



Emerging Mechanisms of Cardiovascular Protection for the Omega-3 Fatty Acid Eicosapentaenoic Acid

R. Preston Mason, Peter Libby, Deepak L. Bhatt

ABSTRACT: Patients with well-controlled LDL (low-density lipoprotein) levels still have residual cardiovascular risk associated with elevated triglycerides. Epidemiological studies have shown that elevated fasting triglyceride levels associate independently with incident cardiovascular events, and abundant recent human genetic data support the causality of TGRLs (triglyceride-rich lipoproteins) in atherothrombosis. Omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), lower blood triglyceride concentrations but likely exert additional atheroprotective properties at higher doses. Omega-3 fatty acids modulate T-cell differentiation and give rise to various prostaglandins and specialized proresolving lipid mediators that promote resolution of tissue injury and inflammation. The REDUCE-IT (Reduction of Cardiovascular Events with Icosapent Ethyl—Intervention Trial) with an EPA-only formulation lowered a composite of cardiovascular events by 25% in patients with established cardiovascular disease or diabetes mellitus and other cardiovascular risk factors. This clinical benefit likely arises from multiple molecular mechanisms discussed in this review. Indeed, human plaques readily incorporate EPA, which may render them less likely to trigger clinical events. EPA and DHA differ in their effects on membrane structure, rates of lipid oxidation, inflammatory biomarkers, and endothelial function as well as tissue distributions. Trials that have evaluated DHA-containing high-dose omega-3 fatty acids have thus far not shown the benefits of EPA alone demonstrated in REDUCE-IT. This review will consider the mechanistic evidence that helps to understand the potential mechanisms of benefit of EPA.

VISUAL OVERVIEW: An online [visual overview](#) is available for this article.

Key Words: eicosapentaenoic acid ■ fatty acids ■ inflammation ■ lipoproteins ■ triglycerides

HIGH-DOSE OMEGA-3 FATTY ACID TREATMENT AND RESIDUAL CARDIOVASCULAR RISK

Despite the success of LDL (low-density lipoprotein)-lowering therapies in reducing cardiovascular risk, individuals with well-controlled LDL still have residual cardiovascular risk associated in part with elevated triglycerides.^{1,2} The REDUCE-IT (Reduction of Cardiovascular Events with Icosapent Ethyl—Intervention Trial) that tested an omega-3 fatty acid (n3-FA)-based therapy of eicosapentaenoic acid (EPA) demonstrated cardiovascular risk reduction in addition to the protection afforded by statins. REDUCE-IT demonstrated that a highly purified ethyl ester of EPA, icosapent ethyl, significantly reduced the risk of

cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization in at-risk patients with triglycerides above ≈ 100 mg/dL despite being treated with statins.^{3–6} First ischemic events fell by 25% ($P=0.00000001$) and total (first and subsequent) ischemic events by 31% ($P=0.0000000004$), with consistent benefits across multiple prespecified subgroups, including primary and secondary prevention. The prespecified subgroup of 3146 patients randomized in the United States demonstrated benefits at least as robust as the overall population, with a notable 30% lower rate of mortality ($P=0.004$) in those randomized to icosapent ethyl.⁷ The EPA treatment used yielded consistent benefits across baseline levels of triglycerides as well, including in the $\approx 10\%$ of participants with normal triglycerides.⁸

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Nonstandard Abbreviations and Acronyms

ApoC-III	apolipoprotein C-III
COX	cyclooxygenase
DHA	docosahexaenoic acid
EC	endothelial cell
eNOS	endothelial isoform of nitric oxide synthase
EPA	eicosapentaenoic acid
GPR-120	G-protein-coupled receptor 120
HDL-C	high-density lipoprotein-cholesterol
hsCRP	high-sensitivity C-reactive protein
IL	interleukin
JELIS	Japan EPA Lipid Intervention Study
n3-FAs	omega-3 fatty acids
n6-FAs	omega-6 fatty acids
NF-κB	nuclear factor-κB
NLRP-3	NLR family pyrin domain-containing 3
NO	nitric oxide
oxLDL	oxidized LDL-C
REDUCE-IT	Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial
RLP-C	remnant-like particle cholesterol
ROS	reactive oxygen species
TGRL	triglyceride-rich lipoproteins
TH	T helper
VCAM-1	vascular cell adhesion molecule 1
VLDL	very-low-density lipoprotein

The large relative and absolute risk reductions in several different types of end points from REDUCE-IT seemed to exceed that expected by the degree of triglyceride lowering, suggesting that other properties of EPA likely contribute to the benefits, as will be discussed^{9–11} (Figure 1). Beyond the effect on lowering triglyceride levels, EPA has cell-membrane stabilizing properties that may explain, in part, the significant reductions seen in death from cardiovascular causes (20% reduction), sudden cardiac death (31% reduction), and cardiac arrest (48% reduction). Of note, the results of REDUCE-IT apply to a broad population of at-risk patients.^{12,13} The findings from REDUCE-IT contrast sharply with previous outcome trials that showed no cardiovascular benefit using EPA/docosahexaenoic acid (DHA) combinations or dietary supplements that may contain oxidized fatty acids and saturated fat.^{14–18} Further trials with high-dose n3-FAs in various groups will provide additional insight into this question.

The REDUCE-IT trial followed the JELIS trial (Japan EPA Lipid Intervention Study), which demonstrated that purified EPA (1.8 g/d) reduced the risk of major coronary

Highlights

- Omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have multiple biological effects. In the REDUCE-IT (Reduction of Cardiovascular Events With Icosapent Ethyl-Intervention Trial), a prescription EPA-only formulation called icosapent ethyl reduced cardiovascular events by 25% in high-risk patients with either established cardiovascular disease or diabetes mellitus plus other risk factors.
- Cardiovascular outcomes trials that have evaluated mixed (DHA-containing) omega-3 fatty acids on top of contemporary medical therapy have thus far not shown the benefits of icosapent ethyl as seen in REDUCE-IT and the JELIS (Japan EPA Lipid Intervention Study), highlighting the importance of understanding molecular mechanisms of action of specific omega-3 fatty acids.
- EPA and DHA differ markedly, with distinct effects on membrane structure, rates of lipid oxidation, inflammatory biomarkers, and endothelial function, as well as tissue distributions.
- Ongoing scientific efforts to understand the mechanism of action for EPA will help usher in a new era of cardiovascular therapeutics.

events in hypercholesterolemic patients receiving statin therapy versus those subjected to statin monotherapy. JELIS did not prespecify a minimum triglyceride level for inclusion into the study.¹⁹ This open-label, randomized trial, showed a 19% reduction ($P=0.011$) in cardiovascular events overall in the trial in the EPA group, with consistent benefits in both secondary and primary prevention. Notably, the median triglyceride level at baseline was only slightly above 150 mg/dL, meaning that approximately half of the patients had normal triglycerides. However, in post hoc analyses, among those subjects with higher triglyceride levels (>150 mg/dL) and low HDL-C (high-density lipoprotein-cholesterol) levels (<40 mg/dL), there was 53% reduction in events with EPA treatment.²⁰

OMEGA-3 FATTY ACIDS AND PROGRESSION OF ATHEROSCLEROSIS IN PATIENTS WITH ELEVATED TRIGLYCERIDES

n3-FAs and their bioactive lipid metabolites produce complex and multifactorial biological effects that remain incompletely understood, especially at higher doses, that may reduce the risk of cardiovascular events. A meta-analysis of observational and randomized trials indicated that circulating levels of n3-FAs associated inversely with modestly lower rates of cardiovascular death.^{21,22} Other

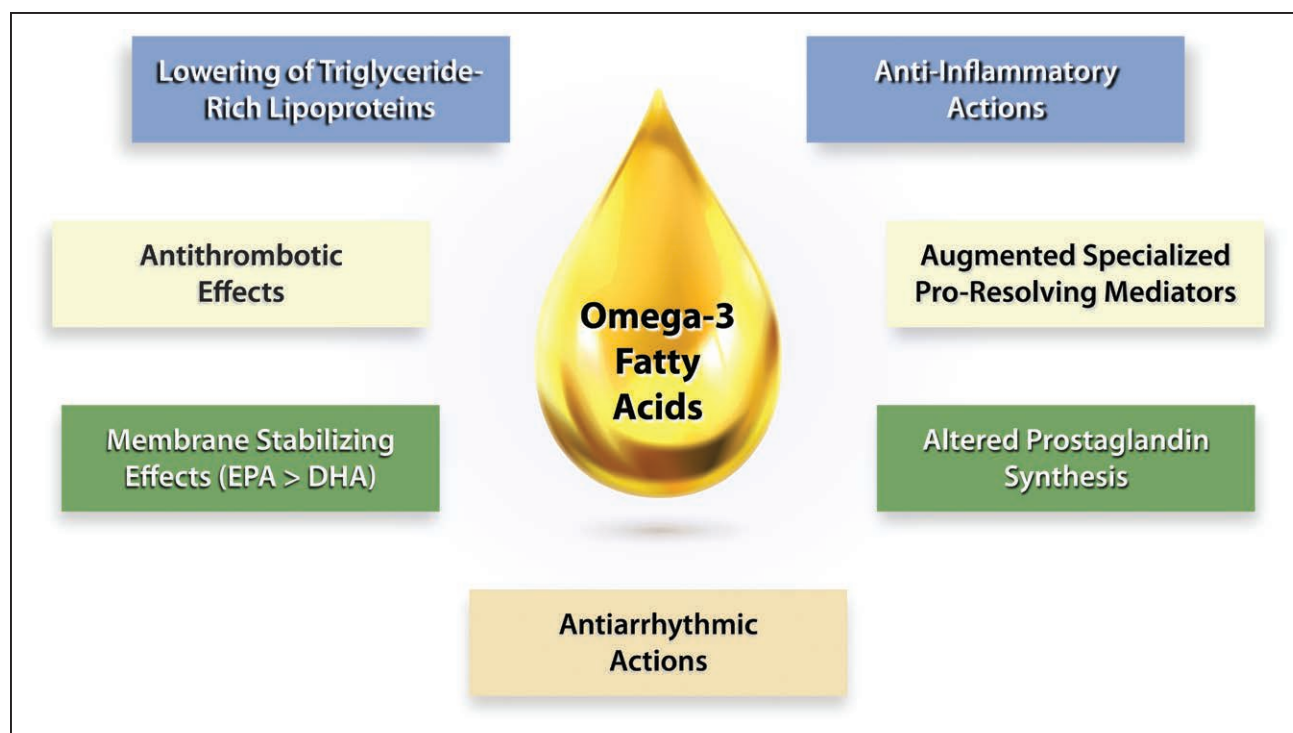


Figure 1. Potential mechanisms of cardioprotection for omega-3 fatty acids.

Omega-3 fatty acids may lessen risk of cardiovascular events through a number of mechanisms that contribute to their overall protective actions. Lowering of TGRL (triglyceride-rich lipoprotein) may account for some but certainly not all of the observed benefits (Figure 2). By boosting the production of anti-aggregatory and vasodilatory prostanoids, such as prostacyclin, omega-3 fatty acids may combat thrombosis as well as vasospasm. Omega-3 fatty acids can incorporate into plasma membranes and those of the mitochondria potentially stabilizing them to resist oxidation and confer protection against arrhythmias. Omega-3 fatty acids and certain prostanoids produced from them can exert anti-inflammatory actions. In addition, the omega-3 fatty acids can provide precursors for the synthesis of specialized proresolving mediators that can combat inflammation, perhaps causing less interference with his defenses than direct anti-inflammatory therapies. A combination of these various mechanisms may contribute to the cardiovascular protection associated with omega-3 fatty acid consumption. DHA indicates docosahexaenoic acid; and EPA, eicosapentaenoic acid.

studies indicated that the cardiovascular benefits of n3-FAs could link to either EPA or DHA but highlighted the need for more definitive evidence.²³ A study of 218 subjects with coronary disease randomized to high-dose EPA and DHA (3.36 g/d) or placebo showed that those with n3-FAs that reached plasma phospholipid levels of at least 4% had a significantly slower progression of coronary plaque as monitored by coronary computed tomographic angiography.²⁴

Clinical studies with high-dose EPA (2–4 g/d) provide some mechanistic insights based on changes in various biomarkers in statin-treated patients with elevated triglycerides that may contribute to the reduction in residual cardiovascular risk due to EPA demonstrated in REDUCE-IT.^{2,5,6} In people with elevated or very high triglycerides, treatment with a highly purified and quality-controlled preparation of EPA (2–4 g/d) reduced the arachidonic acid-to-EPA ratio in blood, hsCRP (high-sensitivity C-reactive protein), RLP-C (remnant-like particle cholesterol), ApoC-III (apolipoprotein C-III), and oxLDL (oxidized LDL-C) concentrations compared with placebo controls.^{5,25–32} EPA generally produced these effects in a dose-dependent manner. Further studies of inflammatory and other biomarkers from REDUCE-IT

and other trials should lead to additional insights into mechanisms of action of highly purified EPA in individuals with elevated triglycerides and cardiovascular risk.

Laboratory and clinical studies suggest that EPA also influences vascular functions related to atherosclerosis such as improved endothelial-dependent vasodilation, membrane stabilization, reduced inflammation, and limiting features of plaques associated with propensity to provoke thrombosis.^{33–40} In a study of patients who underwent carotid endarterectomy, those treated with n3-FAs enriched with EPA (0.81 g/day of EPA and 0.675 g/day of DHA) had fewer foam cells, features of plaque instability, markers of inflammation, and T-cell content.³⁸ HDL isolated from EPA-only treated individuals exhibited enhanced cholesterol efflux from monocytes and augmented antioxidant and anti-inflammatory actions.⁴¹ In cultured human endothelial cells (ECs), EPA-enriched HDL increased levels of specialized proresolving lipid mediators while reducing cytokine-stimulated VCAM-1 (vascular cell adhesion molecule 1) expression.⁴² EPA partitions into the outer monolayer of the HDL particle and exhibits greater antioxidant function than DHA-loaded HDL.⁴³

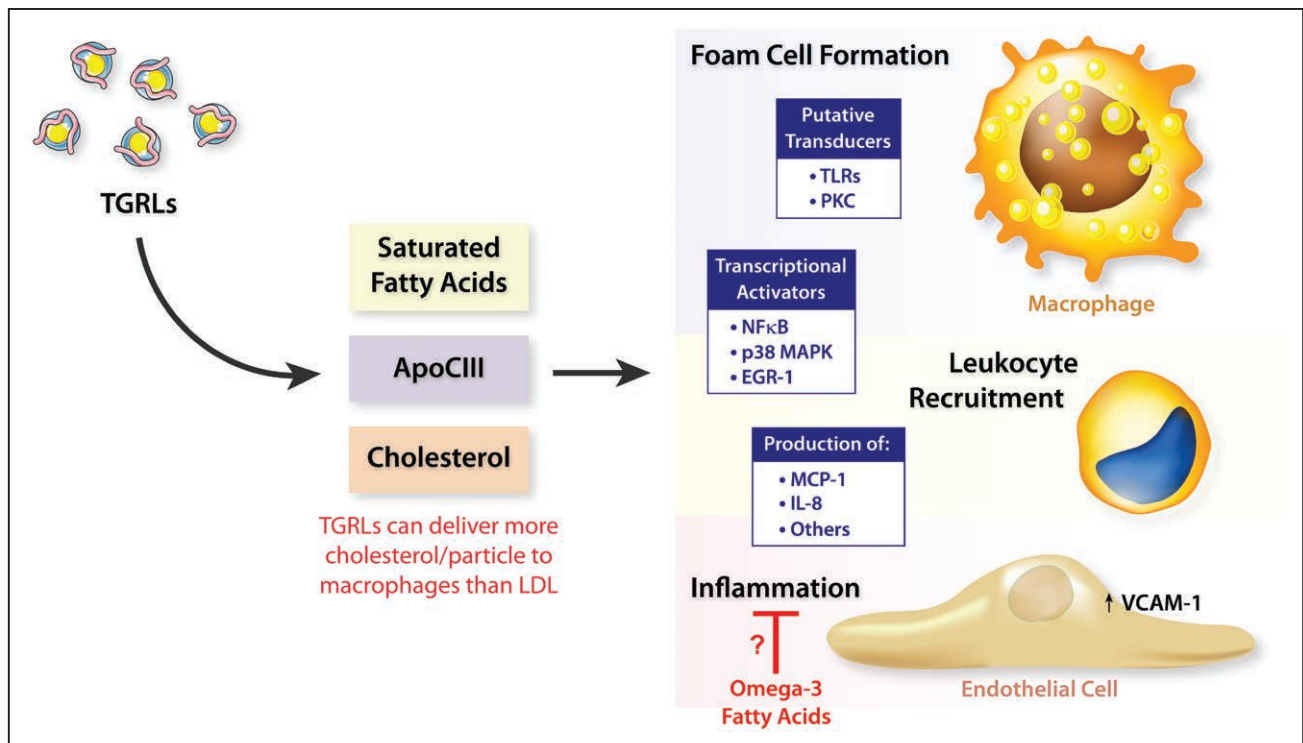


Figure 2. Atherogenic pathways for TGRLs (triglyceride-rich lipoproteins).

TGRL (estimated by serum triglyceride measurements) can promote vascular dysfunction and atherosclerosis through a number of mechanisms. Saturated fatty acids, notably palmitate, can promote inflammation, in part, due to activation of the NLRP-3 (NLR family pyrin domain-containing 3) inflammasome, which produces activated forms of the proinflammatory cytokines IL (interleukin)-1 β and IL-18.⁴⁷ ApoC-III (Apolipoprotein CIII) can exert direct proinflammatory effects of cells involved in atherosclerosis, such as macrophages and endothelial cells. Human genetic studies strongly support the causality of ApoC-III in human atherothrombosis. TGRL particles deliver cholesterol effectively to macrophages and can promote foam cell formation. Omega-3 fatty acids may exert some of their apparent protective effect on atherothrombosis by blocking some of these proinflammatory and other deleterious effects of TGRL. EGR-1 indicates early growth response protein 1; LDL, low-density lipoprotein; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; NF- κ B, nuclear factor- κ B; PKC, protein kinase C; TLR, toll-like receptors; and VCAM-1, vascular cell adhesion molecule 1.

In patients with coronary heart disease, a combination EPA with statin therapy significantly reduced coronary plaque volume compared with statin therapy alone in the CHERRY trial (Combination Therapy of Eicosapentaenoic Acid and Pitavastatin for Coronary Plaque Regression Evaluated by Integrated Backscatter Intravascular Ultrasonography).³³ In 193 patients who were to undergo percutaneous coronary intervention underwent random allocation to the statin pitavastatin (4 mg/d, n=96) or to a pitavastatin/EPA group (pitavastatin 4 mg/d and EPA 1.8 g/d, n=97), for 6 to 8 months. Integrated backscatter intravascular ultrasound assessed coronary plaque volume and character in nonstented lesions. Total atheroma volume fell significantly in the pitavastatin/EPA but not the pitavastatin alone group. An imaging study in a North American population is currently underway with highly purified EPA (4 g/d) to evaluate changes in atherosclerotic plaque characteristics in statin-treated patients with coronary atherosclerosis, triglyceride levels of 135 to 499 mg/dL, and LDL levels of 40 to 115 mg/dL.⁴⁴ This study (EVAPORATE [Effect of Vascepa on Improving Coronary Atherosclerosis in People With High Triglycerides Taking Statin Therapy]) is measuring low attenuation

plaque volume by multidetector computed tomography angiography in 80 enrolled patients at a final analysis at 18 months. A prespecified interim analysis at 9 months was presented at the 2019 AHA Scientific Sessions (Philadelphia, PA) and showed a significant difference in a secondary end point of total plaque volume progression with EPA versus placebo.

EPA can reduce triglyceride concentrations without raising LDL-C levels at 2-4 g/d, compared with formulations that contain another n3-FA, DHA, at similar doses, in patients with very high triglycerides (>500 mg/dL).^{25,27,45} The mechanism for this unexpected finding with EPA treatment may relate to reduced production and faster clearance of TGRL (triglyceride-rich lipoproteins); estimated by serum triglyceride measurements) in concert with more rapid clearance of LDL particles and slower production of VLDL (very-low-density lipoprotein) particles.^{19,27,46} At lower triglyceride levels, mixed n3-FA formulations also do not raise LDL. Through various mechanisms, TGRLs can promote vascular dysfunction and atherosclerosis (Figure 2). Saturated fatty acids, such as palmitate, may promote inflammation through NLRP-3 (NLR family pyrin domain-containing

3) inflammasome activation, leading to activated forms of the proinflammatory cytokines IL (interleukin)-1 β and IL-18.⁴⁷ Clinical trials that have tested other triglyceride-lowering agents (eg, fenofibrate or niacin) have not shown significant cardiovascular benefits when added to statins compared with statin therapy alone.^{48–52} These trials did not prospectively enroll patients with elevated triglyceride levels, an important limitation.

Beyond their effects on levels of LDL, EPA-, and DHA-containing formulations appear to differ with respect to hsCRP reduction. Prescription EPA reduces hsCRP in patients with elevated or high triglycerides, an effect enhanced by combination with more potent statins.²⁶ In contrast, mixed EPA and DHA treatment at a similar dosage (4 g/d) did not reduce hsCRP concentrations in statin-treated patients.⁵³ The EPA and DHA combination did reduce apoC-III levels as previously observed with purified EPA.^{26,31,32} A decrease in apoC-III would lower triglyceride levels through several mechanisms, including reduced inhibition of lipoprotein lipase. EPA administration also inhibits platelet activation and aggregation, which, along with reducing mean platelet count, may contribute to antithrombotic effects.^{54–57} (Figure 1) Finally, both EPA and DHA have effects on hemodynamics, adiponectin, and IL-18 levels as well as platelet function in various clinical and experimental studies.^{9,58–65} Inconsistent effects of EPA and DHA in such studies may result from differences in the experimental conditions, dosage, and patient characteristics.

EPA AND DHA GIVE RISE TO BIOACTIVE LIPIDS THAT MODULATE INFLAMMATION

Atherosclerosis involves inflammatory mechanisms of both the adaptive and innate immune responses.^{66,67} The n3-FAs give rise to signaling molecules that can reduce inflammation in different tissues and vascular beds. EPA and DHA form the cardioprotective and antithrombotic metabolites thromboxane A3/prostacyclin. In contrast, n6-FAs form thromboxane A2, a platelet activator that contributes to atherothrombosis.⁶⁸ The n3-FAs compete with the n6-FA for the COX (cyclooxygenase) enzymes that synthesize the thromboxanes and thus limit the production of these potent proaggregatory and vasoconstrictor mediators.^{28,69} Despite this link between n6-FAs and proinflammatory signaling molecules, a recent pooled analysis of 30 cohort studies showed higher circulating and tissue levels of linoleic acid associate with lower risk of major cardiovascular events.⁷⁰ Compared with other FAs, such as saturated FAs, linoleic acid has favorable effects on lipid metabolism.⁷¹ Another meta-analysis showed the lower cardiovascular risk was observed with n3-FAs and arachidonic acid but not other n6-FAs.²²

Of the various eicosanoids produced by COXes, prostacyclin has particular interest with respect to vascular

protection. Produced by healthy ECs, prostacyclin functions through a paracrine signaling pathway mediated by G protein-coupled receptors on nearby platelets and ECs. Receptor binding leads to inhibition of abnormal platelet activation while counteracting the prothrombotic effects of thromboxane. Prostacyclin also promotes smooth muscle relaxation and endothelial-dependent vasodilation.

Additional bioactive lipids that derive from n3-FAs originate from macrophages and neutrophils include the leukotrienes and resolvins.⁷² The metabolism of n3-FAs produces resolvins, maresins, and protectins. These metabolites, known as specialized proresolving lipid mediators, promote the resolution of inflammation as part of a highly coordinated process that helps to reestablish homeostasis after tissue injury or infection.⁷³ Following acute inflammation, these steps include a reduction in the production of cytokines and extracellular-reactive oxygen species (ROS), along with inhibition of granulocyte trafficking. These bioactive lipids also modulate the inflammatory response by influencing macrophage-mediated clearance of cellular debris.⁷⁴

Emerging lines of evidence indicate that these metabolites of n3-FAs may also limit chronic inflammation and activation of cells of the adaptive immune system in a coordinated fashion. By modulating membrane lipid dynamics, n3-FAs influence the organization of microdomains or lipid rafts enriched in cholesterol and sphingolipids. These highly ordered lipid assemblies, in turn, control the clustering of proteins required for cell signaling during CD4⁺ T-lymphocyte activation and differentiation.⁷⁵ The ability of n3-FAs to reduce inflammation may arise, in part, from favorable changes in the Th1/Th2 balance through polarization of CD4⁺ T cells toward a Th2 slant as evidenced in vitro.⁷⁶ Thus, n3-FAs can regulate T-cell function and regulation, such as the T helper (TH)1/TH2 balance, as well as levels of TH1 and TH17.^{76–78} In general, TH1 or TH17 polarized responses promote inflammation while TH2 and regulatory T cell predominant responses promote repair and resolution. Such findings have important implications for atherosclerosis. In human peripheral blood lymphocytes, resolvins reduced cytokine production by activated CD8⁺ T cells and CD4⁺ TH1 while limiting CD4⁺ T-cell differentiation into TH1 and TH17 cells.⁷⁹ Mice with genetically impaired ability to synthesize n3-FAs have increased TH1/TH17 cells and decreased regulatory T cells compared with wild-type mice.⁷⁹

The n3-FAs and their metabolites associate differentially with experimental atherosclerotic plaques. In atherosclerosis-prone mice (lacking apolipoprotein E, *ApoE*^{-/-}) fed a Western diet supplemented with EPA (1%, w/w) or DHA (1%, w/w) for 3 weeks, EPA treatment reduced plaque volume as compared to DHA.⁸⁰ EPA and its metabolites, especially 12-hydroxy-EPA, associated preferentially with thin-cap plaques and accumulation of

anti-inflammatory M2 macrophages while DHA associated with plaques of various sizes. In the aortic root, total EPA and 12-HETE levels followed a concentration gradient from the vascular endothelium to the media. In addition, n3-FAs can ligate the GPR-120 (G-protein-coupled receptor 120) found on macrophages. Binding these receptors by n3-FAs inhibits the activation of NF- κ B (nuclear factor- κ B), a key regulator of inflammatory gene transcription.^{81,82}

COMPARATIVE BIOPHYSICAL AND ANTIOXIDANT PROPERTIES OF OMEGA-3 FATTY ACIDS

EPA and DHA have direct and indirect cellular actions that differ depending on their particular hydrocarbon length and number of double bonds.¹⁰ In particular, DHA has an additional double bond (6 total) and 2 more carbons compared with EPA. These structural properties influence the interactions of these 2 fatty acids with surrounding membrane lipids that, in turn, can alter membrane lipid raft formation and signal transduction pathways (Figure 3).^{36,83–86} Based on its hydrocarbon length and number of double bonds, EPA inserts into lipoprotein particles and cellular membranes in an extended conformation where it can scavenge ROS through stabilization of the unpaired electrons by its multiple conjugated double bonds, a property known as conjugative resonance stabilization.³⁶ Patients with elevated triglycerides treated with prescription EPA (2–4 g/d) have significantly reduced oxLDL levels in plasma compared with placebo.²⁶

Oxidative modification of LDL can favor endothelial dysfunction, vascular inflammation, and other aspects of atherogenesis.^{87–89} Oxidatively modified LDL, but not native LDL, can foster foam cell formation. Circulating levels of oxLDL and other lipid oxidation products correlate with the severity of acute coronary syndromes and an increased risk for myocardial infarction, vascular procedures, and metabolic syndrome.^{90–93} In laboratory experiments, EPA had potent antioxidant effects in various apolipoprotein B-containing lipoprotein particles (LDL, VLDL, and small dense LDL) and in model membranes, properties not shared by other agents that lower triglycerides under identical experimental conditions.^{83,94,95} These observations argue that EPA has a preferred and energetically favorable location in plasma membranes that facilitates ROS scavenging and stabilizes the membrane structure. EPA may also intercalate into other cellular membranes, such as those of the mitochondria, where it could produce similar effects on membrane structure under conditions of oxidative stress. The antioxidant capacity of DHA wanes more rapidly than that of EPA due to its longer carbon chain length and an additional double bond that leads to rapid isomerization

and overall increased membrane fluidity rather than stability.^{36,83,94} In particular, while EPA had more of an extended orientation and conformation within the cell membranes, DHA interacted with the phospholipid head group region with concomitant disorder in the membrane hydrocarbon core.^{36,83} These differences in membrane dynamics and conformation agree with the greater susceptibility to isomerization observed for DHA by various laboratories.^{83–85,94,96} The antioxidant effects of EPA in cell membranes *in vitro* also pertain under conditions of hyperglycemia, a condition of increased generation of ROS and carbonyl species.⁹⁵ The presence of an active metabolite of atorvastatin that has antioxidant properties *in vitro* enhanced the antioxidant actions of EPA.⁹⁴ The atorvastatin metabolite and EPA have complementary locations in the membrane hydrocarbon core that facilitate free radical stabilizing properties in an additive or even synergistic fashion not observed with DHA.⁹⁴

OMEGA-3 FATTY ACIDS AND STATINS ALTER ENDOTHELIAL FUNCTIONS

EC vasomotor dysfunction generally involves reduced nitric oxide (NO) bioavailability, and associates with vasoconstriction and early plaque development.^{97–99} Arterial stiffness correlates with cardiovascular disease and all-cause mortality independent of traditional risk factors.¹⁰⁰ EPA produces favorable effects on arterial stiffness in patients with cardiovascular disease or its risk factors, including those with diabetes mellitus or receiving statins.^{101–104} The ability of EPA to reduce arterial stiffness did not depend on changes in blood pressure or LDL levels but correlated with reduced biomarkers of inflammation and oxidative stress. EPA may also enhance EC vasodilator function when combined with a statin as will be discussed. Mice deficient in the eNOS (endothelial isoform of nitric oxide synthase) show insulin resistance and reduced NO bioavailability.¹⁰⁵ Such animals display vascular abnormalities associated with insulin resistance, along with hyperinsulinemia. Previous studies have demonstrated that DHA can suppress the expression of cytokine-induced proatherogenic and proinflammatory proteins in human ECs.^{106,107} Similar benefits were observed with EPA as it inhibited lipopolysaccharide-induced monocyte adhesion and expression of adhesion molecules both *in vitro* and *in vivo*.¹⁰⁸

Recent studies investigated the combined effects of n3-FAs and statins on human ECs in culture. The combination of EPA and atorvastatin reduced endothelial dysfunction triggered by either oxLDL or high glucose in a manner not seen with DHA or statin treatment.³⁵ This improvement in endothelial vasodilator function accompanied pronounced increases in the EC ratio of stimulated NO to peroxynitrite (ONOO⁻, a highly oxidant species) release; the effects did not depend on changes

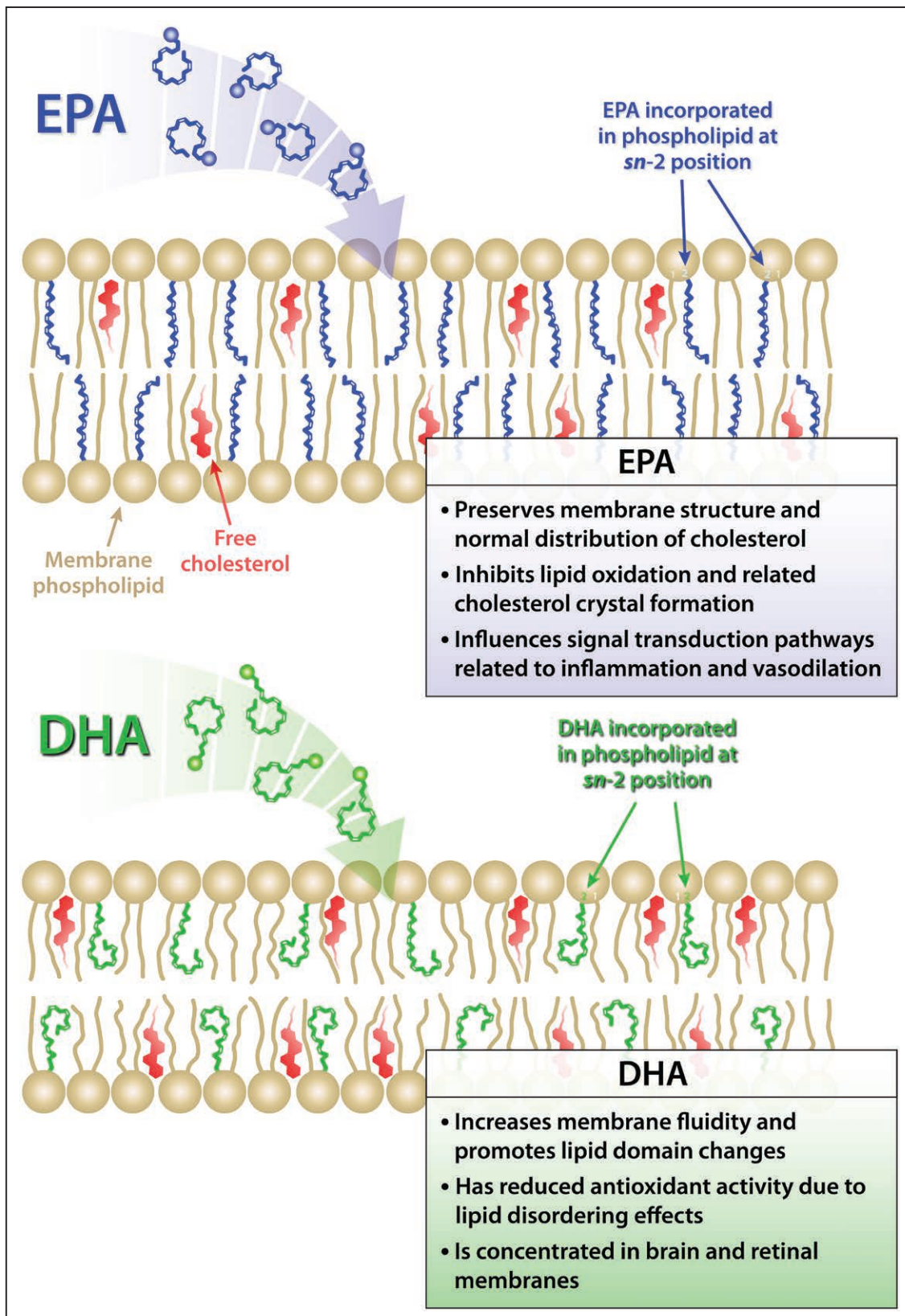


Figure 3. Molecular membrane interactions of omega-3 fatty acids.

Schematic illustration of the proposed location and contrasting effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on membrane structure. The insertion of EPA and DHA affect distinct regions of the membrane lipid bilayer due to differences in their hydrocarbon length and number of double bonds. The longer hydrocarbon length of DHA leads to more rapid isomerization and conformational changes that result in increased membrane fluidity and promotion of cholesterol domains. EPA has a more stable and extended structure that contributes to membrane stability as well as inhibition of lipid oxidation and cholesterol domain formation.

in eNOS protein expression, suggesting an improvement in eNOS activity. The favorable antioxidant interactions between EPA and atorvastatin may relate to common mechanisms.⁹⁵ The effects of EPA and a statin on EC function also pertained to rodent studies.³⁵ While either EPA or atorvastatin showed separate benefits, their combination augmented NO bioavailability under conditions of high glucose concentrations alone or in combination with exposure to oxLDL.³⁵

EFFECTS OF OMEGA-3 FATTY ACIDS ON MEMBRANE FLUIDITY, INCLUDING CHOLESTEROL DOMAIN AND CRYSTAL FORMATION

Excessive cholesterol accumulation in the membranes of vascular smooth muscle cells and macrophages can promote the formation of distinct lipid domains within the cell membrane consisting of bilayers of cholesterol monohydrate.^{109,110} Such cholesterol domains may facilitate the formation of extracellular cholesterol crystals, a hallmark of atherosclerotic plaques.¹¹¹ Oxidative stress and high glucose can also stimulate cholesterol membrane domains independently of lipid changes.¹¹² Such effects depended on glucose concentration and did not apply to iso-osmotic concentrations of another monosaccharide

(mannose), likely due to glucose's ability to promote ROS generation. Cholesterol crystals costimulate inflammasomes, intracellular macromolecular assemblies that contain and regulate caspase-1, the enzyme that processes pro-IL-1 β and pro-IL-18 into their active proinflammatory cytokine products.¹¹³

In *in vitro* studies using model membranes, EPA inhibited glucose-induced cholesterol crystalline domain formation at pharmacologically relevant concentrations due to its antioxidant activity.^{95,114} Neither certain other agents that lower triglycerides nor vitamin E reproduced this action, as they did not inhibit cholesterol domain formation or interfere with oxidative modification of the membrane lipids.⁹⁵ These findings suggest that EPA has a particular hydrocarbon length and number of double bonds that foster preferential intercalation into the alkyl chain core of the membrane bilayer, where it inhibits cholesterol domain formation. By contrast, the longer hydrocarbon length for DHA promotes rapid isomerization or conformational changes in the membrane,¹¹⁵ while EPA preserves a more ordered membrane structure.³⁶ These conformational differences cause DHA to change the normal distribution of cholesterol and even promote membrane cholesterol domains as compared to EPA.^{83,116,117} Although these properties might prove deleterious in vascular cells, such changes in the organization of cholesterol and other membrane lipids may contribute

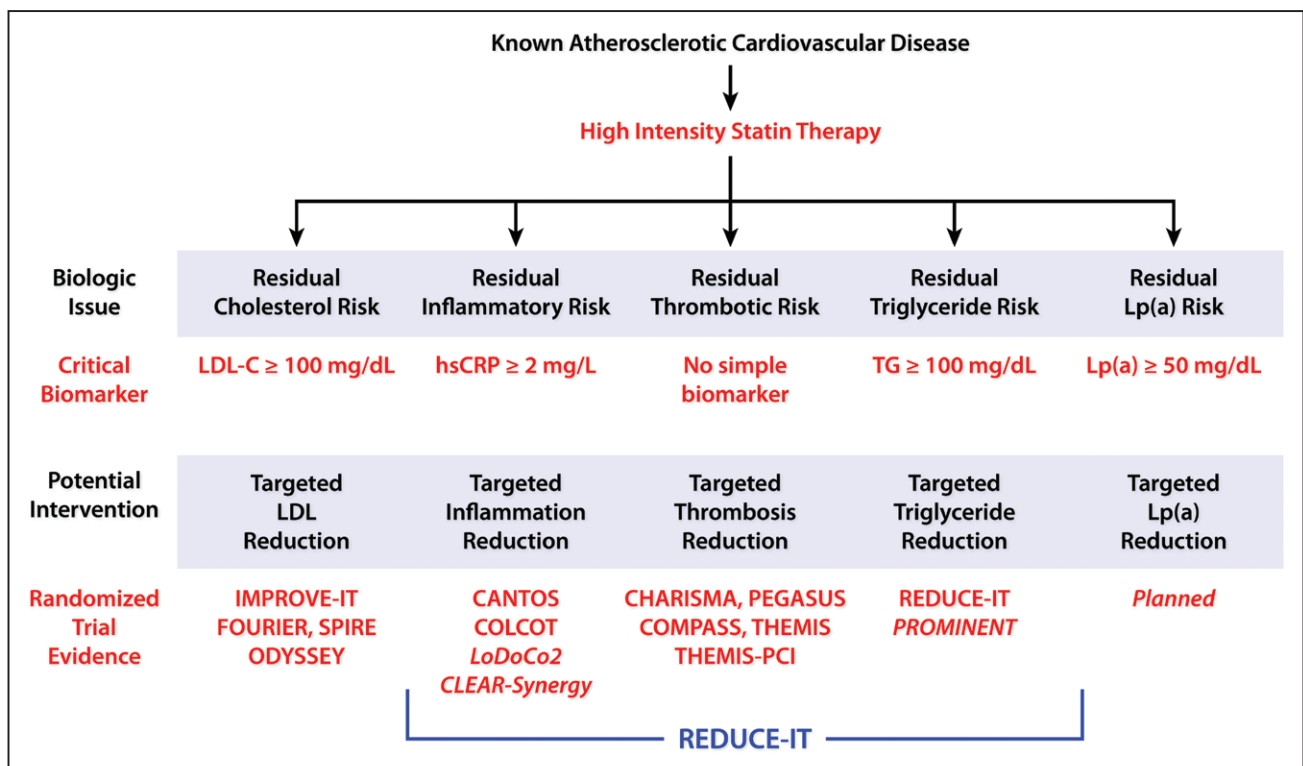


Figure 4. Clinical advances in the management of residual cardiovascular risk.

Beyond a plant-based diet and high-intensity statins, further potential strategies to reduce residual cardiovascular risk include those targeting LDL (low-density lipoprotein)-cholesterol, inflammation, thrombosis, triglycerides (TGs), and Lp(a). hsCRP indicates high-sensitivity C-reactive protein; and REDUCE-IT, Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial.

essentially to maintaining normal membrane structure and lipid raft organization in nervous tissues and in the retina where DHA is the most common polyunsaturated FA in cellular membranes.^{118,119}

Thus, EPA-based therapeutics appear to offer a major advance in cardiovascular risk reduction that adds to the protection afforded by statins. REDUCE-IT demonstrated that high doses of a highly purified ethyl ester of EPA, icosapent ethyl, provided large relative and absolute risk reductions in cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, and coronary revascularization in at-risk patients with triglycerides above ≈ 100 mg/dL despite therapy with statins.^{2,3,5,6,120} Several other medications are in early stages of evaluation, with promising effects on biomarkers such as triglycerides (Figure 4). These compounds may provide similar, lesser, or even greater risk reductions in cardiovascular events than seen in REDUCE-IT, though large, long-term cardiovascular outcome trials will be necessary to establish any clinical benefits. In addition, other trials have focused on inflammation. In patients with previous myocardial infarction, CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcome Study) found a 15% reduction in MACE (major adverse cardiovascular events) with a targeted anti-inflammatory approach using canakinumab, validating the pivotal role of inflammation in provoking ischemic events.¹²¹ COLCOT (Colchicine Cardiovascular Outcomes Trial), using colchicine, confirmed that an anti-inflammatory approach can provide incremental cardiovascular benefit, demonstrating in patients with recent myocardial infarction that there was a 23% reduction in MACE, largely driven by a reduction in coronary revascularization.¹²² Anti-inflammatory mechanisms of EPA may also contribute to some proportion of cardiovascular benefit in REDUCE-IT, albeit further downstream in the inflammatory process, as evidenced by large reductions in multiple types of end points without increased infection rates.

Another trial that tested a mixture of EPA and DHA at 4 g/d, called STRENGTH (A Long-Term Outcomes Study to Assess Statin Residual Risk Reduction With Epanova in High Cardiovascular Risk Patients With Hypertriglyceridemia; URL: <https://www.clinicaltrials.gov>. Unique identifier: NCT02104817), was terminated due to futility at the recommendation of the independent data-monitoring committee. Thus, currently, the only n3-FA proven to be of cardiovascular benefit in outcome trials remains EPA. The failure to show significant benefit in STRENGTH indicates that the addition of DHA may diminish or even negate certain benefits of EPA, or that the benefits shown in REDUCE-IT accrue from the higher dosage of EPA or the specific formulation. As already discussed, DHA or certain other agents that lower triglyceride tested under identical conditions lack certain atheroprotective mechanisms exerted by EPA, including effects on membrane lipid order and cholesterol

crystalline domain formation, along with other important differences. Other potential differences among n3-FAs with respect to mechanisms of atherosclerosis thus merit further study. Several other trials of potent therapies that lower triglycerides are being planned,¹²³ and one with the selective PPAR- α (peroxisome proliferator-activated receptor- α) modifying agent pemafibrate is already underway (PROMINENT [Pemafibrate to Reduce Cardiovascular Outcomes by Reducing Triglycerides in Patients With Diabetes]; URL: <https://www.clinicaltrials.gov>. Unique identifier: NCT03071692).^{124–126} These trials should help to understand the effects of triglyceride lowering on cardiovascular outcomes. In the meantime, the scientific underpinnings behind the mechanisms of action of n3-FAs such as EPA continue to grow and help inform our understanding of this novel axis of cardiovascular risk reduction.

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