

Review

Omega-3 fatty acids and coronary heart disease risk: Clinical and mechanistic perspectives

William S. Harris^{a,*}, Michael Miller^b, Ann P. Tighe^c,
Michael H. Davidson^d, Ernst J. Schaefer^e

^a *Nutrition and Metabolic Disease Research, Sanford Research/USD, Sanford School of Medicine,
University of South Dakota, Sioux Falls, SD, United States*

^b *Medicine, Epidemiology & Preventive Medicine, Center for Preventive Cardiology, Division of Cardiology,
University of Maryland Medical Center, Baltimore, MD, United States*

^c *Scientific and Editorial Services, Scientiae, LLC, New York, NY, United States*

^d *Radiant Research, Chicago, IL, United States*

^e *Tufts University School of Medicine, Lipid Metabolism Laboratory, Jean Mayer USDA HNRCA at Tufts University, Lipid and
Heart Disease Prevention Clinic and Lipid Research Laboratory, New England Medical Center, Boston, MA, United States*

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Abstract

The most common omega-3 fatty acids contain 18–22 carbons and a signature double bond at the third position from the methyl (or *n*, or omega) end of the molecule. These fatty acids must be obtained in the diet as they cannot be synthesized by vertebrates. They include the plant-derived α -linolenic acid (ALA, 18:3 n -3), and the fish-oil-derived eicosapentaenoic acid (EPA, 20:5 n -3) and docosahexaenoic acid (DHA, 22:6 n -3). Normally, very little ALA is converted to EPA, and even less to DHA, and therefore direct intake of the latter two is optimal. EPA and DHA and their metabolites have important biologic functions, including effects on membranes, eicosanoid metabolism, and gene transcription. Studies indicate that the use of fish oil is associated with coronary heart disease risk reduction. A number of mechanisms may be responsible for such effects. These include prevention of arrhythmias as well as lowering heart rate and blood pressure, decreasing platelet aggregation, and lowering triglyceride levels. The latter is accomplished by decreasing the production of hepatic triglycerides and increasing the clearance of plasma triglycerides. Our focus is to review the potential mechanisms by which these fatty acids reduce cardiovascular disease risk.
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Keywords: Omega-3 fatty acids; Coronary heart disease; Arrhythmia; Platelet aggregation; Triglyceride

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* Corresponding author at: Sanford Research/USD, 1400 W. 22nd Street, Sioux Falls, SD 57105, United States. Tel.: +1 816 932 8235; fax: +1 816 932 8278.

E-mail address: Bill.Harris@usd.edu (W.S. Harris).

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1. Introduction

Omega-3 fatty acids have been shown to exert cardioprotective effects in both primary and secondary coronary heart disease (CHD) prevention trials [1,2]. Proposed mechanisms to account for these findings include reduced triglyceride (TG) concentrations, antiarrhythmic effects, decreased platelet aggregation, plaque stabilization, reduced blood pressure, and/or a reduction in heart rate [2]. High TG levels have been shown to be an independent risk factor for CHD in a meta-analysis of 17 large, population-based studies ($N > 56,000$) [3]. After correcting for high-density lipoprotein cholesterol (HDL-C), every 88 mg/dL increase in TGs was associated with an increase in CHD risk of 14% in men and 37% in women. These findings are supported by data from families with familial hypertriglyceridemia and patients with premature familial coronary artery disease (CAD), as well as data from the Copenhagen Male Study. In this study, middle-aged men without overt CHD at baseline showed increasing incidence of ischemic heart disease over 8 years with increasing baseline TGs within each tertile of HDL-C [4]. However, while the data are strong for benefit associated with LDL cholesterol lowering, only very limited data are available to document CHD benefits from lowering of plasma TGs in randomized placebo-controlled trials. The purpose of this paper is to review the literature relating to the possible mechanisms for the TG-lowering effect of omega-3 fatty acids, as well as other potential cardioprotective mechanisms.

2. Treatment of hypertriglyceridemia with omega-3 fatty acids

The National Cholesterol Education Program Third Adult Treatment Panel (NCEP ATP III) recommends that patients with borderline (150–200 mg/dL) and high (>200 mg/dL) TG levels be treated with lifestyle modifications [5]. The NCEP ATP III also indicates that patients with high TG levels (200–499 mg/dL) may need pharmacologic therapy that targets non-high-density lipoprotein cholesterol (non-HDL-C): statins, fibrates, and nicotinic acid. It is not clear whether TG reduction, especially when combined with low-density lipoprotein cholesterol (LDL-C) reduction, contributes more to cardiovascular event rate reduction than that attained through LDL-C lowering alone.

The TG-lowering effect of omega-3 fatty acids in humans is well established [6]. A meta-analysis of 36 crossover and 29 parallel-design studies demonstrated that omega-3 fatty

acids lowered serum TG levels in a dose-dependent manner, with the TG lowering being generally proportional to baseline levels [7]. In trials of subjects with TG levels >150 mg/dL (>1.69 mmol/L) taking the omega-3 fatty acids eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA) in dosages of 3.4–4 g/day, TG levels decreased by an average of 29% (range 16–45%).

In addition to fibrates and nicotinic acid, a highly concentrated, prescription omega-3 (P-OM3) preparation (LovazaTM,¹ Reliant Pharmaceuticals, Inc., Liberty Corner, NJ) is now available for the treatment of hypertriglyceridemia as an adjunct to diet. P-OM3 contains over 90% long-chain omega-3 fatty acid ethyl esters, primarily EPA (465 mg/g) and DHA (375 mg/g) [8]. Multiple randomized, controlled trials confirm the efficacy of P-OM3 as a TG-lowering therapy, including the combined results from two trials (Fig. 1). In these trials a 45% increase in LDL-C produced a final mean LDL-C of 129 mg/dL [6,9]. While these increases were clearly not inconsequential and could theoretically diminish the overall cardioprotection afforded by omega-3 fatty acids, the actual clinical relevance of this finding is uncertain in view of the favorable effects on TG, HDL-C, and associated enrichment of tissue omega-3 fatty acid levels. Regulation of LDL-C levels in subjects with hypertriglyceridemia is complex. In apoB-100 kinetic studies, P-OM3 increased the percent conversion of very low-density lipoprotein (VLDL) to LDL without increasing LDL apolipoprotein B-100 (apoB) levels [10]. Interestingly, weight loss in overweight subjects with hypertriglyceridemia can also raise LDL-C, and it appears to do so by reducing the fractional catabolic rate of LDL [11]. Studies in non-human primates suggest that omega-3 fatty acid-enriched LDL particles have physical, chemical, and biological properties that may render them less atherogenic than control LDL particles [12–14]. Specifically, cultured human THP-1 macrophage cells incubated with acetylated LDL from monkeys fed an omega-3 fatty acid-enriched diet accumulated significantly less cholesteryl ester than cells incubated with similar LDL obtained from animals fed diets enriched with other fats [12]. It must be

¹ LovazaTM was formerly known as Omacor[®]. Reliant Pharmaceuticals, Inc. has changed the name of Omacor to LovazaTM (omega-3-acid ethyl esters). Reliant took this step at the request of the FDA and in response to a limited number of reports of prescribing and dispensing errors [Data on File. Reliant Pharmaceuticals, Inc.] due to similarity in name between the company's Omacor capsules and Xanodyne Pharmaceuticals' Amicar[®] (aminocaproic acid) [Amicar[®] is a registered trademark of Xanodyne Pharmaceuticals, Inc.]. The name change is intended to minimize the potential for such errors in the future.

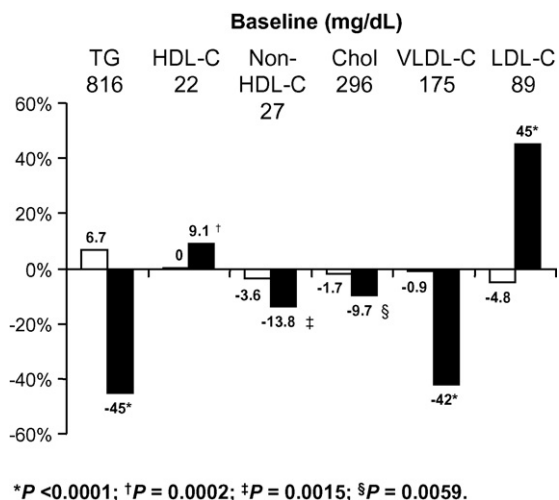


Fig. 1. Prescription omega-3 acid ethyl esters (P-OM3) and lipid levels in subjects with hypertriglyceridemia (≥ 500 mg/dL). Compared with placebo, P-OM3 (4 g/day for 6 weeks [9] or 4 months [6]) significantly reduced triglyceride (TG) levels. Non-high-density lipoprotein cholesterol decreased because the decrease in very-low-density-lipoprotein cholesterol was greater (in absolute values) than the increase in low-density lipoprotein cholesterol (LDL-C). In this patient population, the normal conversion of very-low-density-lipoprotein to low-density lipoprotein is inhibited (which contributes to the severe elevation in TG). In these studies the baseline low-density lipoprotein cholesterol (LDL-C) was 89 mg/dL. This 45% increase in LDL-C produced a final mean LDL-C of 129 mg/dL. Data from studies by Harris et al. [6] ($N = 42$) and Pownall et al. [9] ($N = 40$) were pooled.

appreciated, however, that these monkeys were given doses of omega-3 fatty acids that were about five-fold higher than are used in humans to lower TGs; thus the clinical relevance is unclear. Nevertheless, evidence reviewed below indicates that the rise in LDL in patients with hypertriglyceridemia may be offset by concurrent treatment with statins.

3. Biochemistry of omega-3 fatty acids

Fatty acids are straight chains of carbon atoms (usually 12–24) with an alpha (or carboxylic acid) end and an omega (or methyl or n) end. Fatty acid nomenclature begins with the number of carbons, then after a colon, the number of double bonds, followed by the position of the first double bond counting from the omega (or n th) carbon. The major saturated fatty acids in plasma are palmitic acid (16:0) and stearic acid (18:0); they are called ‘saturated’ because all carbon–carbon bonds are saturated with hydrogens, meaning there are no double bonds. The major monounsaturated fatty acid, oleic acid (18:1 n 9), contains one double bond.

Omega-3 fatty acids are polyunsaturated fats in which the first double bond counting from the omega carbon is at position 3, hence the name omega-3 (or $n-3$). Major omega-3 fatty acids include α -linolenic acid (ALA, 18:3 $n-3$), EPA (20:5 $n-3$), and DHA (22:6 $n-3$), and comprise one of the two classes of essential fatty acids. Preformed EPA and DHA are best

obtained from fatty fish or fish-oil supplements. ALA may be obtained from certain seed oils, but only a small percentage of ALA is converted to EPA in mammals, and further transformation to DHA is very low (Fig. 2). The other class of essential fatty acids is the omega-6 fatty acids, comprised mainly of linoleic acid (LA, 18:2 $n-6$) and arachidonic acid (AA, 20:4 $n-6$).

Essential fatty acids play a key role in many metabolic processes, and cannot be synthesized by mammals because the necessary enzymes to place a double bond at the omega-3 or -6 positions are absent [15–17]. Omega-6 fatty acids and their derivatives play a role in the immune response and in thrombosis, whereas omega-3 fatty acids and their derivatives are less active in these processes. After absorption, fatty acids are incorporated into triglycerides (3 fatty acids on a glycerol backbone), phospholipids (2 fatty acids attached to phosphatidic acid backbone), and cholesteryl esters (1 fatty acid attached to free cholesterol). About 70% of the cholesterol in plasma is in the form of cholesteryl ester. Phospholipids are critical for the formation of every cell membrane in the body. The phospholipid bilayer of the membrane is oriented so that the polar head groups interface with the aqueous environment inside and outside of the cell while the fatty acid chains are oriented towards the interior of the membrane, providing a water-impermeable barrier. In this membrane are embedded cholesterol and a large variety of proteins (e.g., receptors, ion channels, signaling complexes). The fluidity of the membrane may be very important for receptor function and recycling as well as the efficiency of signaling pathways. The fluidity of the membrane is determined in part by the fatty acid content of the membrane phospholipids. Fatty acids with multiple double bonds confer increased fluidity to cell membranes, which may partially account for their benefits in preventing cardiac arrhythmias, as well as in the maintenance of neurologic function. While only about 4% of the fatty acids in the bloodstream are DHA, almost 30% of the fatty acids in phospholipids in the brain and retina are DHA. This observation suggests an important role for DHA in neurologic and visual function.

The final step in the conversion of ALA to DHA is a beta oxidation step converting 24:6 $n3$ to 22:6 $n3$, and this step occurs in liver peroxisomes. Rare patients lacking peroxisomes (Zellweger’s disease) or having peroxisomal dysfunction (neonatal adrenal leukodystrophy) have marked plasma DHA deficiency, develop severe neurologic dysfunction, and die at an early age. Therefore, DHA appears to be important for central nervous system function [18,19]. Cross-sectional studies have linked low DHA levels with dementia, while prospective studies have linked both all-cause dementia and Alzheimer’s disease with decreased fish intake and low plasma phospholipid DHA levels [20–34]. In the Framingham Heart Study subjects who were in the highest quartile of plasma phospholipid DHA levels consumed on average at least 180 mg of DHA per day, and had a 50% reduction in the risk of all-cause dementia and Alzheimer’s disease [28]. Recent data have also linked less rapid progression of coro-

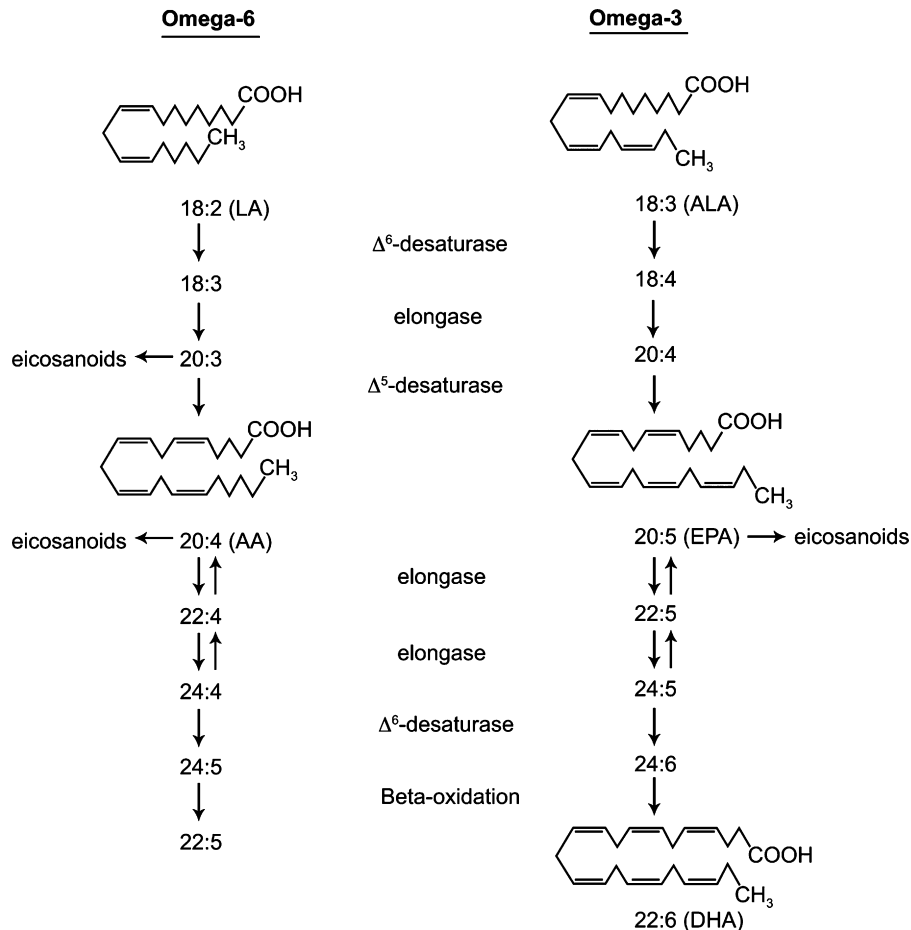


Fig. 2. Synthesis of omega-6 and omega-3 fatty acids in mammals. The primary dietary omega-6 fatty acid is linoleic acid (LA) which has 18 carbons and 2 double bonds (18:2 n -6). α -linolenic acid (ALA) is a short-chain omega-3 fatty acid (18:3 n -3) found in plant products such as flaxseed and soybean oils. Essential fatty acids cannot be synthesized by mammals because the necessary enzymes to place a double bond at the omega 3 or 6 positions are absent. The final step in the conversion of ALA to docosahexaenoic acid (DHA) is a β -oxidation step converting 24:6 n -3 to 22:6 n -3. In adult men, about 1–5% of ALA is converted to eicosapentaenoic acid (EPA), and conversion to DHA is very low (<0.1%). In women, fractional conversion to DHA appears to be somewhat greater. The initial introduction of a double bond into ALA by Δ^6 -desaturase is the rate-limiting reaction of the pathway. Although the affinity of Δ^6 -desaturase is higher for ALA than for LA, the typically higher cellular concentrations of LA result in greater net conversion of long-chain omega-6 fatty acids. Diets high in omega-6 fatty acids can reduce the conversion of ALA to EPA and DHA. Adapted from Jump [43] and Calder [2].

nary atherosclerosis in patients with higher levels of plasma DHA [35].

4. Omega-3 fatty acids: TG-lowering mechanisms

Elevated TG levels may result from genetic or metabolic abnormalities that lead to increased plasma residence time of potentially atherogenic chylomicron and/or VLDL remnants. Hypertriglyceridemia associated with elevations in VLDL can be due to overproduction of VLDL particles by the liver, reduced intravascular lipolysis of VLDL-TG, and/or delayed clearance of small (remnant) VLDL particles from the plasma. VLDL particles are formed in the liver from apo B, cholesterol, cholesteryl ester, phospholipids, and TG, the latter originating from long-chain free fatty acids extracted from the plasma, recycled fatty acids, and/or de novo synthesis from acetyl co-enzyme A (CoA).

The omega-3 fatty acids found in fish oil lower fasting and postprandial plasma TG concentrations without clinically significant effects on fat absorption [36]. In general, clinical studies indicate that both EPA and DHA have similar TG-lowering effects [37]. Treatment with 3.4 g/day of EPA and DHA for 4 months increased EPA and DHA proportions in phospholipids two- to threefold from baseline levels [6].

The molecular mechanisms by which EPA and DHA reduce serum TGs are not completely understood, but several potential mechanisms derived from preclinical studies are illustrated in Fig. 3. These studies provide compelling evidence that these fatty acids can both reduce hepatic VLDL-TG synthesis and secretion and enhance TG clearance from chylomicrons and VLDL particles. It is also known that EPA (and DHA) are preferentially shunted into phospholipid synthesis pathways, compared to other fatty acids (i.e., oleate) which are preferentially incorporated into triacylglycerol [38].

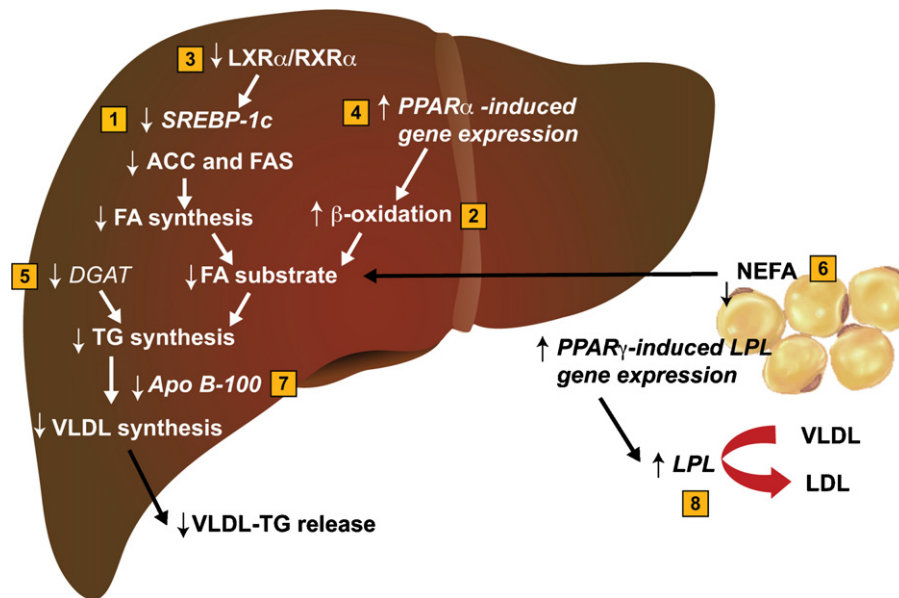


Fig. 3. Potential triglyceride (TG)-lowering mechanisms of the omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid. Reduced very-low-density-lipoprotein cholesterol triglyceride (VLDL-TG) secretion may be due to decreased expression of sterol regulatory element-binding proteins (SREBP)-1c (1) or increased rates of mitochondrial and/or peroxisomal β -oxidation (2), leading to reduced substrate for TG synthesis. (3) Decreased SREBP-1c expression may be mediated by inhibition of liver X receptor (LXR) ligand binding to LXR/retinoid X receptor. Increased rates of peroxisomal β -oxidation may be a consequence of peroxisome proliferator-activated receptor (PPAR)- α -induced increase in acyl-coenzyme A oxidase gene expression (4). Decreased activity of TG-synthesizing enzymes (5), decreased non-esterified fatty acid delivery from adipose tissue (6), and decreased availability of apo B (7) are potential mechanisms for reduced VLDL-TG release. In the periphery, increased lipoprotein lipase activity (8) may lead to increased VLDL-TG clearance, possibly due to increased PPAR- γ and/or PPAR- α gene expression. Adapted from Harris and Bulchandani [39] and Davidson [49].

5. Reduced VLDL-TG synthesis

As noted above, omega-3 fatty acids reduce serum TG concentration in humans partly via inhibition of hepatic VLDL-TG secretion rates secondary to decreased synthesis of TG. Thus, reductions in hepatic TG synthesis will lead to reduced production and secretion of VLDL [39]. Studies using perfused monkey liver system show that EPA and DHA decrease hepatic TG secretion through relatively poor utilization of EPA as a substrate for VLDL-TG [40], resulting in a lipid-poor hepatic VLDL [41]. Omega-3 fatty acid-induced decreases in VLDL-TG synthesis appear to be associated with decreases in transcription factors that control the expression of the enzymes responsible for TG assembly within hepatocytes and for fatty-acid oxidation. EPA and/or DHA can also increase intracellular degradation of apo B in primary rat hepatocytes, resulting in decreased VLDL production [42]; however, the importance of this pathway in humans is not clear. Studies in African green monkeys show that fish oil feeding does not significantly affect hepatic apo B secretion [40]. Omega-3 fatty acids can also lower circulating non-esterified fatty acid (NEFA) concentrations (discussed below). Although all of these mechanisms may play a role in the reduction of VLDL-TG synthesis by omega-3 fatty acids, a systematic review of preclinical studies in rats concluded that EPA and/or DHA is most consistently associated with decreased hepatic lipogenesis [39].

Dietary fat has been shown to affect gene transcription via ligand-activated nuclear transcription factors. All peroxisome proliferator-activated receptor subtypes (PPAR- α , - β , and - γ) bind EPA [43] and it has been postulated that omega-3 fatty acids may modulate fatty acid β -oxidation by interacting with PPAR- α (see below). However, effects of omega-3 fatty acids on lipogenic gene expression were observed in PPAR- α null mice, ruling out an absolute requirement for PPAR- α in omega-3 fatty acid-induced suppression of lipogenic gene expression [44]. Thus, details of the mechanism(s) of action by which PPARs might be involved in the TG-lowering effect of EPA and DHA are still lacking.

Sterol regulatory element-binding proteins (SREBPs) are transcription factors that regulate cholesterol-, fatty-acid-, and TG-synthesizing enzymes. One of the main molecular pathways for hepatic lipogenesis involves activation of the transcription factor SREBP-1c, which in turn stimulates the synthesis of acetyl-CoA carboxylase-1 (ACC1) and fatty-acid synthase (FAS), critical lipogenic enzymes (Fig. 3) [45]. The liver X receptor alpha/retinoid X receptor alpha (LXR α /RXR α) heterodimer regulates expression of the SREBP-1c gene via two LXR-responsive elements (LXREs) in the SREBP-1c promoter. Fish-oil feeding in mice is associated with a significant decrease in plasma TG levels and a marked decrease in the level of hepatic SREBP-1c mRNA [46], an effect that may be due to EPA- and DHA-induced inhibition of binding of the LXR/RXR heterodimer to the LXREs in the SREBP-1c promoter, thereby

suppressing SREBP-1c gene expression [47]. In addition, suppression of SREBP-1c mRNA and the SREBP-1 protein by EPA was associated with decreased TG synthesis in HepG2 human hepatoma cells [48]. However, studies in rats suggest that EPA-induced suppression of SREBP-1c and its targeted lipogenic genes is independent of LXR α (reviewed in Davidson) [49]. Thus, inhibition of LXR α binding to LXREs is likely not the only important cause of EPA- and DHA-induced suppression of SREBP-1c mRNA.

Regulation of SREBP-1c expression may not be specific to long-chain omega-3 fatty acids since levels of SREBP-1c mRNA were decreased in HepG2 cells cultured in medium containing not only EPA and DHA but also ALA and omega-6 fatty acids [46]. Neither of the latter two lowers serum TGs in humans. Another study found no change in the levels of SREBP-1 in HepG2 cells cultured in medium containing EPA and DHA [50]. These discrepant findings may be due to differences in experimental models, and thus additional studies are needed to determine the role of hepatic SREBP-1c in the TG-lowering effects of EPA and DHA.

The farnesoid X receptor (FXR) is a nuclear receptor for bile acids that also plays a central role in lipid homeostasis [51]. Studies in HepG2 cells demonstrate that FXR suppresses hepatic lipase and apo CIII gene expression and induces apo CII and VLDL-receptor gene expression [52–55], all of which may contribute to the TG-lowering action of FXR agonists [49]. Notably, mice lacking a functional FXR protein had a proatherogenic serum lipoprotein profile, including elevated TGs [56]. Since DHA is a ligand for FXR [57], a mechanism for the TG-lowering effects of DHA may involve FXR-induced changes in gene expression [49].

Phosphatidic acid phosphohydrolase (PAP) and acyl-CoA:diacylglycerol acyltransferase (DGAT) are key enzymes in TG biosynthesis, catalyzing the conversion of phosphatidate to diacylglycerol and diacylglycerol to TG, respectively. Although a number of studies have shown that EPA and EPA plus DHA can inhibit the activity of DGAT and PAP in rat liver microsomes, other studies have shown no effect of EPA and DHA on DGAT and PAP activity. Importantly, most of these studies used EPA or EPA and DHA at supraphysiological doses and employed different experimental conditions [39]. Thus, the extent to which the TG-lowering effects of EPA and DHA depend on the inhibition of DGAT and/or PAP remains speculative.

NEFAs, which appear to enter cells via fatty-acid transport proteins [58], are rapidly converted by acyl-CoA synthetases into fatty acyl-CoA thioesters that are potential substrates for TG synthesis [43]. Reduced serum NEFAs could potentially reduce hepatic TG synthesis. However, reduced plasma TG levels may themselves lead to decreased circulating NEFA concentrations, in which case the reduced NEFA levels may be an effect of omega-3 fatty acid-induced TG lowering, not a cause [39]. Alternatively, individual fatty acids may be differentially processed. For example, a study by Parks et al. [40] showed that in livers from monkeys fed fish oil (vs. lard),

there is preferential incorporation of EPA into hepatic phospholipids and a lower percentage incorporated into secreted TG [41]. Although human data are lacking, studies using non-human primates suggest that, compared with other fatty acids, differences in the intrahepatic processing of free EPA and DHA may contribute to their TG-lowering effects.

Slower formation of TG-rich VLDL in rodents that were fed fish oil or EPA has been linked to a faster rate of hepatic fatty-acid oxidation. Evidence suggestive of this effect has also been seen in healthy human subjects receiving dietary supplementation with 9 g of EPA + DHA per day [59]. Of the rat studies that show an EPA- and/or DHA-induced increase in β -oxidation, about half report an increase in peroxisomal oxidation and the other half, mitochondrial (reviewed in Harris and Bulchandani) [39]. In vitro and ex vivo studies have shown that EPA and DHA can induce acyl-CoA oxidase gene expression in rat hepatocytes in a PPAR- α -dependent manner. However, other studies in rats [39] and monkeys [40] have found that EPA and/or DHA had no significant effect on β -oxidation. Hence, the extent to which increased β -oxidation plays a role in reducing the production of VLDL-TG in humans taking 3–4 g of EPA and DHA remains unknown.

Overall, EPA and DHA have demonstrated effects in reducing hepatic VLDL-TG synthesis. While the molecular mechanisms for this noted reduction are not fully understood, they are likely due to the modulation of transcription factors involved in hepatic fatty-acid uptake, synthesis, and oxidation, as well as those involved in TG synthesis and VLDL assembly.

6. Enhanced TG clearance

Chylomicrons and VLDL are competitive substrates for lipoprotein lipase (LPL), a TG hydrolase present on the capillary endothelium of various tissues. EPA and DHA, when given individually (4 g/day), both significantly increased the rate of chylomicron clearance (Fig. 4A), an effect associated with shorter chylomicron TG half-life [37]. The accelerated chylomicron TG clearance was associated with increased pre-heparin LPL activity (Fig. 4B). All of these effects were statistically significant only in the fed, not the fasted state suggesting that insulin may play a role in this phenomenon. Additional studies are needed to determine if omega-3 fatty acids amplify insulin-induced LPL activity and/or enhance blood flow to adipose tissue and muscle, thereby exposing postprandial chylomicrons to tissues enriched with endothelial LPL. Khan et al. demonstrated that the TG-lowering effects of EPA and DHA in subjects with an atherogenic lipoprotein profile were associated with increased LPL gene expression in adipose tissue (Fig. 5) and significantly increased post-heparin plasma LPL activity [60]. EPA was shown to increase PPAR- γ mRNA in isolated adipocytes [61], and PPAR- γ mRNA levels in adipose tissue have been positively correlated with plasma EPA concentra-

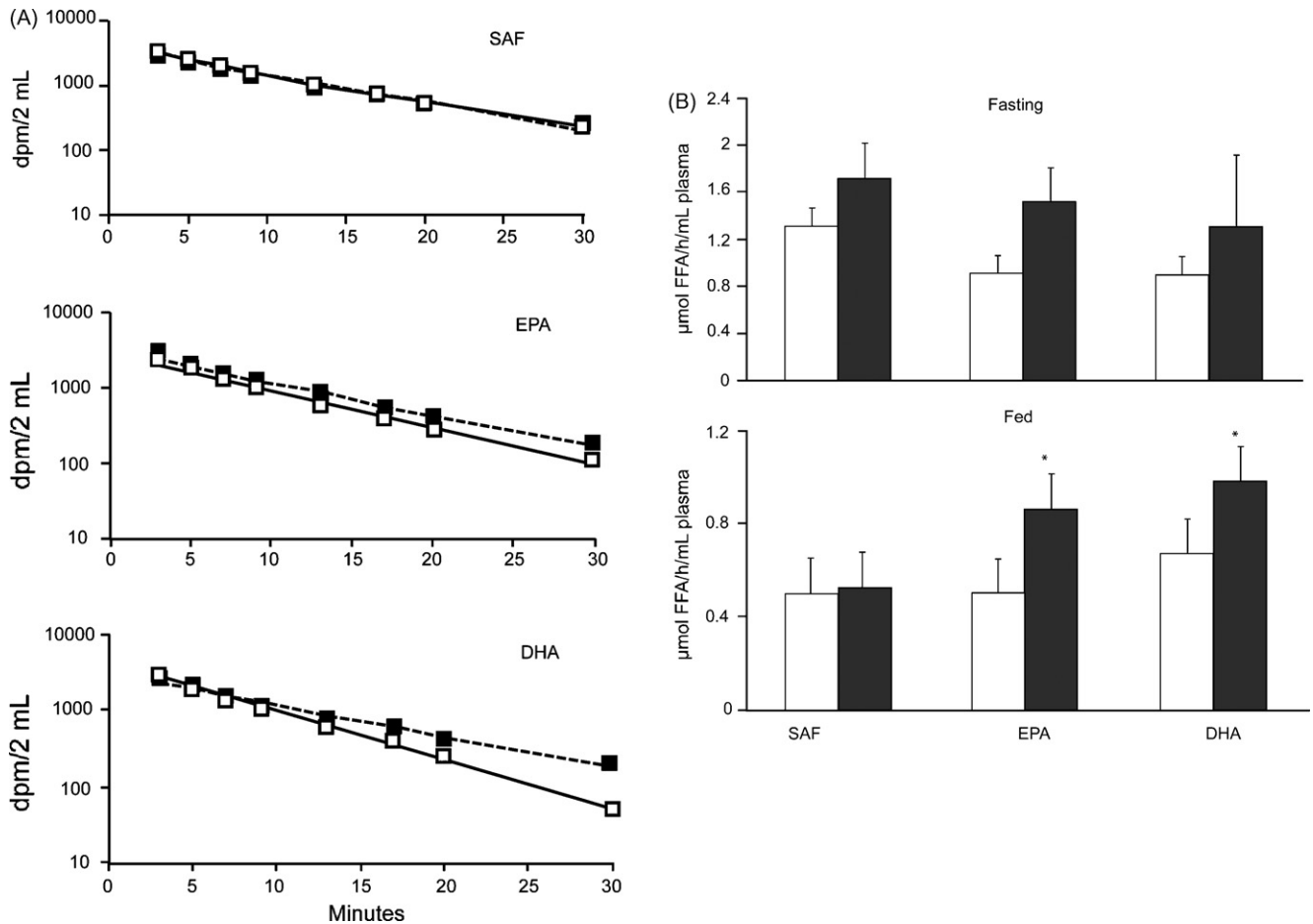


Fig. 4. Effect of omega-3 fatty acids [4 g/day of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), or safflower oil (SAF)] on chylomicron clearance and lipolytic activity in normolipidemic humans. (A) Clearance of $[^3\text{H}]$ triolein-labeled lipid emulsion from chylomicron fraction measured during the fed state during placebo (olive oil) treatment (black boxes) and EPA, DHA, or SAF treatment (white boxes). (B) Pre-heparin lipolytic activities during the fed state increased from placebo (white bars) to active treatment (black bars), with increased activity being observed with both DHA (+47%) and EPA (+73%) treatments compared with SAF. Post-heparin lipoprotein lipase activities were not affected by any treatment (data not shown). * $P < 0.05$ vs. change in SAF group. From Park and Harris [37].

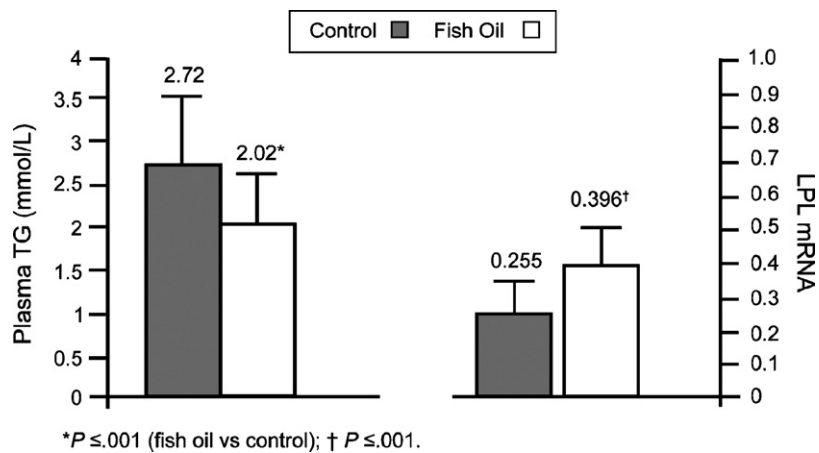


Fig. 5. Triglyceride-lowering effects of eicosapentaenoic acid plus docosahexaenoic acid in subjects with an atherogenic lipoprotein profile are associated with increased lipoprotein lipase (LPL) gene expression in adipose tissue. Not shown: post-heparin plasma LPL activity was significantly increased at 5 min post-injection (+31%, $P < 0.036$). From Khan et al. [60].

tions in obese subjects [61]. Since LPL activity in adipose tissue from obese ob/ob mice was shown to be increased by a PPAR- γ agonist [62], increased LPL activity associated with EPA plus DHA treatment may be a consequence of PPAR- γ induction.

The majority of clinical studies have not demonstrated a significant change in the fractional catabolic rate of apo B particles and chylomicron remnants, which suggests that whole particle clearance rates per se are not accelerated [39]. This does not, however, contradict the data supporting an increased rate of TG removal from VLDL and chylomicron particles in the circulation, which appears to be enhanced via activation of LPL by omega-3 fatty acids.

7. The combined effects of omega-3 fatty acids and statins

Statins (inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase) decrease LDL levels primarily by raising the number of LDL receptors, and thus, enhance the removal of LDL from plasma. Studies suggest that co-administration of P-OM3 with a statin improves the lipid profile in patients with hypertriglyceridemia to a greater extent than statin treatment alone [10,63,64]. Chan et al. showed that in insulin-resistant obese men, P-OM3 lowered TG to a similar extent with or without statin therapy [10]. P-OM3 decreased the rate of VLDL secretion and increased the conversion of VLDL to intermediate-density lipoprotein (IDL) and LDL (Fig. 6). Combined treatment with atorvastatin and P-OM3 also increased conversion of VLDL to IDL or LDL, but the pool sizes of IDL and LDL decreased by 35% and 40%, respectively, because of the statin-induced activation of LDL receptors. The results of this study indicate that there are differential mechanisms by which atorvastatin and P-OM3 reduce plasma TGs, and thus there is a role for combined therapy in insulin-resistant obese subjects with dyslipidemia.

8. Other cardioprotective effects of omega-3 fatty acids

An extensive body of data supports a cardioprotective effect of omega-3 fatty acids [1,65–69]. Diets enriched with omega-3 fatty acids protect against coronary artery atherosclerosis in non-human primates, an effect that appears to be independent of plasma lipoproteins [70]. Indeed, in some European countries, P-OM3 is approved for use in post-myocardial infarction (MI) patients to prevent CHD events [71]. The American Heart Association (AHA) advises ~1 g/day of EPA plus DHA for cardiovascular protection in patients with documented CHD, and in those without documented CHD, the consumption of a variety of fatty fish at least twice per week. The AHA recommends that treatment of elevated TGs with omega-3 fatty acids at higher doses

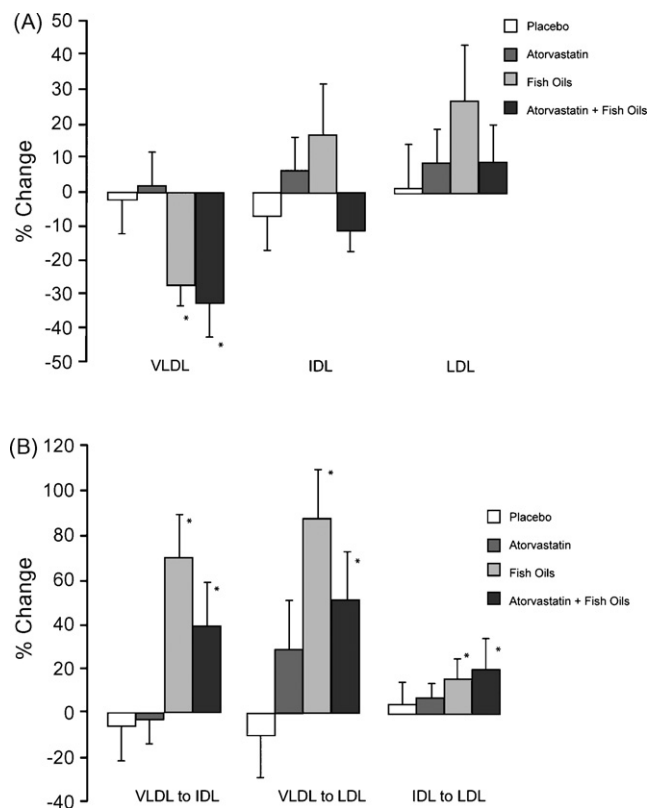


Fig. 6. Effects of omega-3 acid ethyl esters (P-OM3, 4g/day) and atorvastatin (singly and in combination) on apolipoprotein B-100 (apo B) kinetics. (A) Percentage change in the secretion rate of apo B-containing lipoproteins into the plasma. (B) Percentage change in the interconversion of apo B-containing lipoproteins. * $P < 0.01$ compared with placebo group. From Chan et al. [10].

(2–4 g/day) be undertaken under a physician's supervision [68].

Meta-analyses of primary and secondary CHD prevention trials have shown that omega-3 fatty acids can significantly decrease the risk of all-cause mortality, CHD death, and sudden death [1]. The largest single study to test the efficacy of omega-3 fatty acid for secondary prevention of CHD was the GISSI-Prevenzione Study [67]. Patients who had survived a heart attack ($n = 11,324$) were randomized to either 300 mg of vitamin E, 850 mg of omega-3 fatty acid ethyl esters, both, or usual care alone. After 3.5 years, the group given the omega-3 fatty acid alone experienced a 20% reduction in all-cause mortality ($P = 0.01$), and a 45% reduction in sudden death ($P < 0.05$) compared to the usual care group. Vitamin E provided no additional benefit. This trial, although very large and carried out in a relatively "real-life" setting, did not include a placebo arm and drop out rates were high (>25%) in both the omega-3 and the vitamin E groups. Thus, there remains a need for further research to determine the efficacy, the optimal dose and mechanism of action of omega-3 fatty acids for the prevention of CHD death. Further evidence in secondary prevention was observed in a high-fish-consuming population in Japan. The Japan EPA Lipid Intervention Study (JELIS) [69]

Table 1
Factors involved in CHD that may be affected by EPA and/or DHA

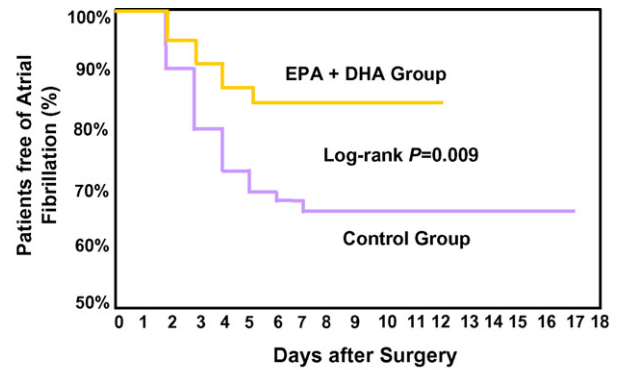
Factor	Effect
Serum TG	↓
Production of chemoattractants	↓
Production of growth factors	↓
Cell surface expression of adhesion molecules	↓
Production of inflammatory eicosanoids	↓
Blood pressure	↓
Endothelial relaxation	↑
Thrombosis	↓
Cardiac arrhythmias	↓
Heart rate variability	↑
Atherosclerotic plaque stability	↑

Adapted from Calder [2]; ↑ = increase; ↓ = decrease.

included 18,645 patients (14,981 patients with no history of coronary artery disease and 3664 patients with a history), all on statin treatment, who were randomized to 1.8 g/day EPA (no DHA) or to usual care and followed for 4.6 years for major coronary events. Compared with the statin-only group, the EPA-plus-statin group demonstrated a 19% reduction in major coronary events ($P=0.011$). The effect was virtually the same in both the primary and secondary subgroups, but reached statistical significance only in the secondary group ($P=0.048$). The fact that effect sizes were the same strongly suggests that EPA was equally effective in both settings, and it was the low number of events in the primary prevention group that prevented the results from reaching statistical significance. The beneficial effects of EPA on CHD events was not associated with changes in the levels of total cholesterol, TG, HDL-C, or LDL-C, indicating that non-lipid factors played a major role in a cardioprotective effect of EPA. Among the proposed factors that may account for the cardioprotective effects of omega-3 fatty acids are antiarrhythmic effects, decreased platelet aggregation, stabilization of atherosclerotic plaques, and blood-pressure lowering (Table 1) [2,68].

9. Cardiac arrhythmia suppression

The cardioprotective effects of fish oil have been attributed to antiarrhythmic effects of EPA plus DHA (reviewed in Reifel and McDonald) [72]. Preclinical data indicate that several mechanisms may account for the antiarrhythmic action of omega-3 fatty acids. Omega-3 fatty acids are incorporated into myocardial cell membranes [73], potentially altering both eicosanoid production and ion-channel function [65]. Atrial fibrillation is the most common cardiac arrhythmia observed clinically and is a cause of particularly costly cardiovascular morbidity (stroke and heart failure). Caló et al. demonstrated that administration of P-OM3 at 2 g/day in patients undergoing coronary artery bypass graft surgery substantially reduced the incidence of postoperative AF (Fig. 7) [74]. Omega-3 fatty acids may produce an antiarrhythmic action by preventing cytosolic free calcium levels from reaching toxic levels in cardiac myocytes. Dhein et al. showed



	Control (n=81)	EPA + DHA (n=79)	P
Post CABG AF	33%	15%	0.013
Hours of AF	24	16	0.12
Hospital Length of Stay After Surgery	8.2 ± 2.6 days	7.3 ± 2.1 days	0.017

Fig. 7. Omega-3-acid ethyl esters reduce the incidence of atrial fibrillation (AF) post-coronary artery bypass graft surgery (CABG). Patients ($N=160$) awaiting CABG were randomized to usual care or prescription omega-3-acid ethyl esters (2 g/day) for at least 5 days pre-surgery through hospitalization. AF, detected by electrocardiography during hospitalization, was defined as an electrocardiography-confirmed episode of AF > 5 min or requiring intervention. From Caló et al. [74].

that infusion of EPA, DHA, or α -linolenic acid in spontaneously beating isolated rabbit heart (Langendorff technique) produced negative inotropic and chronotropic effects [75]. Although omega-3 fatty acids have been shown to suppress both L-type calcium channels and sodium channels in rat cardiomyocytes, omega-6 fatty acids have similar effects [76].

Several recent clinical trials have examined whether omega-3 fatty-acid supplementation suppresses arrhythmias in patients with implantable cardioverter defibrillators (ICD). In the Fatty Acid Antiarrhythmia Trial (FAAT), Leaf et al. randomized 402 patients with ICDs to 2.6 g/day EPA plus DHA vs. placebo and found significant reductions in time to first ICD discharge, with the most benefit observed in patients with preexisting CHD [66]. In contrast, Raitt et al. observed no benefit of EPA plus DHA (1.3 g/day), although the study did exclude patients with a recent MI [77]. The most recent clinical trial was the Study on Omega-3 Fatty Acids and Ventricular Arrhythmia (SOFA), which examined 546 patients with ICDs who were randomized to either 0.8 g/day of EPA and DHA or placebo, to assess appropriate ICD discharges for ventricular tachycardia/ventricular fibrillation [78]. While no difference in the primary endpoint was identified, there was a trend ($P=0.13$) towards longer event-free survival in the EPA and DHA group among the prespecified subgroup with prior MI ($n=342$). The prevention of triggered arrhythmic

afterpotential discharges that accompany ischemia has been proposed as an important mechanism underlying omega-3 fatty acid supplementation [65]. Therefore, these data support the use of omega-3 fatty acids in post-MI patients with or without ICD placement. However, in non-ischemic patients with ICDs, there is little support for the use of fish oils in arrhythmia suppression.

10. Decreased platelet aggregation

The antithrombotic potential of omega-3 fatty acids was one of the first effects reported in Greenlandic Eskimos, who consume large amounts of whale and seal meat. Omega-6 fatty acids and certain of their derivatives can enhance thrombosis, while omega-3 fatty acids and their derivatives have an opposing effect [2]. AA is the precursor for the 2-series eicosanoids, which have a wide range of effects on metabolic pathways relevant to atherosclerosis. Thromboxane A2 stimulates platelet aggregation and produces vasoconstriction, and 5-lipoxygenase metabolites (e.g., leukotrienes) have been linked to inflammation and atherogenesis. On the other hand, the AA-derived prostacyclin is a potent vasodilator and opposes platelet aggregation. These essential metabolic functions of AA metabolites, if internally imbalanced and unopposed by sufficient omega-3 fatty acids, may contribute to a proatherogenic state. Consumption of EPA and DHA can lower tissue levels of AA by inhibiting its synthesis and by taking its place in membrane phospholipids [2,6,73]. EPA-derived 3-series eicosanoids are typically less vasoconstrictive and produce less platelet aggregation than those made from AA [2]. The net result of higher tissue omega-3 fatty acid levels is thus antithrombotic. Although EPA plus DHA have been associated with modest increases in bleeding times, no published studies have reported clinically significant bleeding episodes among patients treated with antiplatelet drugs and relatively high doses (3–7 g/day) of EPA plus DHA [79].

11. Atherosclerotic plaque stabilization

Thies et al. demonstrated that atherosclerotic plaques from patients treated with fish oil were less heavily infiltrated with macrophages than those in the placebo group [80]. Moreover, 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 to plaques from patients given placebo (Fig. 8).

12. Blood pressure and heart-rate reduction

A meta analysis of 36 randomized trials found that fish-oil intake (median dose 3.7 g/day EPA plus DHA) reduced sys-

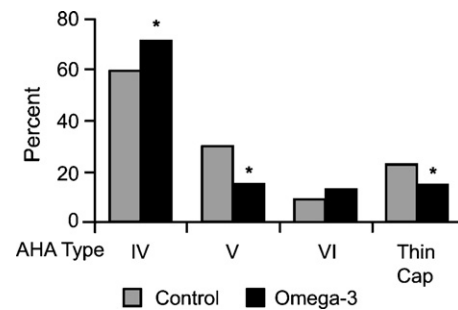


Fig. 8. Effect of omega-3 fatty acids on carotid plaque composition. Patients awaiting carotid endarterectomy were randomized to control (vegetable oil [$n=57$]) or omega-3 fatty acids ($n=53$) for a median of 42 days pre-procedure. AHA, American Heart Association. * $P<0.05$. From Thies et al. [80].

tolic blood pressure by 2.1 mm Hg ($P<0.01$) and diastolic blood pressure by 1.6 mm Hg ($P<0.01$) [81]. At least two mechanisms could account for this effect. First, incorporation of EPA and DHA into membrane phospholipids could increase systemic arterial compliance [82]. Second, EPA and DHA could improve endothelial function [83]. This is consistent with the observation that the antihypertensive effect of fish oil may be greater in populations with arterial stiffness and/or microvascular dysfunction, i.e., populations with hypertension and older populations [81]. In addition, a meta-analysis of 30 randomized trials found that fish-oil intake (median dose 3.5 g/day EPA plus DHA) reduced heart rate by 1.6 bpm compared with placebo ($P=0.002$) [84].

13. Conclusion

Multiple factors that affect CHD risk may be affected by omega-3 fatty acids (Table 1). The TG-lowering effects of omega-3 fatty acids appear to be due to a combination of decreased hepatic TG secretion combined with enhanced clearance of TG from the plasma. Gaps in our understanding of the mechanisms that link omega-3 fatty acids and CHD risk are due, in part, to variability in study designs and animal models, and to the use of supraphysiological doses of fish oil in some animal studies. Very limited mechanistic data in humans are available. Nonetheless, clinical studies with omega-3 fatty acids have demonstrated multiple cardioprotective benefits. Omega-3 fatty acids reduce

Table 2
Effects of omega-3 fatty acids on plasma lipids in patients with mixed dyslipidemia

Lipid parameter	Effect
TGs	↓ (20–50%)
LDL-C	↑/neutral
Total cholesterol	↑/neutral
HDL-C	↑/neutral

TGs, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ↑ = increase; ↓ = decrease. Adapted from Harper and Jacobson [85].

the level of plasma TGs (Table 2), which is an independent risk factor for CHD. In addition, they exert antiarrhythmic effects in ischemic, post-MI patients; decrease platelet aggregation; increase plaque stabilization; reduce blood pressure; and reduce heart rate. Additional studies are needed to define more clearly the cellular and molecular basis for the cardioprotective effects of omega-3 fatty acids in humans.

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References

- [1] Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health: evaluating the risks and the benefits. *JAMA* 2006;296:1885–9.
- [2] Calder PC. *n*-3 fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clin Sci* 2004;107:1–11.
- [3] Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *J Cardiovasc Risk* 1996;3:213–9.
- [4] Jeppesen J, Hein H, Suadicani P, Gyntelberg F. Triglyceride concentration and ischemic heart disease—an eight-year follow-up in the Copenhagen Male Study. *Circulation* 1998;97:1029–36.
- [5] Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *Circulation* 2002;106:3143–421.
- [6] Harris W, Ginsberg H, Arunakul N, et al. Safety and efficacy of Omacor in severe hypertriglyceridemia. *J Cardiovasc Risk* 1997;4:385–91.
- [7] Harris W. *n*-3 fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr* 1997;65:1645S–54S.
- [8] Lovaza® [package insert]. Liberty Corner, NJ: Reliant Pharmaceuticals, Inc. 2007.
- [9] Pownall H, Brauchi D, Kilinc C, et al. Correlation of serum triglyceride and its reduction by ω -3 fatty acids with lipid transfer activity and the neutral lipid compositions of high-density and low-density lipoproteins. *Atherosclerosis* 1999;143:285–97.
- [10] Chan D, Watts G, Beilin L, Redgrave T, Mori T. Regulatory effects of HMG CoA reductase inhibitor and fish oils on apolipoprotein B-100 kinetics in insulin-resistant obese male subjects with dyslipidemia. *Diabetes* 2002;51:2377–86.
- [11] Ginsberg HN, Le NA, Gibson JC. Regulation of the production and catabolism of plasma low density lipoproteins in hypertriglyceridemic subjects. Effect of weight loss. *J Clin Invest* 1985;75:614–23.
- [12] Lada AT, Rudel LL, StClair RW. Effects of LDL enriched with different dietary fatty acids on cholesteryl ester accumulation and turnover in THP-1 macrophages. *J Lipid Res* 2003;44:770–9.
- [13] Parks JS, Gebre AK. Studies on the effect of dietary fish oil on the physical and chemical properties of low density lipoproteins in cynomolgus monkeys. *J Lipid Res* 1991;32:305–15.
- [14] Parks JS, Bullock BC. Effect of fish oil versus lard diets on the chemical and physical properties of low density lipoproteins of nonhuman primates. *J Lipid Res* 1987;28:173–82.
- [15] Jump D. Fatty acid regulation of gene transcription. *Crit Rev Clin Lab Sci* 2004;41:41–78.
- [16] Sampath H, Ntambi J. Polyunsaturated fatty acid regulation of gene expression. *Nutr Rev* 2004;62:333–9.
- [17] Bezard J, Blond J, Bernard A, Clouet P. The metabolism and availability of essential fatty acids in animal and human tissues. *Semin Nephrol* 1994;34:539–68.
- [18] Neuringer M, Anderson GJ, Connor WE. The essentiality of *n*-3 fatty acids for the development and function of the retina and brain. *Annu Rev Nutr* 1988;8:517–41.
- [19] Neuringer M, Connor WE, Lin DS, Barstad L, Luck S. Biochemical and functional effects of prenatal and postnatal omega 3 fatty acid deficiency on retina and brain in rhesus monkeys. *Proc Natl Acad Sci USA* 1986;83:4021–5.
- [20] Soderberg M, Edlund C, Kristensson K, Dallner G. Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease. *Lipids* 1991;26:421–5.
- [21] Conquer JA, Tierney MC, Zecevic J, Bettger WJ, Fisher RH. Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. *Lipids* 2000;35:1305–12.
- [22] Tully AM, Roche HM, Doyle R, et al. Low serum cholesteryl ester-docosahexaenoic acid levels in Alzheimer's disease: a case-control study. *Br J Nutr* 2003;89:483–9.
- [23] Heude B, Ducimetiere P, Berr C. Cognitive decline and fatty acid composition of erythrocyte membranes—the EVA study. *Am J Clin Nutr* 2003;77:803–8.
- [24] Kalmijn S, van Boxtel MP, Ocke M, et al. Dietary intake of fatty acids and fish in relation to cognitive performance at middle age. *Neurology* 2004;62:275–80.
- [25] Morris MC, Evans DA, Bienias JL, et al. Consumption of fish and *n*-3 fatty acids and risk of incident Alzheimer disease. *Arch Neurol* 2003;60:940–6.
- [26] Morris MC, Evans DA, Tangney CC, Bienias JL, Wilson RS. Fish consumption and cognitive decline with age in a large community study. *Arch Neurol* 2005;62:1849–53.
- [27] Huang TL, Zandi PP, Tucker KL, et al. Benefits of fatty fish on dementia risk are stronger for those without APOE epsilon4. *Neurology* 2005;65:1409–14.
- [28] Schaefer EJ, Bongard V, Beiser AS, et al. Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. *Arch Neurol* 2006;63:1545–50.
- [29] Calon F, Lim GP, Yang F, et al. Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. *Neuron* 2004;43:633–45.
- [30] Freund-Levi Y, Eriksson-Jonhagen M, Cederholm T, et al. Omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: OmegAD study: a randomized double-blind trial. *Arch Neurol* 2006;63:1402–8.
- [31] Kotani S, Sakaguchi E, Warashina S, et al. Dietary supplementation of arachidonic and docosahexaenoic acids improves cognitive dysfunction. *Neurosci Res* 2006;56:159–64.

- [32] Cole GM, Frautschy SA. Docosahexaenoic acid protects from amyloid and dendritic pathology in an Alzheimer's disease mouse model. *Nutr Health* 2006;18:249–59.
- [33] Uauy R, Dangour A. Nutrition in brain development and aging: role of essential fatty acids. *Nutr Rev* 2006;64:S24–33.
- [34] Johnson EJ, Schaefer EJ. Potential role of dietary *n-3* fatty acids in the prevention of dementia and macular degeneration. *Am J Clin Nutr* 2006;83:1494S–8S.
- [35] Erkkila AT, Matthan NR, Herrington DM, Lichtenstein AH. Higher plasma docosahexaenoic acid is associated with reduced progression of coronary atherosclerosis in women with CAD. *J Lipid Res* 2006;47:2814–9.
- [36] Harris W. Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. *J Lipid Res* 1989;30:785–807.
- [37] Park Y, Harris WS. Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. *J Lipid Res* 2003;44:455–63.
- [38] Miller M, Motevalli M, Westphal D, Kwiterovich Jr PO. Incorporation of oleic acid and eicosapentaenoic acid into glycerolipids of cultured normal human fibroblasts. *Lipids* 1993;28:1–5.
- [39] Harris W, Bulchandani D. Why do omega-3 fatty acids lower serum triglycerides? *Curr Opin Lipidol* 2006;17:387–93.
- [40] Parks JS, Johnson FL, Wilson MD, Rudel LL. Effect of fish oil diet on hepatic lipid metabolism in nonhuman primates: lowering of secretion of hepatic triglyceride but not apoB. *J Lipid Res* 1990;31:455–66.
- [41] Parks JS, Wilson MD, Johnson FL, Rudel LL. Fish oil decreases hepatic cholesteryl ester secretion but not apoB secretion in African green monkeys. *J Lipid Res* 1989;30:1535–44.
- [42] Fisher EA, Pan M, Chen X, et al. The triple threat to nascent apolipoprotein B. Evidence for multiple, distinct degradative pathways. *J Biol Chem* 2001;276:27855–63.
- [43] Jump DB. The biochemistry of *n-3* polyunsaturated fatty acids. *J Biol Chem* 2002;277:8755–8.
- [44] Ren B, Thelen A, Peters J, Gonzalez F, Jump D. Polyunsaturated fatty acid suppression of hepatic fatty acid. Synthase and S14 gene expression does not require peroxisome proliferator-activated receptor alpha. *J Biol Chem* 1997;272:26827–32.
- [45] Horton J, Bashmakov Y, Shimomura I, Shimano H. Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proc Natl Acad Sci USA* 1998;95:5987–92.
- [46] Le Jossic-Corcoss C, Gonthier C, Zaghini I, et al. Hepatic farnesyl diphosphate synthase expression is suppressed by polyunsaturated fatty acids. *Biochem J* 2005;385:787–94.
- [47] Yoshikawa T, Shimano H, Yahagi N, et al. Polyunsaturated fatty acids suppress sterol regulatory element-binding protein 1c promoter activity by inhibition of liver X receptor (LXR) binding to LXR response elements. *J Biol Chem* 2002;277:1705–11.
- [48] Zaima N, Sugawara T, Goto D, Hirata T. Trans geometric isomers of EPA decrease LXRalpha-induced cellular triacylglycerol via suppression of SREBP-1c and PGC-1beta. *J Lipid Res* 2006;47:2712–7.
- [49] Davidson M. Mechanisms for the hypotriglyceridemic effect of marine omega-3 fatty acids. *Am J Card* 2006;98(Suppl.):27i–33i.
- [50] Yu-Poth S, Yin D, Kris-Etherton PM, Zhao G, Etherton TD. Long-chain polyunsaturated fatty acids upregulate LDL receptor protein expression in fibroblasts and HepG2 cells. *J Nutr* 2005;135:2541–5.
- [51] Edwards PA, Kast HR, Anisfeld AM. BAREing it all: the adoption of LXR and FXR and their roles in lipid homeostasis. *J Lipid Res* 2002;43:2–12.
- [52] Sirvent A, Claudel T, Martin G, et al. The farnesoid X receptor induces very low density lipoprotein receptor gene expression. *FEBS Lett* 2004;566:173–7.
- [53] Sirvent A, Verhoeven AJ, Jansen H, et al. Farnesoid X receptor represses hepatic lipase gene expression. *J Lipid Res* 2004;45:2110–5.
- [54] Kast HR, Nguyen CM, Sinal CJ, et al. Farnesoid X-activated receptor induces apolipoprotein C-II transcription: a molecular mechanism linking plasma triglyceride levels to bile acids. *Mol Endocrinol* 2001;15:1720–8.
- [55] Claudel T, Inoue Y, Barbier O, et al. Farnesoid X receptor agonists suppress hepatic apolipoprotein CIII expression. *Gastroenterology* 2003;125:544–55.
- [56] Sinal CJ, Tohkin M, Miyata M, et al. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell* 2000;102:731–44.
- [57] Zhao A, Yu J, Lew JL, et al. Polyunsaturated fatty acids are FXR ligands and differentially regulate expression of FXR targets. *DNA Cell Biol* 2004;23:519–26.
- [58] Gimeno RE. Fatty acid transport proteins. *Curr Opin Lipidol* 2007;18:271–6.
- [59] Dagnelie P, Rietveld T, Swart G, Stijnen T, van den Berg J. Effect of dietary fish oil on blood levels of free fatty acids, ketone bodies and triacylglycerol in humans. *Lipids* 1994;29:41–5.
- [60] Khan S, Minihane AM, Talmud PJ, et al. Dietary long-chain *n-3* PUFAs increase LPL gene expression in adipose tissue of subjects with an atherogenic lipoprotein phenotype. *J Lipid Res* 2002;43:979–85.
- [61] Chambrier C, Bastard JP, Rieusset J, et al. Eicosapentaenoic acid induces mRNA expression of peroxisome proliferator-activated receptor gamma. *Obes Res* 2002;10:518–25.
- [62] Leibowitz MD, Fievet C, Hennuyer N, et al. Activation of PPARdelta alters lipid metabolism in db/db mice. *FEBS Lett* 2000;473:333–6.
- [63] Durrington P, Bhatnagar D, Mackness M, et al. An omega-3 polyunsaturated fatty acid concentrate administered for one year decreased triglycerides in simvastatin treated patients with coronary heart disease and persisting hypertriglyceridaemia. *Heart* 2001;85:544–8.
- [64] Nordoy A, Bonna K, Nilsen H, et al. Effects of simvastatin and omega-3 fatty acids in plasma lipoproteins and lipid peroxidation in patients with combined hyperlipidaemia. *J Intern Med* 1998;243:163–70.
- [65] Leaf A, Kang J, Xiao Y, Billman G. Clinical prevention of sudden cardiac death by *n-3* polyunsaturated fatty acids and mechanism of prevention of arrhythmias by *n-3* fish oils. *Circulation* 2003;107:2646–52.
- [66] Leaf A, Albert C, Josephson M, et al. Prevention of fatal arrhythmias in high-risk subjects by fish oil *n-3* fatty acids. *Circulation* 2005;112:2762–8.
- [67] GISSI-Prevenzione Investigators. Dietary supplementation with *n-3* polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* 1999;354:447–55.
- [68] Kris-Etherton P, Harris W, Appel L. American Heart Association (AHA) and AHA Nutrition Committee, Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* 2002;106:2747–57.
- [69] Yokoyama M, Origasa H, Matsuzaki M, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet* 2007;369:1090–8.
- [70] Rudel LL, Parks JS, Hedrick CC, Thomas M, Williford K. Lipoprotein and cholesterol metabolism in diet-induced coronary artery atherosclerosis in primates. Role of cholesterol and fatty acids. *Prog Lipid Res* 1998;37:353–70.
- [71] Imazio M, Forno D, Quaglia C, Trincherio R. Omega-3 polyunsaturated fatty acids role in postmyocardial infarction therapy. *Panminerva Med* 2003;45:99–107.
- [72] Reiffel J, McDonald A. Antiarrhythmic effects of omega-3 fatty acids. *Am J Cardiol* 2006;98:50i–60i.
- [73] Harris W, Sands S, Windsor S, et al. Omega-3 fatty acid levels in transplanted human hearts: effect of supplementation and comparison with erythrocytes. *Circulation* 2004;110:1645–9.
- [74] Caló L, Bianconi L, Colivicchi F, et al. *n-3* fatty acids for the prevention of atrial fibrillation after coronary artery bypass surgery: a randomized controlled trial. *J Am Coll Cardiol* 2005;45:1723–8.
- [75] Dhein S, Michaelis B, Mohr FW. Antiarrhythmic and electrophysiological effects of long-chain omega-3 polyunsaturated fatty acids. *Naunyn Schmiedeberg Arch Pharmacol* 2005;371:202–11.
- [76] Xiao YF, Kang JX, Morgan JP, Leaf A. Blocking effects of polyunsaturated fatty acids on Na⁺ channels of neonatal rat ventricular myocytes. *Proc Natl Acad Sci USA* 1995;92:11000–4.

- [77] Raitt M, Connor W, Morris C, et al. Fish oil supplementation and risk of ventricular tachycardia and ventricular fibrillation in patients with implantable defibrillators: a randomized controlled trial. *JAMA* 2005;293(23):2884–91.
- [78] Brouwer I, Zock P, Bocker D, et al. Effect of fish oil on ventricular tachyarrhythmia and death in patients with implantable cardioverter defibrillators: the study on omega-3 fatty acids and ventricular arrhythmia (SOFA) randomized trial. *JAMA* 2006;295:2613–9.
- [79] Harris WS. Expert opinion: omega-3 fatty acids and bleeding—cause for concern? *Am J Cardiol* 2007;99:S44–6.
- [80] Thies F, Garry J, Yaqoob P, et al. Association of *n-3* polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. *Lancet* 2003;361:477–85.
- [81] Geleijnse J, Giltay E, Grobbee D, Donders A, Kok F. Blood pressure response to fish oil supplementation: metaregression analysis of randomized trials. *J Hypertens* 2002;20:1493–9.
- [82] Nestel P, Shige H, Pomeroy S, et al. The *n-3* fatty acids eicosapentaenoic acid and docosahexaenoic acid increase systemic arterial compliance in humans. *Am J Clin Nutr* 2002;76:326–30.
- [83] Chin JP, Dart AM. How do fish oils affect vascular function? *Clin Exp Pharmacol Physiol* 1995;22:71–81.
- [84] Mozaffarian D, Geelen A, Brouwer I, et al. Effect of fish oil on heart rate in humans: a meta-analysis of randomized controlled trials. *Circulation* 2005;112:1945–52.
- [85] Harper C, Jacobson T. An evidence-based approach to the use of combination drug therapy for mixed dyslipidemia. *J Clin Outcomes Manage* 2006;13:57–68.