Basic mechanisms behind the effects of n-3 fatty acids on cardiovascular disease

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\textbf{ABSTRACT}

The epidemiological association between high intakes of n-3 fatty acids (FA) and decreased morbidity and mortality from cardiovascular disease (CVD) can be explained by two main basic mechanisms: (a) an effect on atherothrombosis, and (b) an effect on cardiac arrhythmias. These mechanisms probably reflect different beneficial influences of n-3 FA on cardiovascular biology. Effects on atherothrombosis include the modulation of the expression of pro-atherogenic genes (e.g., endothelial leukocyte adhesion molecules, inflammatory cytokines and cyclooxygenase (COX)-2) and the hepatic synthesis of very low density lipoproteins (VLDL), and are slow in onset, requiring incorporation into cell membrane phospholipids, and usually doses in humans in the order of 3 g/day or higher. Effects on cardiac arrhythmias include complex interactions with ion channels (sodium, potassium and calcium channels), typically requiring the presence of free FA in extracellular fluids and usually occurring with lower doses (around 1 g/day) of nutritional or pharmacological intake. We have focused most of our research effort in unraveling the pathophysiological background of protection by n-3 FA from atherothrombosis. As the result of incorporation of n-3 FA in the sn-2 position predominantly of the phosphatidyl ethanolamine pool in the inner leaflet of the plasma membrane, n-3 FA appear on the one hand to increase the production of bioactive lipid mediators (protectins and resolvins) affecting cytokine-induced signal transduction; and on the other hand to directly interfere with the generation of reactive oxygen species (mostly hydrogen peroxide), directly responsible for the activation of the transcription factor nuclear factor (NF)-\textsuperscript{κ}B, which controls the expression of a variety of pro-inflammatory and pro-atherogenic genes, including those encoding for interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor (TNF)\textsubscript{α}, vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and COX-2. The upstream-direct or indirect-inhibition of cytokine- and other atherogenic trigger-induced signaling pathway may involve interference with the activation of protein kinase (PK) C isoforms and NADP(H) oxidase. Such interference may also explain the blunt anti-inflammatory effect of n-3 FA from atherothrombosis. As the result of incorporation of n-3 FA in the sn-2 position predominantly of the phosphatidyl ethanolamine pool in the inner leaflet of the plasma membrane, n-3 FA appear on the one hand to increase the production of bioactive lipid mediators (protectins and resolvins) affecting cytokine-induced signal transduction; and on the other hand to directly interfere with the generation of reactive oxygen species (mostly hydrogen peroxide), directly responsible for the activation of the transcription factor nuclear factor (NF)-\textsuperscript{κ}B, which controls the expression of a variety of pro-inflammatory and pro-atherogenic genes, including those encoding for interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor (TNF)\textsubscript{α}, vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and COX-2. The upstream-direct or indirect-inhibition of cytokine- and other atherogenic trigger-induced signaling pathway may involve interference with the activation of protein kinase (PK) C isoforms and NADP(H) oxidase. Such interference may also explain the blunt anti-inflammatory effect of n-3 FA in many experimental models and clinical conditions of inflammation. All together, these mechanisms may provide an integrated view of how n-3 FA may affect CVD.

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1. Introduction

Atherosclerosis is the underlying cause of most cardiovascular diseases (CVD), including coronary artery disease, ischemic gangrene, abdominal aortic aneurysms, and many cases of heart failure and stroke. This group of diseases constitutes the main cause of death in the Western world today. The World Health Organization expects CVD to be the main killer worldwide within 15 years, due to the accumulation of several metabolic risk factors, including obesity and diabetes, in both the Western world and developing countries. These facts underscore the need for an intensified research effort in parallel to putting increased preventive measures into action for CVD.

In atherosclerosis, the combined action of frequently coexisting risk factors, such as dyslipidemia, hypertension, hyperglycemia and its consequences, induce the gradual thickening of the arterial wall to form an atherosclerotic plaque, occasionally resulting in the narrowing of the artery lumen. This may cause the chronic onset of organ ischemia, most commonly affecting the heart and the brain. Plaques can however also abruptly rupture, causing thrombosis, the main underlying cause of myocardial infarction or stroke. Intensive studies of the cellular and molecular...
mechanisms that underlie atherogenesis have led to a general agreement on the pathogenesis of this process, recognizing the involvement of chronic inflammation at every step of the process, from its onset, to its progression, to its ultimate clinical manifestations. Most risk factors indeed act by promoting atherogenesis, by inducing or intensifying such underlying inflammatory processes.

The recent recognition that diet strategically affects the majority of modifiable risk factors for CVD has further highlighted the importance of a correct dietary approach to fight atherosclerosis [1], emphasizing the recently appreciated anti-inflammatory properties of specific essential fatty acids (FA), mostly the n-3 FA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as extremely valuable preventive or therapeutic means.

Since the early studies in Greenland Eskimos, many studies have confirmed the association between n-3 FA and decreased risk of CVD [2]. Of particular interest are two recent meta-analyses, suggesting that EPA and DHA significantly reduce the rates of cardiac and sudden death, and possibly stroke also for modest consumption of fish (1–2 servings/week), thus remarking that the anti-inflammatory and lipid-regulating effects exerted by n-3 FA exceed the potential health risks from consumption of fish (1–2 servings/week), thus remarking that the beneficial contribution of an “unorthodox” generation of alternative eicosanoid derivatives of n-3 FA has been recently appreciated. Such alternative compounds are typically produced during the resolution of self-limited inflammation. These compounds were first identified by Serhan et al. with the trivial name of resolvins (resolution phase interaction products), to emphasize their original isolation and production during the resolution phase of acute inflammation and to signify the frequent contribution of the transcellular biosynthesis of these new mediators. The production of resolvins is mediated by the serial combined activities of acetylated COX-2 (or cytochrome P-450 monooxygenase) and 5-LO on EPA, to produce the E-series resolvins (Resolvin E1 and 2 or RvE1 and RvE2), and on DHA, to produce the 17R D-series resolvins (RvD1 through RvD4). Upon tissue

2. Metabolism n-3 FA

Usually esterified in the sn-2 position of the phospholipid n-6 FA arachidonic acid (AA) as well as the n-3 FA EPA and DHA, can be released through the action of phospholipase A2 and metabolized through “orthodox” and “unorthodox” pathways (Fig. 1).

The “orthodox pathway” involves reactions catalyzed by cyclooxygenase (COX) and lipooxygenases (LO) to eicosanoids, including prostaglandins (PG), thromboxanes (TX), and leukotrienes (LT), which are mediators of a vast number of biologic effects. AA is the precursor of the prostanoids of the 2-series (including PGI2 (prostacyclin) and TXA2), whereas EPA is the precursor of prostanoids of the 3-series (PGI3 and TXA3).

Increasing the content of n-3 FA in the diet causes a partial substitution of the FA of the 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 FA are worse substrates for the metabolizing enzymes) and favors the synthesis of generally less biologically active prostanoids, especially TXA3, which, contrary to AA-derived TXA2, has minimal platelet-aggregating and vasoconstricting activity. The results of these changes in eicosanoid production are vasodilatation and inhibition of platelet aggregation. In leukocytes and monocytes, AA and EPA are substrates of 5-LO for the synthesis of LT. LTBA derived from AA, has potent chemotactic and other leukocyte-activating properties, whereas sulphido-peptide LT (LTCA, LTDA, LTE4) have vasocostructive effects and can increase vascular permeability. Through 5-LO, EPA gives rise to LT of the 5-series, namely LTBA, LTC5, LTD5, and LTE5, which have weaker pro-inflammatory and vasoconstrictive activities than those of the 4-series. On the contrary, in endothelial cells (EC), AA and EPA are precursors of the almost equipotent PGI2 and PGI3, respectively, both endowed with anti-aggregating and vasodilating properties.

In addition to these metabolic pathways, the existence and the beneficial contribution of an “unorthodox” generation of alternative eicosanoid derivatives of n-3 FA has been recently appreciated. Such alternative compounds are typically produced during the resolution of self-limited inflammation. These compounds were first identified by Serhan et al. with the trivial name of resolvins (resolution phase interaction products), to emphasize their original isolation and production during the resolution phase of acute inflammation and to signify the frequent contribution of the transcellular biosynthesis of these new mediators. The production of resolvins is mediated by the serial combined activities of acetylated COX-2 (or cytochrome P-450 monooxygenase) and 5-LO on EPA, to produce the E-series resolvins (Resolvin E1 and 2 or RvE1 and RvE2), and on DHA, to produce the 17R D-series resolvins (RvD1 through RvD4). Upon tissue

Fig. 1. Metabolism of n-3 FA versus n-6 FA by COX and lipooxygenases. AA = fatty acid(s); PGI2 = prostaglandin H2; PGI3 = prostacyclin; TXA2 = thromboxane A2; PGI4 = prostaglandin D2; PGE4 = prostaglandin E2; PGF4 = prostaglandin F2; EPETE = hydroperoxy eicosatetraenoic acids; EET = epoxyeicosatrienoic acids; LT = leukotrienes; PGI3 = prostaglandin H3; PGI5 = prostaglandin A5; TXA3 = thromboxane A3; PGF5 = prostaglandin F5; GSH = glutathione; PLA2 = phospholipases A2; AA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.
specialization, DHA can be also metabolized by 15-LO to produce the 17S D-series resolvins, with potent anti-inflammatory activities [5] (Fig. 1).

In addition, the formation of another set of compounds, formed by the nitration of unsaturated FA, called nitrolipids, has been shown to occur in vivo and to have potent biological actions. Such derivatives are known as nitro-FA (NO2-FA) and display both cGMP-independent and receptor-dependent signaling actions, as well as robust electrophilic reactivity. Recent data indicate that NO2-FA can signal predominantly via nitric oxide (NO)-independent mechanisms, acting via electrophilic and receptor-mediated reactions to stimulate smooth muscle relaxation, block platelet activation, inhibit human neutrophil function, superoxide generation, nuclear factor (NF)-κB activation, integrin and immunoglobulin expression, thus globally suppressing inflammation [6] (Fig. 1).

3. Biological and molecular effects of n-3 FA on cardiovascular diseases

The reduced morbidity and mortality from CVD by n-3 FA can be explained by two main basic mechanisms: (a) the suppression of cardiac arrhythmias and (b) reduced atherogenesis, occurring through the modulation of specific atherothrombotic risk factors, decreased platelet aggregation, reduced plasma triglycerides and blood pressure, and, above all, the regulation of systemic and local inflammation underlying plaque inception, progression and instability.

3.1. Suppression of cardiac arrhythmias

Several epidemiological studies have supported an important antiarrhythmic effect by n-3 FA, including sudden death, arrhythmic coronary heart disease death and atrial fibrillation [7]. However, recent findings also support the view that such protective effect by n-3 FA occur not only in patient with prior myocardial infarction, but importantly also at a population level in primary prevention, for a fish intake >300 g/week [8]. There are several mechanisms able to explain how n-3 FA may prevent arrhythmias. It is long known that n-3 FA inhibit voltage-gated sodium channels, resulting in a longer relative refractory period and in an increased voltage required for membrane depolarization, which reduces heart rate [9,10]. Furthermore, n-3 FA also maintain the integrity of L-type calcium channels, thus preventing cytosolic calcium overload during periods of ischemic stress [11]. On the other hand, n-3 FA may indirectly contribute to the decrease in the heart rate by improving left ventricular efficiency and reducing blood pressure [12].

3.2. Decreased platelet aggregation

n-6 FA are precursors for the 2-series eicosanoids, which have a wide range of potential effects on metabolic pathways relevant to thrombosis. In fact, although the AA derivative PGL2 is a potent vasodilator, TXA2 stimulates platelet aggregation and produces vasoconstriction, and 5-LO metabolites, LT, have been linked to inflammation and atherogenesis [13]. Consumption of EPA and DHA lower tissue levels of AA by inhibiting its synthesis and by taking its place in membrane phospholipids [14]. EPA-derived 3-series eicosanoids are typically less vasoconstrictive and produce less platelet aggregation than those made from AA [14]. Fish oil could, therefore, be considered as “positive conditioners” of the thromboxane–prostaglandin balance. In general, however, the reduction in platelet function by EPA and DHA, when measurable, is limited in extent. In addition, many described effects of fish oil intervention on surrogate in vitro end point hemostatic indices, such as hemostatic factors, were variable and overall inconsistent [15].

3.3. Reduced triglycerides synthesis

Although the importance of plasma triglycerides in atherogenesis remains controversial [16], recent data reveal an enhancement of inflammatory response by the endothelium exposed to triglyceride–rich lipoproteins [17], thus re-proposing a role of these unmodified lipoproteins as possible critical contributors to inflammation in atherogenesis. One of the most consistent and best recognized anti-atherogenic properties of n-3 FA is in the reduction of fasting and post-prandial serum triglycerides and free FA (FFA) [18]. Such effect occurs by a reduction in the synthesis of triglycerides, and hence the secretion rates of hepatic very low density lipoproteins (VLDL), through the interference with most of the transcription factors that control the expression of enzymes responsible for both triglyceride assembly and FA oxidation. It was shown, for example, that n-3 FA reduce the expression of the sterol regulatory-element-binding proteins (SREBP), transcription factors that regulate cholesterol-, FA-, and triglyceride-synthesizing enzymes [19]. This effect seems to be due to EPA- and DHA-induced inhibition of liver X receptor alpha/retinoid X receptor alpha (LXRα/RXRα) heterodimer binding to the promoter of the SREBP-1c gene [20]. Similarly well documented is the increase by n-3 FA of the mitochondrial and peroxisomal FA β-oxidation rates. This effect is mediated by n-3 FA activation of the peroxisome proliferator-activated receptor (PPAR)α, a ligand-activated transcription factor [21] that up-regulates the expression of acyl-coenzyme A oxidase, the rate-limiting enzyme in the β-oxidation pathway [22,23]. Finally, a possible effect by n-3 FA on the farnesoid X receptor (FXR) activity was also postulated. FXR is a nuclear receptor for bile acids that plays a central role in lipid homeostasis [24]. Studies in hepatoma cell lines have demonstrated that the activation of FXR suppresses the gene expression of hepatic lipase and apo CII, and induces apo CII and VLDDL-receptor gene expression, all of which may contribute to the triglyceride-lowering action of FXR agonists [25]. Notably, mice lacking of a functional FXR protein had a pro-atherogenic serum lipoprotein profile, including elevated triglycerides. Since DHA is a ligand for FXR, it was postulated that a mechanism for the triglyceride-lowering effects by DHA may involve FXR-induced changes in gene expression [25].

3.4. Antihypertensive effects

The antihypertensive effect is viewed as another potential cardioprotective effect by n-3 FA. By a combined analysis of 36 randomized older trials, fish-oil intake (median dose 3.7 g/day EPA plus DHA) has been found to reduce systolic blood pressure by 2.1 mmHg and diastolic blood pressure by 1.6 mmHg [26]. These findings have been corroborated by those obtained in a large international cross-sectional epidemiologic study conducted on 4680 middle age men and women, in which n-3 FA intake was found to be inversely related to blood pressure, both in hypertensive and in non-hypertensive subjects [27]. At least two mechanisms may account for this effect. Firstly, incorporation of EPA and DHA into membrane phospholipids was shown to increase systemic arterial compliance [28]. Secondly, EPA and DHA seem to improve endothelial function by enhancing the release of NO [29]. This effect seems to be due to the lipid and structural modification of caveolae, plasma membrane microdomains that act as regulators of endothelial nitric oxide synthase.
counterpart, i.e., a reduced monocyte adhesion to cytokine-interleukin(IL)-6 and IL-8, and was accompanied by a functional soluble proteins macrophage-colony stimulating factor (M-CSF), This effect was not limited to the expression of transmembrane positively related to the extent of n-3 FA, we postulated an anti-angiogenic activity and a reduction of MMP release by fish oil as putative mechanisms. We therefore investigated the effects of n-3 FA firstly on in vitro angiogenesis models and then on the MMP release by macrophages. In agreement with what observed by Kanayasu [38] and Tsuji [39], we found that the exposure of EC to DHA reduced the tube-like network formation in collagen matrix (Matrigel) in vitro (Fig. 2A).

As postulated by many authors and confirmed by Jones et al. [40], we inferred a role for COX-2 activity in the orchestration of angiogenesis by EC, since cell treatment with NS-398, an inhibitor of COX-2 activity, abolished such vascular organization. It was therefore plausible that DHA exerts an anti-angiogenic effect at least in part by reducing stimulated COX-2 expression. To confirm this hypothesis, we recently observed that treatment of EC with DHA before PMA stimulation highly reduced COX-2 expression and activity [41].

The release of MMP is also regulated by COX-2-derived prostaglandins [42]. In the attempt to fully explain the findings on plaque stabilization [35], we tested the effect of DHA on the release of MMP by human macrophages in culture. We observed that cell treatment with DHA for 48 h before stimulation resulted in a significant inhibition of MMP-9 release (Fig. 2B) [43], thus potentially having a plaque-stabilizing effect [35].

3.7. The modulation of NF-κB activation as the common denominator for the anti-inflammatory and anti-atherogenic effect by n-3 FA

Adhesion molecules, soluble cytokines, COX-2 and MMP-9, which are all reduced in their expression by DHA, are structurally and functionally different molecules that share however common features, namely the inducibility by pro-inflammatory and pro-atherogenic stimuli and the presence of at least one consensus binding site for the transcription factor NF-κB. For this reason, in an attempt at elucidating the mechanism of n-3 FA action, we focused our interest on whether and how DHA might interfere with the pathway leading to NF-κB activation.

Active NF-κB complex are dimers of various combinations of the Rel family polyepitides, consisting of p50, p52, c-Rel, v-Rel, p65, and Rel B [44]. In resting cells, NF-κB is retained in the cytoplasm by binding to one of the inhibitory IκB proteins (IκBα, IκBβ, IκBε, p105, and p100), which blocks the nuclear translocation of NF-κB [44]. NF-κB is activated in response to a wide variety of pro-inflammatory and pro-angiogenic stimuli each promoting the dissociation of IκB through its phosphorylation, followed by ubiquitination and degradation. Thus, the unmasking of the nuclear localization sequence of NF-κB allows NF-κB to enter the nucleus and bind to κB-regulatory elements. The phosphorylation of IκB is catalyzed by an IκB kinase(IKK) complex [44]. The core of the IKK complex consists of a heterodimer of IKKα and IKKβ, and two IKKγ subunits. IKKα and IKKβ mediate the phosphorylation of IκB, whereas IKKγ links the core to the upstream signaling molecules [44]. Also the activation of the IKK complex is dependent on phosphorylation, and multiple upstream kinases, some of which are redox-sensitive, have been suggested to act as IKK kinases [44]. Many agents that activate mitogen activated protein kinase (MAPK) also induce an overproduction of

(eNOS) activity [30]. Such effects are however quantitatively quite modest.

3.5. Modulation of inflammatory steps in early and late atherogenesis

It is now widely recognized that atherogenesis is a process largely overlapping the classical inflammatory reaction. In its initial stages, atherogenesis is indeed featured by the intimal recruitment of selected populations of leukocytes, especially monocytes and some T-lymphocytes, and, secondarily, by the gradual accumulation of lipids, at the beginning inside the monocytes, with the consequent foam cell generation, and then extracellularly, after the occurrence of apoptosis and/or necrosis of the same foam cells. Monocyte recruitment is therefore considered a “critical event” in the onset of atherosclerosis [31] and many efforts have been made to identify agents able to reduce the endothelial de novo expression of adhesion molecule for leukocytes. Using human EC in culture activated by cytokines as an in vitro model of early atherogenesis, we assessed the effects of various FA on the surface expression of several adhesion molecules, and subsequently characterized mechanisms and functional relevance of such effects. DHA and—to a lesser extent—EPA, when added to cultured EC hours to days before the stimulation with cytokines, and long enough to allow a significant incorporation of this FA in cell membrane phospholipids, significantly inhibited the expression of both vascular cell adhesion molecule (VCAM)-1 and E-selectin after stimulation with virtually any stimulus able to elicit the coordinated expression of such genes [32]. The inhibition of adhesion molecule expression occurred in a range of n-3 FA concentrations compatible with nutritional supplementation of these FA to a normal Western diet, positively related to the extent of n-3 FA incorporation into total cell lipids, and were inversely related to the content of n-6 FA [32]. 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 macrophage-colony stimulating factor (M-CSF), interleukin(IL)-6 and IL-8, and was accompanied by a functional counterpart, i.e., a reduced monocyte adhesion to cytokine-activated endothelium [32]. Experiments following the fate of 14C-labelled DHA into cell phospholipids showed a significant incorporation of DHA into the phosphatidyl ethanolamine pool, i.e., in a specific and not particularly abundant phospholipid pool, likely in the inner plasma membrane, and therefore in a possibly strategic position to alter intracellular signal transduction pathways. However, whether n-3 FA protective effect occurs directly, for example by quenching cytokine-elicited reactive oxygen species (ROS), which act as second messengers in endothelial activation [33], or indirectly, through the production of metabolically active oxidized products, still remain under scrutiny. For example, our data do not exclude the possibility that products of n-3 FA oxidation might inhibit cytokine-induced VCAM-1 expression by a PPARα-dependent mechanism [34].

3.6. Modulation of angiogenesis and plaque stability: effects on COX-2 expression

A recent study by Thies et al. has highlighted a potential role of n-3 FA in decreasing the risk of atherosclerotic plaques rupture in patients awaiting carotid endarterectomy [35]. In this study, plaques from patients taking fish oil featured a net incorporation of n-3 FA into plaque lipids, and this correlated with a reduced macrophage infiltration and thicker fibrous caps compared with plaques from patients assuming sunflower oil-enriched control capsules [35]. In recent years, supportive experimental evidence has indicated that plaque neovascularization and exacerbated release of metalloproteinases (MMP) by the activated endothelium and macrophages play a pathogenetic role in plaque progression [36] and instabilization [37]. In an attempt to find out the targetable mechanistic basis of the plaque-stabilizing effect by n-3 FA, we postulated an anti-angiogenic activity and a reduction of MMP release by fish oil as putative mechanisms. We therefore investigated the effects of n-3 FA firstly on in vitro angiogenesis models and then on the MMP release by macrophages. In agreement with what observed by Kanayasu [38] and Tsuji [39], we found that the exposure of EC to DHA reduced the tube-like network formation in collagen matrix (Matrigel) in vitro (Fig. 2A).

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ROS besides activating NF-κB, suggesting that a cross-talk occurs between these pathways (Fig. 3). However the exact site of action of these MAPK along the pathway leading to NF-κB activation, as well as the site of interference by DHA with ROS production, likely triggered by the activation of nicotinamide adenine dinucleotide (phosphate) [NAD(P)H] oxidase (Fig. 3), are still elusive.

By transient transfection experiments using full-length human COX-2 promoter constructs, we showed that DHA inhibited promoter activity independent of the pro-inflammatory stimuli used, and that the DHA inhibitory effect was abrogated only when promoter sequences were lacking, by deletion or site-mutation, the functional NF-κB sites. This, together with the observed reduced activation of NF-κB and the reduced nuclear translocation of p65, suggested an interference by DHA with the activated cytoplasmic signaling pathway leading to NF-κB activation [41].

It is well known that n-3 FA, modifying lipid composition, can alter membrane lipid microdomains, such as lipid rafts and caveolae, involved in the compartmentalization, modulation, and integration of cell signaling components [45]. In our experimental conditions, DHA accumulates in membrane phospholipids. Therefore, in an attempt at exploring which molecular target(s) upstream of NF-κB was (were) affected by DHA, we first focused our attention on the NAD(P)H oxidase, an enzyme system producing ROS involved in the activation of NF-κB by IL-1 [46], the assembly and activation of which is potentially sensitive to membrane phospholipid composition [47]. We demonstrated that DHA reduces the p47phox subunit membrane translocation and hence NAD(P)H oxidase activity and intracellular ROS production [41]. Since PKC activities are involved in NAD(P)H oxidase activation and COX-2 expression [48], and since PKC signaling activities are specifically involved in the activation of NF-κB, we next explored the possibility that PKC could be another molecular switch affected by DHA membrane conditioning. We monitored membrane translocation of the main PKC isoforms in EC stimulated by PMA and IL-1 in the presence of DHA. All such isoforms were activated by PMA, as demonstrated by their translocation to plasma membrane; but only the translocation of PKCβ was reduced by DHA treatment [41]. We therefore concluded that DHA, possibly modifying the plasma membrane lipid composition and hence membrane microdomain organization, inhibits at least two molecular switches involved in NF-κB activation: NAD(P)H oxidase and PKCβ activities. DHA might do this as precursor of various anti-inflammatory lipid mediators [5]. To verify if any of these lipid derivatives is involved in the observed DHA inhibitory effect, we finally evaluated the reversion of DHA protective effect in the presence of chemical and/or molecular inhibitors of several polyunsaturated fatty acid-metabolizing enzymes such as 5-, 12-, and 15-LO and the cytochrome P450 system. We observed that only the inhibition of 15-LO partially reverted DHA inhibitory effect, thus suggesting the involvement of resolvins in mediating, at least in part, the anti-inflammatory effects by DHA [41]. An integrative molecular model of dietary n-3 FA interference with the IL-1 signaling pathway leading to adhesion molecules and COX-2 induction in EC is proposed in Fig. 3.

4. Conclusions and perspectives

The vascular endothelium plays a key role in the progression of CVD. On the other hand, n-3 FA have emerged as an effective tool in primary and secondary prevention of CVD and some forms of cancer. Although they exert multiple actions [49], the
transcriptional control of several endothelial pro-inflammatory genes, including those encoding for adhesion molecules, chemokines and other soluble cytokines in the endothelium, likely plays an important role. The recent findings showing inhibition of COX-2 expression and activity by n-3 FA allows a deeper understanding of the therapeutic potential of these nutrients, being COX-2 overexpression pathogenetically involved in several inflammatory/regenerative diseases besides atherosclerosis (from rheumatoid arthritis to inflammatory bowel disease and possibly—Alzheimer’s disease). Most or all such effects of n-3 FA, involving a blunted activation of inflammatory genes in response to activating stimuli, appear to have the inhibition of NF-κB activation as a common denominator. Such inhibition appears to be the final result of an interference by n-3 FA with early signaling events in response to inflammatory stimuli.

In general, therefore, n-3 FA appear to finely tune the response of our genes to dangerous environmental challenges (nutrigenomics), by curbing physiological responses without abrogating them totally. By decreasing the endothelial responsiveness to pro-inflammatory, pro-atherogenic and pro-angiogenic stimuli, n-3 FA appear to favorably impact molecular events not targeted by any other drugs or interventions, thus allowing their proposition in a therapeutic role complementary to those of already implemented pharmacological treatments in several “inflammatory” diseases, including atherosclerosis.

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