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N-3 Fatty Acids and Cardiovascular Disease: Actions and Molecular Mechanisms

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Summary

Cardiovascular disease and atherosclerosis are a leading cause of morbidity and mortality worldwide. Epidemiological studies and randomized control intervention trials have reported that n-3 fatty acids reduce cardiovascular events. A variety of biologic and molecular effects of n-3 fatty acids can modulate the mechanisms of development and progression of atherosclerosis. These include n-3 fatty acid effects on inflammation, cardiac excitability, platelet function; triglycerides blood levels, blood pressure and the stability of atheroma plaques. The molecular mechanisms are still not fully defined; but might involve changes in membrane fluidity, receptor responses and binding to intracellular receptors regulating gene transcription. Understanding and elucidating these mechanisms is important to development future strategies for prevention and treatment of cardiovascular disease.

Keywords

cardiovascular disease; omega-3 fatty acids; atherosclerosis

Overview on Omega-3 Fatty Acids and Cardiovascular Disease

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality in the world, both in developed and developing countries [1]. Diet can affect the majority of modifiable risk factors for CVD [2]. In the last decade it is increasingly recognized that the very long chain n-3 polyunsaturated fatty acids (n-3 FA) can modulate the mechanisms of development and progression of atherosclerosis. Since the early studies in Greenland Eskimos [3] demonstrated that the ingestion of the long chain n-3 FA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), protected them from CVD many subsequent studies show associations between n-3 FA and decreased risks of CVD [4–8].

EPA and DHA are the major bioactive n-3 FA. The α -linolenic acid (ALA) precursor of EPA and DHA is generally far less effective at inducing biological effects, in part because of its inefficient conversion to EPA and DHA in humans [9]. Wang et al. [10] in a systematic review of the literature on the effects of n-3 FA (consumed in fish or fish oils rich in EPA and DHA or as ALA) on CVD suggests that EPA and DHA, but not ALA, reduces the rates of all-mortality cardiovascular cause, cardiac and sudden death, and possibly stroke. Mozafarian et al. [11] in a recent meta-analysis described that the benefit of fish or fish-oil consumption for prevention of cardiac death is non-linear and that modest consumption of fish (e.g., 1–2 servings/week), especially species higher in the n-3 FA EPA and DHA, reduces the risk of

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coronary death by 36% and total mortality by 17%. Above this threshold, little additional benefit was seen.

In support of the epidemiological studies, which indicate associations, the hypothesis that ingestion of n-3 FA protects individuals from CVD has been tested in a few randomized control intervention trials (RCT) [12–14]. In the DART trial [12] men who had recovered from myocardial infarction (MI) were randomized to either increased fish intake or fish oil. After 2 years subjects advised to eat fatty fish had a 29% reduction in all-cause mortality in comparison with the no diet advice group. The investigators concluded that the intake of 2 or 3 portions/week of fatty fish may reduce mortality in men who have recovered from MI. GISSI-Prevenzione investigators [13] showed that dietary supplementation with n-3 FA (1g/day) very rich in EPA and DHA in patients after MI reduce total mortality by 21% and sudden death by 45%. Yokoyama et al. [14] showed that pharmacological doses of purified EPA reduce the frequency of major coronary events in hypercholesterolaemic Japanese patients, thus demonstrating benefit even in a population with relatively high intakes of n-3 FA.

Thus, based on existing evidence the American Heart Association, in 2002, revised its recommendations for dietary intake of n-3 FA and has recommended that adults without known CVD consume at least two preferably oily fish meals/week to reduce risk for cardiac events and 1 g/day of n-3 FA rich oil should be ingested in patients with known CVD [15]. Similar recommendations have been made by other international health organizations [16,17]. However there are still a number of questions to be answered for more specific recommendations, regarding specific dosages, the role of EPA vs DHA, and whether guidelines need to be different in primary as compared to secondary CVD prevention [18].

Biological effects of n-3 FA related to CVD

The biological effects of n-3 FA on atherosclerosis include preventing arrhythmias [8,12,19–24], decreasing platelet aggregation [27–29], lowering plasma triglycerides [30–35], increasing HDL cholesterol and LDL particle size [36–40], decreasing blood pressure [42–45], reversing cholesterol accumulation from atheromatous plaques [46,47] and decreasing inflammation [48–50].

Epidemiological studies provide support for an antiarrhythmic effect of n-3 FA, including sudden death, arrhythmic coronary heart disease death, and atrial fibrillation. In the GISSI trial, Macchia et al. [19] found that the effect of n-3 FA on sudden death reduction was greater in patients with left ventricular systolic dysfunction, suggesting that one primary effect of n-3 FA therapy in patients with prior myocardial infarction is antiarrhythmogenic. Subsequent studies have suggested that the major impact of n-3 FA on arrhythmias is in patients with prior MI [12,20,21]. Chrysoshoou et al. [22] in a sample of Greek adults found that patients who consumed 300 g fish/week or more had a 29.2% lower likelihood of having QTc intervals >0.45 s ($P=0.03$), therefore n-3 FA could provide antiarrhythmic protection at a population level. On other hand Mozzafarian et al. [23] evaluated the association between dietary fish intake and several cardiac electrocardiographic parameters in humans relevant to arrhythmic risk. They found intakes of higher 1 g/day were associated with 2.3 beats/min lower heart rate, a 7.6 ms longer PR interval and 46% lower likelihood of prolonged QT, which suggests that dietary fish intake links to cardiac electrophysiology in humans, with potential implications for arrhythmic risk. This association has been corroborated in recent meta-analyses [8,24].

However contrasting results have been reported in patients with implantable cardioverter defibrillators [24–26] and it is possible that n-3 FA increase risk of re-entrant tachycardia in a small and specific subset of individuals with a slow re-entrant arrhythmic loop around a region of scarred myocardium [23].

The n-3 FA have also an antithrombotic effect; it could explain in part the potent protective effect of these towards acute cardiovascular events [27]. Knapp et al. [28] reported that high doses of eicosapentaenoic acid supplementation (10 g/day) for one month altered the pattern of synthesis of thromboxanes and prostacyclins and DeCaterina et al. [29] patients with angiographically proven coronary artery disease find that n-3 FA supplementation (3 g/day EPA and 1.3 g/day DHA) for 28 days decreased serum thromboxane A₂ in 36% of subjects and bleeding times increased 40% from baseline.

One of the most consistent effects of n-3 FA is the reduction in fasting and postprandial serum triglycerides and free fatty acids [30,31]. The importance of plasma triglycerides levels in cardiovascular disease remains controversial unlike total cholesterol, which is well-established independent risk factor for CVD [32]. Sarwar et al. [33] in a meta-analysis with 29 Western prospective studies (262,525 participants and 10,158 CVD cases) showed that the adjusted odds ratio for coronary heart disease was 1.72 in a comparison of individuals in the top third with those in the lower third of usual log-triglyceride values. In two prospective recent studies Bansal et al. [34] and Nordestgaard et al. [35] find strongest associations between non-fasting triglyceride levels and incident cardiovascular events. These findings suggest that this particular aspect in cardiovascular risk prevention may be optimized with n-3 FA supplementation.

N-3 FA, especially DHA, can increase HDL cholesterol [36,37] as described by Mori and Woodman [38] in overweight, hypercholesterolemia subjects and in treated hypertensive subjects with type 2 diabetes. They reported that EPA and DHA are equally effective at reducing serum triglyceride, but that DHA only raised HDL cholesterol (specifically the HDL₂ fraction). Furthermore they also find that LDL particle size increased significantly with DHA supplementation, a finding reported by other authors [39,40]. Since small, dense LDL particles are associated with an increased risk of coronary artery disease [41] these changes might be expected to contribute to a reduction in atherogenic risk.

The antihypertensive effect of n-3 FA has been shown in RCT [42,43]. Morris et al. [43] reported in a meta-analysis of 31 placebo-controlled trials, an overall reduction of -3.0/-1.5 mmHg with a significant dose-response effect estimated at -0.66/-0.35 mmHg/g n-3 FA. In another more recent meta-analysis of 36 trials, Geleijnse et al. [44] showed that systolic blood pressure was reduced by -2.1/-1.6 mmHg and diastolic blood pressure -1.6 mmHg. These effects tended to be greater in populations that were older (> 45 years) and in hypertensive populations (≥ 140/90 mmHg). Furthermore Ueshima et al. [45] in an international cross-sectional epidemiologic study of 4680 men and women ages 40 to 59 from 17 population-based samples in China, Japan, United Kingdom, and United States reported that n-3 FA intake related inversely to blood pressure, not only in hypertensive person but also in non hypertensive persons.

The effects of n-3 FA on late stages of atherosclerosis have been less studied. Studies in patients who underwent coronary angiography (n=223) and were given EPA plus DHA at a dosage of 3 g/day for 3 months followed by 1,5 g/day for 21 months had less progression and more regression of preexisting lesions than did those given a placebo [46]. Furthermore, the patients who underwent coronary artery bypass grafting and were given 4 g/day of EPA plus DHA had smaller vein graft occlusion rates of 27% compared with 33% in the control group [47]. Thus, these authors suggested that dietary intake of n-3 FA, approximately 1.5 g/day (EPA+DHA) for 2 years, modestly mitigated the course of human coronary atherosclerosis, as assessed by angiography. However, other studies with EPA plus DHA supplementation have failed to find beneficial effects in regression of atheroma plaque, but these studies have major variations in study design compared to the positive end-point studies [51,52].

Atherosclerosis has traditionally been viewed to reflect the deposition of lipids within the arterial wall of medium-sized and large arteries. This concept has recently been modified as atherosclerosis is considered now to have major inflammatory components in its pathogenesis towards disease [53]. Activated endothelial cells express selective adhesion molecules (CAMs) such as E-selectin, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1) that recruit inflammatory cells to the arterial wall. Soluble forms of these molecules (sCAMs) are thought to reflect the amount of molecules expressed on the endothelial surface [54]. Patients with manifest or future CVD have an elevated serum concentration of these inflammatory markers [55,56]. EPA and DHA supplementation in human studies decrease sCAMs [48,49] and pro-inflammatory cytokines IL-1 β , IL-6 and TNF α [50]. Thus, these findings suggest that anti-inflammatory effects of n-3 FA will be important contributors to their effects in CVD.

Molecular mechanism of n-3 FA related to CVD

Molecular mechanisms by which n-3 FA act on CVD while still not fully defined, include a spectrum of events varying from altering membrane fluidity [57] and receptor responses following their incorporation into cell membranes to direct binding to intracellular receptors regulating gene transcription [58,59]. Many of these effects are depicted in Figure 1.

a) Antiarrhythmic effects—There are several mechanisms through which n-3 FA could prevent arrhythmias. The n-3 FA are incorporated into myocyte membranes and may influence ion channel function. N-3 FA inhibit voltage-gated sodium channels in cardiomyocytes, resulting in a longer relative refractory period and an increased voltage required for depolarization which reduce heart rate [60]. N-3 FA also maintained the integrity of L-type calcium channels, preventing cytosolic calcium overload during periods of ischemic stress [61]. On other hand n-3 FA improves left ventricular efficiency and reduces blood pressure which could indirectly decrease heart rate [62].

b) Dyslipidemia—N-3 FA are important regulators of many genes critical for controlling lipid homeostasis pathways. n-3 FA have been reported to bind to peroxisome proliferator-activated receptor- α PPAR α and peroxisome proliferator-activated receptor- γ PPAR γ to induce physiologic responses such as promoting β -oxidation and adipogenesis [63]. n-3 FA inhibit sterol response element-binding protein (SREBP) conversion from its inactive form, thus potentially altering the transcription of a variety of genes involved in cholesterol, triglyceride and fatty acid synthesis, leading in part to decreases in plasma triglyceride concentration [64,65].

Chan et al. [36] reported that fish oils decreased the fractional catabolic rates of HDL. This was related to the decrease in levels of plasma triglycerides, which stabilizes HDL particles as they become larger, retain more cholesterol and are less susceptible to catabolism by hepatic and renal clearance pathways.

c) Atheroma plaque development, cholesterol delivery and inflammation—LDL cholesteryl ester (LDL-CE) can independently be delivered to cells without the concomitant uptake of the whole LDL particle, a process called “selective uptake” (SU) [66,67]. Seo et al. [68] showed that arterial LDL-CE delivery via SU can be an important mechanism in vivo in mouse models and that a saturated fat-rich diet enhances total arterial LDL delivery and SU, and in an atherosclerosis prone mouse model this could account for almost 60% of LDL-CE delivery to the artery. Furthermore saturated fat-rich diets markedly increased levels of arterial lipoprotein lipase (LPL) especially in the medial layer. This enzyme has a pro-atherogenic action since as well as producing hydrolysis of chylomicrons and very low-density lipoprotein, it acts as a molecular bridge for various lipoproteins and promotes their binding and

internalization through their receptors or SU promoting foam cell formation [69,70]. N-3 FA decrease LPL activity [71] and its expression in macrophages [72] which leads us to propose that EPA and DHA could influence SU and delay the development of atherosclerosis, in part, by decreasing delivery of LDL cholesterol into the arterial wall.

Another potential mechanism by which fish oils may prevent the development of atherosclerosis or restenosis could be inhibition of the mitogen induced smooth muscle cell (SMC) proliferation and migration of these into the vessel wall intima [73,74]. Pakala et al. [75] in cell culture showed that EPA and DHA inhibit smooth muscle cells proliferation induce by serotonin (5HT) and thromboxane A₂.

Importantly, DHA and EPA have long been recognized to modulate inflammatory responses [76]. N-3 FA addition, are associated with a reduction in the content of arachidonic acid (AA) in membrane phospholipids in platelets, endothelial cells and inflammatory cells. This decreases the amount of substrate available for eicosanoid synthesis resulting in decreased production of prostaglandin E₂ (PGE₂), thromboxane B₂ (TXB₂), leukotriene B₄ (LTB₄), hydroxyeicosatetraenoic acid (5-HETE) and leukotriene E₄ (LTE₄), potent pro- inflammatory agents. On the other hand EPA also acts as a substrate for cyclooxygenase and lipoxygenase enzymes, thus giving rise to a different family of eicosanoids: the 3-series prostaglandins and thromboxanes, which have less biological activity, and decrease inflammation [76].

However these fatty acids not only are the precursors of eicosanoides and other lipid mediators, but also can modify the imbalance Th1/Th2 that has been observed in atherosclerosis. This shift in the Th1/Th2 balance could be through the modulation of signaling molecules and transcription factors [77]. N-3 FA suppress production of Th1 cytokines (IL1, IL2, IL8, INF- γ and TNF- α) in mononuclear cells in response to lipopolysaccharide (LPS) [78,79] and in lymphocytes of mice fed with different types of fatty acid [80]. This is important because TNF- α and IL-1 β induce MCP-1 and VCAM-1 expression in endothelial cells, these attract to macrophage and other inflammatory cells [81,82].

N-3 FA effects on inflammatory cytokine expression could be at least in part through direct actions on the intracellular signaling pathways that lead to inactivation of one or more transcription factors such as nuclear factor κ B (NF- κ B) [83]. Fish oil decreased endotoxin induced activation of NF- κ B in human monocytes [84], and this was associated with decreased I κ B phosphorylation [85]. The inhibition of NF- κ B activation can be caused by mechanisms that produce the activation of PPAR or inhibition of Toll-like receptors [50].

Furthermore n-3 FA can also act on other inflammatory cells. Thies et al. [86] found that a moderate amount of EPA, but not other n-6 or n-3 FA can decrease NK cell activity in healthy subjects (>55 years). Peterson et al reported similar findings in rat spleen NK cell activity [87]. Other studies showed that increased intakes of EPA can decrease T lymphocyte functions such as proliferation and interleukin 2 productions in certain populations [88].

Some of these effects could be produced by the generation of alternative eicosanoid derivatives of n-3 fatty acids (EPA and DHA), recently identified by the groups of Micheselli, Serhan and Bazan [89] with the suggested names of resolvins for the E series generated from EPA and resolvin of series D and protectins for those generated from DHA. These catabolic products of n-3 FA suppress inflammation and help in the resolution of inflammatory events, including leukocyte infiltration and clearance of the cellular debris from sites of inflammation [90,91].

These anti-inflammatory actions of n-3 FA not only could prevent atheroma plaque development, but also could contribute to stabilization of atherosclerotic plaques. A thin fibrous cap and raised numbers of inflammatory cells such as macrophages in atheroma plaque are characteristics of an unstable plaque [92]. Thies et al. [93] reported that in patients awaiting

carotid endarterectomy the n-3 FA supplementation induced changes that can enhance stability of atherosclerotic plaques (thick fibrous caps, no signs of inflammation, lower number of macrophages). They suggest that atherosclerotic plaques are responsive to dietary modification such as n-3 FA which will affect plaque stability.

c) Blood pressure and platelet aggregation—N-3 FA alter vascular function by enhancing the release of nitric oxide (NO) [94,95], as well as modifying the release of ADP [96], decreasing vasoactive prostanoids (such as thromboxane A₂ which is a potent platelet aggregator and vasoconstrictor) [97] and improving arterial compliance [98]. Therefore n-3 FA can decrease blood pressure [99]. Furthermore n-3 FA can influence gene regulation by down regulating gene expression of platelet-derived growth factors and suppress platelet activating factor, a potent platelet aggregator and leukocyte activator [100].

In conclusion, a growing body of evidence, encompassing human to cellular and molecular studies are defining the roles for n-3 FA as bioactive agents for reducing the risks of and treating CVD.

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Figure 1. Different mechanisms by which n-3 FA can potentially modulate atherosclerosis. Pathways marked by an asterisk (*) are likely targets for n-3 FA regulation