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CARDIOMETABOLIC DISEASE AND TREATMENT (R. SANTOS, SECTION EDITOR)

Controversies in the Use of Omega-3 Fatty Acids to Prevent Atherosclerosis

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Abstract

Purpose of Review We discuss current controversies in the clinical use of omega-3 fatty acids (FA), primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and examine discrepancies between recent trials. Furthermore, we discuss potential side effects reported in these studies and the role of mixed omega-3 FA dietary supplements and concerns about their use.

Recent Findings REDUCE-IT showed that addition of icosapent ethyl, a highly purified form of EPA, can reduce risk of cardiovascular events among statin-treated individuals with high triglycerides. Additional supportive evidence for EPA has come from other trials and meta-analyses of omega-3 FA therapy. In contrast, trials of mixed EPA/DHA products have consistently failed to improve cardiovascular outcomes. Discrepancies in results reported in RCTs could be explained by differences in omega-3 FA products, dosing, study populations, and study designs including the placebo control formulation. Evidence obtained from highly purified forms should not be extrapolated to other mixed formulations, including "over-the-counter" omega-3 supplements.

Summary Targeting TG-rich lipoproteins represents a new frontier for mitigating ASCVD risk. Clinical and basic research evidence suggests that the use of omega-3 FA, specifically EPA, appears to slow atherosclerosis by reducing triglyceride-rich lipoproteins and/or inflammation, therefore addressing residual risk of clinical ASCVD.

Keywords Omega 3 · Fish oils · Triglycerides · Icosapent ethyl

Introduction

Elevated plasma triglycerides (TG) result from an excess of triglyceride-rich lipoproteins (TRL) of several types, mainly VLDL and their remnants. Compelling evidence exists supporting the hypothesis that elevation of triglycerides is

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associated with increased atherosclerotic cardiovascular disease (ASCVD) risk. Current treatment for hypertriglyceridemia includes intensive diet and lifestyle changes such as weight loss and increased physical activity, and statins are also commonly used. Other pharmacological options have been considered, such as fibrates and niacin, with inconsistent risk reduction of ASCVD.

The Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial (REDUCE-IT) [1••] showed evidence for the role of eicosapentaenoic acid (EPA) in its highly purified ethyl ester derivative, icosapent ethyl (IPE), in addition to statin treatment, for ASCVD risk reduction. However, more recent studies have tested other formulations of omega-3 FA with mixed results.

In this article, we discuss current controversies in the clinical use of omega-3 FA, including EPA and docosahexaenoic acid (DHA) as well as examine discrepancies between recent trials. Finally, we discuss potential side effects reported in these studies and the role of mixed omega-3 FA dietary supplements and concerns in their use.

Physiological Mechanisms for EPA and DHA

Omega-3 FA have complex cellular effects and are incorporated into the cellular phospholipid bilayer replacing arachidonic acid, a precursor of pro-inflammatory mediators [2]. Due to its unique structural shape and longer carbon construction, DHA is associated with an increase in the fluidity of the phospholipid bilayer as compared to EPA [3]. As a result, the presence of DHA in the membranes allows for free radical movement within the lipid core and wider lipid oxidation [3]. While the presence of EPA in the phospholipid bilayer leads to homogenous cholesterol distribution in the membrane, DHA enhances the formation of cholesterol rafts and subsequent cholesterol crystals [2, 3].

Omega-3 FA have favorable effects in reducing inflammation [2]. This is mediated through inhibiting reactive oxygen species (ROS), leading to a reduction in the activation of Ik-B and NF-KB and several other transcription factors [4]. As a result, omega-3 FA are associated with downregulation of pro-inflammatory cytokine genes such as IL-8 and TNF-α [5]. Omega-3 FA also downregulate the expression of adhesion molecules and reduce the recruitment of macrophages in the vascular wall [5].

Furthermore, omega-3 FA are the precursors of specialized pro-resolving lipid mediators (SPM), a key set of molecules involved in resolving inflammation [6]. A higher level of SPM to pro-inflammatory mediators ratio was associated with slowing of plaque progression as well as possible plaque regression [7]. EPA and DHA also improve efferocytosis in macrophages and help recruit anti-inflammatory M2 macrophages [5].

In patient with hypertriglyceridemia, higher EPA levels are associated with a reduction in high-sensitivity C-reactive protein (hsCRP), while a similar effect was not observed with DHA [8, 9]. Of note, in the REDUCE-IT trial, the benefit with high-dose EPA supplementation was largely independent of baseline or achieved TG levels, although the risk reduction was the greatest in those with a baseline TG ≥ 250 mg/dL [1••]. In addition to TG lowering, EPA is more potent in inhibiting the oxidation of ApoB-containing lipoproteins including low-density lipoprotein (LDL), small dense LDL, and very low-density lipoproteins (VLDL) [10–12].

In the vascular wall, EPA is a more potent inducer of nitric oxide (NO) release from the endothelium than DHA [13]. This effect of EPA on NO release may be further potentiated in the presence of an atorvastatin active metabolite [14]. EPA has been proposed to stabilize coronary plaque by reducing the formation of cholesterol crystals, which in turn may reduce the likelihood of plaque disruption [5, 12].

Furthermore, omega-3 FA may exert cardiovascular benefits by slowing atherosclerotic plaque progression [15]. Among those individuals without diabetes, an omega-3 index ≥ 4% was associated with no progression of fibrous, non-calcified, calcified, and total plaque [15]. In the same study, those in the highest quartile of EPA levels, but not DHA levels, had significantly lower fibrous, non-calcified, and total plaque volume compared with those in the lowest tertile [15]. The effects of EPA supplementation were also demonstrated in the Effect of Vascepa on Improving Coronary Atherosclerosis in People With High Triglycerides Taking Statin Therapy (EVAPORATE) trial where high-dose EPA supplementation was associated with regression of low-attenuation plaque volume compared with placebo. However, the placebo arm in EVAPORATE was noted to have almost doubling of low-attenuation plaque volume at follow-up [16•]. Other differences are summarized in Table 1.

Current Evidence for Omega-3 FA—Can Differences in Design Explain Differences in Results?

Detailed summaries of the design and findings of the existing trials for omega-3 FA are shown in Tables 2 and 3, respectively. The differences in omega-3 formulations, population choice, variability in study endpoints, and trial designs have led to significant controversy on the cardiovascular benefits of omega-3 supplementation.

EPA Trials

One of the early notable studies, Japan EPA Lipid Intervention Study (JELIS) [17], was an open-label trial that looked

Table 1 Comparison between eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)

EPA	DHA
• Greater reduction in hsCRP	• Longer hydrocarbon length, promoting conformational changes in the membrane of vascular smooth muscle cells and macrophages
• Inhibits oxidation of ApoB-containing particles	• Increases LDL-C levels
• Greater induction of nitric oxide release	• Does not decrease ApoB levels
• Greater lowering of linoleic and arachidonic acid plasma levels	• Favors reduction of platelet aggregation in females
• Induces endothelium-dependent vasorelaxation and enhances endothelial function	• Greater anti-thrombotic effect
• Favors reduction of platelet aggregation in males	• Greater increase in adiponectin levels

at 18,645 Japanese patients with hypercholesterolemia randomized for treatment with 1800 mg per day of EPA plus a low-intensity statin or statin alone (control group). The investigators found a 19% relative reduction in non-fatal coronary events ($p = 0.015$) but observed no effect on non-cardiac or cardiac sudden death in patients treated with omega-3 FA compared to controls. The effect on mortality was arguably blunted by the choice of population given that the Japanese population has higher-than-average omega-3 consumption through high fish intake in the diet. This could possibly explain the overall low rates of cardiac death in the JELIS study and marginal additional decrease in cardiac death with EPA supplementation. The open-label nature of the trial allowed space for further bias. Lastly, the demonstrated benefit was largely driven by unstable angina and revascularization events, which may be less objective components of the composite end point.

REDUCE-IT [1••] randomized 8179 patients with known atherosclerotic heart disease or diabetes with an additional risk factor, on baseline statin therapy, and with relatively well-controlled LDL-C but residual TG elevation, to therapy with IPE (a highly purified ethyl ester form of EPA) at 2 g twice daily versus a placebo control. The results of the trial indicated that IPE was successful not only at reducing mean TG levels, but also cardiovascular events and death by 25%. Given that the cohort was already on baseline statin treatment and had well-controlled LDL levels (< 100 mg/dL), the findings indicate an additive benefit to using IPE. The findings contrast with prior negative trials, which might be due to the use of a high dose form of purified EPA in REDUCE-IT. Additionally, there was a high proportion of participants with known atherosclerotic disease, and subgroup analyses showed considerably greater benefits in this group.

Mixed EPA/DHA Formulations

The A Study of Cardiovascular Events in Diabetes (ASCEND) trial [18] was a large mail-based randomized trial that focused on EPA + DHA supplementation among patients with diabetes with no known cardiovascular disease and found no reduction in the primary outcome of vascular events, which included non-fatal myocardial infarction or stroke, transient ischemic attack, or vascular death. It showed no reduction in incidence of atrial fibrillation or ventricular arrhythmia with omega-3 fatty acid supplementation. The trial did, however, note a significant reduction in vascular death in the omega-3 arm.

The Vitamin D and Omega-3 Trial (VITAL) [19] investigated the supplementation of 840 mg omega-3 fatty acids with both DHA and EPA in 25,871 healthy participants followed over 5 years and found no effect on CV death, stroke, or atrial fibrillation. The investigators demonstrated that patients with omega-3 FA supplementation had lower rates of myocardial infarction, but analyses did not correct for multiple comparisons. Overall, the trial was considered null because of the lack of statistical significance on the predefined primary endpoint; however, with the relatively low dose of omega-3 fatty acids used in a baseline healthy cohort, the significant reduction in myocardial infarction should not be overlooked. It is important to note that subgroup analyses showed a possible lower incidence of the primary cardiovascular endpoint in participants with baseline low fish intake.

The Statin Residual Risk Reduction with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridemia (STRENGTH) trial [20••] evaluated the effect of a 4-g combination of EPA/DHA carboxylic acid vs corn oil among statin-treated patients with dyslipidemia and high cardiovascular risk. The double-blind trial findings showed a neutral effect on a composite of CV death, non-fatal MI or stroke, coronary revascularization, or unstable angina requiring hospitalization. Higher rates of atrial fibrillation and gastrointestinal adverse events were seen with omega-3 FA supplementation. Despite using a higher dose of EPA/DHA than used in prior trials, STRENGTH was terminated early due to futility of benefit, supporting prior findings of null clinical effects of mixed EPA/DHA formulations.

Notably, subgroup analysis of participants with highest DHA levels did not find poorer outcomes. As opposed to REDUCE-IT, EPA levels did not correlate with outcomes.

Table 2 Design of studies

Author, year (name of RCT)	Design	Treatment period	Treatment group	Control	Number of participants
Yokoyama, 2007 (JELIS) [17]	Prospective, randomized open-label, blinded endpoint evaluation	November 1996–November 1999	1.8 g EPA with statin (5 mg simvastatin or 10 mg pravastatin)	Statin alone (5 mg simvastatin or 10 mg pravastatin)	18,645 randomly assigned patients
Manson, 2018 (VITAL) [76]	Randomized, double-blind, placebo-controlled trial, with a two-by-two factorial design	November 2011–March 2014	2000 IU of vitamin D_3 and 1 g/day Ω-3 fatty acids	Placebo vitamin D or Ω-3 fatty acids	25,871 underwent randomization
Bhatt, 2019 (REDUCE-IT) [1••]	Phase 3b randomized, double-blind, placebo-controlled trial	November 2011–August 2016	IPE (2 g twice daily with food [total daily dose, 4 g])	Placebo	8179 patients were randomly assigned
Budoff, 2020 (EVAPORATE) [16•]	Multicenter, randomized, double-blind, placebo-controlled trial		4 g/day IPE	Placebo	80 patients were enrolled
Nicholls, 2020 (STRENGTH) [20••]	A double-blind, randomized, multicenter trial	October 2014–June 2017	4 g/day of omega-3 carboxylic acid formulations	Corn oil	13,078 patients randomized
Kalstad, 2020 (OMEMI) [23]	Randomized, double-blind, placebo-controlled trial	November 2012–June 2020	1.8 g EPA + DHA	Corn oil	1027 elderly patients with recent acute myocardial infarction

Other than using a different intervention (omega-3 FA formulation) in STRENGTH compared to REDUCE-IT, another key difference exists between these two trials. The control group in STRENGTH received corn oil, whereas the REDUCE-IT control group received mineral oil. It has been suggested that mineral oil could interfere with statin absorption; indeed, the placebo group in REDUCE-IT had higher levels of LDL-C (11%), non-HDL-C, ApoB, and C-reactive protein (32%) at the end of the study period [21]. In contrast, such changes were minimal in the control arm in STRENGTH. The use of mineral oil has also been suggested to be responsible for an increase in blood pressure and possibly in atrial fibrillation (1.4% absolute increase) seen in REDUCE-IT control arm. A recent narrative review identified 80 studies that used some form of mineral oil as placebo and did not find any clinically relevant effect other than a lubricant laxative effect in the gastrointestinal tract [22].

More recently, the Omega-3 Fatty acids in Elderly with Myocardial Infarction (OMEMI) [23] investigators showed that among 1027 elderly patients with recent myocardial infarction on high statin dose with relatively low levels of triglycerides, supplementation with 1.8 g of n-3 PUFA (EPA and DHA) did not significantly reduce all-cause death, nonfatal MI or stroke, heart failure hospitalization, or unscheduled revascularization at 24 months. This study uniquely informs the approach to secondary prevention in the elderly population, which is often not included in trials.

These contrasting results of omega-3 FA trials have raised significant controversy on the contribution of different supplement formulations and their advantage in specific populations. In a recent meta-analysis of thirty-eight randomized clinical trials, including 149,051 participants, Khan et al. found with moderate certainty that omega-3 fatty acids reduced cardiovascular mortality and events, and that EPA did so to a greater extent than EPA + DHA formulations [24]. With improving primary prevention and increase in use of statins, the question remains as to whether the meta-analyses should have excluded the early trials (GISSI-P, JELIS, etc.) to better represent today's populations.

Physiological Mechanisms of Potential Side Effects

At low doses (< 1 g per day), omega-3 FA supplementation is generally well tolerated. However, clinical trials using higher doses of omega-3 FA supplementation have raised concerns regarding bleeding and arrhythmia and gastrointestinal side effects.

Omega-3 FA inhibit platelet aggregation by suppressing the effects of COX-1 on arachidonic acid. This results in a reduction in the production of thromboxane A2 [25]. DHA also increase the production of protectin DX which

Table 3 Findings of the studies

Author, year (name of RCT)	Major findings
Yokoyama, 2007 (JELIS) [17]	• The 5-year cumulative rate of major coronary events was 2.8% in the EPA group and 3.5% in controls, resulting in a significant relative risk reduction of 19% in the EPA group ($p=0.011$) • EPA treatment was associated with a significant reduction of 24% in the frequency of unstable angina • The occurrence of coronary death or myocardial infarction was not significantly lower (22%) in the EPA group than in controls • The frequency of fatal or non-fatal myocardial infarction was not significantly reduced (23%) in the EPA group
Manson, 2018 (VITAL) [76]	• No difference between the intervention and placebo groups at a median of 5.3 years • Statistically significant reduction in total MI which carried a hazard ratio (HR) of 0.71; 95% confidence interval (CI) 0.59–0.9
Bhatt, 2019 (REDUCE-IT) [1••]	• A primary endpoint* event occurred in 17.2% of the patients in the IPE group, as compared with 22.0% of the patients in the placebo group (hazard ratio, 0.75; 95% confidence interval [CI], 0.68 to 0.83; $p<0.001$), an absolute between-group difference of 4.8 percentage points (95% CI, 3.1 to 6.5); the number needed to treat to avoid one primary endpoint event was 21 (95% CI, 15 to 33) over a median follow-up of 4.9 years *A primary endpoint was defined as a cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, coronary revascularization, or unstable angina
Budoff, 2020 (EVAPORATE) [16•]	• There were no significant differences in basic lipid measures of total cholesterol, LDL, HDL, and triglyceride level from baseline to follow-up • At the 9-month interim analysis, there was no significant change in low-attenuated plaque (LAP) between active and placebo groups
Nicholls, 2020 (STRENGTH) [20••]	• The primary endpoint of cardiovascular death, myocardial infarction, stroke, coronary revascularization, or unstable angina requiring hospitalization occurred in 785 patients (12.0%) treated with omega-3 FA and 795 (12.2%) treated with corn oil (HR, 0.99 [95% CI, 0.90–1.09]; $p=.84$)
Kalstad, 2020 (OMEMI) [23]	• No difference between the intervention and placebo groups after 2 years • Primary endpoint of non-fatal MI, unscheduled revascularization, stroke, all-cause death, and heart failure hospitalization occurred in 108 (21.4%) patients treated with n-3 EPA + DHA vs 102 (20%) in the placebo arm (HR, 1.08 [95% CI, 0.82–1.41]; $p=0.60$)

inhibits COX-1 and COX-2 [25]. Altogether, these effects on platelets may in turn increase the risk of bleeding. In the REDUCE-IT trial, the rates of serious adverse bleeding events were numerically higher in the treatment arm but not statistically significantly different from placebo (2.7% vs 2.1%; $p=0.06$) [1••]. Moreover, none of the serious adverse bleeding events resulted in death in either treatment arm. The rates of hemorrhagic stroke, serious central nervous system bleeding, or gastrointestinal bleeding were similar in both treatment groups. In both STRENGTH [20••] and OMEMI [23] trials, there was no trend in excess bleeding events in those randomized to EPA + DHA compared with placebo.

Concerns of higher incidence of atrial fibrillation have also arisen in trial participants receiving omega-3 FA. In REDUCE-IT, 5.3% of those randomized to EPA developed atrial fibrillation compared with 3.9% in the placebo group [1••]. It is important to note that most of those developing an atrial fibrillation adverse event were those with prior history of atrial fibrillation. Also, 3.1% in the EPA group were hospitalized due to atrial fibrillation compared with 2.1% in the placebo group [1••]. However, there was a reduction in the rates of cardiac arrests and sudden cardiac deaths, suggesting a potential beneficial effect on ventricular arrhythmias. In STRENGTH, those randomized to EPA + DHA had a 62% higher hazard of new-onset atrial fibrillation compared with placebo. Similar to REDUCE-IT and STRENGTH, the OMEMI trial showed a significantly higher incidence of atrial fibrillation in those randomized to EPA + DHA compared with placebo (HR = 1.84; $p=0.006$) [23]. Despite the increase in rate of atrial fibrillation in REDUCE-IT, STRENGTH, and OMEMI, this was not associated with an increase in the risk of stroke. In fact, in REDUCE-IT, IPE supplementation was associated with a 28% risk reduction of fatal or non-fatal stroke. A recent systematic review found that omega-3 FA supplementation was associated with an increased risk of atrial fibrillation, which is particularly higher when the dose of omega-3 FA formulation is greater than 1 g/day [26].

Gastrointestinal side effects are the most common type of side effect observed with omega-3 FA supplementation. Among the adverse events in STRENGTH, gastrointestinal-related adverse events were the leading cause of study drug discontinuation [20••]. By contrast, in REDUCE-IT, the rate of adverse gastrointestinal events was significantly lower in the IPE arm (33%) compared with mineral oil placebo

(35.1%) [1••]. The frequency of gastrointestinal adverse effect seems to be dose-dependent and mediated via bacterial metabolism of omega-3 fatty acids resulting in gas formation and secretory diarrhea [27].

"Over-the-Counter" Omega FA

Figure 1 displays the main concerns regarding the use of these over-the-counter formulations. About 1 out of 2 adults in the USA and more than 70% of adults aged ≥ 65 years use dietary supplements according to a study published using the National Health and Nutrition Examination Survey (NHANES) 2011–2012 data [28]. Dietary supplements are intended for general consumer health but not to treat medical conditions. It is important to highlight this statement because dietary supplements, like omega-3, do not meet regulatory standards required for regular over-the-counter medications, which do undergo rigorous FDA oversight, and do obtain FDA approval for marketing and to be used as treatment for medical conditions [29].

Just like other dietary supplements such as multivitamins, omega-3 supplements are widely available and popular among consumers; indeed, a survey of patients with ASCVD reported that 86% of them were taking omega-3 supplements without the recommendation of a health care professional [30]. These formulations have variable EPA and DHA concentrations, usually much lower than the high-dose formulations studied in randomized clinical trials (RCT). Furthermore, they are typically mixed products, unlike the EPA-only formulation in REDUCE-IT, and therefore, evidence from REDUCE-IT cannot be extrapolated to over-the-counter omega-3 products. However, patients may be more inclined to use omega-3 dietary supplements instead of prescribed high-dose formulations given the lower cost and easier availability of the former compared to the latter. It has been estimated that patients would need to take from 3 to 112 times the number of supplement doses if attempting to reach prescription doses [31]. Notable, as discussed above, DHA only can increase LDL-C levels with undesired effects [32].

There is also significant concern for potential poor quality and purity, given that some of these supplements have been found to include contaminants and oxidation products (i.e., oxidized lipids) in addition to saturated fats and cholesterol [31, 33–36]. It is important to note that oxidized lipids have been associated with negative effects on atherogenic lipid biomarkers and potential implications particularly for those patients at high-risk [33, 37]. Some other studies have suggested that other contaminants such as polychlorinated biphenyls may counteract any potential benefits obtained from dietary intake of omega-3 FA [38].

Omega-3 FA and Effect on Metabolism of TG-Rich Lipoproteins and Other Lipid Measures

Omega-3 FA have different effects on lipids and lipoprotein measures. For instance, DHA has been shown to increase high-density lipoprotein cholesterol (HDL-C) [39, 40]. A crossover trial showed that DHA led to a greater increase in LDL-C and HDL-C levels compared to EPA [39], but had only a modestly greater effect in reducing TG; no differences were noted in apolipoprotein B (ApoB) levels between the two arms [39]. These results were confirmed by a recent meta-analysis [41]. A small trial showed that DHA increased levels of LDL-C, LDL particle size, and the

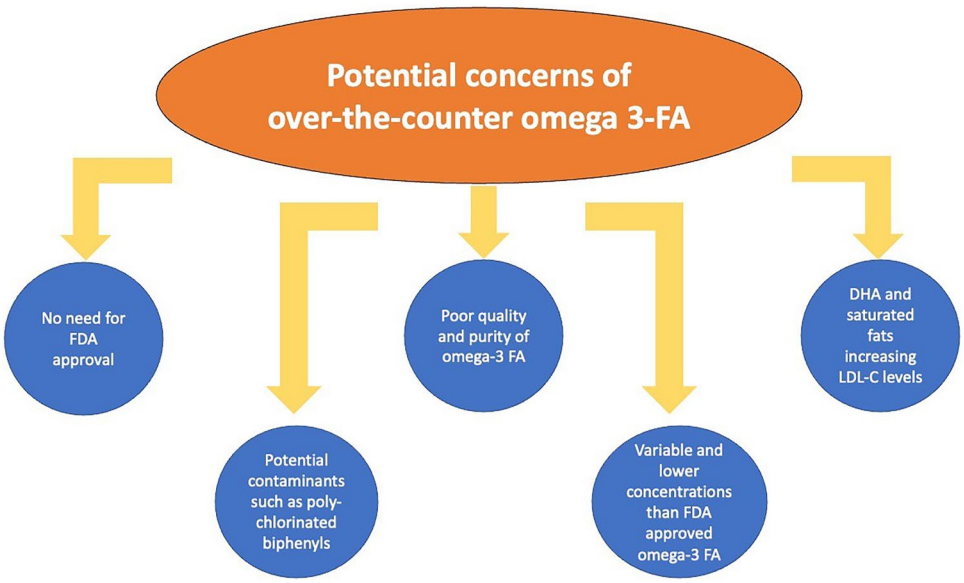

Fig. 1 Main concerns regarding the use of over-the-counter omega-3 FA formulations

HDL-2 subfraction, which were not observed in EPA supplementation [42]. In REDUCE-IT, participants in the IPE arm had a median reduction of 12.2% in non-high-density lipoprotein cholesterol (non-HDL-C) and 9.7% in ApoB [1••] compared to placebo arm. Omega-3 FA have been shown in kinetic studies to reduce postprandial hypertriglyceridemia, as well as remnant lipoprotein cholesterol and the TG content of chylomicrons and VLDL [43–47].

EPA may reduce TRL production by inhibiting transcription of the gene for sterol regulatory element-binding protein-1 (SREBP) [5, 48, 49], a transcriptional factor that plays a major role in synthesis of FA and TG, as well as inhibition of its activation via posttranslational mechanisms [50, 51] and degradation of its active form [52]. These transcriptional factors can "sense" the abundance of FA and cholesterol; for instance, the mature form of SREBP is released from the endoplasmic reticulum for nuclear translocation when the cell is deprived of FA and cholesterol, and binds to the sterol response element region of the downstream lipogenic genes. Some studies have shown that administering omega-3 FA suppresses expression of the SERBP-1 gene at both transcriptional and posttranscriptional levels via two mechanisms: (1) inhibits the formation of the liver X receptor (LXR)/retinoid X receptor (RXR) complex and subsequent SREBP-1 precursor gene expression, and (2) omega-3 FA accelerate the decay of SREBP-1 mRNA [53].

It has also been suggested that this process is mediated by increasing fatty acid oxidation and TG catabolism in adipose and muscle tissues and enhanced TRL clearance by regulation of peroxisome proliferator-activated receptor (PPAR) gene activity [49]. PPARs are transcription factors that function as a heterodimer with RXR and bind to the specific sequence of the promoter region of target genes called PPAR response elements (PPREs) [54]. Three PPAR isotypes have been identified: PPARα, PPARβ/δ, and PPARγ. Activation of PPARα—mostly expressed in the liver—can increase FA oxidation and reduce the excess TG load in the body. PPARβ/δ—which have higher activity in skeletal and cardiac muscles—activate genes involved in FA oxidation. PPARγ is expressed in adipose tissue and increases the expression of genes involved in preadipocyte differentiation into adipocytes and stimulates fatty acid deposition in adipocytes [55]. Omega-3 FA can bind to all three isotypes of PPARs [56], and induce the transcription of genes encoding mitochondrial and peroxisomal enzymes involved in lipid oxidation [57], as well as enhance the lipoprotein lipase (LPL) gene expression in both adipose and muscle tissues, therefore increasing the clearance of TRL [58, 59].

Another effect that has been proposed is the inhibition of diacylglycerol acyl transferase, which is the most important TG-synthesizing hepatic enzyme [60]. Other studies suggested that omega-3 FA have inhibiting effects on phosphatidic acid phosphatase, a regulatory enzyme that catalyzes the conversion of phosphatidate to diacylglycerol [61]. Omega-3 FA supplementation can also promote intracellular degradation of ApoB particles via post-Golgi oxidation [62], which also leads to reduction of the assembly and secretion of VLDL particles in the hepatocyte [63, 64]. Others postulate that omega-3 FA can increase peripheral TG clearance by upregulation of LPL with subsequent reduction in intrahepatic fatty acid pools, the primary source for hepatic TG synthesis [45]. Finally, omega-3 FA may also reduce levels of apolipoprotein C3, which can mediate their TG-lowering effects [65].

Omega-3 FA in the Current Guidelines

In the 2002 American Heart Association (AHA) statement, EPA and DHA were recommended at a dose of 2–4 g/day for reducing TG in patients with hypertriglyceridemia under the direct supervision of a physician with no prescription omega-3 FA available at that time [66]. Since 2004, two types of prescription omega-3 FA have been approved by the US Food and Drug Administration (FDA) for the treatment of hypertriglyceridemia: Lovaza® and Vascepa®. Lovaza® is prescribed as four 1-g capsules or two 2-g capsules, which provide approximately 3 g/day of EPA and DHA combined. Vascepa® is prescribed as two 1-g capsules or four 0.5-g capsules twice daily to provide 4 g/day of IPE. Many dietary supplements are also on the market, but not reviewed or approved by the FDA [67].

The current 2015–2020 dietary guidelines for the American general population recommend consumption of 8 oz per week of seafood which contains an approximate of 250 mg of EPA and DHA. The amount of 250 mg per day is thought to be associated with decreased cardiac death in patients with and without pre-existing cardiovascular disease. The AHA recommends 8 oz of seafood or 2 servings per week as a quantity which is also deemed to be environmentally sustainable [68].

The 2019 AHA/American College of Cardiology (ACC) guidelines for primary prevention of cardiovascular disease give a class I recommendation for a diet rich in vegetables, fruits, nuts, whole grains, legumes, and fish to decrease cardiovascular disease risk factors [69]. An AHA 2018 advisory in regard to omega-3 FA recommends non-fried seafood 1–2 times per week [68]. More recently, a study of Pesco-Mediterranean diet which is a traditional Mediterranean diet with fish being the primary protein source with concomitant suggested a benefit in cardiovascular and neurological health which argues for the role of omega-3 FA [70].

A recent ACC Consensus Statement suggested that the use of IPE would be reasonable as a next step in patients with clinical ASCVD and LDL-C < 70 mg/dL and persistent fasting TG between 150 and 500 mg/dL who are on

Table 4 Summary of guideline recommendations

Professional society	Recommendation
National Lipid Association (NLA) [73]	• 45 years old with clinical ASCVD or 50 years old with diabetes mellitus requiring medication + 1 or more additional risk factors • TG 135–499 mg/dL
European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) [74]	• Patients with high- and very high-risk on statin therapy • TG 135–499 mg/dL
American Diabetes Association (ADA) [77]	• Patients with diabetes mellitus and ASCVD/other risk factors on statin therapy and controlled LDL-C • TG 135–499 mg/dL
American Association of Clinical Endocrinologists (AACE) [78]	• Patients with high-risk on maximally tolerated statin therapy • TG 135–499 mg/dL

maximally tolerated statin therapy [71]. The AHA released an advisory statement identifying IPE as the first non-LDL-lowering therapy with proved cardiovascular benefits and suggested it to be considered first-line therapy for patients with diabetes mellitus and coronary artery disease with TG > 135 mg/dL despite maximally tolerated statin therapy and lifestyle changes [72].

The National Lipid Association (NLA) recommends treatment with IPE for patients older than 45 years old with clinical ASCVD or 50 years old with diabetes mellitus requiring medication plus ≥ 1 additional risk factor (based on entry criteria in REDUCE-IT), with fasting TG 135–499 mg/dL on high-intensity or maximally tolerated statin therapy [73]. Finally, the European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) guidelines give a class IIa recommendation for IPE for patients at high- or very high-risk with TG levels between 135 and 499 mg/dL despite statin treatment [74]. Society guidelines' recommendations for omega-3 FA are summarized in Table 4.

Open Questions and Future Directions

The efficacy of omega-3 FA, however, is an active field of research because their mechanism of action is not fully elucidated, and the inconsistency between the primary endpoints achieved in only some trials. The differences between trials in the use of EPA or mixed EPA + DHA, as well as designs, participant characteristics, doses of active interventions, and placebo arms, could help to explain these discrepancies.

There are additional questions with regard to omega-3 FA. For instance, it would be important to identify the group of individuals who would benefit the most from its use, such as those with low dietary intake of omega-3 FA or genetically predisposed to have low omega-3 FA blood levels. This information would be important to provide future directions aiming to personalize therapy even further.

Additionally, it is unclear the degree of ASCVD risk reduction that can be obtained by IPE supplementation in the setting of aggressive LDL-C lowering (i.e., well below 70 mg/dL). In REDUCE-IT, LDL-C derived from last visit was above 80 mg/dL in both arms. Future trials will need to consider more aggressive LDL-C approaches in the control group, including other more potent therapies such as proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors [75].

Conclusions

Targeting TG-rich lipoproteins represents a new frontier for modulating ASCVD risk. Clinical and basic research evidence suggests that the use of omega-3 FA, specifically EPA, appears to mitigate atherosclerosis by reducing TRL and/or inflammation, therefore addressing the residual risk of clinical ASCVD that remains in the statin era. Discrepancies in results reported in RCTs could be explained by differences in study designs (i.e., placebo arm, omega-3 FA doses and formulations), which may also help to provide mechanistic insights for omega-3 FA.

Declarations

Conflict of Interest Under a license agreement between Corrie Health and the Johns Hopkins University, the University owns equity in Corrie Health and the University, Dr. Marvel and Dr. Martin are entitled to royalty distributions. Additionally, Dr. Marvel and Dr. Martin are co-founders of and hold equity in Corrie Health. This arrangement has been reviewed and approved by the Johns Hopkins University in accordance with its conflict of interest policies. Dr. Marvel has received research support from Apple and iHealth.
Dr. Martin also reports personal fees from Amgen, AstraZeneca, Esperion, 89bio, Sanofi-Aventis, Novo Nordisk, iHealth, Novartis, and DalCor; non-financial support from Apple and iHealth; grants and non-financial support from Google; and grants from Maryland Innovation Initiative, American Heart Association, Aetna Foundation, PJ Schafer

Memorial Fund, David and June Trone Family Foundation, National Institutes of Health, and FH Foundation, outside the submitted work. The other authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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• Of importance
•• Of major importance

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