n-3 FA, which mostly substitute less unsaturated FA in the membrane phospholipid pools. If extended to cell types different from endothelial cells, such as the monocyte-macrophage, also undergoing activation phenomena upon cytokine or LPS stimulation, they may provide a coherent explanation for a number of previous observations, such as the inhibition of cytokine formation from LPS-activated macrophages [42]. Preliminary results of our studies (De Caterina, Unpublished data) also indicate that similar effects are involved in the transcriptional regulation of the proinflammatory gene COX-2, providing a further explanation for the anti-inflammatory properties of n-3 FA. As to the mechanism(s) involved, these effects might be intrinsically linked to polyunsaturated FA peroxidability, usually regarded as a detrimental effect of higher unsaturated FA, but which could simply be the other side of the same coin. Future research will have to further elucidate molecular aspects of these phenomena as well as the greater scope of this research line in explaining many biologic effects of unsaturated FAs as modulators of biologic responses to cytokines.

Conclusions

Omega-3 FAs have emerged over the past 20 years as an effective and attractive way to reduce the burden of cardiovascular disease both in secondary and in primary prevention. Although their effects on vascular disease are multiple, antiatherogenic effects are most likely an important component of the benefit. Here, the effects of n-3 FA in controlling the transcription of a number of proinflammatory genes (including those encoding for adhesion molecules, chemokines, other soluble cytokines, and COX-2) likely play an important role. In addition, n-3 FAs modulate the production of proinflammatory leukotrienes and may counteract the full-blown expression of atherosclerosis-prone genotypes due to altered activity of 5-lipoxygenase [30••]. The control of gene expression by n-3 FA is an example of how important nutrients are in modulating gene expression. Far from being only the source of energy and building blocks of our body, nutrients fine tune the response of our genes to the environment, and n-3 FAs are a fine example of such properties. By decreasing the endothelial responsiveness to proinflammatory and proatherogenic stimuli, n-3 FAs act on molecular events not

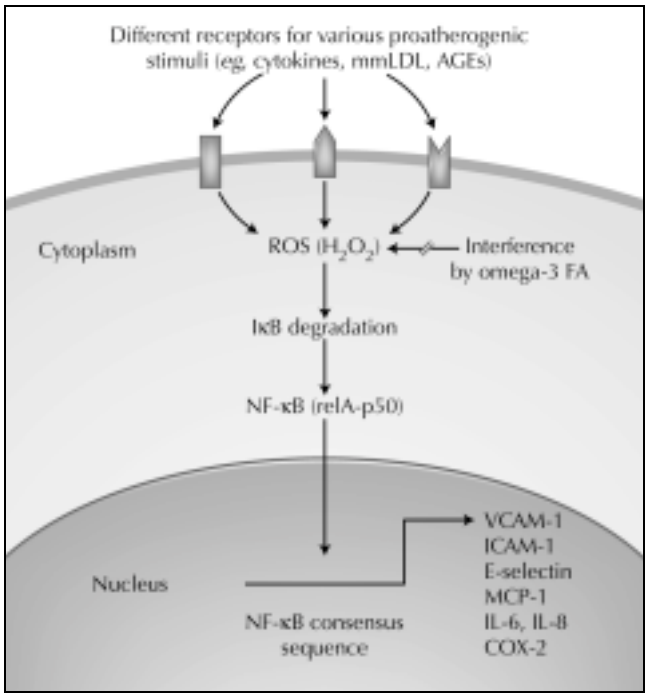

Figure 2. A scheme of the putative site of action of omega-3 fatty acids (FA) on endothelial activation, at the level of generation of reactive oxygen species. (AGE—advanced glycation end products; COX—cyclooxygenase; ICAM-1—intercellular adhesion molecule-1; IκB—NF-κB inhibitor; IL—interleukin; MCP-1—monocyte chemoattractant protein-1; M-CSF—macrophage-colony stimulating factor; mmLDL—minimally modified low-density lipoproteins; NF-κB—nuclear factor-kB; ROS—reactive oxygen species; VCAM-1—vascular cell adhesion molecule-1.)

targeted by any other drugs or interventions, and thereby complementary to those of already fully implemented pharmacologic treatments.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. De Caterina R, Zampolli A: **n-3 fatty acids: antiatherosclerotic effects.** *Lipids* 2001, **36:**S69–S78.
2. Davilgus M, Stamler J, Orencia A, *et al.*: **Fish consumpion and the 30-year risk of fatal myocardial inarction.** *N Engl J Med* 1997, **336:**1046–1053.
3. Albert C, Campos H, Stampfer M, *et al.*: **Blood levels of long-chain n-3 fatty acids and the riesk of sudden death.** *N Engl J Med* 2002, **346:**1113–1118.
4. • Kris-Etherton P, Harris W, Appel L, for the AHA Nutrition Committee: **Omega-3 fatty acids and cardiovascular disease—new recommendations from the American Heart Association.** *Arterioscler Thromb Vasc Biol* 2003, **23:**151–152.

This paper reviews the scientific basis for the recent American Heart Association Recommendation on the adequate intake of n-3 FA in the diet for prevention of cardiovascular disease.

5. Middaugh J: **Cardiovascular death among Alaskan natives 1980–1986.** *Am J Public Health* 1990, **80:**282–285.
6. Newman W, Middaugh J, Propst M, Rogers D: **Atherosclerosis in Alaskan natives and non-natives.** *Lancet* 1993, **341:**1056–1057.
7. Sacks F, Stone P, Gibson C, *et al.*: **Controlled trial of fish oil for regression of human coronary atherosclerosis. HARP Research Group.** *J Am Coll Cardiol* 1995, **25:**234–237.
8. von Shacky C, Angerer P, Kothny W, *et al.*: **The effect of dietary omega-3 fatty acids on coronary atherosclerosis. A randomized, double-blind, placebo-controlled trial.** *Ann Intern Med* 1999, **130:**554–562.
9. Angerer P, Kothny W, Stork S, von Schacky C: **Effect of dietary supplementation with omega-3 fatty acids on progression of atherosclerosis in carotid arteries.** *Cardiovasc Res* 2002, **54:**183–190.
10. Angerer P, von Shacky C: **n-3 polyunsaturated fatty acids and the cardiovascular system.** *Curr Opin Lipidol* 2000, **11:**57–63.
11. Eritsland J, Arnesen H, Gronseth K, *et al.*: **Effect of supplementation with n-3 fatty acids on grft patency in patients undergoing coronary artery bypass operation. Results from the SHOT study [abstract 351].** *Eur Heart J* 1994, **15:**29.
12. Slack J, Pinkerton C, VanTassel J: **Can oral fish oil supplement minimize re-stenosis after percutaneous transluminal angioplasty? [abstract].** *J Am Coll Cardiol* 1987, **9:**64.
13. Dehmer GJ, Popma JJ, van den Berg EK, *et al.*: **Reduction in the rate of early restenosis after coronary angioplasty by a diet supplemented with n-3 fatty acids.** *N Engl J Med* 1988, **241:**733–740.
14. Grigg L, Kay T, Valentine P, *et al.*: **Determinants of restenosis and lack of effect of dietary supplementation with eicosapentaenoic acid on the incidence of coronary artery restenosis after angioplasty.** *J Am Coll Cardiol* 1989, **13:**665–672.
15. Milner M, Gallino R, Leffingwell A, *et al.*: **Usefulness of fish oil supplements in preventing clinical evidence of restenosis after percutaneous transluminal coronary angioplasty.** *Am J Cardiol* 1989, **64:**294–299.
16. Reis G, Boucher T, Sipperly M, *et al.*: **Randomised trial of fish oil for prevention of restenosis after coronary angioplasty.** *Lancet* 1989, **11:**177–181.
17. Nye E, Ilsley C, Ablett M, *et al.*: **Effect of eicosapentaenoic acid on restenosis rate, clinical course and blood lipids in patients after percutaneous transluminal coronary angioplasty.** *Aust NZ J Med* 1990, **20:**549–552.
18. Bairati I, Roy L, Meyer F: **Double-blind, randomized, controlled trial of fish oil supplements in prevention of recurrence of stenosis after coronary angioplasty.** *Circulation S-I* 1992, **85:**950–956.
19. Kaul U, Saghvi S, Bahl V, *et al.*: **Fish oil supplements for prevention of restenosis after coronary angioplasty.** *Int J Cardiol* 1992, **35:**87–93.
20. Franzen D, Schannwell M, Oette K, Hopp H: **A prospective, randomized, and double-blind trial on the effect of fish oil on the incidence of restenosis following PTCA.** *Cathet Cardiovasc Diagn* 1993, **28:**301–310.
21. Bellamy C, Schofield P, Faragher E, Ramsdale D: **Can supplementation with omega-3 polyunsaturated fatty acids reduce coronary angioplasty restenosis rate?** *Eur Heart J* 1992, **3:**1626–1631.
22. Leaf A, Jorgensen M, Jacobs A, *et al.*: **Do fish oils prevent restenosis after coronary angioplasty?** *Circulation S-I* 1994, **90:**2248–2257.
23. Cairns J, Gill J, Morton B, *et al.*, for the EMPAR Collaborators: **Fish oils and low-molecular-weight heparin for the reduction of restenosis after percutaneous transluminal coronary angioplasty. The EMPAR Study.** *Circulation S-I* 1996, **94:**1553–1560.

24.• Maresta A, Balduccelli M, Varani E, *et al.*: **Prevention of post-coronary angioplasty restenosis by omega-3 fatty acids: main results of the Esapent for Prevention of Restenosis Italian Study (ESPRIT).** *Am Heart J* 2002, **143:**1–10.

The latest in a long series of studies aimed at demonstrating effects of n-3 FA on restenosis after percutaneous coronary angioplasty. At variance from some previous studies, this one, conducted with a long (4 to 6 weeks) pretreatment and a relatively high dose of n-3 FA, did demonstrate decreased restenosis in patients allocated to the n-3 FA supplement. Because most angioplasties are now conducted with the use of stents, the issue of whether n-3 FAs prevent post-angioplasty restenosis remains unsettled.

25. De Caterina R, Gimbrone MA Jr: **Leukocyte-endothelial interactions and the pathogenesis of atherosclerosis.** In *n-3 Fatty Acids—Prevention and Treatment in Vascular Disease.* Edited by Kristensen SD, Schmidt EB, De Caterina R, Endres S. London: Springer Verlag; 1995:9–24.

26. Li H, Cybulsky MI, Gimbrone MA Jr, Libby P: **An atherogenic diet rapidly induces VCAM-1, a cytokine-regulatable mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium.** *Arterioscler Thromb* 1993, **13:**197–204.

27. Cybulsky MJ, Iiyama K, Li H, *et al.*: **A major role for VCAM-1, but not ICAM-1, in early atherosclerosis.** *J Clin Invest* 2001, **107:**1255–1262.

28. Libby P: **Inflammation in atherosclerosis.** *Nature* 2002, **420:**866–874.

29. Libby P: **Vascular biology of atherosclerosis: overview and state of the art.** *Am J Cardiol* 2003, **91(Suppl):**3A–6A.

30.•• De Caterina R, Zampolli A: **From asthma to atherosclerosis—5-lipoxygenase, leukotrienes, and inflammation.** *N Engl J Med* 2004, **350:**4–7.

An updated and concise overview of the biologic steps in atherosclerosis, keyed to the role of leukotrienes and their modulation by diet.

31. De Caterina R, Cybulsky MI, Clinton SK, *et al.*: **The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of pro-atherogenic and pro-inflammatory proteins in human endothelial cells.** *Arterioscler Thromb* 1994, **14:**1829–1836.

32. De Caterina R, Cybulsky M, Clinton S, *et al.*: **Omega-3 fatty acids and endothelial leukocyte adhesion molecules.** *Prostagl Leukotr Ess Fatty Acids* 1995, **52:**191–195.

33. Weber C, Erl W, Pietsch A, *et al.*: **Docosahexaenoic acid selectively attenuates induction of vascular cell adhesion molecule-1 and subsequent monocytic cell adhesion to human endothelial cells stimulated by tumor necrosis factor-alpha.** *Arterioscler Thromb Vasc Biol* 1995, **15:**622–628.

34. Collins T: **Endothelial nuclear factor-kB and the initiation of the atherosclerotic lesion.** *Lab Invest* 1993, **68:**499–508.

35. Collins T, Read M, Neish A, *et al.*: **Transcriptional regulation of endothelial cell adhesion molecules: NF-kB and cytokine-inducible enhancers.** *FASEB J* 1995, **9:**899–909.

36.•• Kang JX, Wang J, Wu L, Kang ZB: **Transgenic mice: fat-1 mice convert n-6 to n-3 fatty acids.** *Nature* 2004, **427:**504.

Brilliant demonstration of the possibilities offered by transgenic mouse technology. The transgenic mouse here described has a very low n-6 to n-3 FA ratio, close to the ideal 1:1 in the diet of our hunter-gatherer forebears. Numerous possibilities are opened by this technology to study the effects of n-3 FA in models of atherosclerosis and inflammation.

37. De Caterina R, Bernini W, Carluccio M, *et al.*: **Structural requirements for inhibition of cytokine-induced endothelial activation by unsaturated fatty acids.** *J Lipid Res* 1998, **39:**1062–1070.

38. Carluccio M, Massaro M, Bonfrate C, *et al.*: **Oleic acid inhibits endothelial activation—a direct vascular antiatherogenic mechanism of a nutritional component in the Mediterranean diet.** *Arterioscler Thromb Vasc Biol* 1999, **19:**220–228.

39. De Caterina R, Libby P, Peng HB, *et al.*: **Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines.** *J Clin Invest* 1995, **96:**60–68.

40.• Massaro M, Basta G, Lazzerini G, *et al.*: **Quenching of intracellular ROS generation as a mechanism for oleate-induced reduction of endothelial activation and early atherogenesis.** *Thromb Haemost* 2002, **88:**335–344.

This paper shows that n-3 fatty acids blunt the activation of the transcription factor NF-kB by reducing the production of reactive oxygen species by endothelial cells in response to proinflammatory stimuli.

41. Dyerberg J, Bang HO, Stofferson E, *et al.*: **Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis.** *Lancet* 1978, **2:**117–119.

42. Endres S, Ghorbani R, Kelley VE: **The effect of dietary supplementation with n-3 polyunsaturated fatty acid on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells.** *N Engl J Med* 1989, **320:**265–271.