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Review article

Biologic plausibility, cellular effects, and molecular mechanisms of eicosapentaenoic acid (EPA) in atherosclerosis

Kenneth M. Borow [a, *], John R. Nelson [b], R. Preston Mason [c]

[a] MediMergent, LLC and The National Medication Safety, Outcomes and Adherence Program, 407 Wyntre Lea Drive, Bryn Mawr, PA 19010, USA
[b] UCSF School of Medicine, Fresno-Medicine Residency Program—Volunteer, 7061 N. Whitney Street, Suite 101, Fresno, CA 93720, USA
[c] Harvard Medical School, 100 Cummings Center, Suite 135L, Beverly, MA 01915, USA

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ABSTRACT

Residual cardiovascular (CV) risk remains in dyslipidemic patients despite intensive statin therapy, underscoring the need for additional intervention. Eicosapentaenoic acid (EPA), an omega-3 polyunsaturated fatty acid, is incorporated into membrane phospholipids and atherosclerotic plaques and exerts beneficial effects on the pathophysiologic cascade from onset of plaque formation through rupture. Specific salutary actions have been reported relating to endothelial function, oxidative stress, foam cell formation, inflammation, plaque formation/progression, platelet aggregation, thrombus formation, and plaque rupture. EPA also improves atherogenic dyslipidemia characterized by reduction of triglycerides without raising low-density lipoprotein cholesterol. Other beneficial effects of EPA include vasodilation, resulting in blood pressure reductions, as well as improved membrane fluidity. EPA's effects are at least additive to those of statins when given as adjunctive therapy. In this review, we present data supporting the biologic plausibility of EPA as an anti-atherosclerotic agent with potential clinical benefit for prevention of CV events, as well as its cellular effects and molecular mechanisms of action. REDUCE-IT is an ongoing, randomized, controlled study evaluating whether the high-purity ethyl ester of EPA (icosapent ethyl) at 4 g/day combined with statin therapy is superior to statin therapy alone for reducing CV events in high-risk patients with mixed dyslipidemia. The results from this study are expected to clarify the role of EPA as adjunctive therapy to a statin for reduction of residual CV risk.

1. The importance of biologic plausibility

Biologic plausibility has been defined as evidence that a surrogate biochemical, anatomic and/or morphologic, or pathophysiologic end point is on the causal pathway to the adverse outcome or is a regular finding associated with that outcome and is plausibly related to a common causal factor [1]. It has also been suggested that the persuasiveness of a surrogate end point in supporting effectiveness of a drug is based on a history of successful intervention with pharmacologically related agents [1]. Other factors that have been described as potential components of biologic plausibility include the criteria of strength, specificity, consistency, and coherence of the data [2]. Recently, Mendelian randomization studies have demonstrated that causality can be ascribed to specific pathways that may or may not directly correlate with changes in surrogates of interest [3,4].

Over the past decade, multiple large, randomized, comparative cardiovascular (CV) trials conducted in statin-treated patients have had disappointing results, thereby raising questions about the biologic plausibility of certain lipid biomarkers as surrogate measures of atherosclerotic disease [3]. The omega-3 polyunsaturated fatty acid (omega-3 PUFA) eicosapentaenoic acid (EPA) represents a potential therapeutic option based on the biologic plausibility of its effects on multiple key atherosclerosis processes. Herein, we review the integrated effects of EPA on the cellular and molecular mechanisms of atherosclerotic plaque development, plaque rupture, and thrombus formation, and then discuss how the biologic plausibility of EPA as an anti-atherosclerotic agent supports its potential clinical benefits for prevention and/or treatment of CV disease.

* Corresponding author.
E-mail addresses: kborow@gmail.com (K.M. Borow), JR4Nelson@yahoo.com (J.R. Nelson), rpmason@elucidaresearch.com (R.P. Mason).

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2. Reducing cardiovascular residual risk in patients with atherosclerosis

Atherosclerosis is a progressive inflammatory process responsible for adverse CV outcomes. The goals of treatment are to prevent, regress, and/or stabilize atherosclerotic plaques in order to reduce risk of acute plaque rupture and acute coronary syndrome (ACS), thereby increasing life span and quality of life. It is important to emphasize that <20% of coronary culprit lesions associated with ACS have sufficient prior luminal stenosis to warrant coronary revascularization [5]. Statins significantly reduce CV events and improve survival in primary and secondary prevention settings. However, a high level of residual risk (defined as risk of CV events persisting despite achievement of low-density lipoprotein cholesterol [LDL-C], blood pressure, and glycemic treatment goals) is still evident in many dyslipidemic patients [6]. Increases in obesity, metabolic syndrome, and type 2 diabetes mellitus have added to the challenges of managing residual risk, supporting the need for effective adjunctive therapy [6]. The JELIS study demonstrated that adding EPA to statin therapy significantly reduced major coronary events compared with statin therapy alone in hypercholesterolemic patients [7].

Since publication of the 2013 American College of Cardiology/American Heart Association Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults, which questioned the beneficial effects of add-on therapy to statins [8], data from 3 clinical trials have been reported that suggest that add-on therapy to statins is indeed a viable approach for reducing residual CV risk. The first was the IMPROVE-IT study, for which final results showed that adding ezetimibe to simvastatin significantly reduced major CV events in patients with ACS despite excellent control of LDL-C with simvastatin alone [9,10]. Interim 1-year exploratory results from the other 2 trials utilized inhibitors of proprotein convertase subtilisin-kexin type 9 (PCSK9) [11,12]. Overall, these data provide important contemporary information on the value of lowering LDL-C levels regardless of the agent used [13]. With this as a background, the integrated beneficial lipid/lipoprotein-altering and pleiotropic effects of EPA on atherosclerosis offer a potential effective approach to reducing CV residual risk when used as add-on therapy to a statin.

The omega-3 PUFAs EPA and docosahexaenoic acid (DHA), as well as omega-6 PUFAs such as arachidonic acid (AA), are long-chain, highly unsaturated fatty acids that are incorporated into membrane phospholipids due to their lipophilic nature [14–16]. They serve as precursors for bioactive lipid mediators including eicosanoids, prostaglandins, leukotrienes, protectins, and resolvins [15]. In general, AA-derived mediators have pro-inflammatory effects whereas EPA-derived mediators have anti-inflammatory effects [15].

Omega-3 PUFAs have a broad range of beneficial CV effects including reducing triglycerides, very-low-density lipoprotein (VLDL), inflammatory markers, remnant-like lipoparticle cholesterol (RLP-C), oxidized low-density lipoprotein (ox-LDL), heart rate, blood pressure, and possibly arrhythmia risk [17–20]. Importantly, these benefits are observed with omega-3 PUFAs alone or as add-on therapy to statins. Statins, like the omega-3 PUFAs, have pleiotropic effects. This was recently emphasized by Blaha and Martin, who proposed that multiple simultaneous statin-associated mechanisms may be necessary to reduce CV risk [21]. These authors extended their interpretation to suggest that effects beyond specific lipids are important and may be requisite for any anti-atherosclerotic drug. Therefore, effective treatments may need to target more than a single mechanism associated with the causal pathway for atherosclerosis [21].

3. Overview of key athero-inflammatory-thrombotic processes

Endothelial dysfunction is a common denominator underlying multiple CV risk factors including hypertension, diabetes, smoking, and lipid disorders, and is evident as an early manifestation of atherogenesis [22]. Key events in the atherogenic process are summarized in Fig. 1 [23].

Evidence from recent genetic studies suggests that triglycerides and triglyceride-rich lipoproteins (TRLs) are also causally involved in coronary atherosclerosis [24–27]. In these studies, loss of function mutations in apolipoprotein (Apo) C-III, which associates with TRLs and impairs their hepatic uptake, were associated with low triglyceride levels and reduced coronary heart disease (CHD) risk. Hydrolysis of TRLs produces RLPs including chylomicron, VLDL, and intermediate-density lipoprotein (IDL) remnants. RLP levels and the cholesterol content they carry are predictive of future coronary events in patients with CHD independent of diabetes or traditional risk factors such as high-density lipoprotein cholesterol (HDL-C) and LDL-C levels [28,29]. Moreover, remnant cholesterol from TRLs is a causal risk factor for ischemic heart disease [30]. The hydrolysis of TRLs also causes endothelial dysfunction and increases endothelial permeability [31,32]. RLPs are also involved in foam cell formation, but unlike LDL, oxidative modifications are not required [33]. An estimated 36% of the cholesterol content from plaque removed from patients undergoing aortic reconstruction was derived from VLDL and IDL [34]. Elevated RLPs promote a procoagulant state by inducing tissue factor in endothelial cells as well as by tissue factor–independent mechanisms [35,36]. VLDL particles generate thrombin at rates near that of activated platelets, but unlike platelets, do not require an activation step [37]. Finally, in serial angiographic studies, progression of coronary artery lesions showed greater association with IDL than LDL as measured by analytical ultracentrifugation [38,39].

4. Effects of EPA on athero-inflammatory-thrombotic processes

4.1. Effects of EPA on endothelial function/dysfunction

There is substantial evidence that EPA has a beneficial effect on endothelial function. The release of nitric oxide (NO) by vascular endothelial cells serves to regulate vasomotor tone in response to acetylcholine and other vasoactive agonists [40]. Under normal conditions, these agents induce endothelium-dependent vasodilation via NO release. However, in the presence of significant endothelial dysfunction, NO release is reduced or abolished, a situation that is further aggravated by vasoconstriction that occurs via direct activation of vascular smooth muscle. The beneficial effects of NO are balanced by the toxic effects of reactive oxygen species including peroxynitrite ($ONOO^{-1}$) [22,40].

In human umbilical vein endothelial cells (HUVECs) that were exposed to ox-LDL, EPA improved the balance between NO and $ONOO^{-1}$, acting synergistically with statins [41]. EPA attenuated palmitic acid–induced generation of reactive oxygen species, expression of adhesion molecules and cytokines, activation of apoptosis-related proteins, and apoptosis in HUVECs [42,43]. EPA also inhibited lipid peroxidation in membrane vesicles with normal or elevated cholesterol levels; this effect was also augmented by the presence of a statin [44]. Glucose contributes to lipid peroxidation, resulting in pathologic changes in lipid structural organization, including development of cholesterol crystalline domains. EPA significantly inhibited glucose-induced lipid peroxidation and cholesterol crystalline domain formation in

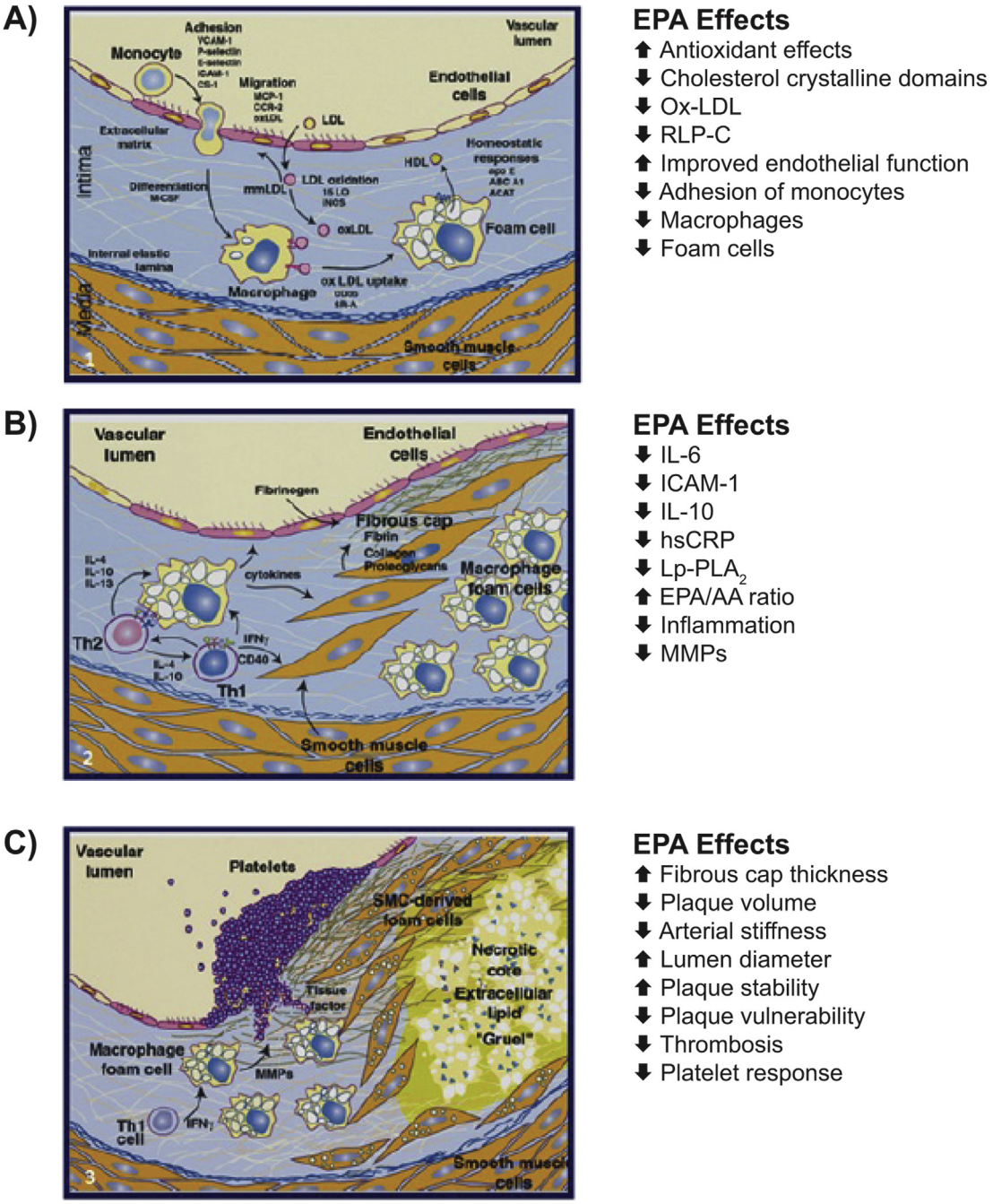

Fig. 1. Cellular and molecular mechanisms of atherosclerosis and role of EPA. Mechanisms are depicted in the illustrations and described below; effects of EPA are listed to the right of each figure indicating increases (↑) or decreases (↓). A) Low-density lipoprotein (LDL) is subject to oxidative modifications in the subendothelial space, progressing from minimally modified LDL (mm-LDL) to extensively oxidized LDL (ox-LDL). Monocytes attach to endothelial cells that have been induced to express cell adhesion molecules by mm-LDL and inflammatory cytokines. Adherent monocytes migrate into the subendothelial space and differentiate into macrophages. Uptake of ox-LDL via scavenger receptors leads to foam cell formation. Ox-LDL cholesterol taken up by scavenger receptors is subject to esterification and storage in lipid droplets, is converted to more soluble forms, or is exported to extracellular high-density lipoprotein (HDL) acceptors via cholesterol transporters, such as *ABC-A*. B) Interactions between macrophage foam cells, T helper (Th) 1 cells, and Th2 cells establish a chronic inflammatory process. Cytokines secreted by lymphocytes and macrophages exert both pro- and anti-atherogenic effects on each of the cellular elements of the vessel wall. Smooth muscle cells (SMCs) migrate from the medial portion of the arterial wall, proliferate, and secrete extracellular matrix proteins that form a fibrous plaque. C) Necrosis of macrophage and SMC-derived foam cells leads to the formation of a necrotic core and accumulation of extracellular cholesterol. Macrophage secretion of matrix metalloproteinases (MMPs) and neovascularization contribute to weakening of the fibrous plaque. Plaque rupture exposes blood components to tissue factor, initiating coagulation, the recruitment of platelets, and the formation of a thrombus. When the thrombus is of sufficient size to obstruct the coronary artery lumen, ischemic symptoms are precipitated, leading to ACS. ACS, acute coronary syndrome; ACAT, acyl CoA:cholesterol acyltransferase; Apo E, apolipoprotein E; CCR, C–C chemokine receptor; CD, clusters of differentiation; CS, connecting segment; EPA/AA, eicosapentaenoic acid/arachidonic acid ratio; hsCRP, high-sensitivity C-reactive protein; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; LO, lipoxygenase; Lp-PLA_2, lipoprotein-associated phospholipase A_2; MCP, monocyte chemotactic protein; RLP-C, remnant-like lipoparticle cholesterol; VCAM, vascular cell adhesion molecule. Adapted with permission from Glass and Witztum [23].

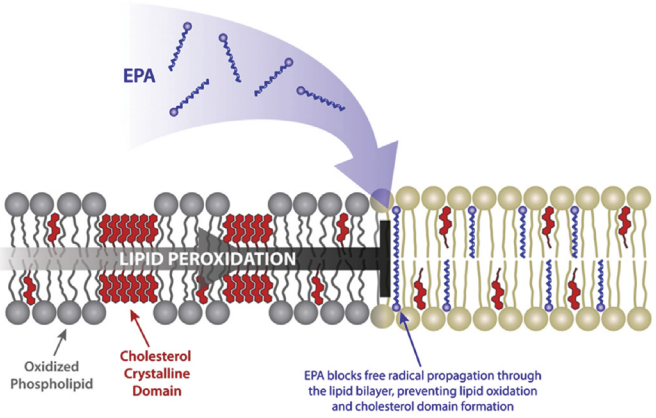

Fig. 2. Emerging Antioxidant Mechanisms for EPA. The phospholipid bilayer of the cell membrane is shown with the outer hydrophilic polar headgroups (circles) and hydrophobic tails pointing inward. Cholesterol (shown in red) and eicosapentaenoic acid (EPA; shown in blue) intercalate into the membrane lipid bilayer due to their hydrophobicity. This hydrophobicity also promotes incorporation of EPA and cholesterol into atherosclerotic plaques. Within the phospholipid bilayer, EPA may interfere with the propagation of free radicals. Reproduced with permission from Mason and Jacob [18].

membrane lipid vesicles [18]. These antioxidant effects may be attributed to the intercalation of EPA into the membrane lipid bilayer where it may interfere with the propagation of reactive oxygen species and preserve membrane lipid structural organization (Fig. 2) [18]. EPA has been shown to attenuate LDL oxidation and glucose-induced lipid peroxidation [18]. In addition, recent data have suggested that EPA induces neovasculogenesis in human endothelial progenitor cells by modulating c-kit protein and PI3-K/Akt/eNOS signaling pathways, thereby exerting a preventive effect against ischemic injury [45]. EPA at a dose of 4 g/day for 12 weeks reduced ox-LDL compared with placebo by 13.3% ($P < 0.0001$) in statin-treated patients with high triglycerides (200 to <500 mg/dL) in the ANCHOR study and by 6.6% ($P = 0.055$) in patients with very high triglycerides (500 to ≤2000 mg/dL) in the MARINE study [17]. The addition of EPA (1.8 g/day for 6 months) to optimal statin therapy significantly improved endothelial function as measured by the duration of reactive hyperemia in patients with type 2 diabetes mellitus ($P = 0.01$) [46], and by flow-mediated dilation in patients with CHD ($P = 0.02$) [47]. Similarly, administration of EPA (1.8 g/day for 3 months) restored endothelium-dependent vasodilation (measured by peak forearm blood flow during reactive hyperemia) in hyperlipidemic patients to a level comparable to that observed in normolipidemic controls [48]. The addition of EPA (1.8 g/day for 48 weeks) to statin therapy compared with statin monotherapy inhibited progression of arterial stiffness as measured by the stiffness parameter β-index of the carotid in CHD patients ($P = 0.02$) [49].

RLPs impair endothelial function via direct and indirect effects on endothelial NO synthase [50]. EPA (4 g/day for 12 weeks) significantly reduced RLP-C by 25.8% in statin-treated patients with high triglycerides ($P = 0.0001$) and by 29.8% in patients with very high triglycerides ($P = 0.0041$) compared with placebo [51]. In a subanalysis of the ANCHOR study, EPA significantly reduced RLP-C by 25.0% ($P < 0.0001$) and VLDL-triglycerides by 28.9% ($P < 0.01$) compared with placebo in the subset of statin-treated patients with high triglycerides and type 2 diabetes mellitus [19]. In another study, EPA (1.8 g/day for 3 months) significantly reduced small dense LDL particles ($P < 0.01$) and RLP-triglycerides ($P < 0.05$) compared with baseline in patients with type 2 diabetes mellitus and the metabolic syndrome, which may have been due in part to a reduction in cholesteryl ester transfer protein activity [52].

4.2. Effects of EPA on monocytes, macrophages, and foam cells

The differentiation of monocytes into macrophages and subsequently into foam cells is a key step in the atherogenic process and in the maladaptive immuno-inflammatory responses involved in atherosclerosis (Fig. 1A) [53,54]. EPA has been shown to have beneficial effects on each of these cell types. In hyperlipidemic patients with type 2 diabetes mellitus, EPA (1.8 g/day for 6 months) significantly increased circulating levels of adiponectin, a protein that stimulates NO production and suppresses monocyte attachment to endothelial cells ($P < 0.01$) [55]. After treatment, adiponectin levels in the patients with diabetes approached those seen in non-diabetic controls. In experimental models, EPA reduced monocyte adhesion whereas the omega-6 AA increased monocyte adhesion to endothelial cells in the presence or absence of an inflammatory stimulus (ie, tumor necrosis factor-α [TNFα]) [56]. In an in vitro assay under physiologic flow conditions, EPA inhibited lipopolysaccharide (LPS)-induced and TNFα-induced monocyte rolling and adhesion to HUVECs [57]. Mechanistically, EPA inhibited LPS-induced intracellular signaling pathways, leading to a reduction in vascular cell adhesion molecule-1 expression [57].

Furthermore, fewer macrophages were found in the atherosclerotic plaques of patients at the time of carotid endarterectomy who received omega-3 PUFAs (fish oil) compared with omega-6 PUFAs (sunflower oil) or a control oil until surgery ($P < 0.0001$ and $P < 0.0016$, respectively) [58]. Across all 3 groups, plaques with the highest infiltration of macrophages contained significantly less EPA than plaques with moderate infiltration [58]. After percutaneous coronary intervention (PCI) for ACS, patients randomized to EPA (1.8 mg/day) plus rosuvastatin had a lower incidence of macrophage accumulation in non-culprit thin-cap fibroatheroma lesions compared with those receiving rosuvastatin alone when assessed by serial optical coherence tomography at 9 months (13% vs 46%; $P = 0.02$) [59]. Fibrous cap thickness at 9 months was significantly greater in the group receiving the EPA plus statin combination (102 vs 70 μm; $P < 0.0001$). Since EPA is highly lipophilic, its ability to be readily incorporated into advanced atherosclerotic plaques may help explain some of its beneficial actions.

LDL is subject to oxidative modifications in the subendothelial space, leading to formation of extensively oxidized LDL (Fig. 1A). Uptake of ox-LDL by macrophages leads to foam cell formation. As noted previously, EPA significantly reduced ox-LDL in patients with very high triglycerides and in statin-treated patients with high triglycerides [17]. This might result in better clearance of LDL and contribute to the differential effects observed in patients for EPA and DHA, where EPA results in no change or a decrease in LDL-C while DHA increases LDL-C [60]. RLPs also cause foam cell formation; as noted above, EPA has been shown to reduce RLPs in several studies.

4.3. Effects of EPA on inflammation and cytokines

Atherosclerosis is a chronic inflammatory disease (Fig. 1B) [61,62]. The eicosanoids are a family of lipid mediators that modulate inflammatory and immune responses and play a critical role in platelet aggregation and cell proliferation and differentiation [15]. The eicosanoids are derived from PUFAs found in membrane phospholipids. PUFAs serve as substrates for cyclooxygenase (COX) enzymes, giving rise to prostanoids and lipoxygenase (LOX) enzymes producing leukotrienes, lipoxins, and other lipid products [15]. Arachidonic acid is the major PUFA in cell membranes, and therefore most eicosanoids produced are 2-series prostanoids (eg, prostaglandin E_2, thromboxane A_2 containing 2 double bonds) or 4-series leukotrienes (eg, leukotriene B_4 containing 4 double bonds) [15]. In contrast, EPA is converted into 3-series prostanoids (ie,

containing 3 double bonds) and 5-series leukotrienes (ie, containing 5 double bonds). These structural differences have a profound impact on the biologic activities of the eicosanoids, with AA-derived molecules generally having pro-inflammatory and/or pro-thrombotic effects whereas those derived from EPA exert anti-inflammatory and/or anti-thrombotic effects [15,63].

EPA may reduce AA-derived eicosanoids through several mechanisms including competition with AA for incorporation into membrane phospholipids and by direct inhibition of the COX-2 and 5-LOX enzymes thereby shifting production to omega-3 PUFA–derived eicosanoids [63]. EPA may also promote resolution of vascular inflammation by producing resolvins and protectins [64,65]; resolvins are formed by aspirin-acetylated COX-2 in vascular endothelial cells and 5-LOX in leukocytes whereas protectins are formed by 15-LOX in multiple cell types [63]. Both resolvins and protectins reduce neutrophil recruitment, thereby helping to resolve inflammatory processes and correct the impaired resolution of vascular inflammation seen in atherosclerosis [63].

Although the primary route of EPA metabolism is through beta-oxidation, EPA can also be converted via cytochrome P450 pathways to epoxides [66–68]. Cytochrome P450 epoxygenase-derived eicosinoids have been investigated as potential mediators of some of the pleiotropic beneficial CV effects of omega-3 fatty acids [67]. They have been shown to have important beneficial roles in vascular tone as well as nonvasodilatory anti-inflammatory CV effects [68,69].

EPA has been demonstrated to have a favorable impact on markers of inflammation in clinical studies. In the ANCHOR and MARINE trials, EPA (4 g/day for 12 weeks) significantly reduced high-sensitivity C-reactive protein (hsCRP) by 22% in statin-treated patients with high triglycerides ($P = 0.0005$) and by 36% in patients with very high triglycerides ($P = 0.0012$) compared with placebo [17]; similar results were observed in a subanalysis of patients from MARINE and ANCHOR with metabolic syndrome [70]. In both studies, EPA also significantly reduced lipoprotein-associated phospholipase A_2 (an enzyme that facilitates enzymatic modification of ox-LDL in plaques) by 19.0% ($P < 0.0001$) in ANCHOR and by 13.6% ($P = 0.0003$) in MARINE [17].

In patients who underwent percutaneous coronary intervention after myocardial infarction, early treatment with EPA (1.8 g/day for 1 month) significantly reduced peak hsCRP levels compared with the control group ($P = 0.001$), and also significantly reduced composite cardiac end points ($P = 0.01$), particularly the incidence of ventricular arrhythmias ($P = 0.03$) [71]. In obese adolescents, changes in arterial stiffness were inversely correlated with plasma EPA levels following treatment for 3 months with omega-3 PUFAs ($r = -0.47$; $P = 0.025$) [72]. The reactive hyperemic response improved with omega-3 treatment compared with placebo ($P = 0.01$). Omega-3 supplementation also reduced lymphocyte and monocyte levels as well as levels of the pro-inflammatory cytokines TNFα, interleukin (IL)-1β, and IL-6 [72]. Thus, omega-3 improved vascular function and reduced inflammation in these obese adolescents. In dyslipidemic obese adults, EPA (1.8 g/day for 3 months) significantly increased serum levels of the anti-inflammatory cytokine IL-10 ($P < 0.01$) and IL-10 expression by peripheral blood monocytes ($P < 0.05$) compared with untreated controls [73]. In another study of patients awaiting endarterectomy who received omega-3 PUFAs, the proportion of EPA in carotid plaque phospholipids was found to be inversely correlated with plaque inflammation ($r = -0.263$; $P = 0.011$) and the number of T-cells in the plaque ($r = -0.268$; $P = 0.010$) [74]. The patients who received omega-3 PUFA treatment had significantly lower plaque messenger RNA (mRNA) levels for the pro-inflammatory cytokine IL-6 ($P = 0.040$) and intercellular cell adhesion molecule-1 ($P = 0.014$) than patients in the control group. Plaque TNFα and IL-10 mRNA levels did not differ significantly between groups [74].

The plasma EPA/AA ratio has been reported to correlate with atherosclerosis progression and CV outcome. In patients with angina pectoris who received statins for 8 months after PCI, the EPA/AA ratio was negatively correlated with the percentage change from baseline in both plaque volume ($r = -0.19$; $P = 0.05$) and plaque fibrous component volume ($r = -0.21$; $P = 0.04$) [75]. A low EPA/AA ratio was identified as an independent risk factor for ACS in patients ≥50 years of age as well as in younger adults [76]. In ACS patients assessed by optical coherence tomography, a low EPA/AA ratio was associated with vulnerable coronary plaque morphology, showing a significant correlation with fibrous cap thickness ($r = 0.37$; $P = 0.002$) [77]. On multivariate analysis, low EPA/AA was independently associated with thin-cap fibroatheroma. Treatment with EPA leads to higher EPA/AA ratios [78]. In patients undergoing elective PCI and receiving either EPA plus a statin or statin monotherapy for 6 months, the EPA/AA ratio was negatively associated with atherosclerosis progression (P for trend = 0.044) [79]. Treatment with EPA plus rosuvastatin significantly increased EPA/AA compared with rosuvastatin alone in patients undergoing PCI for ACS (1.11 vs 0.42; $P = 0.0001$) [59]. After 9 months of treatment, EPA/AA levels were negatively correlated with pentraxin-3 levels, a marker of arterial inflammation. Taken together, the greater suppression of arterial inflammation by EPA plus statin compared with a statin alone may be a potential mechanism for stabilization of vulnerable plaques and ultimate reduction in CV residual risk [59].

In a study conducted in Japanese patients with dyslipidemia, EPA treatment for 4 weeks led to significantly higher EPA concentrations in the HDL fraction, with an EPA/AA ratio comparable to that in serum ($P < 0.05$). The EPA-rich HDL fraction had significantly increased activity of the anti-oxidative enzyme paraoxonase-1 ($P < 0.05$), and it also significantly improved endothelial cell migration ($P < 0.05$) and inhibited cytokine-induced cell adhesion molecule expression in HUVECs [80].

4.4. Effects of EPA in atherosclerotic plaque

Fibrous cap thickening may help stabilize sites of atherosclerotic plaques and thereby prevent plaque rupture (Fig. 1C). EPA (1.8 g/day for 8 months) significantly increased fibrous cap thickness in ACS patients compared with baseline and compared with untreated controls as measured by optical coherence tomography (both $P \leq 0.001$) [81]. In patients undergoing PCI for ACS, treatment with EPA plus rosuvastatin for 9 months produced a greater increase in fibrous cap thickness (55 vs 24 μm; $P < 0.0001$) and greater decrease in plaque lipid arc (−34 vs −13°; $P = 0.007$) and length (−2.8 vs −1.2 mm; $P = 0.009$) compared with rosuvastatin alone [59]. Comparable results were observed with EPA plus statin versus statin alone in a small group of patients with stable angina [82].

EPA has been shown to reduce plaque volume in several studies. The addition of EPA (1.8 g/day) to high-intensity statin therapy, but not statin therapy alone, significantly reduced lipid plaque volume and significantly increased fibrous plaque volume after 6 months (both $P < 0.05$) as measured by intravascular ultrasound [83]. Similarly, EPA (1.8 g/day) plus pitavastatin significantly reduced coronary plaque volume after 8 months compared with pitavastatin alone (−24% vs −2%, $P < 0.01$) in patients with impaired glucose tolerance and angina pectoris [84]. Significant reduction in soft plaque volume as measured by 64-slide multi-detector row computed tomography was also reported in patients with suspected CHD who were treated with EPA for 1 year but not in those treated with ezetimibe [85].

In patients with type 2 diabetes mellitus, treatment with EPA (1.8 g/day) for a mean of 2.1 years significantly reduced the annual change in mean carotid intima media thickness (IMT; $P = 0.029$),

maximum carotid IMT ($P = 0.0008$), and brachial-ankle pulse wave velocity ($P = 0.021$) compared with a control group that did not receive EPA [86]. EPA was also shown to decrease carotid IMT in patients with hypertriglyceridemia and improve maximal IMT in patients with atherosclerosis risk factors despite therapy of the underlying conditions [87,88]. Following elective PCI, the addition of EPA to statin therapy significantly reduced the minimum coronary lumen diameter (−0.104 vs −0.078 mm; $P = 0.02$) and percent diameter of stenosis (−0.27% vs 1.60%; $P = 0.026$) compared with statin monotherapy as measured angiographically at 6 months [79].

As noted earlier, EPA is readily incorporated into advanced atherosclerotic plaques due to its lipophilic nature. Higher levels of EPA in plaques have been associated with decreased plaque inflammation and increased stability [74]. Conversely, a low serum EPA/AA is associated with plaque vulnerability [77]. With regard to neovascularization, the presence of intimal microvessels tended to be less frequent in EPA-treated patients on optimized statin therapy than in patients treated with statins alone ($P = 0.08$) in a study of non-culprit thin-cap fibroatheroma lesions [59].

Finally, the effect of omega-3 PUFAs on the extracellular matrix of plaque was evaluated in patients awaiting endarterectomy [74]. Plaque from patients who received omega-3 PUFAs had significantly lower mRNA levels for MMP-7 ($P = 0.006$), MMP-9 ($P = 0.005$), MMP-12 ($P = 0.004$), and tissue inhibitor of MMP-2 ($P = 0.014$) compared with plaque from patients in a control group. Moreover, the proportion of EPA in carotid plaque phospholipids was inversely correlated with the median plaque feature summation score, which included 7 plaque characteristics known to be adversely associated with the progression of atherosclerosis (ie, lipid core, foam cells, hemorrhage, overall inflammation, fibrous cap inflammation, plaque macrophages, fibrous cap macrophages) ($r = -0.211$; $P = 0.043$) [74].

4.5. Effects of EPA on thrombus formation

Thromboxane A2 derived from AA is a potent mediator of platelet aggregation. In contrast, EPA inhibits platelet aggregation through metabolically derived prostanoid intermediates which are rapidly converted to prostaglandin D3 [89]. These observations suggest that incorporation of EPA into platelet membranes may reduce platelet aggregation. In hyperlipidemic patients with type 2 diabetes mellitus, EPA (1.8 g/day for 6 months) significantly decreased platelet-derived microparticles ($P < 0.05$) [55]. Thus, in the situation of a ruptured atherosclerotic plaque leading to acute coronary syndrome, EPA may be able to help limit the size of the overlying thrombus by reducing platelet aggregation, thereby limiting the amount of myocardium placed at ischemic risk.

5. Biologic plausibility of EPA therapy for reducing cardiovascular events

As described in the preceding sections, EPA has multiple mechanisms of action that may play beneficial roles at each step in the atherosclerosis pathway from endothelial dysfunction through plaque rupture and thrombus formation. These potential benefits, which remain evident when EPA is added to statin therapy compared with statin therapy alone, help to support the likelihood that EPA is a biologically plausible agent for having therapeutic effects on the adverse causal pathway and clinical outcomes associated with atherosclerosis. This statement is further supported by evidence from recent genetic studies of Apo C-III that point to triglycerides as being causally involved in atherosclerosis and CHD [24–27]. EPA has been shown to significantly reduce triglycerides and Apo C-III without raising LDL-C in patients with very high triglycerides and in statin-treated patients with high triglycerides [90–92]. Although the exact mechanism by which EPA lowers triglyceride levels is not known, EPA has been shown to reduce hepatic VLDL-triglyceride synthesis and/or secretion and enhance triglyceride clearance from circulating VLDL particles [93]. Possible mechanisms for these effects include increased β-oxidation, inhibition of acyl-CoA:1,2-diacylglycerol acyltransferase, decreased lipogenesis in the liver, and increased plasma lipoprotein lipase activity [66].

As noted earlier, a history of successful intervention with a pharmacologically related agent has been suggested as one of the required criteria for biologic plausibility. This has occurred with EPA, as demonstrated in the JELIS study, in which 18,645 hypercholesterolemic Japanese patients were randomly assigned to receive EPA (1.8 g/day) plus a statin or the statin alone [7]. Ninety percent of the patients received pravastatin 10 mg or simvastatin 5 mg once daily. After a mean follow-up of 4.6 years, EPA plus statin significantly reduced risk of a major coronary event by 19% compared with statin monotherapy (hazard ratio [HR]: 0.81; 95% confidence interval [CI]: 0.69–0.95; $P = 0.011$) [7]. EPA treatment was associated with a significant 19% risk reduction for major coronary events in the secondary prevention subgroup ($P = 0.048$) and a nonsignificant 18% risk reduction in the primary prevention subgroup ($P = 0.132$) [7]. A series of pre-specified sub-analyses further documented the benefit of adding EPA to statin therapy [94–99]. In the 957 patients with baseline triglyceride levels ≥150 mg/dL and HDL-C <40 mg/dL, there was a 53% reduction with EPA in the cumulative incidence of major adverse CV events ($P = 0.043$) [94]. In the secondary prevention cohort, adding EPA compared with statin monotherapy reduced risk of major coronary events by 27% among the 1050 patients with a previous MI ($P = 0.033$) and by 41% among the 537 patients with prior MI and coronary intervention ($P = 0.008$) [95]. Limitations of the JELIS study include its Japanese-only population with relatively high baseline plasma EPA levels due to dietary fish consumption, low baseline triglyceride levels (~150 mg/dL), and open-label design. However, clinical end points were adjudicated by a committee blinded to patient treatment.

Patients on chronic hemodialysis represent a population at very high risk for CV events. EPA was shown to significantly reduce risk of CV events independent of triglyceride and hsCRP levels in a controlled trial of 179 chronic hemodialysis patients randomized in a 1:1 ratio to EPA (1800 mg/day) or control [100]. Patients were followed for 2 years with significant reductions in CV death (HR: 0.20; 95% CI: 0.04–0.91; $P = 0.037$), CV events including acute myocardial infarction, stroke, and aortic disease–related events (HR: 0.50; 95% CI: 0.26–0.96; $P = 0.039$), and combined outcome (HR: 0.49; 95% CI: 0.26–0.90; $P = 0.021$). Another study of 176 patients receiving chronic hemodialysis showed that EPA treatment resulted in reduced all-cause death compared with chronic hemodialysis patients not receiving EPA treatment ($P < 0.05$; multivariate Cox proportional hazards regression) [101]. EPA was also found to improve lipid profiles and RLP-C, triglyceride, and ox-LDL levels in patients receiving hemodialysis [102,103]. The beneficial effects of EPA in chronic hemodialysis patients support the concept that EPA therapy can improve CV outcomes in enriched high-risk populations with significant unmet clinical need.

Treatment with omega-3 PUFA formulations containing both EPA and DHA has been evaluated in a number of other outcome studies. However, the results were inconsistent, possibly due to use of relatively low doses of PUFAs as well as differences in patient populations including baseline CV risk profiles, dietary omega-3 PUFA intake, and/or variability in baseline triglyceride levels [104–110]. Furthermore, the addition of DHA to EPA can result in increases of approximately 15%–49% in LDL-C [111–113].

REDUCE-IT (NCT01492361), an ongoing, randomized, controlled

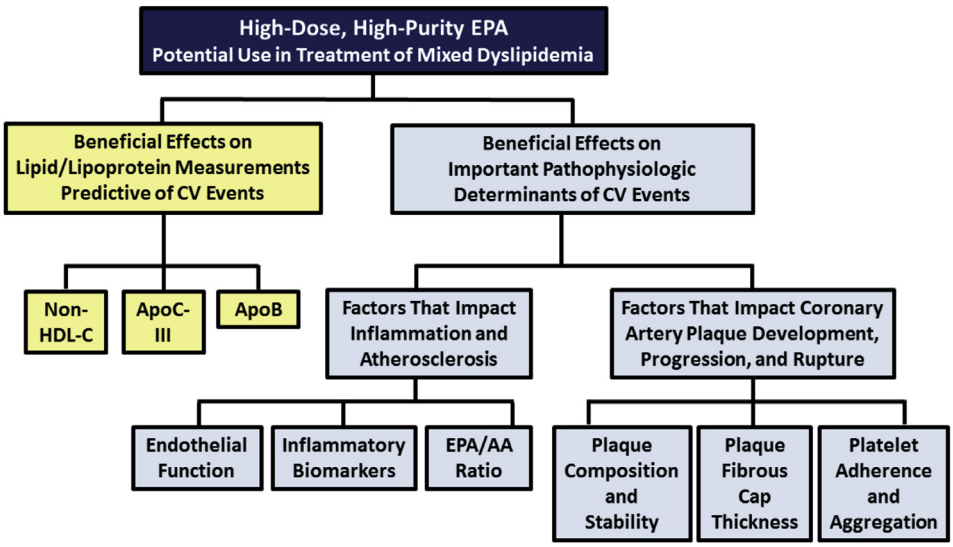

Fig. 3. Potential beneficial effects of eicosapentaenoic acid (EPA) on clinical cardiovascular (CV) end points. Apo, apolipoprotein; AA, arachidonic acid; non-HDL-C, non-high-density lipoprotein cholesterol.

clinical trial, is specifically designed to evaluate whether prescription strength (4 g/day) highly purified EPA combined with a statin is superior to a statin alone in reducing CV events in high-risk patients with persistently high triglycerides. The drug being tested in the REDUCE-IT trial is icosapent ethyl, a high-purity prescription formulation containing the ethyl ester of EPA, which is already indicated as an adjunct to diet for reducing triglycerides in adults with severe hypertriglyceridemia (≥500 mg/dL) [66]. The recommended dose is 4 g/day taken as two 1-g capsules twice daily with food. Treatment with icosapent ethyl has resulted in beneficial lipid effects in the MARINE and ANCHOR trials in addition to the reductions in inflammatory markers and RLP-C noted earlier. In the MARINE study conducted in patients with very high triglycerides, treatment with icosapent ethyl at a dose of 4 g/day for 12 weeks significantly reduced triglycerides by 33.1% ($P < 0.0001$), VLDL-triglycerides by 25.8% ($P = 0.0023$), VLDL-cholesterol (VLDL-C) by 28.6% ($P = 0.0002$), non-high-density lipoprotein cholesterol (non-HDL-C) by 17.7% ($P < 0.0001$), and Apo B by 8.5% ($P = 0.0019$) compared with placebo [90]. Importantly, LDL-C was not increased (it was reduced by 2.3%; $P = 0.677$ vs placebo) unlike what may occur with omega-3 PUFA products that contain both EPA and DHA [111–113]. In the ANCHOR study conducted in statin-treated patients with high triglyceride levels, treatment with icosapent ethyl at a dose of 4 g/day for 12 weeks significantly reduced triglycerides by 21.5% ($P < 0.0001$), LDL-C by 6.2% ($P = 0.0067$), VLDL-triglycerides by 26.5% ($P < 0.0001$), VLDL-C by 24.4% ($P < 0.0001$), non-HDL-C by 13.6% ($P < 0.0001$), and Apo B by 9.3% ($P < 0.0001$) compared with placebo [91].

Evidence is mounting that a raised concentration of remnant cholesterol—marked by elevated triglyceride levels—is an additional causal risk factor for CV disease and all-cause mortality, and that low HDL-C may only be a long-term marker of raised triglycerides and remnant cholesterol [3]. Triglycerides, TRLs, and particularly RLPs have been convincingly and causally implicated in the development of CV risk [26,114]. The results from REDUCE-IT are expected to clarify whether EPA's beneficial effects on triglycerides and other lipid parameters in conjunction with its pleiotropic effects on atherosclerotic plaque will result in a reduction of major CV events in statin-treated patients with high CV risk and mixed dyslipidemia. In a recent preliminary study, a novel pharmacoeconomic model revealed that combining EPA with a statin for secondary prevention of CV disease was associated with cost savings and improved utilities compared with statin alone [115]. The key factors supporting the possibility that REDUCE-IT may show beneficial effects on CV end points have been described in preceding sections and are summarized in Fig. 3.

6. Expert opinion

As noted previously, residual CV risk remains in many patients despite statin therapy, even at high doses. This underscores the medical need for effective add-on therapy. The IMPROVE-IT and recent PCSK9 trials demonstrated the plausibility of add-on therapy to a statin as a means to address residual CV risk and the importance of the level of LDL-C in managing that risk (ie, "lower LDL-C is better") [10,13]. Thus, use of agents that have the potential to raise LDL-C may not be desirable or optimal for reducing CV risk. High-dose, high-purity prescription icosapent ethyl reduces key atherogenic parameters including triglycerides, non-HDL-C, Apo B and Apo C-III, and RLP-C, but does not raise LDL-C in patients with high or very high baseline triglycerides [51,90,92]. In contrast, omega-3 PUFA products that contain DHA may raise LDL-C [111–113]. In view of the favorable lipid and lipoprotein effects of icosapent ethyl in conjunction with the beneficial pleiotropic effects of EPA on the atherosclerosis processes described in this review, we look forward to the results of the REDUCE-IT trial to help clarify whether icosapent ethyl can add benefit in reducing CV events in statin-treated patients. Icosapent ethyl may potentially be an important addition to the clinician's armamentarium for prevention and treatment of atherosclerotic vascular disease, especially given the existing excellent safety and tolerability profile of icosapent ethyl.

7. Conclusions

EPA has beneficial effects on multiple atherosclerosis processes including endothelial function, oxidative stress, foam cell formation, inflammation/cytokines, plaque formation/progression, platelet aggregation, thrombus formation, and plaque rupture. EPA reduces atherogenic dyslipidemia including triglycerides and RLP-C, while also having other beneficial effects arising from its intercalation into membrane phospholipids. Interestingly, the effects of EPA are maintained when added to statin therapy. On the basis of

this profile and EPA's biologic plausibility, the results of REDUCE-IT are eagerly anticipated as they will clarify the potential role of EPA in reducing CV events in statin-treated patients.

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