OMEGA-3 AND CARDIOVASCULAR PREVENTION – IS THIS STILL A CHOICE?

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ABSTRACT

There is currently growing attention being paid to the role of elevated triglycerides (TGs) as important mediators of residual atherosclerotic cardiovascular disease (ASCVD) risk. This role is supported by genetic studies and by the persistent residual risk of ASCVD, even after intensive statin therapy. Although TG lowering drugs have shown conflicting results when tested in cardiovascular outcome trials, data from the REDUCE-IT study with the ethyl ester of ω-3 eicosapentaenoic acid (EPA) have revived hope in this area of research. The aim of the present review is to critically discuss the most recent large trials with ω-3 fatty acids (FAs) trying to elucidate mechanistic and trial-related differences, as in the case of REDUCE-IT and STRENGTH studies. The ω-3 FAs may lower cardiovascular risk through a number of pleiotropic mechanisms, *e.g.*, by lowering blood pressure, by mediating antithrombotic effects, by providing precursors for the synthesis of specialized proresolving mediators that can inhibit inflammation or by modulating the lipid rafts enriched in cholesterol and sphingolipids. In conclusion, in a field fraught with uncertainties, the ω-3 FAs and especially high dose icosapent ethyl (the ethyl ester of EPA) are at present a most valuable approved therapeutic option to reduce the ASCVD risk.

Keywords. ω-3 fatty acids, REDUCE-IT, STRENGTH, icosapent ethyl, inflammation

1. INTRODUCTION

The central role of high-intensity statins to reduce atherosclerotic cardiovascular disease (ASCVD) events has been established in both primary and secondary prevention [1, 2]. However, a substantial residual risk persists despite significant reductions in low-density lipoprotein cholesterol (LDL-C) regardless of the lipid lowering treatment, namely, statins alone or in association with ezetimibe or PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitors [3]. This has sparked interest in treating individuals with residually elevated triglycerides (TGs), who have higher concentrations of atherogenic cholesterol carried by circulating TG-rich lipoproteins [4].

At present, the bioactive ω-3 fatty acids (ω-FAs) eicosapentaenoic (EPA; 20:5 ω-3) and docosohexaenoic (DHA; 22:6 ω-3) acids are attracting increasing attention. Besides exerting significant TG-lowering effects, there is a strong and consistent evidence from large cohort studies that ω-3 FAs reduce incidence and mortality from the main noncommunicable diseases through pleiotropic mechanisms [5]. They also improve outcomes in hospitalized patients receiving artificial nutrition support [6]. Due to their long hydrocarbon chains, EPA and DHA are sometimes termed “very long-chain ω-3 fatty acids” to be differentiated from the 18-carbon-plant-derived ω-3 FAs. EPA and DHA are present in high amounts in most seafood, in the blubber and tissues of sea mammals, in supplements like fish oils, cod liver oil and krill oil [7], and in some algal oils [8]. EPA and DHA are transported in the bloodstream esterified into TGs, phospholipids and cholesteryl esters as components of lipoproteins, although they can be also found in the bloodstream in the non-esterified form non-covalently bound to albumin. Through different mechanisms, EPA and DHA alter cell and tissue responsiveness resulting in more optimal conditions for growth, development and maintenance of health [9]. Many of these effects seem to be mediated by the incorporation of EPA and DHA into the phospholipids of cell membranes, although they can also directly activate membrane G-protein coupled receptors [10]. EPA and DHA have been associated with cardiovascular (CV) and non-CV benefits, *e.g.*, in the case of rheumatoid arthritis [11], asthma [12] or psoriasis [13] as well as in the case of inflammation-driven depression [14]. Relevant to inflammation, EPA and DHA are substrates for the generation of specialized pro-resolving lipid mediators (SPMs) which act to inhibit pro-inflammatory signalling. SPMs include resolvins, protectins and maresins [15].

Pertaining to the cardiovascular benefit of ω-3 FAs, clinical trials evaluating ω-3 FAs can be differentiated into those that studied mixed formulations (EPA + DHA) and those evaluating EPA alone. Interestingly amongst the earliest and most positive outcome studies

was the JELIS (Japan EPA Lipid Intervention Study) randomized open label trial testing EPA ethyl ester at a dose of 1.8 g of EPA per day. In this study enrolling participants with a history of coronary artery disease, elevated TGs and median LDL-C of 136 mg/dl on predominantly low intensity statins, a 19% relative risk (RR) reduction was observed in major coronary events (HR 0.81, 95%CI 0.69, 0.95). The effect was mainly driven by reduction in hospitalizations for unstable angina (HR 0.76, 95%CI 0.62, 0.95) [16]. A secondary analysis of the JELIS study showed that EPA was effective also in reducing the incidence of coronary artery disease (CAD) events for patients with this dyslipidemic pattern (*e.g.*, with abnormal TG and HDL-C levels) [17]. Considering that numerous studies with ω-3 FAs, including primary and secondary prevention trials and meta-analyses, have not hitherto provided entirely consistent results [18, 19], the aim of this review is to critically discuss the most recent outcome trials with ω-3 FAs trying to highlight mechanistic and trial-related differences.

2. Fatty acids and cardiovascular prevention

2.1 Linoleic acid

The health association of polyunsaturated fatty acid (PUFA) intake has been evaluated starting from the 1950s in particular investigating cardiovascular (CV) health in Southern Italian populations [20]. Ancel Keys and a number of other investigators pointed out that the so-called Mediterranean diet - a diet characterized by a high intake of fruits and vegetables, low intake of meat and high content of unsaturated fatty acids – was associated with increased longevity and lower CV risk. In these studies, the relative benefit from monounsaturates such as oleic acid from olive oil and the PUFA linoleic acid (LA; 18:2ω–6) from vegetables and other dietary oils was not investigated in further detail. Support for these early observations came from the Helsinki Study, where a comparative evaluation of diets followed in two psychiatric hospitals led to the conclusion that PUFAs, represented by LA, most likely had CV benefit based on the observation of a significant reduction of CV deaths in the hospital following a more LA-enriched diet [21].

In the following years, however, the prevalence of a Western type of diet - characterized by a high dietary intake of saturated fats, animal proteins, sucrose and a low intake of fibre - together with the availability of data coming from large US and European studies on the link between plasma and adipose tissue LA, led to some scepticism on the real association

between intake of LA and CV risk [22]. Although some reports indicated that a major metabolic effect attributable to LA is the reduction of LDL-C [23], the resulting association with reduced CV risk is still disputed by some. A meta-analysis of observational studies showed that higher LA exposure was associated with a moderately lower risk of CVD (overall pooled Relative Risk (RR) 0.86; (95% CI 0.77, 0.97)) [24]. In pooling data from 30 prospective studies from 13 countries (with median follow-up ranging from 2.5 to 31.9 years) evaluating the association between circulating and adipose tissue levels of LA with incident total CVD, Marklund et al. found a statistically significant reduction of total CVD (Hazard Ratio (HR) 0.93; 95%CI 0.88, 0.99), cardiovascular mortality (HR 0.78; 95%CI 0.70, 0.85), and ischemic stroke (HR 0.88, 95%CI 0.79, 0.98). Conversely, significant changes in CHD risk were not detected (HR 0.94; 0.88, 1.00) [25]. Adipose tissue concentrations of LA are particularly responsive to dietary LA, as demonstrated by diet modification studies and, as such, it is used as a biomarker of dietary LA intake [26].

Other studies did not report a significant correlation between LA content in adipose tissue and CHD risk. Kark et al. showed that a very high LA intake did not confer a lower risk of nonfatal myocardial infarction (MI) [27]. Further evidence even found a raised risk of MI associated with LA when exposure was measured as adipose tissue concentration [28]. A similar conclusion was reported by Pedersen et al. showing that LA and alpha-linolenic acid (ALA; 18:3ω–3) in adipose tissue were intercorrelated and associated with an increased Odds Ratio (OR) of 2.10 (95%CI 0.87, 5.07) and of 1.96 (95%CI 0.83, 4.61), for the risk of myocardial infarction (MI)**,** respectively [29]. Most recently Nielsen et al. evaluated a large cohort of 57,053 middle-aged subjects in a Danish prospective study [30]. A random sample of the full cohort (n = 3,167) and all incident MI cases appearing during 16 years of follow-up (n = 2,819) were considered. Content of LA in adipose tissue was not associated with the risk of MI; evaluation of men and women combined or separately, led to the same conclusion [30].

2.2. From LA to ω-3 FA

From the evaluation of the whole spectrum of FAs in biological samples, it has become more and more apparent that ω-3 FAs but not LA are most strongly linked with lower CV risk. From evaluation of biomarkers in the CHS (Cardiovascular Health Study), it was evident that the greatest risk reduction was found for ω-3 FAs such as EPA and DHA [31]. Individuals

with higher plasma levels of ω-3 FA biomarkers had a lower total mortality: HR 0.83 (95%CI 0.71, 0.98) for EPA; HR 0.80 (95%CI 0.67, 0.94) for DHA, and HR 0.73 (95%CI 0.61, 0.86) for total ω-3 PUFAs. Similar conclusions have been reached in an analysis of the Framingham Heart Study. Participants in the highest (>6.8%) compared to those in the lowest Omega-3 Index (O3I) quintiles (<4.2%) had a 34% lower risk of death from any cause and 39% lower risk of incident CVD [32]; O3I is the sum of EPA plus DHA in erythrocyte (red blood cell; RBC) membranes.

The measurement of the RBC FA fingerprint to predict risk of all cause and CV mortality has been a major advance. RBC ω-3 FAs are representative of both body membrane fatty acid composition and long-term intake of EPA + DHA [33]. By this relatively simple approach, it has been possible to demonstrate that in patients with acute coronary syndrome (ACS), RBC FA profiles contribute significantly to the discrimination of ACS cases, especially when combined with standard risk factors [34]. In the Framingham Offspring Cohort without prevalent CVD, in whom RBC FA levels were evaluated at baseline and after 11 years of follow up, findings were consistent with the hypothesis that ω-3 FAs have anti-inflammatory properties [35]. In these individuals, a FA matrix including saturated and monounsaturated fatty acids and the O3I allowed successful prediction of all-cause mortality, with predictivity as good as a model including classical standard risk factors such as lipid levels, blood pressure, smoking, and diabetes [36].

All these findings have pointed out with certainty that ω-3 FAs are most likely responsible for the beneficial activity of a PUFA-rich diet and thus it is, today, most reasonable to provide individuals with extra ω-3 FAs allowing them to reach optimal blood and tissue levels.

2.3 ω-3 Fatty acids

Recognising the potential for long chain ω-3 FAs (i.e. EPA and DHA) to lower incidence of ASCVD dates back almost 50 years after the observation that native populations in Greenland, Northern Canada and Alaska, consuming their traditional diet, were found to have much lower rates of death from ASCVD than predicted, despite a high dietary fat intake [37-39]; these populations had a low incidence of ischemic heart disease and a complete absence of diabetes mellitus [40]. Such observations were confirmed in a variety of follow-up studies reporting that high levels of EPA and DHA had the potential of reducing or

antagonizing platelet reactivity, of ameliorating oxidative stress, and reducing biomarkers of chronic inflammation such as C-reactive protein and tumour necrosis factor-α (TNFα) [41, 42]. Interest in more recent years has been stimulated by the observed ability of ω-3 FAs to lower plasma TGs [43]. This may occur by reducing very-low density lipoprotein production, by stimulating catabolism of TG-rich lipoproteins by way of lipoprotein lipase [44-47], or through formation of a biliary metabolite that can limit lipid absorption and prevent hepatic lipid accumulation [48]. Besides these effects, ω-3 FAs have demonstrated a blood pressure-lowering effect in individuals at higher CV risk, namely, those with hypertension or hyperlipemia. A dose response meta-analysis showed a nonlinear dose-response relationship for systolic and diastolic blood pressure. The J-shaped curves suggested that dosages of combined EPA+DHA between 2 and 3 g/day were associated with the strongest effect [49]. Among the several mechanisms by which ω-3 FAs can reduce blood pressure, are the reduction of oxidative stress through downregulation of nicotinamide adenine dinucleotide phosphate oxidase, suppression of the xanthine oxidative pathway, and activation of the antioxidant enzyme superoxide dismutase [50-52].

2.3.1 Evidence from the most recent clinical trials

ω-3 PUFA supplements are available in a variety of formulations, including TGs, modified phospholipids, ethyl esters and free fatty acids of the major ω-3s (EPA, DHA, and docosapentenoic acid (DPA; 22:5ω–3)) in different combinations. The most frequently used regimens are daily doses of approx. 1 g of EPA+DHA in 1.5:1 to 3:1 ratios, or EPA monotherapy of 2 to 4 g/day [53]. Several trials on large population samples have used combinations of EPA+DHA such as the ORIGIN (Outcome Reduction With Initial Glargine Intervention) [54], ASCEND (A Study of Cardiovascular Events iN Diabetes) [55] and VITAL (Vitamin D and Omega-3 Trial) [56]. In all of these, a daily dose of a 1 g capsule containing almost 840 mg of EPA+DHA as ethyl esters, came up with essentially non-superiority vs placebo. These studies differ somewhat, the ASCEND and ORIGIN trials enrolling, respectively, patients with dysglycemia and diabetes in primary prevention, whereas the VITAL tested ω-3 FAs vs placebo or vitamin D in healthy people with the aim of reducing cancer or CVD. Although null for its primary outcome, a secondary analysis of VITAL showed that when ω-3 FAs were compared with placebo, the HR for first heart failure (HF) hospitalization was 0.69 (95%CI 0.50, 0.95) in participants with prevalent type 2 diabetes and 1.09 (95%CI 0.88, 1.34) in those without type 2 diabetes [57]. In the VITAL-FH trial

(using a single 1 g capsule/day containing 840 mg EPA + DHA), ω-3 FAs reduced recurrent but not initial, HF hospitalizations by 14% (p = 0.048) [58]. This dosage was the same as that used in the earlier GISSI-HF trial, that showed the superiority of ω-3 FAs vs placebo in reducing death or hospitalization for CV reasons by 8% (number-needed-to-treat 44) and death for any cause by 9% (number-needed-to-treat 56) [59].

Two outcome trials published in 2020 of secondary CV prevention, using different formulations of EPA + DHA, the large STRENGTH (The Long-Term Outcomes Study to Assess Statin Residual Risk with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridemia) and the OMEMI (Omega-3 Fatty Acids in Elderly Patients With Acute Myocardial Infarction) trial on a smaller sample (1,027 participants of age 75 $\pm$ 3.6 y; 29% females) showed neutral results. In the latter trial, treatment with the combination of EPA+DHA was again ineffective, with the composite outcome occurring in 21.4% of participants in the ω-3 group and in 20% of those on placebo, and no difference in total mortality or LDL-C levels [60]. The OMEMI trial had limitations that could have biased the final endpoint: the advanced age of participants and the allowance to consume supplemental cod liver oil for the duration of the trial; assumptions for the power calculation proving too optimistic, largely because of a lower event rate than expected; the addition of the endpoint component of HF hospitalization did not add the number of events that might be expected in an elderly population; the dosage used in OMEMI was about twice that used in comparable earlier trials, although considerably lower than the dosage used in REDUCE-IT and also in STRENGTH.

STRENGTH recruited 13,078 patients with hypertriglyceridemia (TGs $\geq$ 240 mg/dL) and/or diabetes (70%) and was designed with the primary endpoint of testing the efficacy of 4 g/d of a carboxylic acid formulation of EPA and DHA (providing 2.2 g EPA + 0.8 g DHA daily) or corn oil on the composite of cardiovascular death, nonfatal MI, nonfatal stroke, coronary revascularization, or unstable angina requiring hospitalization (Table 1). The study was halted prematurely for futility (HR 0.99, 95% CI 0.90, 1.09) despite a 20% reduction in TG and hsCRP concentrations. An increased rate of new-onset atrial fibrillation (HR 1.69, 95%CI 1.29, 2.21) and of gastrointestinal adverse events was observed [61].

REDUCE-IT (A Study of AMR101 to Evaluate Its Ability to Reduce Cardiovascular Events in High-Risk Patients With Hypertriglyceridemia and on Statin) was an event-driven trial

enrolling patients with mean LDL-C of 75 mg/dL on optimal statin treatment but with moderately elevated TG levels (median value 216 mg/dL) [62] (Table 1). The 8,179 participants had a high CV risk (71% with established CV disease and 58% with type-2 diabetes). Patients receiving 4 g of icosapent ethyl (IPE) per day (2 g twice daily with meals: ~3.6 g EPA as ethyl ester daily) for a median follow up of 4.9 years had an absolute between group difference in primary CV endpoints of 4.8% *vs* placebo, with a number-needed-to-treat of 21; the benefit was consistent in patients with or without a diagnosis of HF [63]. The RR for the primary endpoint was 25%, 26% for the key secondary endpoint, and 32% RR for total ischemic events. The benefit began early in the study and persisted across prespecified interim analyses through to the final analysis [64].

A subsequent evaluation of the trial findings reported a significant decrement in the occurrence of first and all recurrent major CV events by 30%. Specifically, first events fell by 25%, second by 32%, third by 31% and fourth by 48% [65]. Total key secondary endpoint events (cardiovascular death, nonfatal MI, or nonfatal stroke) were reduced by 28%. As reported by the authors, it should be considered that the REDUCE-IT patients represent a population at high risk for ischemic events, as suggested by the annualized placebo primary endpoint event rate (5.74%), which was expected per study design and is consistent with historical data for similar high-risk statin-treated patient populations [65].

Findings of REDUCE-IT indicate that TG reduction (18.3% fall from baseline to one year) may be an important target of ω-3 FA therapy, although possibly not all the benefits reported in the trial were explained by TG lowering. Indeed, even the subset of patients with baseline TGs ≤ 150 mg/dL benefitted [62]. On this matter, it is worth considering that IPE 4 g/day significantly reduced remnant-like particle cholesterol levels versus placebo in patients with elevated TG levels in the MARINE (Efficacy and Safety of AMR101 (Ethyl Icosapentate) in Patients With Fasting Triglyceride (TG) Levels ≥ 500 and ≤ 2000 mg/dL) and ANCHOR (Effect of AMR101 (Ethyl Icosapentate) on Triglyceride (TG) Levels in Patients on Statins With High TG Levels (≥ 200 and < 500 mg/dL)) studies [66]. High remnant-like particle cholesterol or remnant cholesterol levels are strong markers of coronary risk and are likely causal factors for ischemic heart disease [67].

In REDUCE-IT, IPE, the ethyl ester of EPA, was also superior to placebo in reducing CV events and mortality in patients with prior MI. In these patients, the RR pertaining to the

primary composite endpoint dropped by 35% (RR 0.65; 95%CI 0.56, 0.77) and that pertaining to incidence, namely, time to first events, fell by 26% (RR 0.74; 95%CI 0.65, 0.85) [68]. The superiority of IPE was also evident in the case of revascularization. First, revascularizations were reduced to 9.2% compared to 13.3% with placebo (HR 0.66; 95%CI 0.58, 0.76) with a number-needed-to-treat for 4.9 years equal to 24. PCI and coronary artery bypass grafting were also dramatically reduced, respectively, by 32% (HR 0.68; 95%CI 0.59, 0.79) and 39% (HR 0.61; 95%CI 0.45, 0.81) [69]. This conclusion was supported by the results of the EVAPORATE (Effect of Vascepa on Improving Coronary Atherosclerosis in People With High Triglycerides Taking Statin Therapy) trial in 80 coronary artery disease patients. They were allocated into two groups, given a similar dose of IPE as in REDUCE-IT or a placebo and followed for 18 months [70]. Coronary computed tomographic scans showed that IPE was superior to placebo in reducing plaque volume (primary endpoint) by 17%, and fibrofatty and fibrous plaques by 34% and by 20%, respectively, whereas dense calcium did not change between groups. Conversely, data of the HEARTS (Slowing HEART Disease With Lifestyle and Omega-3 Fatty Acids) study, evaluating the superiority of ω-3 FAs (1.86 g EPA and 1.5 DHA daily) on top of statins in reducing progression of noncalcified coronary artery plaque did not meet the primary endpoint [71].

Comparison of the STRENGTH (2.2 g EPA + 0.8 g DHA daily) *vs* the REDUCE-IT (3.64 g EPA only daily) studies raised the question of possible deleterious effects of DHA in STRENGTH or the use of mineral oil as the placebo in REDUCE-IT [72], with the potential of the latter to raise LDL-C and hsCRP, might be potentially responsible for the different findings [73]. In the STRENGTH trial, free fatty acids rather than ethyl esters were used with the hypothesis of bioavailability advantage, because ω-3 carboxylic acids are freely absorbed and have less dependence than ethyl ester formulations on coadministration with a fat-containing meal [74]. The choice of the placebo and the dose of placebo was another aspect given high consideration [75]. Pharmaceutical-grade mineral oils are composed of saturated hydrocarbons that are highly refined to achieve extremely low levels of aromatic hydrocarbon impurities, to ensure elimination of carcinogens and compliance with international Pharmacopoeia monographs (Figure 1). A recent narrative review reported that among eighty studies in which mineral oil was used as a comparator, adverse events were generally gastrointestinal and consistent with use as a lubricant laxative. Changes in TG, LDL-C, HDL-C, hsCRP and other biomarkers were inconsistent and generally not statistically significant or clinically meaningful with mineral oil, as were changes in blood

pressure. Moreover, analyses prepared for an FDA public Advisory Committee showed that LDL-C levels did not influence time to primary and secondary endpoints in REDUCE-IT [76]. Changes in LDL-C found in the placebo arm of the REDUCE-IT and calculated by the Martin/Hopkins formula were far below the threshold of 40 mg/dL which is generally considered to translate to a 22% rise in CV risk. However, relative to other biomarkers involved in pathways known to associate with atherosclerosis risk [77-80], these were raised in participants allocated to mineral oil. At the last visit, median changes were +9.5% (+1.12 μmol/L) for homocysteine, +7.6% (1.15 mg/dL) for lipoprotein(a), +5.1% (2301.8 mU/L) for oxidized LDL, +26.3% (0.73 pg/mL) for interleukin 6, +48.3% (0.03 pg/mL) for interleukin 1β, +25.8% (33.4 nmol/min*mL) for lipoprotein-associated phospholipase A2 and +30.1% (0.42 mg/L) for hsCRP [81].

The hypothesis that contrasting results in REDUCE-IT *vs* STRENGTH could be possibly explained by differences in effect of active oils and comparators (mineral oil *vs* corn oil) on lipid traits and hsCRP, or in study populations (high *vs* moderate risk patients) was the issue of a cohort mimicking trial using data extrapolated from the Copenhagen General Population Study. The conclusion was that the contrasting results between the two trials could be explained by a difference in effect of comparator oils but not active oils [82], Steg and Bhatt pointed out that analyses of mimicking trials correlated baseline levels of lipids and hsCRP to differences in risk without taking into consideration follow-up and confounding differences, namely, baseline characteristics [83]. This aspect among others was reported among the limitations of the study, *e.g.*, exact statin doses during follow-up were unknown; the use of non-fasting lipid profiles; the use and adherence to medications that could affect the outcomes were not taken into consideration [82]. Furthermore, in the accompanying editorial pertaining to the Copenhagen General Population Study [84], it was described that the corn oil comparator of the STRENGTH was high in LA, contributing roughly 1% of daily energy. It should be recalled that at least in observational studies, each 1% increment in energy from PUFAs was associated with an 3-7% lower incidence of CHD [85]. To sum-up, as hypothesized by Ridker et al. the resolution of this controversy can only be addressed by running a biomarker trial randomly allocating patients to icosapent ethyl and a non-mineral oil comparator [81].

Comparison of differences in progression of total plaque and total non-calcified plaque volumes in mineral oil placebo patients from EVAPORATE *vs* the non-mineral oil placebo

arm of the Garlic 5 (Effect of Aged Garlic Extract (AGE) on Improving Coronary Atherosclerosis in People With Type 2 Diabetes Mellitus) study showed no significant relationship between mineral oil placebo consumption and progression of coronary plaque volumes [86, 87]. This evidence reinforced results from the previous CHERRY (Combination therapy of eicosapentaenoic acid and pitavastatin for coronary plaque regression evaluated by integrated backscatter intravascular ultrasonography) study that combination of EPA and pitavastatin significantly reduced coronary plaque volume compared to pitavastatin alone in patients with coronary heart disease [88].

Other possible factors explaining differences between the results achieved in the two trials could be the larger reductions in apoB and hsCRP achieved in REDUCE-IT vs STRENGTH (compared to comparators), respectively, -9.7% vs -2% and -39.9% vs -20%. As each apoB-containing lipoprotein contains a single molecule of apoB, concentrations of apoB are a direct measure of the total number of atherogenic lipoproteins and believed to be the most predictive parameter of CV risk [89]. Relative to hsCRP, although Mendelian randomization studies failed to prove a causal link between genetic variants of CRP affecting protein level and risk of coronary heart disease [90], it is undisputable that hsCRP adds prognostic information on CV risk comparable to blood pressure or cholesterol [91]. Interestingly, although the results of the PREPARE-IT 1 (Prevention and Treatment of COVID19 With EPA in Subjects at Risk – IntErvention Trial) indicated that use of high-dose IPE (8 g/day) for 60 days did not prevent incident SARS-CoV-2 infection among healthy participants who did not have prior known infection or vaccination against COVID-19, levels of hsCRP were not changed in either arm, including the placebo arm, which received mineral oil placebo.

To address whether achieving high levels of EPA after treatment with a ω-3 carboxylic acid formulation of EPA and DHA could be associated with CV benefit or whether achieving high levels of plasma DHA undermined the benefit of EPA, a secondary analysis of the STRENGTH study was performed. Achieving the highest tertiles of EPA and DHA was associated with neither benefit (HR 0.98; 95%CI 0.83, 1.16) nor harm in patients at high CV risk [92]. These data provide no evidence that achieving higher levels of EPA or DHA was associated with a CV benefit. Evaluation of plasma EPA levels, 12 months after randomization, showed that individuals in the top tertile reached levels of 151 (132-181) μg/mL, that were close to the median levels observed in REDUCE-IT (144 μg/mL). Overall, median EPA levels in STRENGTH were 89 μg/mL (38% lower than in REDUCE-IT) with a

+269% rise vs placebo, differently from the median +400% rise found in REDUCE-IT. This difference likely reflects the lower EPA dose used in STRENGTH than in REDUCE-IT (3.84 vs 2.2 g/day). In line with this evidence, a *post-hoc* analysis of REDUCE-IT emphasized the strong association between plasma EPA concentrations and ASCVD risk reduction, particularly in those achieving levels between 140 and 200 μg/mL. Benefits were beyond those that could be explained by the degree of TG lowering or other biomarker changes, such as in LDL-C, HDL-C or hsCRP [93]. This observation is in line with a secondary analysis of the JELIS trial in which individuals with EPA levels ≥ 150 μg/mL were those who benefitted the most. The risk of major coronary events was significantly decreased (20%) in the group reaching on-treatment plasma EPA concentrations of 150 μg/mL or more compared with those achieving EPA levels ≤ 87 μg/mL [94]. A secondary analysis of the OMEMI study showed that greater on-treatment increases in EPA levels were associated with lower risk of MACE (HR 0.86; 95%CI 0.75, 0.99), the lowest risk being found in the top three quartiles of EPA increase (HR 0.39; 95%CI 0.19, 0.79). Conversely, changes in DHA did not associate to CV outcomes (HR 0.84; 95%CI 0.66, 1.06) [95]. Overall, as elsewhere reviewed, there may be a required threshold of EPA to be reached, namely 100 μg/mL, to achieve a clinical benefit [96]. In this context, evaluation of the link between blood ω-3 FAs and total and cause-specific mortality over a follow-up of 16 years showed that, among 17 prospective studies, risk for death from all causes was significantly lower (by 15-18%) in the highest vs the lowest quintile for circulating long chain (20-22 carbon) ω-3 FAs (EPA, DPA, and DHA) [97]. In line with this evidence, the main conclusions of the MERLIN-TIMI 36 (Metabolic Efficiency With Ranolazine for Less Ischemia in Non-ST Elevation Acute Coronary Syndromes) trial were that in patients after non-ST-segment-elevation-acute coronary syndrome, plasma long-chain ω-3 FAs were inversely associated with lower odds of sudden cardiac death, independent of traditional risk factors and blood lipids [98].

Finally, the higher percentage of bleeding found in REDUCE-IT *vs* STRENGTH appears to indicate a stronger antiplatelet activity exerted by IPE, again perhaps because of the higher dose of EPA. On this matter, as elsewhere hypothesized, it seems that IPE could be a therapeutic approach to reduce residual cardiovascular risk by targeting not only TGs, but also inflammation and thrombosis (Figure 2) [43].

Further analyses of REDUCE-IT (namely, REDUCE-IT RENAL) indicate that treatment was effective also in patients with impaired kidney function, the largest reduction in CV deaths

occurring in patients with the lowest eGFR [99]. However, 4.2% of patients given IPE experienced atrial fibrillation/flutter vs 3.0% of those given placebo, with an overall HR of 1.42 and 5.4% had serious bleeding vs 3.6% (placebo) with a HR of 1.40. The study therefore supports a significant preventive activity of IPE 4 g daily in renal patients, albeit with care.

A similar maintenance of benefit was found in patients with a history of previous coronary artery bypass grafting (REDUCE-IT CABG). In a total of 1,837 of the REDUCE-IT participants with a history of CABG, randomization to EPA was associated with a significant reduction in the primary (HR 0.76) and key secondary endpoints (HR 0.69) as well as in the total (first, subsequent or recurrent) ischemic events (HR 0.64) [100]. This absolute risk reduction of 6.2% (95% CI 2.3, 10.2) in the first events, leads to a number-needed-to-treat of 16 (95% CI 10, 44) during a median follow up time of 4.8 years.

Finally, a meta-analysis comprising 149,051 individuals relative to the CV benefit of ω-3 FAs - as monotherapy (EPA) or in association (EPA+DHA) - concluded that ω-3 FAs reduce CV mortality and other CV outcomes. Specifically, for CV mortality the RR was 0.93 (95%CI 0.88, 0.98), for non-fatal MI the RR was 0.87 (95%CI 0.81, 0.93), for CHD the RR was 0.91 (95%CI 0.87, 0.96), for MACE the RR was 0.95 (05%CI 0.92, 0.98) and for revascularization the RR was 0.91 (95%CI 0.87, 0.95). Interestingly, the protective effect of EPA+DHA supplementation on CVD events and MI appears to increase with dosage [101], namely, every 1 g/day EPA+DHA corresponded to 9% and 5.8% lower risk of MI and CVD events, respectively. For all these endpoints, a greater RR reduction in CV outcomes was found when EPA was given as monotherapy, *e.g.*, for MACE, the RR was 0.78 (95%CI 0.71, 0.85) with EPA monotherapy while EPA+DHA was associated to a RR of 0.99 (95%CI 0.95, 1.02).

Concerning safety issues, the potential onset of atrial fibrillation (AF) associated with ω-3 FA treatment was the object of a meta-analysis by Gencer et al. [102]. Among 81,210 patients, the majority (72.6%) were enrolled in trials testing ≤ 1 g/d ω-3 FAs and 27.4% in trials testing ≥ 1g/d. In a weighted analysis with an average follow up of 4.9 years, ω-3 FA supplements were associated with increased risk of AF; the HR was greater in those on ≥ 1 g/d (HR 1.49) compared with dose on ≤ 1 g/d (HR 1.25). In a meta-regression analysis, the HR for AF increased by 1.11 for every 1 g of higher dosages of ω-3 FA (thus indicating some care in the use of the high dose ω-3 FAs). However, since the CV benefit of ω-3 FAs appears to be

dose-dependent, the associated AF risk should be balanced against the benefit on overall CV outcomes. Finally, pertaining to the OMEMI study, a secondary analysis showed an independent association between larger on-treatment increases in EPA and lower incidence of CV events in elderly post-AMI patients. Conversely, there was no significant association with changes in DHA [95]. ω-3 FAs (as free fatty acids) cause acute atrial conduction slowing, suppress AF inducibility, at times simulating atrial flutter and enhancing atrial flutter inducibility. Free EPA and DHA, in contrast to esterified forms, cause significant atrial conduction slowing with minimal effect on tissue refractoriness [103]. Furthermore, in the myocardium, ω-3 FAs influence ion channel activity in a manner dependent on lipid composition and fluid dynamics [104]. Finally, when postoperative AF is considered, ω-3 FAs might have a protective effect with a 16% reduction [105].

4. In search of mechanistic insights

4.1. Structural features

The availability of ω-3 FAs as seemingly powerful tools to reduce CV risk has led to investigations on structural and functional features of the two major molecules, EPA and DHA. They differ in structure, DHA (22:6) having an additional double bond (6 in total) and two more carbons compared with EPA having five double bonds (20:5). DHA and EPA are esterified in the phospholipid bilayer of cell membranes, but, because of their structural differences, DHA and EPA differ physicochemically, such that they can have different effects on lipid raft formation, oxidation rate and signal transduction [106]. DHA and EPA segregate differently between raft and non-raft domains of membranes and the resulting changes in molecular organization within the plasma membrane modulate the conformation, movement and interactions, and thereby the activity, of signalling proteins [107].

Relative to membrane structure, EPA and DHA affect distinct regions of the membrane lipid bilayer due to differences in hydrocarbon length and number of double bonds. The longer hydrocarbon length and higher number of double bonds for DHA leads to 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 [43]. After being added to cell membranes, DHA undergoes rapid conformational transitions (in less than 50 ns), whereas EPA does not undergo such similar rapid changes, allowing EPA to be freely distributed throughout the

membrane [108]. EPA is inserted into lipoprotein particles and membranes in a manner that scavenges reactive oxygen species (ROS), an effect due to its peculiar conjugative resonance stabilization [109]. The antioxidant effect mediated by EPA creates a unique ability to quench ROS associated with cellular membranes and lipoproteins. This feature limits the amount of oxidized LDL in plasma, a property that is increased in combination with atorvastatin. These two amphipathic molecules further stabilize unpaired lipid free radicals and thereby reduce oxidative damage and formation of oxidized LDL [110].

Cholesterol plays a major role in determining and modulating membrane fluidity. If excessively accumulated in membranes, cholesterol promotes the formation of extracellular cholesterol crystals, a constant feature within the necrotic core of the atherosclerotic plaque [111] (Figure 3). Cholesterol crystals induce inflammasome activation that induces autocleavage and activation of caspase 1. Active caspase 1 can then cleave pro-IL-1β and the constitutively expressed pro-IL-18 to their mature, secretable forms [112]. Intriguingly the shape of cholesterol crystals produced by macrophages depends on the lipid composition of cellular membranes involved in the process of cholesterol crystallization [113]. When membrane cholesterol content is high, EPA and DHA exhibit different effects on the apparent area expansion module of membranes [114]. Overall, it seems that DHA promotes membrane cholesterol domains, compared to EPA. Namely, DHA promotes rapid isomerization or conformational changes in the membrane due to the longer hydrocarbon length, whereas EPA preserves a more ordered membrane structure [115, 116]. By using small-angle x-ray diffraction approach, Sherrat et al. showed that EPA has a well-defined location in the hydrocarbon core region of the membrane bilayer, whereas DHA gives rise to changes in electron density consistent with a location in the phospholipid head group corresponding to an increased molecular volume or disorder [109]. However, as noted elsewhere, when EPA and DHA were combined in equal amounts, their separate membrane effects were attenuated [117]. The same authors compared the effects of the phospholipid-linked EPA and DHA on membrane structure in the presence of cholesterol and/or phospholipids (PLs) with heterogeneous acyl chains at different concentrations [118]. PLs containing esterified ω-3 FAs (PL-EPA and PL-DHA) have pronounced effects on membrane structure that highly depend on the surrounding lipid composition, including cholesterol and saturated FAs. With PL-EPA, the membrane hydrocarbon electron density is raised over an area of ± 0-10 Ångström (Å) from the center, indicative of an extended orientation, whereas

PL-DHA only raises electron density in the PL head group region with disorder in the hydrocarbon core.

Concerning plaque formation/progression and the incorporation into thin-cap atherosclerotic plaques, a head-to-head comparison between EPA and DHA showed that in *ApoE*$^{-/-}$ mice EPA is distributed more densely in the thin-cap plaques than in the thick-cap plaques, whereas DHA is more evenly distributed. Moreover EPA, but not DHA, significantly reduces the intimal thickness of macrophage-rich plaques; cholesteryl ester EPA levels were 36-fold higher in the plaque compared to 4.4-fold for cholesteryl ester DHA and EPA was colocalized with anti-inflammatory M2 macrophages in thin-cap plaques [119]. In this context, considering that fibrous cap thickening may help stabilize atherosclerotic plaques, higher levels of EPA in plaques have been associated with decreased plaque inflammation and raised stability [120].

Of note is also the effect of EPA to prevent HDL oxidation as well as of EPA-enriched HDL to enhance the HDL cholesterol efflux capacity [121], a property of HDL linked to atheroprotection [122] (Figure 3). Remarkably, EPA, but not DHA, incorporation, stimulates ABCA1-mediated cholesterol efflux from human THP-1 macrophages [123].

In atherosclerosis, endothelial cell dysfunction associates with abnormal vasomotor control and loss of nitric oxide (NO) bioavailability. Studies in human umbilical vein endothelial cells (HUVECs) showed that treatment with ω-3 FAs (EPA, DHA) and ω-6 FAs (ARA) had differential effects on NO bioavailability. HUVECs treated with EPA had significantly greater NO release with a concomitant reduction in release of $ONOO^-$; ARA did not significantly alter either NO or $ONOO^-$ release while DHA only raised NO [124].

4.2. ω-3 FAs and inflammatory processes

Inflammation is an obligatory marker of atherosclerotic cardiovascular disease, resulting from the pro-inflammatory activity of cholesterol itself as well as from other well-established molecular mechanisms [125]. Considering that membrane FAs can be converted to active molecules regulating inflammatory processes, the basic anti-inflammatory mechanism associated to FAs is the incorporation of ω-3 FAs into cell membranes at the expense of arachidonic acid (ARA), resulting in inhibited ARA metabolism and consequent decreased

expression of the COX gene and, as a final consequence, reduction of ARA-derived eicosanoids [126].

These processes are activated by inflammatory stimuli through enzyme activation, such as phospholipase A2 (PLA2). Human PLA2, responsible of the hydrolysis of the *sn*-2 acyl chain of glycerol phospholipids, has differential preference between specific ω-3 FAs (EPA and DHA) compared to the ω-6 ARA. PLA2 constitutes a superfamily of enzymes composed of six different types that contribute to numerous biological functions by hydrolysing membrane glycerophospholipids producing lysophospholipids and free fatty acids [127]. In particular, three different PLA2 forms, *i.e.*, cytosolic cPLA2, calcium independent iPLA2 and secreted sPLA2 are crucial in the processes of inflammation-driven ARA release, concomitant with the anti-inflammatory features of EPA and DHA. Human cPLA2 selectively prefers ARA, whereas iPLA2 prefers EPA and sPLA2 prefers DHA as the substrate [128].

ARA is usually the major PUFA in membranes of cells involved in inflammation, being a precursor to a number of potent pro-inflammatory mediators including prostaglandins (PGs) and leukotrienes (LTs). Thus, it is commonly believed that increasing dietary intake of the ω-6 fatty acids ARA or its precursor LA raises inflammation [129]. While the metabolism of ARA mainly leads to inflammatory products, since when released from cells it is a substrate for cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P-450 enzymes resulting in eicosanoids that regulate inflammatory processes including PGs, thromboxanes (TXs) and LTs [130], the case of ω-3 FAs is more complex (Figure 4).

Incorporation of EPA and DHA is partly at the expense of ARA [131] and EPA has been shown to inhibit ARA metabolism [132]. Thus, a number of studies in healthy humans or in patients with chronic inflammatory conditions, *e.g.*, rheumatoid arthritis (RA) or inflammatory bowel disease, have shown a reduced production of ARA-derived PGs, TXs and LTs following use of supplements with EPA and DHA for weeks to months [133]. It appears that a threshold for EPA intake should be considered because at a daily intake of 1.35 g/ day for 3 months EPA did not influence neutrophil or monocyte phagocytosis and respiratory burst, whereas an intake of 4 g/day proved optimal, particularly in elderly people [134]. One more reason to believe that EPA doses may have a significant influence on their anti-inflammatory activity, with an apparent contrasting role of ARA, is most likely related to the mononuclear cell membrane EPA content. An important benefit of increasing the EPA intake is, in fact, an

increase in the EPA:ARA ratio in monocyte membranes, leading to a decrement in PGE_2 levels as a function of the increasing EPA:ARA ratio [134].

In the inflammatory process [135], the 4-series LTs and the 2-series PGs derived from ARA are proinflammatory and mediate key events in the development of atheromatous plaques. Conversion of EPA by COX, LOX and P-450 enzymes follows a similar pathway to that of ARA with, however, notable differences, since EPA gives rise to the 3-series PGs and TXs and to the 5-series LTs, all generally less biologically active than those produced from ARA; for example LTB_5 is 10 to 100-fold less potent as a neutrophil chemoattractant compared to LTB_4 from ARA. Moreover, it should be considered that EPA-derived mediators often have less affinity for eicosanoid receptors compared to the ARA-derived ones [136]. Consistently, numerous studies have reported that in healthy volunteers, supplementation with ω-3 FAs leads to a reduction in the 2-series PGs and 4-series LTs [137, 138].

Aside from the antagonism to ARA, EPA and DHA reduce production of major inflammatory cytokines, such as tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and IL-8 although relatively high doses (> 2 g EPA + DHA per day) may be necessary. A similar antagonism has been also described in the case of endothelial adhesion molecules like vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) [133]. A direct evaluation of the two fatty acids (EPA and DHA) was carried out by Allaire et al. [139], testing in a double-blind fashion supplementation of EPA and DHA (both 2.7 g/d) vs corn oil (control group) for a period of 10 weeks. Compared with EPA, DHA appeared to lead to a larger reduction of IL-18 (-7.0 vs -0.5%), hsCRP (-7.9% vs -1.8%) and TNF-α (-14.8% vs -7.6%) and to raised adiponectin (+3.1% vs -1.2%) vs EPA; effects on IL-6 (-12.0% vs -13.4%) were similar. Interestingly DHA led to a more pronounced reduction of TGs (-13.3% vs -11.9%) and a greater increase in HDL-C (+7.6% vs -0.7%) and LDL-C (+6.9% vs +2.2%) vs EPA. Different conclusions were reached by Pisaniello et al. reporting instead that supplementation with EPA, more than DHA, ameliorates acute and chronic vascular inflammation. EPA (4 g/day) reduced the expression of the C-C motif chemokine ligand 2 (CCL2) by 25% and vascular inflammation compared with placebo [140]. Additionally, available reports suggest that purified EPA may affect red cell distribution width (RDW) associated with ischemic heart disease and chronic inflammation with raised RBC deformability [141, 142].

In the search for other biological mechanisms, there is now clear evidence that EPA-driven effects on the inflammatory environment may be consequent in part to inactivation of the NF-κB signal transduction pathway and in part to reduction in the phosphorylation of inhibitors of κB (IκB) [143]. NF-κB exists in the cytoplasm in an inactive form associated with the regulatory protein IκB, this latter being an important step in NF-κB activation. NF-κB is clearly one of the most important regulators of proinflammatory gene expression, such as TNF-α, IL-1β, IL-6, and IL-8 [144]. A similar antagonistic effect on NF-kB activation is exerted by DHA in response to lipopolysaccharides [145].

A further mechanism linking ω-3 FAs and NF-κB activation involves peroxisome proliferator-activated receptor γ (PPAR-γ) which physically interferes with the translocation of NF-κB into the nucleus. Moreover, activation of PPAR-γ by ω-3 FAs is at least partly responsible for the inhibition of the proinflammatory cytokines TNF-α and IL-6 following LPS stimulation [146]. Besides the activation of PPAR-γ, it is worth mentioning that ω-3 FAs exert a large fraction of their metabolic activity by an overall activation of the PPAR system. Activation of the PPAR-α system by ω-3 FAs falls in the same pharmacological area of other molecules activating PPAR-α, such as fibrates and similar molecules. Years ago our group proposed the term ”fraudulent fatty acids” for these molecules [147]. ω-3 FAs, particularly EPA, activate the peroxisomal oxidative system in preference to the mitochondrial system, because of the weaker recognition by carnitine palmitoyltransferase. Accordingly, ω-3 FAs act as “fraudulent fatty acids” leading to the classical cellular morphological changes exerted by PPAR-α agonists [148].

These initial observations on the physiology and possible pharmacological effects of ω-3 FAs have led to a wide series of investigations indicative of an activity of these agents on glucose homeostasis in prediabetes and type 2 diabetes mellitus. One of the possible effects of EPA is to minimize insulin resistance as shown by the analysis of adipose tissue adipokine levels, proteomic studies in cultured adipocytes, and animal studies indicating that including EPA in high-fat diets is associated with reduced adipose inflammation and lipogenesis and elevated markers of fatty acid oxidation [149]. However, whereas mice receiving one week of treatment ≥96% EPA ethyl ester or 46.5% EPA and 37.5% DHA ethyl esters followed by 6 weeks on a high fat regimen inducing diabetes had an improvement in insulin resistance and fasting insulin, an improved glucose tolerance was found only with 96% EPA ethyl ester.

This improved pancreatic β cell function and increased the number of small sized islets. 96% EPA ethyl ester also reduced liver TGs and increased the expression of liver FA oxidation genes [150].

The full explanation for the potent anti-inflammatory activity of ω-3 FAs, in addition to the activity on ARA-derived eicosanoids and inflammatory gene expression, has been provided by the identification of the so-called pro-resolving lipid mediators from EPA and DHA. **Resolvins** (short for resolution phase interaction products), produced from EPA (E-series) and DHA (D-series) and **protectins** and **maresins** (short for macrophage mediators in resolving inflammation) from DHA have been well characterized [151, 152]. Collectively these are term specialized pro-resolution mediators or SPMs. Extensive investigations on resolvins, protectins and maresins in cell culture and animal models have provided convincing information on their anti-inflammatory activities; for example, resolvin E1, resolvin D1 and protectin D1 all inhibit trans-endothelial migration of neutrophils, preventing their infiltration to the site of inflammation. Moreover, resolvin D1 inhibits the production of IL-1β whereas protectin D1 inhibits TNF-α and IL-1β production [153, 154]. Pertaining to atherosclerosis [155], most SPMs were detected in advanced atherosclerotic plaque, but resolvin D1 was higher in stable than unstable plaques although protectin D1 was not different [156].

All these biological activities, similar to the case of the classical eicosanoids, are mediated by specific G protein coupled receptors. Reactivity of these SPMs has raised considerable interest in the case of atherosclerosis characterized by persisting chronic vascular inflammation [157]. SPMs are autacoids, which by definition suggests that local tissue microenvironments play important roles in the production and function of these mediators. The balance of pro-inflammatory mediators and SPMs during acute inflammation will regulate the duration of inflammatory responses and the timing of tissue resolution, *e.g.*, by promoting apoptosis and efferocytic clearance of inflammatory cells and other processes that dampen inflammation and repair collateral damage [158].

The balance between SPMs and proinflammatory mediators has emerged in the raised risk of plaque rupture in atherosclerosis with advanced plaque progression, an event that can be suppressed by ‘normalizing’ resolution:inflammation imbalance during a critical period of plaque development. Reduced levels of SPMs, including resolvin D1, in blood, saliva, and

locally in plaques, correlates with atheroprogression and plaque severity [159, 160]. Mechanistically, administration of resolvin D1 to *Ldlr*$^{-/-}$ mice during plaque progression promotes plaque stability, including decreased lesional oxidative stress and necrosis, improves lesional efferocytosis, and leads to thicker fibrous caps [156]. Among other possible mechanisms in the link between ω-3 FAs and anti-inflammatory activity, the role of G protein-coupled receptor 120 (GPR120) as an ω-3 FA receptor/sensor has been recently uncovered. Stimulation of GPR120 with ω-3 FAs caused broad anti-inflammatory effects in monocytic RAW 264.7 cells and in primary intraperitoneal macrophages, all changes abrogated by GPR120 knock-down [10]. Conversely the resolvin D1 receptor GPR32 transduces inflammation resolution and atheroprotection [161].

5. Conclusion

It is undeniable that there has been a changing landscape of atherosclerosis that is no longer LDL-C centric [162]. Indeed, atherosclerosis now affects younger people, more women and individuals from a diverse range of ethnic backgrounds [163]. Specifically related to the topic of the present review, although the knowledge of TG metabolism is substantial, the understanding of the pathobiology relating hypertriglyceridemia and raised CV risk is still a work-in-progress [164]. In the meantime, although the use of EPA ethyl ester to reduce ASCVD risk seems beneficial, it should be borne in mind that different outcome trials evaluating ω-3 FA supplementation have produced varying results. On this matter, it is, thus, imperative to understand differences in the impact of formulation and the distinct effects of the ω-3 FAs on lipid oxidation, inflammation, membrane structure/organization, cholesterol domain formation, and endothelial cell function [165]. Thus, it becomes of interest to look forward to the results of the RESPECT-EPA (Randomized trial for Evaluation in Secondary Prevention Efficacy of Combination Therapy – Statin and Eicosapentaenoic Acid) trial which is an open-label study assessing the efficacy of 1.8 g/day of EPA as ethyl ester in Japanese adults with known CHD. This study will help to further clarify the contribution of EPA to ASCVD reduction [166].

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Figure legends

Figure 1. Mineral oil grade classifications. Asterisks and arrows represent refinement/hydrogenation processing steps: removal of impurities such as aromatic compounds, unsaturated compounds, and nitrogen- or sulfur-containing compounds. Reproduced with permission of Oxford University Press [76].

Figure 2. Possible impact of icosapent ethyl in the management of residual cardiovascular risk – hypothesis based on data of the REDUCE-IT (A Study of AMR101 to Evaluate Its Ability to Reduce Cardiovascular Events in High-Risk Patients With Hypertriglyceridemia and on Statin) study. Reproduced and modified with permission of Elsevier [43].

Figure 3. Scheme of pleotropic effects driven by EPA. EPA, eicosapentaenoic acid.

Figure 4. The pathway of biosynthesis of ω-6 and ω-3 fatty acids.

Table 1. Major characteristic of ω-3 FAs outcome studies

	JELIS	**REDUCE-IT**	**STRENGTH**	**OMEMI**
Patients enrolled (n)	18,645 (Hypercholesterolemic)	8179 (High-CV risk)	13,078 (High CV risk, raised TG and low HDL-C)	1027 (Elderly patients with recent acute MI)
Age (years)	61	64	62.5	74
Baseline LDL-C (mg/dL)	178	75	75	75
Baseline HDL-C (mg/dL)	57	40	36	49
Baseline TG (mg/dL)	153	216	240	115
Reduction in TG	9% from baseline	17%[&]	19%[&]	8% from baseline
Baseline EPA	97 μg/mL	26.1 μg/mL	21 μg/mL	2.8 weight%
Achieved EPA	169 μg/mL	144 μg/mL	89.6 μg/mL	5.1 weight%
Rise in EPA levels (%)	70	~ 400	269	87
Secondary prevention	19.7%	71%	56%	96.6% were on statin
Follow-up (years)	4.6	4.9	3.5 (stopped)	2
Comparator	Statin alone	Mineral oil	Corn oil	Corn oil
Active oil	1.8 g/d EPA	Icosapent Ethyl (4 g/d providing ~3.6 g EPA as ethyl ester)	ω-3 carboxylic acids 4 g/d (providing ~2.2 g EPA + 0.8 g DHA)	EPA/DHA (1.8 g/d): 930 mg EPA and 660 mg DHA
Primary endpoint	Major coronary events[$]	MACE	MACE	MACE[£]

Hazard ratio	0.81 (0.69-0.95)	0.75 (0.68-0.83)	0.99 (0.90-1.09)	1.08 (0.82-1.41)

CV, cardiovascular; EPA, eicosapentaenoic; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.
[&] Between Group Difference (Median % changes from baseline)
[$] Major coronary event, including sudden cardiac death, fatal and non-fatal myocardial infarction, and other non-fatal events; MACE= composite of cardiovascular death, nonfatal MI, nonfatal stroke, coronary revascularization, or unstable angina.
[£] a composite of nonfatal MI, unscheduled revascularization, stroke, or all-cause death.