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Omega-3 fatty acids: mechanisms underlying "protective effects" in atherosclerosis

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Abstract

Purpose of review—This article provides an updated review on mechanistic and molecular studies relating to the effects of n-3 fatty acids (FA) on inhibiting atherogenesis.

Recent findings—The effects of n-3 FA on modulating arterial lipoprotein lipase (LpL) levels link to changes in lipid deposition in the arterial wall. LpL expression in the arterial wall also relates to local macrophage-mediated inflammatory processes. Increasing evidence suggests that n-3 FA ameliorate inflammation, another key component in the development of atherosclerosis, including decreases in pro-inflammatory cytokine production. n-3 FA inhibit atherogenic signaling pathways and modulate the phenotypes of inflammatory leukocytes and their recruitment in the arterial wall.

Summary—New mechanistic insights into the anti-atherogenic action of n-3 FA have emerged. These studies may contribute to future therapeutic advances in preventing mortality and morbidity associated with atherosclerosis.

Keywords

Atherosclerosis; inflammation; lipoprotein lipase; macrophages; n-3 fatty acids

Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States [1] and many countries globally. CVD risk factor control remains a challenge for many. Dietary fatty acids (FA) play an important role in the development or prevention of CVD. Diets high in saturated fats increase atherosclerotic CVD morbidity and mortality [2]. On the other hand, omega-3 fatty acids (n-3 FA), especially fish oil-derived eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have emerged as potentially preventive and therapeutic agents to decrease CVD by acting on pathways related to atherosclerosis and myocardial infarction [3]. While controversy continues [4**, 5*], many mechanistic studies point towards beneficial effects of n-3 FA on inhibiting adverse pathways in the pathogenesis of CVD. The aim of this review article is to provide an overview of recent findings on underlying mechanisms of potential anti-atherogenic action of n-3 FA.

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Conflict of interest
None.

Overview of cardio-protective mechanisms of n-3 FA

Dietary n-3 FA are a class of essential polyunsaturated FA (PUFA) with the double bond in the third carbon position (ω-3) from the methyl terminal. n-3 FA can be further converted into signaling molecules, such as eicosanoids and the more recently identified docosanoids which are docosatrienes, protectins, and resolvins [6]. The longer chain n-3 FA, EPA and DHA are generally far more bioactive than their n-3 essential FA precursor, alpha linolenic acid [7]. n-3 FA may be cardio-protective by changing several intermediate determinants of CVD risk [8]. n-3 FA ameliorate hypertriglyceridemia, reduce blood pressure and protect against arrhythmias [6, 9, 10*]. A number of pathways can be mediated directly by incorporation of n-3 FA into cell membrane phospholipids; this results in changes in membrane fluidity by altering the properties of lipid rafts and caveolae, leading to modulation of membrane-associated proteins and receptor activities [11]. Membrane incorporation of n-3 FA decreased the generation of intracellular reactive oxygen species with a subsequent diminished activation of redox-sensitive transcription factors, such as nuclear factor-κB (NF-κB) [12]. n-3 FA signaling through a G-protein coupled receptor, GPR120, is reported to modulate inflammation and insulin-sensitizing effects in monocytes and macrophages [13].

Another action of n-3 FA relating to cardiovascular health is via its interference with cell-membrane ion channels that results in a number of biological effects, including reducing arrhythmias [14, 15]. Although findings on the effects of n-3 FA on reducing recurrent atrial fibrillation in patients are controversial [16, 17], a recent report has demonstrated that n-3 FA were effective in primary prevention of atrial fibrillation [18*].

Other biological effects of n-3 FA are likely mediated through the release of bioactive mediators that suppress pro-inflammatory cytokines and produce anti-inflammatory catabolites, such as protectins and resolvins [8, 19]. n-3 FA have been reported to reduce platelet reactivity, in part, through the modification of platelet fatty acid composition and decreasing production of pro-aggregatory eicosanoids to potentially provide protective action against plaque rupture [20, 21]. Moertl et al reported a role of n-3 FA in controlling thrombosis in patients with chronic heart failure by reducing the levels of mediators that promote thrombosis [22]. However, n-3 FA had little or no effect on markers of platelet and endothelial functions in patients with peripheral arterial disease (PAD) [23]. Higher intake of n-3 FA increased the stability of atherosclerotic plaques in humans [24]. Incorporation of n-3 FA into advanced atherosclerotic plaques increased plaque stability in humans [24, 25] and apoE−/− mice [26] by inducing structural changes of plaques with lower plaque inflammation and by increasing the thickness of fibrous caps [9, 27].

Effects of dietary n-3 FA on arterial lipid deposition and lipoprotein lipase

Lipid deposition is an initial key step in atherogenesis that begins with the entry of lipoproteins (mainly LDL) into the arterial wall. Lipid deposition initiates a pro-inflammatory cascade that attracts monocytes into the subendothelial space [28]. Infiltrated monocytes differentiate into macrophages that take up the lipoproteins and become foam cells. These foam cells make up the fatty streak that can be the precursor of an atherosclerotic plaque [9].

Incorporation of dietary FA into chylomicron remnants influenced lipid accumulation in arterial macrophages [29]. When compared with remnants rich in n-3 FA, chylomicron remnants rich in saturated FA were taken up more rapidly by cultured macrophages and resulted in greater arterial lipid accumulation [30]. FA can also affect binding and uptake of lipids in the arterial wall by macrophages. We have reported that regulatory effects of dietary FA on arterial lipid deposition were related to expression and distribution of arterial

lipoprotein lipase (LpL) [31–33]. In addition to its catalytic activity on triglycerides, LpL can also serve as a bridging or anchoring molecule for LDL and other lipoproteins to cell surfaces [34, 35]. Seo et al reported that a high saturated fat diet increased arterial cholesterol delivery via total LDL and selective LDL-cholesterol uptake in mice, and that this was associated with increased arterial wall LpL levels [31]. However, little is known about how n-3 FA might affect pathways in the early stage of atherosclerosis, i.e., cholesterol delivery and LpL at the levels of arterial wall.

We investigated pathways underlying regulation of arterial LpL and the role of LpL in mediating arterial lipid deposition in the development of atherosclerosis in several mouse models. We demonstrated that specific conditions, such as high intakes of saturated FA and insulin resistance, altered recruitment of different cell populations to the arterial wall, particularly accumulation of macrophages that secrete LpL, and thus favor the development of atherosclerosis [32, 33]. n-3 FA decreased the presence of inflammatory cells and hence, macrophage-secreted arterial LpL, which was associated with decreases in arterial cholesterol delivery, inflammation and atherosclerosis. The presence of arterial LpL itself also appears important for the presence of macrophages in the arterial wall [33]. We recently used LDLR−/− mice to evaluate impact of dietary saturated FA being replaced with n-3 FA on the progression of atherosclerosis and arterial LpL levels and localization. Our preliminary data demonstrate that increasing replacement of saturated FA by n-3 FA intake abrogated the adverse effects mediated by saturated FA by improving mouse plasma lipid profiles and lowering total and LDL cholesterol levels. Incremental inclusion of n-3 FA also decreased aortic macrophage-associated LpL, as well as aortic macrophage markers and pro-inflammatory markers [36].

Emerging mechanisms relating to anti-inflammatory actions of n-3 FA in atherosclerosis

Higher intakes of dietary n-3 FA decreased serum levels of pro-inflammatory biomarkers, including interleukin-6 (IL-6), soluble E-selectin, ICAM-1, VCAM-1 and C-reactive protein (CRP) [37]. As well, a number of studies highlight the importance of leukocyte recruitment in atherosclerosis. Leukocytes recruited during the inflammatory processes include neutrophils, monocytes and T cells, and to a less extent, B cells, dendritic cells (DC) and mast cells [38]. Specific leukocyte subsets are recruited by a unique combination of chemokines and their corresponding receptors. Studies on arterial monocytes and macrophages, and their contributions to innate versus adaptive immunity have been previously reviewed [39]. We will now focus on recent aspects of n-3 FA-mediated effects on monocytes and macrophage recruitment and phenotypes and on previously less appreciated cellular players, such as neutrophils and DC. These highlight the emergence of additional pathways related to murine atherosclerosis that may guide future studies relating to effects of n-3 FA.

Effects of n-3 FA on monocytes and macrophages in atherosclerosis

Monocytes/macrophages are heterogeneous populations of cells that play a key role in innate or adaptive immune response in atherosclerosis. Subpopulations of monocytes and macrophages function differently and are identified by differential expression of selected surface markers in the inflammatory processes [40]. In humans, the presence of CD16 classifies a pro-inflammatory monocyte subset. Mouse monocyte profiling is characterized mainly by the expression of Ly6C; $CCR2^{+}$ (MCP-1 receptor) $Ly6C^{hi}$ monocytes are recruited to inflamed tissues, whereas the $CCR2^{-}Ly6C^{lo}$ subset is recruited to non-inflamed tissues. Monocyte subsets with expression of Ly6C and CCR2 were readily recruited to atherosclerotic lesions and developed into pro-inflammatory macrophages in mice [41, 42].

n-3-rich diets reduced Ly6C^{hi} monocyte subset populations and monocyte recruitment to aortic lesions, resulting in reduced atherosclerosis in LDLR−/− and apoE−/− mice, independent of plasma cholesterol levels [43*].

Effects of n-3 FA on the chemotaxis of monocytes have been reported. Grenon et al demonstrated that n-3 EPA, but not n-6 arachidonic acid (AA), decreased monocyte adhesion to endothelial cells, independent of the stimulation of TNF-α, and that this was associated with reduced mRNA expression of adhesion molecules, such as ICAM-1, VCAM-1, and E-selectin, and pro-inflammatory mediators, such as IL-6 and TNF-α [44*]. Still, Luu et al has reported little effect on migration and adhesion of monocytes isolated from patients with peripheral arterial disease (PAD) receiving fish oil supplementation [45].

Macrophages that reside in the arterial wall mediate inflammatory processes. Jung et al has demonstrated that n-3 EPA attenuated pro-inflammatory markers in cultured murine macrophages by decreasing mRNA expression levels of pro-inflammatory cytokines, such as IL-6; pathways inhibited in part, by decreasing PPARγ levels [46*]. The specific mechanisms on how and which dietary FA influence macrophage-mediated inflammatory responses are still not well-understood. Earlier studies reported that saturated FA triggered the accumulation of macrophages in arterial wall which contributeed to atherogenesis by local secretion of pro-inflammatory mediators.[47, 48]. Saturated FA can activate inflammation through the activation of toll-like receptor (TLR)/ NF-κB-mediated signaling in macrophages [49, 50]. On the other hand, EPA and DHA blocked LPS-induced NF-κB activation in macrophages [50]. DHA inhibited TLR4/NF-κB activation by inhibiting TLR2 dimerization with TLR6 or TLR1 [51].The incorporation of DHA into membranes can alter lipid composition in lipid rafts, resulting in disruption of TLR4 recruitment into lipid rafts and reduced downstream signaling [52]. Interestingly, other pro-inflammatory mediators, such as ceramide [53] and NADPH oxidase (NOX), [54], have been linked to saturated FA exacerbating vascular injury. The specific interaction between n-3 FA and ceramide and/or NOX has yet to be established.

Changes of macrophage phenotypes have been observed during the development of atherosclerosis [55]. As atherosclerotic lesions progress, macrophages exhibit a pro-inflammatory phenotype (M1 macrophages) and produce inflammatory cytokines that are associated with “classical activation”. Alternatively, macrophages in the presence of cytokines such as IL-4 or IL-13 are activated to promote angiogenesis and matrix remodeling while suppressing adverse inflammatory responses (M2 macrophages) [56]. Recently, subsets of macrophages, including Mres, have been identified during the resolution phase of inflammation, that are associated with elevated cAMP levels and feature the properties of both M1 and M2 [57]. Another subset, Mox, develops in response to oxidized phospholipids and is related to a redox-sensitive transcription factor- NF-E2-related factor 2 (Nrf2) [58].

It is possible that macrophage polarization is primed by circulating monocytes. Macrophages produce a class of DHA-derived anti-inflammatory and pro-resolving products, maresins, in resolving inflammation. Lipoxin A4, resolvin E1/ D1/E2, and maresin1 enhance phagocytosis of apoptoic neutrophils by macrophages without inducing pro-inflammatory gene expression [59, 60]. Resolvins produced in human vasculature reduced or blocked polymorphonuclear leukocyte transmigration in vivo [61]. Details on the effects of n-3 FA on macrophage phenotypes in atherosclerosis are still limited. Still, the potential action of n-3 FA on M2 skewing and M1 inhibition of macrophages has been demonstrated in adipose tissue and this linked to macrophage cell-surface receptor, GPR120 [13]. Carotid endarterectomy patients with EPA supplementation had decreased plaque inflammation with fewer T cells and foam cells [25].

Transcriptional control of macrophage function has been reported. Szanto et al showed that activation of M2 macrophages with IL-4 stimulated the activity of PPARγ. This effect was likely associated with a signal transducer and activator of transcription 6 (SATA6), which acted as a facilitating factor for PPARγ by promoting DNA binding and consequently increased the levels of regulated genes and responses [62]. Global gene expression analysis validated the cross-link of IL-4 and PPARγ in mouse and human macrophages as well as in DC [63]. In response to IL-4, STAT6 and PPARγ-coactivator-1β (PGC-1β) induced macrophage FA oxidation and attenuated macrophage inflammation [64]. Potential regulatory effects of n-3 FA on modulating PPARγ levels that affect inflammatory responses in macrophages have been reported [46*]. Additional studies on the relation of n-3 FA and PPARγ should provide further insights into the regulation of macrophage phenotypes in atherosclerosis.

Effects of n-3 FA on emerging contributors to atherogenesis

Transient neutrophilic adhesion to the vasculature endothelium is termed rolling and is a first step in leukocyte migration across the endothelial wall. Hyperlipidemia-induced neutrophilia correlated with increased lesion formation in apoE−/− mice [65]. Neutropenic mice displayed a rescued phenotype with decreased lesion formation at early but not at the late stages of atherosclerotic lesion development [66]. Doring et al demonstrated that neutrophil secondary granule protein cathelicidin directly promoted atherosclerosis by enhancing the recruitment of inflammatory monocytes [67*]. The relationships of n-3 FA with arterial neutrophils are still not clear in atherogenesis. Neutrophils typically have high contents of AA, but oral administration of EPA and DHA resulted in proportional increases of n-3 FA levels in humans [68]. Tull et al reported that n-3 FA reduced the transient migration of neutrophils across endothelial cell monolayers through the modulation of cellular phospholipid composition and production of eicosanoids, such as cyclooxgenase-2 (COX-2) and prostaglandin D2 (PGD2) or PGD3 [69].

Dendritic cells (DC) are antigen-presenting cells that represent an important cellular link between innate and adaptive immunity. They also have a role in tolerance for self-antigens [38]. The impact of n-3 FA on DC maturation and DC-derived pro-inflammatory cytokines has been investigated. EPA and DHA attenuated LPS-induced DC maturation by suppressing IL-12 production and expression of CD40, CD80, CD86 and MHCII, while increasing IL-10 production and expression of IL-10 [70]. These effects were likely modulated through suppressing NF-κB-mediated signaling pathway. Similar findings on reduced DC maturation mediated by n-3 FA in spleen and central nerve system have been reported in mice [71, 72]. Likely, n-3 FA impaired p38 mitogen-activated protein kinase (MAPK) activity that blocked LPS-induced DC maturation [73]. Nakajima et el has further demonstrated that a 5% EPA diet was able to induce atherosclerotic lesion regression by increasing immature arterial DC in LDLR−/− mice [74]. In addition, it has been shown that when bone marrow-derived DC were exposed to n-3-derived resolvin E1 and pathogens, these DC remained at the inflammatory sites, instead of migrating to lymph nodes, and induced apoptosis of activated arterial CD4+ T cells [75].

Conclusion

Findings outlined in this review suggest that anti-atherogenic effects of n-3 FA can affect multiple steps in atherosclerotic lesion development. Pathways reviewed herein also contribute to mechanisms important for arterial plaque stability and rupture [76]. As summarized in Figure 1, these effects include modulation of arterial initial uptake and binding of LDL by modulating arterial LpL and macrophage levels. n-3 FA inhibit pro-inflammatory processes by reducing the production of inflammatory mediators and the recruitment of inflammatory leukocytes. Systems that can be used to study the underlying

mechanisms include the use of various agonists, antagonists or genetic modifications to elucidate further the role of n-3 FA in different steps in the development of atherosclerosis. These findings of "beneficial effects" of dietary n-3 FA on processes related to atherosclerosis are relevant to future strategies for prevention and treatment of CVD.

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Key points

- Molecular and cellular anti-atherogenic targets of n-3 FA have been identified.
- n-3 FA modulate arterial lipid deposition and inflammatory responses through regulating arterial LpL levels early in development of atherosclerosis.
- Growing understanding of the inflammatory pathways and mediators has unveiled new mechanisms that can be considered as part of strategic approaches to decrease risks for CVD using n-3 FA and related molecules in humans.

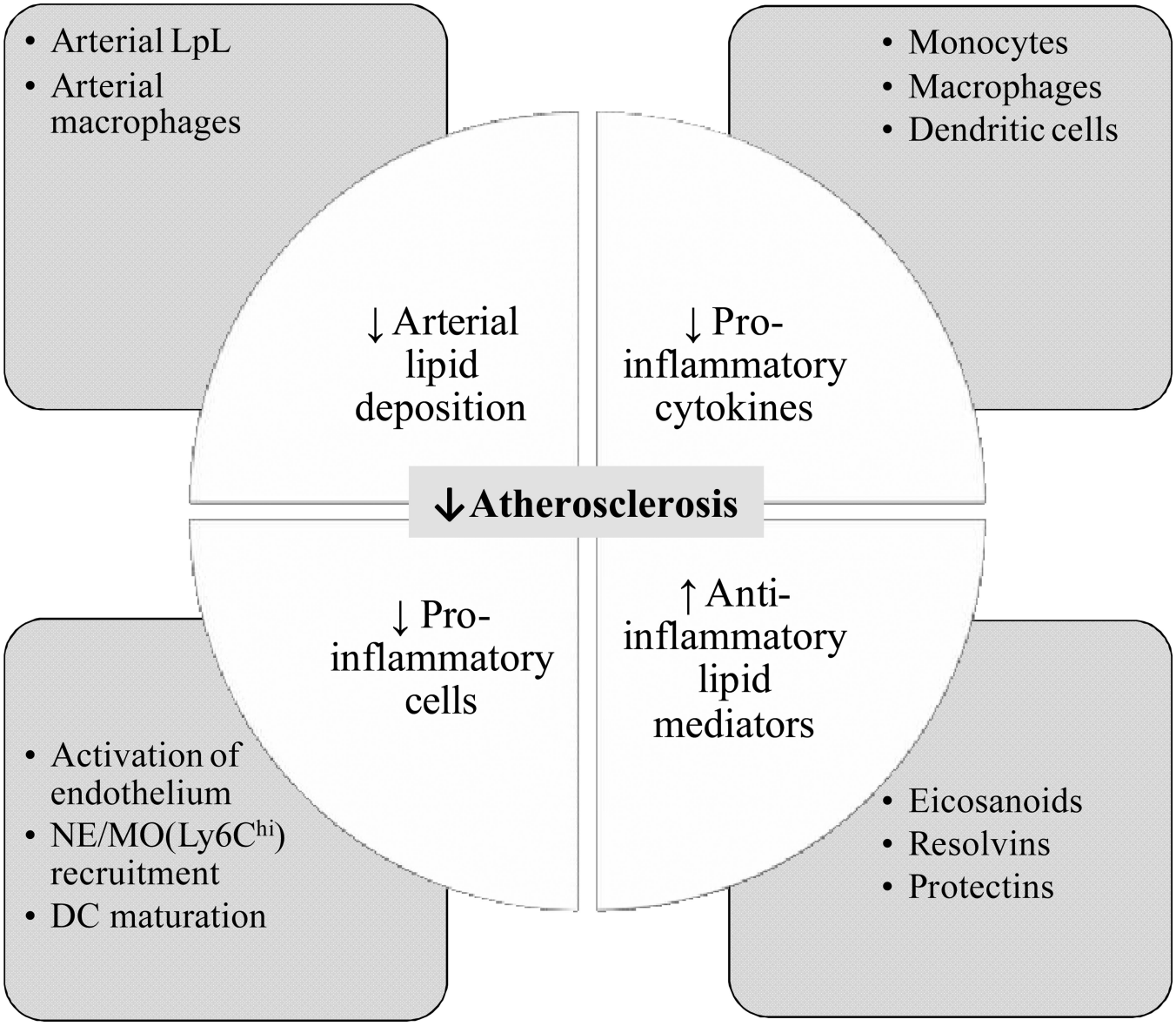

Figure 1. Summary of potential "beneficial effects" of n-3 FA in atherosclerosis

n-3 FA modulate atherosclerosis by affecting uptake and binding of LDL to the arterial wall. This is associated with reduced arterial LpL and macrophage levels. n-3 FA are protective against inflammation in the arterial wall by reducing the production of pro-inflammatory cytokines in monocytes (MO), macrophages or dendritic cells (DC) and decreasing the recruitment of inflammatory leukocytes to the arterial wall, including neutrophils (NE) and MO. Lastly, n-3 FA and n-3-derived eicosanoids, resolvins and protectins are potential anti-inflammatory lipid mediators in atherosclerosis. Specific examples of pathways related to each area are shown in the square boxes.