n–3 Fatty acids and cardiovascular disease: mechanisms underlying beneficial effects1–4

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ABSTRACT
Dietary n–3 fatty acids, particularly eicosapentaenoic acid and docosahexaenoic acid, are important nutrients through the life cycle. Evidence from observational, clinical, animal, and in vitro studies indicates a beneficial role of n–3 fatty acids in the prevention and management of cardiovascular disease. Although the precise mechanisms are still unclear, clinical and preclinical studies indicate that the cardioprotective effects of n–3 fatty acids may be attributed to a number of distinct biological effects on lipid and lipoprotein metabolism, blood pressure, platelet function, arterial cholesterol delivery, vascular function, and inflammatory responses. Am J Clin Nutr 2008;87(suppl):2003S–9S.

INTRODUCTION
Diet has a substantial effect on the progression of atherosclerosis and cardiovascular disease (CVD). For example, dietary sources of n–3 fatty acids, such as fish and certain nuts and vegetable oils, are important components of a healthy diet because they contribute to eicosanoid metabolism, cell membrane phospholipid composition, cell membrane function, and gene expression. There is increasing evidence from primary and secondary intervention studies that increased consumption of n–3 fatty acids reduces the risk of CVD, including myocardial infarction, cardiac arrhythmias, sudden cardiac death, atherosclerosis, and hypertension (1–5). The very-long-chain n–3 fatty acids, especially eicosapentaenoic acid (EPA, 20:5n–3) and docosahexaenoic acid (DHA, 22:6n–3), are believed to be particularly important in the prevention of CVD (5). Mozaffarian and Rimm (6) indicated that modest consumption of fish (1–2 servings/wk), corresponding to about 250 mg per day of EPA and DHA, was associated with a reduced risk of coronary death by 36%, a decrease in total mortality by 17% in the general population, and appeared sufficient for primary prevention. Preformed EPA and DHA are found predominantly in fish and fish oils. α-Linolenic acid (ALA, 18:3n–3), a shorter-chain n–3 fatty acid, is present in various plant-based foods such as flaxseed, walnut, soybean, and canola; can be metabolically converted to EPA and DHA; and is also of interest for CVD prevention. The essential fatty acid precursors are linoleic acid, an n–6 fatty acid, and n–3 ALA. These fatty acids cannot be synthesized by mammals because the necessary enzymes to place a double bond at the appropriate n–3 or n–6 positions are absent. Wang et al (5) summarized that increased intakes of n–3 fatty acids from fish or fish oil supplements, but not of ALA, reduced the rates of all-cause mortality, cardiac and sudden death, and stroke. Similarly, Wilkinson et al (7) found that dietary ALA is not equivalent to EPA and DHA in its effects on CVD risk factors. EPA and DHA, but not ALA, induced changes in plasma levels of HDL cholesterol and small, dense LDL. In addition, ALA supplementation led to a small decrease in fibrinogen levels and fasting plasma glucose, but most cardiovascular risk factors did not appear to be affected (8).

Several mechanisms have been proposed to explain the cardioprotective effects of EPA and DHA (9). These include preventing arrhythmias (10), lowering plasma triacylglycerol (11), decreasing blood pressure (12), decreasing platelet aggregation (13), decreasing arterial cholesterol delivery (14), improving vascular relaxation (15), decreasing arterial inflammatory responses (16), and/or increasing heart rate variability (17). This article provides an overview of the evidence relating to the benefits of n–3 fatty acids in CVD and describes physiologic and molecular mechanisms by which n–3 fatty acids may confer benefits on cardiovascular risk.

n–3 FATTY ACIDS EFFECTS ON CARDIOVASCULAR DISEASE AND INTAKE RECOMMENDATIONS
Diets enriched with n–3 fatty acids protect against coronary artery atherosclerosis in nonhuman primates, an effect that appears to be independent of plasma levels of lipoproteins (18). In humans, n–3 fatty acids have been shown to exert cardioprotective effects in both primary and secondary coronary heart disease (CHD) prevention trials (1–5, 7, 17).

Some studies do not support a beneficial association between n–3 fatty acid intake and CVD risk (19, 20). One possible explanation for this may be that these studies were performed in a population with a high baseline intake of n–3 fatty acids (21). In the Japan EPA Lipid Intervention Study (JELIS), most of the population had a baseline intake of fish above the threshold for

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preventing cardiac death but, still, benefits from EPA supplementation were observed (4). One would expect low baseline rates of cardiac death and little further reduction in cardiac death with additional fish oil consumption. Other possible confounding factors include the consumption of alcohol, vitamin supplement use, differences in exercise habit, exclusion of fish oil supplement use in the assessment of dietary intake of n−3 fatty acid, and misclassification of dietary saturated fatty acid or n−6 fatty acid (22–24). Nevertheless, most epidemiologic studies and randomized control trials have provided a strong body of evidence for cardioprotective effects of n−3 fatty acids (5).

European and American cardiac societies include EPA and DHA in recent treatment guidelines for myocardial infarction, prevention of CVD, and prevention of sudden cardiac death (25). The American Heart Association (AHA) recommends 1 g/d EPA + DHA (preferably from oily fish, and use of EPA plus DHA supplements in consultation with a physician) for individuals with known CHD and the consumption of 2 meals/wk oily fish plus oils and foods rich in ALA for persons without CHD (26). Recent guidelines for women from the AHA state that, as an adjunct to diet, n−3 fatty acid capsules −0.85 to 1 g/d EPA and DHA may be considered in women with CHD, and doses of 2 to 4 g/d EPA and DHA may be used to treat high triacylglycerol levels (≥200 mg/dL) (27). The AHA recommends that treatment with these doses of n−3 fatty acids be administered only under a physician’s supervision. The US Food and Drug Administration has advised that no >3 g/d n−3 fatty acids be provided by dietary supplements and conventional foods (28). Additional clinical studies are needed to develop more specific recommendations and to distinguish between the requirements for DHA and/or EPA (21). Dr. Mozaffarian and colleagues (6, 29–32), as summarized in this supplement, suggest that daily intakes of EPA and DHA of 250 mg or more are needed to decrease the risk of CVD.

Mechanisms for cardioprotective actions of n−3 fatty acids

The exact mechanisms by which n−3 fatty acids exert a cardioprotective effect are unclear but are being better defined. n−3 Fatty acids can influence many aspects of the pathogenesis of CVD, including arrhythmias, lipid concentrations, blood pressure, platelet aggregation, vascular relaxation, inflammation, and likely arterial cholesterol delivery. Overall, the effects of n−3 fatty acids are related to multiple interactive mechanisms, including modulation of eicosanoid and other immune pathways, which leads to alteration of inflammatory responses (33); modulation of molecules or enzymes associated with various signaling pathways involving normal and pathologic cell function (33); incorporation of n−3 fatty acids into membrane phospholipids (34); and direct effects on gene expression (35, 36). For example, n−3 fatty acids affect the expression of several key proteins as modulators of many genes involved in lipid metabolism, inflammation, and smooth muscle cell proliferation, genes that can play a pivotal role in prevention and treatment of CVD and atherosclerosis (Figure 1).

Antiarrhythmic effects

Epidemiologic, clinical, animal, and in vitro studies have demonstrated antiarrhythmic actions of n−3 fatty acids (10). In the GISSI Prevention trial (37), treatment with EPA and DHA (1 g/d) was associated with reductions in risk of sudden cardiac death in patients surviving a recent (<3 mo) myocardial infarction. The relatively immediate and strong effect on sudden coronary death suggests that EPA and DHA act to prevent fatal arrhythmias (38). More recently, in a population-based cohort, usual dietary intake assessed at baseline in 1989–1990 was compared with atrial fibrillation incidence over 12 y of follow-up on the basis of hospital discharge records and annual electrocardiograms (30). Consumption of tuna or other broiled or baked fish was associated with a 28% reduced incidence in persons ingesting these foods 1–4 times/wk (P = 0.005) and a 31% reduced incidence in those ingesting these foods ≥5 times/wk (P = 0.008).

Animal and in vitro studies also support and help in the understanding of the antiarrhythmic action of fish oils. Prevention of ischemia-induced ventricular fibrillation by n−3 fatty acids has been reported in rat (39), nonhuman primate (40), and dog (41) models. Leaf et al (10) found that EPA and DHA prevent the arrhythmias by affecting the excitability of cultured neonatal rat cardiomyocytes, inhibiting fast, voltage-dependent sodium and L-type calcium currents. They offered the following hypothesis. After injury, including ischemia, a gradient of depolarization of cardiomyocytes occurs, and eliciting action potentials during a vulnerable period of the cardiac electrical cycle can initiate arrhythmias. However, in the presence of n−3 fatty acids, a voltage-dependent shift to more hyperpolarized potentials occurs, primarily as a result of inhibition of a fast, voltage-dependent sodium current. This prevents the sodium channel from contributing to the generation of an action potential in partially depolarized cardiomyocytes. Moreover, Schrepf et al (42) demonstrated that n−3 fatty acids may prevent arrhythmias acutely with reduction of sustained ventricular tachycardia. Several recent clinical trials have examined whether n−3 fatty acid supplementation suppresses arrhythmias in patients with implantable cardioverter defibrillators (ICDs) (43–45). In a double-blind, randomized control trial, patients (n = 402) with...
ICDs were randomly assigned to fish oil (total dose of EPA plus DHA of 2.6 g/d) or olive oil for 12 mo (43). Treatment with fish oil showed a trend toward a more prolonged time to the first ICD event for ventricular tachycardia, fibrillation, or death; of note, 35% of patients in this trial discontinued n−3 supplements, which may have impacted results. This treatment has recently been reviewed again by Leaf (46, 47), where he emphasized that n−3 fatty acids may be potent antiarrhythmic agents for reducing ventricular tachycardia/ventricular fibrillation in ICD patients. Some studies show little, if any, protective effect of intake of n−3 fatty acids against ventricular arrhythmia in patients with ICDs (44, 45).

Effects of n−3 fatty acids on plasma lipids and lipoproteins

High triacylglycerol levels have been shown to be an independent risk factor for CHD in a meta-analysis of 17 large, population-based studies (48). Several studies have shown the potent triacylglycerol-lowering effect of n−3 fatty acids in both normolipidemic and hyperlipidemic subjects. According to a meta-analysis by Harris (11), consumption of 3–4 g/d EPA and DHA for 2 wk is followed by a decrease in plasma triacylglycerol concentrations of 25% in normolipidemic subjects (triacylglycerol <2 mmol/L) and 25–34% in hyperlipidemic subjects (triacylglycerol ≥2 mmol/L). More recently, Balk et al (49) reported that ∼0.1–5.4 g/d EPA and DHA decreased fasting triacylglycerol concentrations in subjects who were healthy; subjects who had type 2 diabetes, hypertension, or dyslipidemia; or subjects who had been diagnosed with CVD.

Treatment with n−3 fatty acids have been shown to increase or have no effect on both HDL cholesterol and LDL cholesterol concentrations (50, 51). Harris (11) and Balk et al (49) have demonstrated that there are no significant effects of fish oil supplementation on HDL cholesterol or total cholesterol concentration. There was a 5% and 10% increase in LDL cholesterol in normolipidemic and hyperlipidemic individuals, respectively. Huff and Telford (52) suggested that the rise in LDL cholesterol concentrations by fish oil resulted from enhanced conversion of very-low-density lipoprotein to LDL. A similar finding has been reported following fish oil supplementation in which LDL cholesterol concentration shows a tendency to increase in patients with type 2 diabetes (53). These increases in LDL level could theoretically diminish the overall cardioprotection provided by n−3 fatty acids. However, studies in nonhuman primates fed large quantities of fish oil suggest that n−3 fatty acid–enriched LDL particles may be less atherogenic than control LDL particles (54–56). The latter does not appear to be the result of differences in small compared with large LDL (56) but might be the result of the decreased binding of n−3 fatty acid–enriched LDL to arterial proteoglycans, as observed in vitro (57).

Recent studies provide an attractive explanation for the diverse and variable effects of n−3 fatty acid on homeostatic and lipid factors related to transcription regulation of multiple genes. Both n−6 and n−3 fatty acids can inhibit the expression of genes involved in fatty acid and triacylglycerol syntheses, either as free fatty acids or as part of a triacylglycerol molecule (58). Dietary n−3 fatty acids or their metabolites such as 13-HODE, 15-HETE, prostaglandin J₂, and 15-deoxy-δ (12, 14)-prostaglandin J₂ have been shown to bind to peroxisome proliferator-activated receptor (PPAR)α and PPARγ to influence the transcription of genes involved in β-oxidation, ketogenesis, and adipogenesis (59). Fatty acids are degraded by the β-oxidation pathway. Studies in rats (60) showed that EPA and/or DHA increased free fatty acid β-oxidation in peroxisomes and mitochondria, leaving less substrate for triacylglycerol and very-low-density lipoprotein synthesis. In addition, n−3 fatty acids may decrease hepatic levels of sterol regulatory element-binding protein-1c messenger RNA, which regulates several key lipogenic genes (61). Thus, n−3 fatty acids may exert lipid-lowering effects by modulating the expression of genes encoding proteins involved in fatty acid oxidation while simultaneously inhibiting the expression of lipogenic genes. However, n−3 fatty acids suppressed expression of the hepatic lipogenic genes in mice lacking functional PPAR-α, thereby ruling out a requirement for PPAR-α (62). n−3 Fatty acids also exert effects on the removal of triacylglycerol-rich lipoproteins through stimulation of lipoprotein lipase (LPL), a triacylglycerol hydrolase present on the capillary endothelium of various tissues, via changes in gene expression in adipose tissue, and increases of postheparin plasma LPL activity (63).

Effects of n−3 fatty acids on blood pressure

Human and animal studies have shown that EPA and DHA can lower blood pressure. In a meta-analysis of controlled trials, treatment with 7.7 g/d of n−3 fatty acids supplementation lowered systolic and diastolic blood pressure by 4 and 3 mm Hg, respectively, in hypertensive patients (64). At doses between 3 and 5.6 g/d, EPA and DHA reduced blood pressure in hypertensive individuals by up to 5.5/3.5 mm Hg (12). In prehypertensive rats, DHA treatment for 6 wk attenuated the development of hypertension by 34 mm Hg (65).

Increased arterial wall thickness is characteristic of hypertension and is caused by abnormal growth and hypertrophy of vascular smooth muscle cells (66). Treatment with DHA for 6 wk reduced vascular wall thickness in the coronary artery and aorta of the hypertensive rat (66). Other potential mechanisms for the blood pressure—lowering effect of DHA include blunting of the rennin-angiotensin-aldosterone system by decreasing adrenal synthesis of aldosterone (65), changes in renal arachidonic acid metabolism (65), modulation of calcium release from and influx into vascular smooth muscle cells, and activation of vascular ATP-sensitive potassium channels by vasodilatory prostanooids (67). A meta-analysis of 30 randomized trials found that fish oil intake reduced heart rate by 1.6 beats/min compared with placebo (P = 0.002) (68).

Effects of n−3 fatty acids on platelet function

A common cause of an acute coronary event is atherosclerotic plaque rupture, which triggers thrombus formation and occlusion of the arterial lumen. n−3 Fatty acids may reduce the risk of thrombosis by affecting platelet aggregation and hemostasis. Antiplatelet effects of n−3 fatty acids are primarily mediated by competitive reduction of arachidonic acid conversion to thromboxane A₂, a potent promoter of platelet aggregation (17). Increased n−3 fatty acids, particularly EPA, replace arachidonic acid in membrane phospholipids of platelets (34), with a subsequent reduction in substrate pools for cyclooxygenase 2 to generate thromboxane A₂ and PGI₂. Higher n−3 fatty acid pools, with increased n−3 fatty acyl groups in phospholipids, lead to the production of thromboxane 3 and prostacyclin 3. Thromboxane A₃ has a less potent effect on platelet aggregation than does
thromboxane A₂, whereas prostacyclin 3 retains similar antiaggregation properties as prostacyclin 2. These shifts in the eicosanoid pathways induced by n–3 fatty acids lead to overall antithrombotic effects. In addition, fish oils reduce the levels of one or more coagulation factors, including reductions in factors VII and X (69). Fibrinogen levels in blood, an independent cardiovascular risk factor and a vitamin K-independent protein, are also decreased by fish oil (70). Even minor reductions in fibrinogen levels are potentially clinically important in reducing the risk of atherosclerosis (71).

Atherosclerotic plaques from patients treated with fish oil were shown to be less heavily infiltrated with macrophages than those in the placebo group (72). In addition, plaques from patients treated with fish oil were more likely to be fibrous-cap atheromas (type IV plaque, considered more resistant to rupture), and less likely to be thin, inflamed-cap atheromas (type V plaque) compared with plaques from patients given placebo (72).

Effects of n–3 fatty acids on arterial cholesterol delivery

The LDL receptor (LDLR) is a major contributor to cholesterol delivery to many cells, such as liver (73). However, accumulation of cholesterol in cells and tissues with little or no LDLR, such as the arterial wall, must be mediated by mechanisms distinct from the LDLR. Various mechanisms that might contribute to LDL cholesterol accumulation in the arterial wall have been proposed. These include initial anchoring to cell surface proteoglycans that are expressed ubiquitously and mediate LDL uptake via a low-affinity but high-capacity process (74). Macrophage surface receptors such as CD36 and other scavenger receptors have been shown to bind normal or modified LDL and affect the formation of atherosclerotic plaque (75).

Cholesterol is also delivered to cells and to the arterial wall via selective uptake (SU) (76), a process that mediates delivery of lipoprotein core lipids to cells in tissues without concomitant uptake of whole particles. This action leads to the accumulation of cholesterol in cells and tissues that exceeds cholesterol delivery mediated by a whole particle uptake. SU from HDL via scavenger receptor type B-1 has been well characterized and is involved in reverse cholesterol transport (77). LPL stimulates SU from LDL via scavenger receptor type B-1-independent pathways (76). Consistent with these findings, mice overexpressing human LPL in muscle have significant increases in LDL SU in muscle (76). Because LPL is secreted by arterial macrophages and smooth muscle cells and is increased in atherosclerotic lesions (78), arterial LPL expression might lead to progression of atherosclerosis.

Consistent with this hypothesis, arterial LPL was substantially increased in mice fed a high saturated fat diet (14). A high saturated fat diet increases LPL activity, whereas n–3 fatty acids reduce LPL activity (79) and LPL expression in macrophages (80). These data indicate that dietary fatty acids differentially influence arterial LPL expression and, thus, modulate SU and the development of atherosclerosis, in part, by increasing or decreasing delivery of LDL cholesterol into the arterial wall.

Effects of n–3 fatty acids on vascular function

n–3 Fatty acids have a direct effect on vascular function through uptake and incorporation into vascular smooth muscle and endothelial cells. Vascular smooth muscle cell proliferation plays an important role in the pathogenesis of atherosclerosis. n–3 Fatty acids can inhibit smooth muscle cell proliferation by modulating growth signals or DNA synthesis (81, 82), and this is related to the amount of lipid peroxides formed in the cells (83). Terano et al (81) suggested that EPA inhibits vascular smooth muscle cell proliferation through signal transduction pathways related to a number of growth factors. For example, EPA has been shown to inhibit the binding of platelet-derived growth factor to its surface receptor, to suppress protein kinase C activation, and to inhibit expression of c-fos. In addition, n–3 fatty acids inhibit cyclins and their catalytic subunits (cyclin-dependent kinases), which control the progression of the cell cycle via DNA synthesis, and suppress transforming growth factor-β (82).

The endothelial cell is also important for smooth muscle cell function, vascular remodeling, and maintenance of vascular tone through both vasoconstriction and vasodilation (84). Nitric oxide (NO) is the primary compound responsible for vasodilation in arteries and has inhibitory effects on platelet aggregation and adhesion, leukocyte adhesion, and smooth muscle cell proliferation (85). Endothelial dysfunction is associated with CHD risk factors and is believed to be the key initiating event in atherosclerosis. n–3 Fatty acids enhance production of endothelium-derived vascular relaxing factor, which is reduced in atherosclerotic vessels (86). This result is supported by the observation that vasodilation in response to acetylcholine intra-arterially can be restored in coronary arteries of patients who had received heart transplants and received fish oil supplements for 3 wk, whereas vasoconstriction still occurred in control subjects (87). In another study, both EPA and DHA improved endothelium-dependent vasodilation in subjects with hypercholesterolemia (15). Furthermore, studies in vitro suggest that endothelium-dependent relaxation of vessel preparation by n–3 fatty acids is due to the enhancement of NO release (88). This conclusion is supported by the fact that EPA stimulates NO production by endothelial cells in situ and induces endothelium-dependent relaxation of bovine coronary arteries (89). These studies suggest that n–3 fatty acids increase endothelium-dependent vasodilation in patients with CHD through NO-dependent and NO-independent pathways.

Effects of n–3 fatty acids on CVD-related inflammatory responses

Inflammation is now recognized as a central process in the development of atherosclerosis and CHD (90). In the arterial wall, endothelial activation can be triggered by various inflammatory stimuli such as oxidized LDL, free radical species, lipopolysaccharide, and cytokines. Circulating monocytes are attracted to the endothelium by chemokines, bind to the adhesion molecules, adhere to the endothelium, and transmigrate to the subendothelial space, where they become macrophages. Within the subendothelial macrophages scavenger oxidized LDL, become foam cells, and contribute to the development of the fatty streak in the early stage of atherosclerosis (90). Proliferation of vascular smooth muscle cells may also enhance plaque development with the expression of proinflammatory molecules, including monocyte chemoattractant protein 1 and vascular cell adhesion molecules (91).

n–3 Fatty acids, particularly DHA, have been shown to reduce adhesion and migration of monocytes and influence leukocyte-endothelial cell interactions in atherosclerosis and inflammation, processes that involve increased endothelial expression of leukocyte adhesion molecules or endothelial activation (92).
Caterina et al (93) reported that DHA reduced endothelial expression of vascular cell adhesion molecule-1, E-selectin, intercellular adhesion molecule 1, IL-1 (interleukin)-6, and IL-8 in response to IL-1, IL-4, tumor necrosis factor-α (TNF-α), or bacterial endotoxin (Figure 1). In addition, DHA and EPA reduced TNF-α and IL-1β expression in human THP-1 macrophages (94). Supplementing the diet of volunteers with 7 g/d n-3 fatty acids for 4 wk decreased monocyte chemoattractant protein 1 messenger RNA levels in unstimulated human monocytes (95). At present, there are no clear effects of n-3 fatty acids on levels of IL-10 (95, 96).

Activated transcription factor nuclear factor (NF)-κB is associated with atherosclerotic progression by up-regulating cytokine gene expression (97). Activated NF-κB and its target genes are found in the atherosclerotic vessel wall. Another transcription factor, PPARγ, also plays a role in acute inflammation control (98). Potential antiinflammatory and antiatherogenic properties of PPARγ in monocyte/macrophages, endothelial cells, and vascular smooth muscle cells includes reduction of cytokines such as IL-1, IL-6, and TNF-α release into blood circulation. Because n-3 fatty acids decrease agonist-induced activation of NF-κB and increase PPARγ, it is possible that this is one pathway whereby n-3 fatty acids exert their antiinflammatory effects.

CONCLUSIONS

Substantial evidence supports n-3 fatty acids as a practical, therapeutic adjuvant for promoting cardiovascular health and preventing and treating disease. n-3 Fatty acids modulate a number of important physiologic responses that can contribute to their cardioprotective effects. The multiple and complex mechanisms through which DHA and EPA exert their action appear to be distinct but also complementary. However, more studies are needed to quantify their protective effects and to define exact mechanisms of action.

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