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N-3 Polyunsaturated Fatty Acids and Their Role on Cardiovascular System

Savina Nodari and Francesco Fioretti

Abstract

The interest in n-3 polyunsaturated fatty acids (n-3 PUFAs), their favorable effects on the cardiovascular (CV) risk profile and prevention of CV events has been growing over the years, leading to their recommendation for secondary prevention in post myocardial infarction and hypertriglyceridemia. However, years later conflicting results provided by clinical trials have generated some doubts about their CV benefits, leading to a limited indication for the treatment of hypertriglyceridemia. Only recently, after the REDUCE-IT Trial results on CV events and mortality, n-3 PUFAs have recovered an indication in the international guidelines for hypertriglyceridemia in patients with Atherosclerotic Cardiovascular Disease (ASCVD) or with type 2 diabetes mellitus (T2DM) and other CV risk factors, already on statin therapy. Multiple beneficial CV effects have been highlighted, in addition to the well-known lipid-lowering function, such as anti-inflammatory, anti-thrombotic and endothelial function protective properties. Three formulations of n-3 PUFAs are currently available on the market, sharing some pharmacokinetic and pharmacodynamic characteristics, but also exhibiting peculiar mechanisms. Three major clinical trials evaluated the efficacy and safety of different formulations of n-3 PUFA: JELIS, REDUCE-IT and STRENGTH, with controversial results attributable to various factors. For the future, it could be useful to perform comparative studies between different formulations and placebo, in order to clarify these doubts.

Keywords: n-3 PUFAs, cardiovascular diseases, cardiovascular prevention, coronary artery disease, IPE

1. Introduction

Fatty acids (FA) have the typical RCOOH structure, containing a methyl group, a hydrocarbon chain (R) and a carboxyl group (**Figure 1**). The carbon chain (C) can vary greatly in length, with a range between 2 and 36 C atoms. They are divided into saturated, monounsaturated and polyunsaturated according to the number of double bonds C = C present in their molecule (respectively none, one or more than one). FA have both a systematic and common name (e.g., octadecanoic or stearic acid) [2] and are often expressed with a schematic formula (abbreviated notation): CN: p n-x,
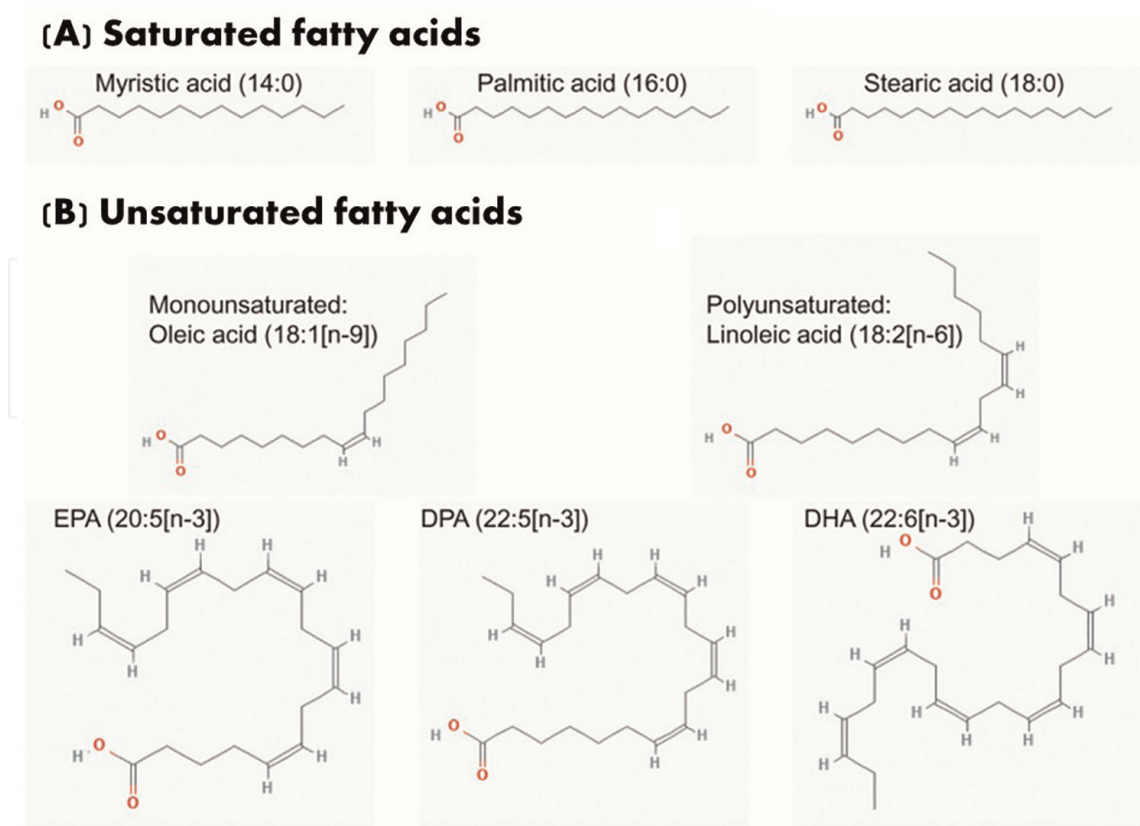
(A) Saturated fatty acids

Myristic acid (14:0)

Palmitic acid (16:0)

Stearic acid (18:0)

(B) Unsaturated fatty acids

Monounsaturated:
Oleic acid (18:1[n-9])

Polyunsaturated:
Linoleic acid (18:2[n-6])

EPA (20:5[n-3])

DPA (22:5[n-3])

DHA (22:6[n-3])

Figure 1.
Chemical structure of key saturated (a) and unsaturated (B) fatty acids. Modified from Wu H et al. [1]. DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid.

where CN represents the total number of atoms of C, p the number of double bonds, x the position of the first double bond from the methyl end (n) [3].

Polyunsaturated (PUFA) n-3 (Omega 3, PUFA n-3) and n-6 (Omega 6, PUFA n-6 Fatty Acids), whose first double bond is respectively on the third and sixth atom of C (numbered by the methyl group of the carbon chain), have a particular biological and medical interest [3].

Linoleic (AL) and alpha-linolenic (ALA) acids are defined as "essentials," because they cannot be synthesized *de novo* in humans and must, therefore, be taken with the diet (**Table 1**). Moreover, they are considered the precursors of the n-3 and n-6 PUFA families [4].

PUFA n-3. They include ALA (18,3 n-3) of plant origin and FA of animal origin (in particular seafood—fish oil), mainly represented by eicosapentaenoic acids (EPA; 20:5 n-3), docosahexaenoic acids (DHA; 22:6n-3) and docosapentaenoic acids (DPA, 22:5 n-3). It has been shown that ALA induces CV benefits in observational studies [5] and fish intake protects against coronary heart disease risk [6]. Moreover, a negative correlation between n-3 PUFAs and CV mortality has been reported in clinical trials and meta-analyses [7].

PUFA n-6. The main n-6 PUFAs are AL and arachidonic acid (AA). AL is the main n-6 PUFA derived by food and is contained in vegetable oils, nuts and seeds; AA is mainly taken from red meat, eggs, seaweed and fish oil [1]. A high intake of n-6 PUFAs, predominantly AL, would appear to be associated with a reduced risk of ischemic heart disease, ischemic stroke and CV mortality [8].

Several studies have shown the importance to maintain the balance between n-6 and n-3 PUFAs rather than the absolute quantity of each individual molecule. The centrality of this balance has been emphasized not only in the cardiology context, but

Product	LA	ALA	AA	EPA + DHA
n − 6 FA rich foods				
Corn oil	50,000	900		
Cotton seed oil	47,800	1000		
Peanut oil	23,900			
Soybean oil	53,400	7600		
Sunflower oil	60,200	500		
Safflower oil	74,000	470		
Margarine	17,600	1900		
Lard	8600	1000	1070	
Chicken egg	3800	220		
Bacon	6080	250	250	
Ham	2480	160	130	
Soya bean	8650	1000		
Maize	1630	40		
Almond	9860	260		
Brazil nut	24,900			
Peanut	13,900	530		
Walnut	34,100	6800	590	
n − 3 FA rich foods				
Canola oil	19,100	8600		
Linseed oil	13,400	55,300		
Herring	150	61.66	36.66	1700
Salmon	440	550	300	1200
Trout	74		30	500
Tuna	260	270	280	400
Cod	4	2	3	300

[a]*Data reported as mg/100 g.*
[b]*Content of* n − 6, n − 3 *FAs may slightly vary according to species, sources and analytical methods.*
Modified from Russo GL et al. [10]. LA, Linoleic Acid; ALA, Alfa-linolenic acid; AA, Arachidonic acid; EPA,
eicosapentaenoic acid; DHA, docosahexaenoic acid.

Table 1.
Dietary sources of principal polyunsaturated fatty acids (PUFAs)[a, b].

also in the pathogenesis of oncological, inflammatory and autoimmune diseases. The pleiotropic effects of PUFAs are summarized in **Table 2** [9].

For example, a high n-6/n-3 ratio is considered harmful to human health, while a value close to 1 is considered protective against degenerative diseases [10].

In healthy subjects taken a typical Swedish diet, a serum PUFA n-6/n-3 ratio of 4.72:1 was observed to be associated with an increase in the number of leukocytes and platelets and VEGF levels, when compared with an n-6/n-3 PUFA ratio of 2.6:1, observed in individuals following a Mediterranean diet. This dietary comparison and

the respective clinical implications are the basis for the recognition of the importance of the Mediterranean diet in maintaining a better state of health, enough to be included in the list of Intangible Heritage of Humanity of UNESCO [11].

2. Molecular mechanisms of n-3 PUFAs

N-3 PUFAs produce multiple effects, as anti-inflammatory, anti-thrombotic properties and actions on endothelial function, in addition to the well-known lipid lowering and hypotriglyceridemic effects (**Figure 2**).

The molecular mechanisms by which n-3 PUFAs exert their pleiotropic activity are complex and not yet fully defined.

Firstly, n-3 PUFAs are embedded in the cell wall and organelles, altering the lipid environment of membranes and their fluidity. This involves n-3 PUFAs in various cellular functions, such as signal transduction and protein transport. The beneficial effects of n-3 PUFAs observed in patients with hypertriglyceridemia seem to derive from their specific actions on inflammation, platelet function and monocyte phenotypes.

In particular, the production of specialized mediators, such as E-series resolvins derived from EPA and protectins and D-series resolvins derived from DHA, can play an important role in the resolution of inflammation. The reduction of the inflammatory response by n-3 PUFAs contributes to inhibit the development of intracellular second messengers, such as diacylglycerol and ceramide. In addition, n-3 PUFAs bind and activate different nuclear receptors and transcriptional factors involved in regulating the expression of different genes. These genes participate in functions related to atherosclerosis, such as inflammation, glucose-insulin homeostasis, lipid metabolism and adipocytokine production.

Finally, the activation of the peroxisome proliferator-activated receptor-γ by n-3 PUFAs results in the reduction of the translocation of NF-κB in the nucleus, decreasing the generation of multiple inflammatory cytokines [1].

3. Mechanisms underlying hypolipemic effect of n-3 PUFAs

Although the effect of n-3 PUFA-based drugs to reduce plasma triglycerides (TG) is widely recognized, their mechanism of action is not fully understood. The results of preclinical and clinical studies suggest that n-3 PUFAs reduce plasma TG concentrations by limiting their synthesis, but also by decreasing their incorporation into very low density lipoproteins (VLDLs), reducing their secretion and improving the clearance of TG by VLDLs [13, 14].

It has been proposed that n-3 PUFAs exert these effects through several mechanisms (**Figure 3**):

1. decrease in hepatic lipogenesis through suppression of the expression of sterol regulatory element-binding protein-1c (SREBP-1c). This leads to a decrease in the expression of enzymes responsible for the synthesis of cholesterol, fatty acids and TG;

2. increase in the β-oxidation of FA, with consequent reduction in the availability of the substrate necessary for the synthesis of TG and VLDL [13];

3. inhibition of key enzymes involved in hepatic TG synthesis, such as acid phosphatidic phosphatase and diacylglycerol acyltransferase [16];

Antithrombotic and antiplatelet
Decreased platelet aggregation: EPA decreased platelet aggregation and inhibited AA-induced thromboxane A2 formation
Decreased platelet adhesion: • EPA reduced platelet adhesion to collagen type I by 60–65% • Fish oil equivalent to 6 g EPA/day reduced platelet pseudopodia
Decreased thromboxane A2/increased thromboxane A3 levels: • EPA added to human thrombocytes incubated with AA decreased thromboxane A2 and increased thromboxane A3 • In human platelet-rich plasma, EPA did not induce platelet aggregation
Anti-inflammatory
Decreased hsCRP: • In ANCHOR, IPE 4 g/day decreased hsCRP vs. placebo by 21.5% ($P < 0.01$) • In REDUCE-IT, IPE decreased hsCRP vs. placebo at year 2 by 39.9% (last visit, 37.6%); $P < 0.001$
Decreased pentraxin-3: In patients with CAD treated with PCI, EPA added to statin therapy after 9 months reduced pentraxin 3 levels vs. statin alone
Decreased Lp-PLA$_2$: In ANCHOR, IPE 4 g/day decreased Lp-PLA$_2$ by 19.6% in patients with hsCRP >2.0 vs. placebo
Decreased NLRP3 inflammasome activation: • EPA blocked NLRP3 inflammasome activation in an animal model of ischemic stroke • EPA decreased NLRP3 gene expression in adipose tissue and in classically activated THP-macrophages
Decreased toll-like receptor 4: EPA reduced gene expression of toll-like receptor 4 by >50% in mice fed a high-fat diet vs. control
Decreased NFKb: EPA reduced the genetic expression of NFKb in a cell culture with THP-1 macrophages
Decreased inflammatory cytokines: • EPA reduced gene expression of IL-1b, TNF-α, and MCP-1 in cell culture with THP-1 macrophages • EPA downregulated expression of IL-6 mRNA in IL-1b–stimulated C6 glioma cells
Increased anti-inflammatory cytokine IL-10: • EPA significantly increased the production of IL-10 in lipopolysaccharide-activated monocytes ($P < 0.05$) • EPA increased IL-10 expression in peripheral blood monocytes in obese patients with dyslipidemia
Decreased endothelial adhesion molecules: • EPA inhibits monocyte adhesion to endothelial cells • EPA decreases plasma concentrations of soluble ICAM and VCAM
Increased "resolution of inflammation": specialized pro-resolving mediators
RvE1 was found to bind to the LTB4 receptor, BLT1, on human PMNs and attenuated LTB4-induced proinflammatory signals and PMN migration in an *in vitro* study
RvE2 was found to enhance phagocytosis of human macrophages and anti-inflammatory cytokine (IL-10) production
RvE3 was found to have potent inhibitory action on neutrophil chemotaxis both *in vitro* and *in vivo*
Anti-oxidant
Increased paraoxonase in patients with type 2 DM: • EPA 2 g increased PON1 activity and levels over 8 weeks vs. placebo • EPA over 8 weeks significantly increased gene expression of PON2 vs. placebo
Inhibition of lipid/lipoprotein oxidation: • EPA inhibited the oxidation of apoB lipoproteins (LDL-C, sdLDL, VLDL-C) in an *in vitro* study • EPA inhibited the oxidation of HDL isolated from the plasma of healthy volunteers; the sustained antioxidant effects of EPA on HDL oxidation were not replicated by DHA

- EPA inhibited oxidation of sdLDL, model membranes, and cholesterol crystal domain formation

Improved endothelial function
EPA significantly increased NO production and reduced glucose inhibition of NO in cultured human endothelial cells
EPA significantly improved FMD by 51% in patients with elevated TG ($P < 0.0001$); EPA/AA ratio was found to be significantly associated with the change in FMD ($P = 0.010$)
EPA improved PFBF during reactive hyperemia to the level in normolipidemic controls, recovery of PFBF correlated positively with EPA levels ($P < 0.05$) and the EPA/AA ratio ($P < 0.01$)
EPA improved vascular function as measured by strain gauge plethysmography in patients with type 2 DM on statin therapy
EPA improved endothelial function in patients with CAD, LDL-C < 100 mg/dL on statin therapy, and impaired FMD
EPA with an atorvastatin metabolite improved endothelial function through increased NO in an *in vitro* study
Increased cholesterol efflux
Increasing EPA phosphatidylcholine content of reconstituted HDL-C was demonstrated to increase cholesterol efflux
EPA inhibited glucose-induced membrane cholesterol crystalline domain formation in an *in vitro* study using multilamellar vesicles
EPA and DPA inhibited oxidation of membrane cholesterol domains in an *in vitro* study utilizing multilamellar vesicles
Increased adiponectin
EPA after 3 months significantly increased adiponectin vs. placebo among obese patients with dyslipidemia ($P < 0.01$)
Change in pulse wave velocity, a measure of arterial stiffness, was negatively correlated with change in adiponectin ($P < 0.01$) and in IL-10 expression of monocytes ($P < 0.05$)
Anti-arrhythmic
EPA may reduce the risk of ventricular arrhythmia by inhibiting the fast sodium current (I_{Na}), L-type calcium inward current (I_{Ca}) and enhancing the slowly activating delayed rectifying outward potassium current (I_{KS}), thus shortening cardiac action potential
EPA reduces cytosolic Ca^{2+} overload, thus reducing the risk of triggered induced arrhythmia

Modified from Trivedi K et al. [11]. AA, arachidonic acid; apo, apolipoprotein; BLT1, leukotriene B4 receptor BLT1; Ca2+, calcium; CAD, coronary artery disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DM, diabetes mellitus; EPA, eicosapentaenoic acid; FMD, flow-mediated dilation; hsCRP, high-sensitivity C-reactive protein; HDL-C, high-density lipoprotein cholesterol; ICAM, intercellular adhesion molecule; IL, interleukin; IPE, icosapent ethyl; LTB4, leukotriene B4 receptor 1; LDL-C, low-density lipoprotein cholesterol; Lp-PLA2: lipoprotein- associated phospholipase A2; MCP, monocyte chemoattractant protein; mRNA, messenger ribonucleic acid; NFKb, nuclear factor kappa-light- chain-enhancer of activated B cells; NLRP3, NLR family pyrin domain containing 3; NO, nitric oxide; PCI, percutaneous coronary intervention; PFBF, peak forearm blood flow; PON, paraoxonase; PMNs, polymorphonuclear leukocytes; REDUCE-IT, Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial; Rv, resolvin; sdLDL, small dense LDL; THP-1, monocyte/macrophage; TNF-α, tumor necrosis factor alpha; VLDL-C, very-low-density lipoprotein cholesterol; VCAM, vascular cell adhesion molecule.

Table 2.
Select pleiotropic effects of EPA.

4. increase the expression of lipoprotein lipase (LPL), a key component of the biosynthetic pathways of TG-rich lipoproteins (TRLs), resulting in greater removal of TG by VLDLs and circulating chylomicrons.
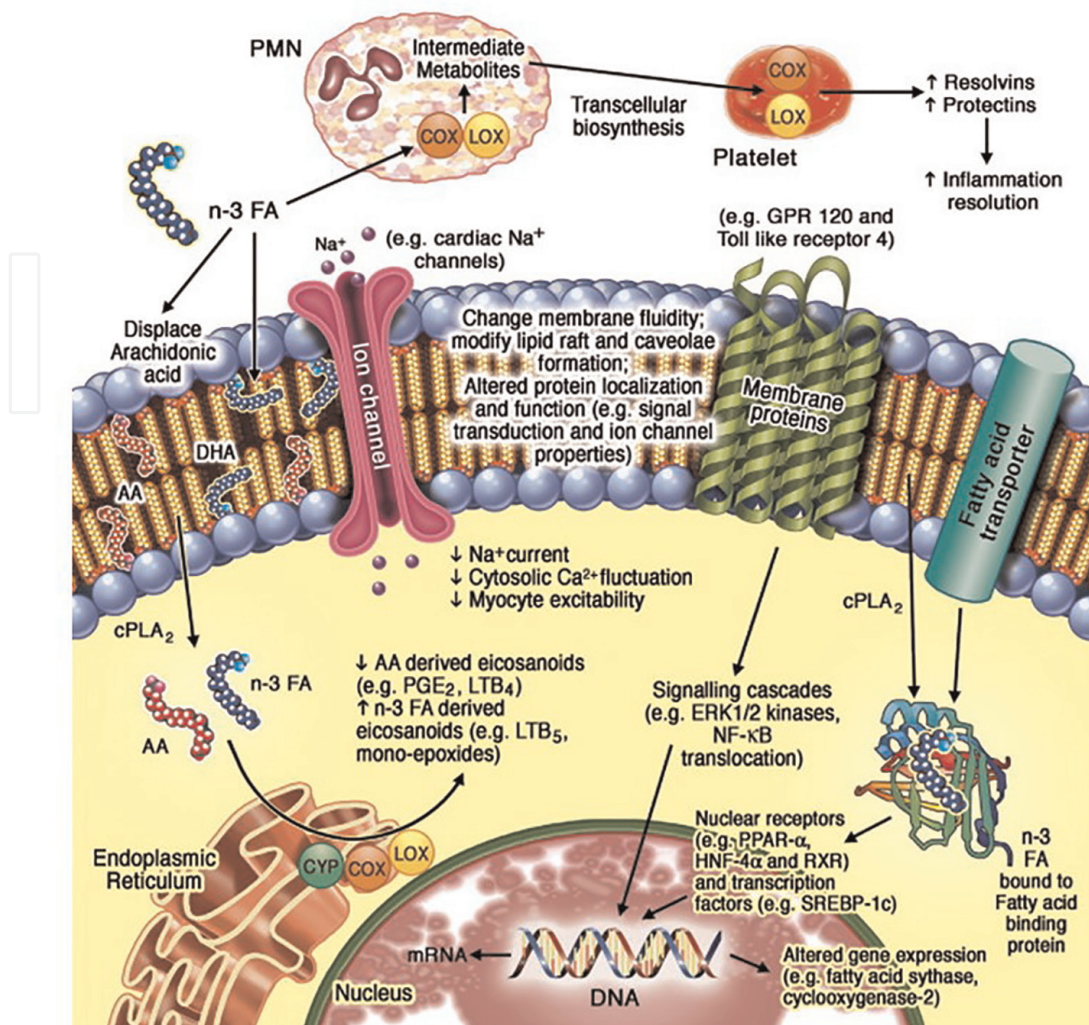
Figure 2.
Molecular pathways affected by n-3 PUFA. Taken from Mozaffarian D et al. [12]. DNA = deoxyribonucleic acid; ERK = extracellular signal-regulated kinase; mRNA = messenger ribonucleic acid; PMN = polymorphonuclear leukocyte.

The primary constituents of n-3 PUFA formulations, EPA and DHA, have both been shown to reduce TG. However, the two n-3 PUFAs are known to produce different effects on LDL-C and HDL-C [17]. In a meta-analysis of studies in which the effects of DHA and EPA were directly compared, DHA was associated with a greater reduction in TG, but also with a greater increase in LDL-C compared to EPA. In addition, DHA was associated with an increase in HDL-C compared to placebo, but not compared to EPA. Further studies are needed to clarify the mechanisms and significance of these differences [17].

N-3 PUFAs have also been shown to reduce circulating levels of apolipoprotein CIII (ApoCIII), believed to be involved in the progression of atherosclerosis and CV diseases (CVDs) through multiple mechanisms, such as activation and potentiation of pro-inflammatory pathways. In addition, it inhibits the receptor-mediated absorption of TRLs and their remnants, slowing their clearance and promoting the formation of small dense LDL particles from VLDLs. ApoCIII also provides a key contribution to the pathogenesis of hypertriglyceridemia, mainly due to its inhibitory action on LPL that hydrolyzes plasma TRLs, producing free FA, chylomicron remnants and intermediate density lipoproteins (IDL). Some IDL particles may undergo further LPL-mediated hydrolysis, converting to LDL.
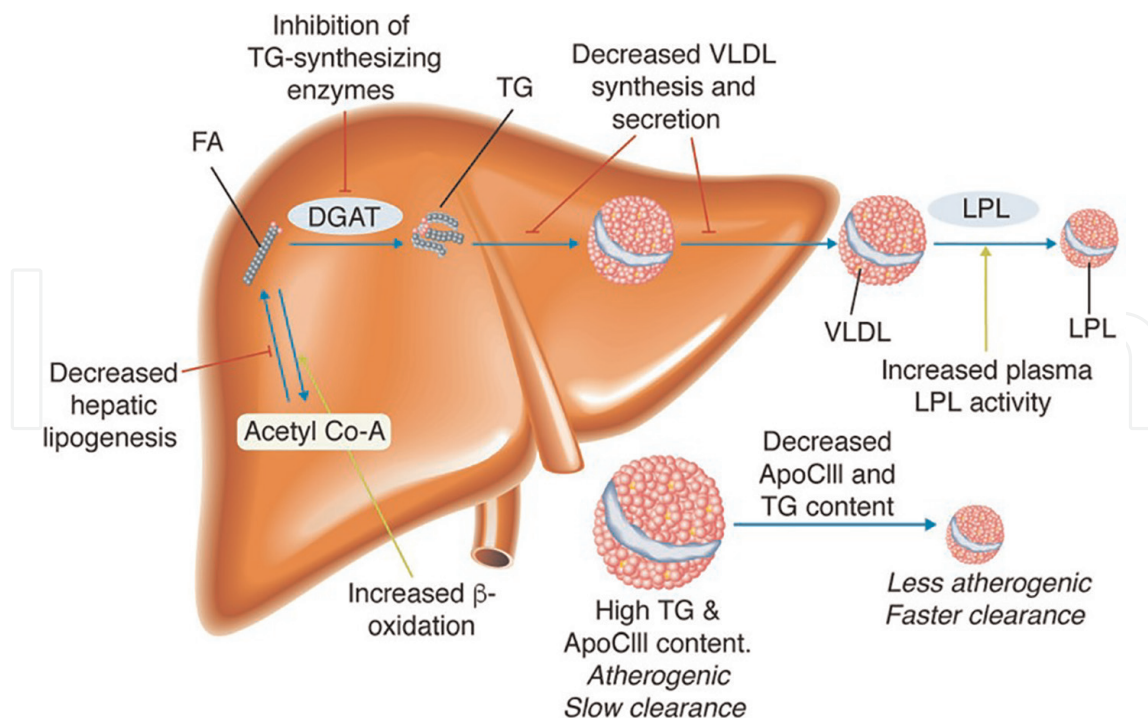
Figure 3.
Proposed mechanisms of action of prescription formulations of long-chain omega-3 fatty acids. Taken from Backes J et al. [15]. ApoCIII apolipoprotein CIII, acetyl Co-a acetyl coenzyme a, DGAT diglyceride acyltransferase; FAfatty acid, LPL lipoprotein lipase, TG triglyceride, VLDL very-low-density lipoprotein.

It has been hypothesized that the different effects of DHA and EPA on the lipid profile can be explained with their different interactions with ApoCIII synthesis. DHA is believed to reduce ApoCIII synthesis by regulating some hepatic transcriptional factors, such as hepatic nuclear factor-4-alpha (HNF4A) and forkhead box-O transcription factor O1 (FOXO1). In this context, DHA promotes more hydrolysis of VLDLs, resulting in greater conversion of VLDLs into LDL, ultimately resulting in the formation of larger, less dense LDL particles. Further studies are necessary to clarify these properties.

In addition to the TG-reducing effect, n-3 PUFAs have beneficial effects on cardiometabolic risk factors, having proved to significantly reduce markers of inflammation associated with atherosclerosis and the development of CVDs.

Clinical studies showed that levels of lipoprotein-associated phospholipase A2 (Lp-PLA2) are reduced by n-3 PUFAs and icosapent ethyl (IPE) compared to placebo, and that the concentration of high-sensitivity C-reactive protein (CRP) is decreased by IPE [18, 19]. Preclinical and clinical studies have also shown that EPA and DHA have antiarrhythmic and antioxidant effects, improving endothelial function and promoting a less atherogenic lipoprotein profile when administered in combination with statin therapy [20]. Moreover, EPA and DHA reduce biomarkers of platelet activity compared to placebo, regardless of concomitant intake of aspirin and statins, and this is supposed to be associated with inhibition of platelet aggregation. Regarding this latter property, DHA is believed to possess more intensive antiplatelet activity than EPA. In addition, DHA has been shown to reduce blood pressure and heart rate, and to play a protective effect on cognitive decline.

Although most studies on the therapeutic effects of n-3 PUFAs focused on EPA and DHA, a recent study investigated the mechanism of action of DPA. DPA levels were independently associated with a reduced risk of developing acute myocardial

infarction (AMI) and coronary artery disease (CAD), while reduced circulating levels of DPA were shown to be associated with the development of lipid-rich plaques and peripheral arterial disease (PAD).

EPA, DHA and DPA counteract platelet aggregation in a dose-dependent manner. However, DPA seemed to be the most intensive inhibitor of platelet activity, compared with EPA and DHA. As DHA and EPA, also DPA can reduce the expression of pro-inflammatory genes.

The mechanisms underlying the reduction of TG by long-chain n-3 PUFAs differ from those of other lipid-lowering drugs, such as statins. Therefore, if administered in combination, different lipid-lowering drugs could potentially have complementary beneficial effects on the lipid profile [13]. As proof of this, incremental reductions in TG levels were observed when n-3 PUFAs are administered with statins [21].

3.1 n-3 PUFAs and atheromatous plaques

The inverse relationship found between the intima-media thickness of the carotid artery and the treatment with n-3 PUFAs suggested their endothelium protective activity and anti-atherosclerosis effect. There is specific evidence that this result is achieved through a reduction in inflammation and increased plaque stability.

The relationship between the vulnerability of atheromatous plaque and the serum n-3/n-6 PUFA ratio was evaluated in a study that enrolled patients undergoing percutaneous coronary angioplasty [22]. The EPA/AA ratio was examined at the time of the patient's admission. An angioscopy examination of the culprit coronary lesion evaluated the color tone of the plaque (white 0; light yellow 1; yellow 2; intense yellow 3) and the possible presence of a thrombus. Patients with stable angina (n = 38) were divided into 2 groups based on a reduced (<0.37; n = 19) or elevated (≥0.37; n = 19) EPA/AA ratio. In the group with reduced EPA/AA, the maximum yellow tone of the plaques was significantly higher than in patients with stable angina and high EPA/AA ratio. Moreover, the number of non-culprit yellow plaques with a thrombus tended to be higher, without reaching statistical significance. Multivariate analysis revealed an association between a lower serum EPA levels with lower EPA/AA ratio and the finding of more vulnerable grade 3 yellow plaques.

In another trial, 49 thin-cap non-culprit atheromatous plaques (TCFA) were evaluated in 30 patients with untreated dyslipidemia [23]. Patients were randomized to EPA (1.8 g/day) + rosuvastatin (n = 15, 23 TCFA) or rosuvastatin alone (n = 15, 26 TCFA). Percutaneous interventions and a 9-month follow-up OCT were performed to assess morphological changes in TCFA. The EPA/AA ratio and pentraxin-3 (PTX3) levels were also evaluated.

Despite LDL-C levels during follow-up were comparable in the two groups, subjects treated with EPA + statin had a higher EPA/AA ratio and lower PTX3 levels than those receiving the statin alone. The OCT also showed that in the EPA + statin group there was a greater increase in the thickness of the fibrotic cap, a more evident reduction of the lipid arch and its length, as well as a lower macrophage accumulation. Such evidence suggested that concomitant use of EPA and rosuvastatin contributes to the stabilization of a vulnerable plaque compared with the use of statin alone and, presumably, through inhibition of arterial wall inflammation.

The recent randomized, double-blind controlled clinical trial EVAPORATE [24] evaluated the volume of atheromatous plaque, using multidetector CT (MDCT), after treatment with 4 g/day of IPE in combination with diet and statin therapy versus statin alone. The study enrolled 80 patients with coronary atherosclerosis documented

by MDCT (one or more stenosis with narrowing >20%), already on statin therapy and with elevated TG levels (median value in both groups of 259.1 ± 78.1 mg/dL). MDCTs follow-up were performed after 9 and 18 months. The primary endpoint was low attenuation volume changes (LAP volume) at 18 months. In the IPE group the LAP plaque volume was reduced by 17%, while in the control group it was more than doubled (+109%; P = 0.0061). Significant differences were also observed in the progression index of other plaques, such as fibrous and fibro-adipose plaques, whose volumes decreased in the IPE group and progressed in the placebo group (P < 0.01).

4. Different pharmacological formulations (OM3EE, OM3CA, IPE, EPA/DPA/DHA EE)

4.1 General aspects

PUFA n-3 formulations are recommended, in combination with diet, for the reduction of serum TG in adults with hypertriglyceridemia.
Mainly three formulations of PUFA n-3 are currently available [13]:

- based on ethyl esters of long-chain fatty acids (OM3EE), composed mainly of EPA and DHA;

- CA-based of PUFA n-3 (OM3CA), composed of long-chain free FA, including EPA, DHA and DPA;

- IPE-based, composed of more than 96% of the purified and stabilized ethyl ester of EPA [12]. IPE is, therefore, a stable and highly purified compound containing a single active ingredient. It has been recently approved in the United States by the FDA for the prevention of Major CV events (MCV) in high-risk individuals (on statin therapy, with triglyceridemia >150 mg/dL, CV disease (CVD) or diabetes mellitus, and 2 or more CV risk factors). This approval is derived from numerous evidences. For example, the REDUCE-IT trial, in which a 25% reduction in the risk of CV events was observed in patients treated with IPE. In contrast, the other two formulations have a combination of n-3 PUFA products (e.g., DHA + EPA), with limited evidence in terms of CV event risk reduction [12]. Nevertheless, at approved doses all three commercially available formulations have been shown to be effective in reducing triglyceridemia and serum VLDL levels.

4.2 Bioavailability

The absorption of DHA and EPA has been shown to vary between formulations.
The OM3CA formulation has a bioavailability (area under the "plasma concentration-time" curve from zero to the last measured concentration value [$AUC_{(0-t)}$]) of fourfold greater for both EPA and DHA during low-fat consumption than observed with OM3EE [12]. Absorption of OM3EE and IPE requires pancreatic lipase-mediated hydrolysis. Since pancreatic lipase levels depend on both the amount and type of lipids ingested, the absorption of OM3EE is thought to depend strongly on the fat content of meals. In contrast, n-3 PUFAs in the free FA form, contained in OM3CA formulations, are not dependent on pancreatic lipase hydrolysis. Therefore, the bioavailability of EPA and DHA present in this formulation is less dependent on

meal fat content than in OM3EE formulations. In one study (n = 54), conducted under low-fat intake conditions, 59% of subjects treated with OM3CA maintained an $AUC_{(0-t)}$ for EPA and DHA ≥50% compared to the $AUC_{(0-t)}$ observed under high-fat intake. In contrast, this finding was present in only 6% of OM3EE-treated subjects. This observation could be useful from a therapeutic point of view, as current guidelines recommend a very low fat diet in patients with severe hypertriglyceridemia [25].

4.3 Efficacy

All n-3 PUFA formulations, administered at a dose of 4 g/day, have been shown to significantly reduce TG, VLDL-C, non-C-HDL, and ApoB levels in hypertriglyceridemic and high CV risk patients [13]. Higher doses of n-3 PUFAs and higher baseline triglyceridemia levels are associated with greater percentage of reductions in TG [26]. This was also observed in the MARINE trial, in which different dosages of highly purified EPA ethyl ester (Vazkepa) were compared (**Figure 4**). This effect appears to be independent from the severity of hypertriglyceridemia.

In the JELIS study, the use of a formulation of highly purified EPA ethyl ester (Epadel) was shown to reduce CV events when combined with statin and a reduction in atheromatous plaque thickness. It has also demonstrated a good safety and tolerability profile.

In REDUCE-IT trial, on the other hand, 4 g/day of EPA ethyl ester was shown to result in favorable effects in terms of reducing ischemic events and CV mortality.

In contrast, in the STRENGTH trial, which used the OM3CA (EPA + DHA) formulation, a neutral effect in terms of CV outcome (MACE) was observed, thus not supporting the hypothesis of using n-3 PUFAs for CV event risk reduction.

DHA-containing formulations (OM3CA and OM3EE) may cause an increase in C-LDL levels, especially in patients with severe hypertriglyceridemia (TG >500 mg/dL). However, this is not associated with increased levels of non-HDL C or ApoB, which seems to be a better predictor of CV risk than C-LDL values [13]. This is particularly true for patients with hypertriglyceridemia, who often have low LDL-C levels [25].

Non-DHA-containing formulations (EPA and IPE) appear not to cause an increase in C-LDL levels [12], as shown in the MARINE [27] and ANCHOR [28] clinical trials, which studied efficacy and safety of IPE.

These different effects of EPA versus DHA on C-LDL levels can be attributed to one or more mechanisms:

- Firstly, DHA (but not EPA) may down-regulate C-LDL receptor (LDL-R) expression and LDL-R-mediated clearance by the liver, which is an important reverse regulator of LDL-C levels. This appears to occur through suppression of the expression of *sterol regulatory element binding protein-2* (SREBP-2) by DHA.

- Secondly, DHA may increase the activity of *cholesteryl ester transfer protein* (CETP), which promotes the transfer of lipid cores between lipoproteins, resulting in an increase of LDL-C and decrease of HDL-C levels. EPA, on the other hand, has a neutral effect on CETP activity.

- Finally, DHA appears to up-regulate lipoprotein lipase enzyme activity to a greater extent than EPA, thereby promoting the conversion of VLDL to LDL with consequent increase of LDL-C levels.
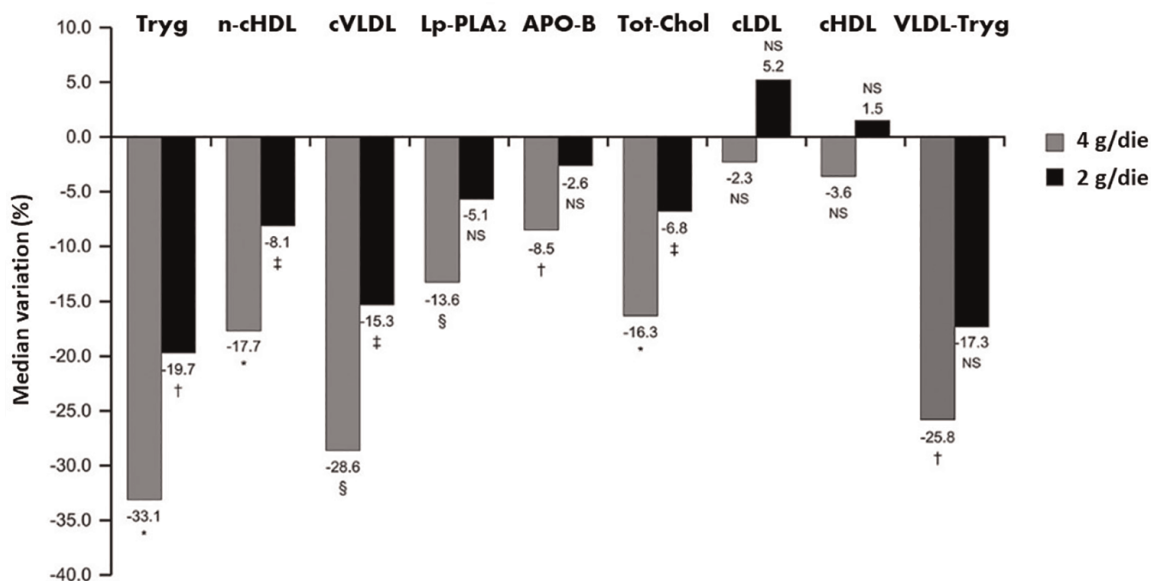
Figure 4.
Change in lipid levels, baseline to week 12 (intent-to-treat population). Percentage of change from baseline to study end of placebo-corrected lipid levels in patients with very high TG levels receiving AMR101 4 g/day or AMR101 2 g/day. Modified from Bays HE et al. [17]. APO-B = apolipoprotein B; cHDL = high-density lipoprotein cholesterol; cLDL = low-density lipoprotein cholesterol; Lp-PLA2 = lipoprotein associated phospholipase A2; n-cHDL = non-high-density lipoprotein cholesterol; NS = not significant; Tot-Chol = total cholesterol; Tryg = triglyceride; cVLDL, very-low-density lipoprotein cholesterol; VLDL–TG, very-low-density lipoprotein–triglycerides.

Interestingly, in patients with triglyceridemia >200 mg/dL but <500 mg/dL, no increase in LDL-C was observed with any of the formulations. This is probably because in the various clinical trials on n-3 PUFAs, such patients had lower baseline serum TG levels and were already on statin therapy.

It was also observed that, unlike IPE, DHA-containing formulations increased HDL-C levels. However, the clinical relevance of increasing HDL-C through pharmacological agents has not been clarified yet.

In contrast, to date, the effect of any n-3 PUFA formulation on pancreatitis has not been determined [13].

EPA and DHA are also believed to act in different or conflicting ways at the cell membrane level. Specifically, the insertion of EPA and DHA occurs in distinct regions of the lipid membrane bilayer as a consequence of the different lengths of the hydrocarbons involved. Their insertion causes conformational changes that result in increased membrane fluidity and promotion of cholesterol domains. Among the 2 n-3 PUFAs, EPA has a more stable and extended structure, which contributes to membrane stability as well as inhibition of lipid oxidation and formation of new cholesterol domains [29]. Their main effects are summarized in **Figure 5**.

4.4 Safety and tolerability

Commercially available n-3 PUFA formulations are generally well tolerated, with similar rates of treatment discontinuation between treatment and placebo groups [13]. In clinical trials, gastro-intestinal events were the main adverse effects with DHA-containing formulations, while arthralgia was the main adverse event reported with EPA [12].

In the REDUCE-IT trial, it was observed a higher incidence of atrial fibrillation and peripheral edema in the treatment group versus placebo group (5.3% vs. 3.9% and 6.5% vs. 5.0%, respectively). The possible pathogenetic mechanisms could be related to their effects on myocyte membrane ion channels. Previous observational and randomized studies have examined the potential antiarrhythmic effects of n-3 PUFAs, leading to conflicting results [30, 31]. However, in the REDUCE-IT trial, the increased incidence of atrial fibrillation in the EPA-treated group was not associated with a higher incidence of heart failure or stroke. Therefore, further studies are required to clarify these aspects.

In both JELIS and REDUCE-IT, modest increases in bleeding episodes were observed in the EPA-treated group compared with controls. Bleeding rates were lower in JELIS (1.1% in EPA vs. 0.6% in control group) than in REDUCE-IT (2.7% with EPA vs. 2.1% with placebo). In JELIS study antiplatelet therapy, at baseline, was present in 13% and 14% in treatment and placebo groups, respectively, compared with 79.7% and 79.1%, respectively, in the REDUCE-IT (26). Although no serious bleeding events were observed in REDUCE-IT or in other clinical trials with n-3 PUFAs, even in those who were on aspirin or warfarin therapy and in those undergone surgery or percutaneous interventions, the potential interaction between EPA and antithrombotic drugs warrants further investigation.

It is not known whether patients with allergies to fish and/or shellfish are at increased risk of allergic reactions to n-3 PUFAs. Therefore, these formulations should be used with caution in patients with known hypersensitivity to fish and/or shellfish [13].

4.5 Differences from n-3 PUFA-based dietary supplements

PUFA n-3 dietary supplements are widely used and they are among the most popular dietary supplements worldwide. However, they are not subject to the strict regulations required for prescription drugs. As a result, the EPA and DHA content of dietary supplements can be variable or too low [13].

Physicians often tend to erroneously consider n-3 PUFA dietary supplements to be adequate and reliable. However, a recent analysis of individual fish oil supplements has shown that they contain an inadequate dose of EPA and DHA, on average equivalent to only 68% of that required. The same analysis found that most supplements exceed the recommended levels of oxidation markers. The oxidative process undergone by n-3 PUFAs results in a gradual reduction in their concentration, leading to reduced efficacy. One study showed that a median intake of 11 servings of fish oil dietary supplements per day was needed to obtain a dose of 3.4 g/day of n-3 PUFAs. The same study showed that dietary supplements often contain other fats and cholesterol, and that their content varies widely among different products. Such variability can be confusing for patients, as well as for physicians, resulting in a drug dosage potentially inadequate to effectively reduce serum TGs in patients with hypertriglyceridemia [29]. Based on this evidence, we conclude that, to date, the benefits of n-3 PUFA-based nutraceuticals are uncertain and their use controversial [13].
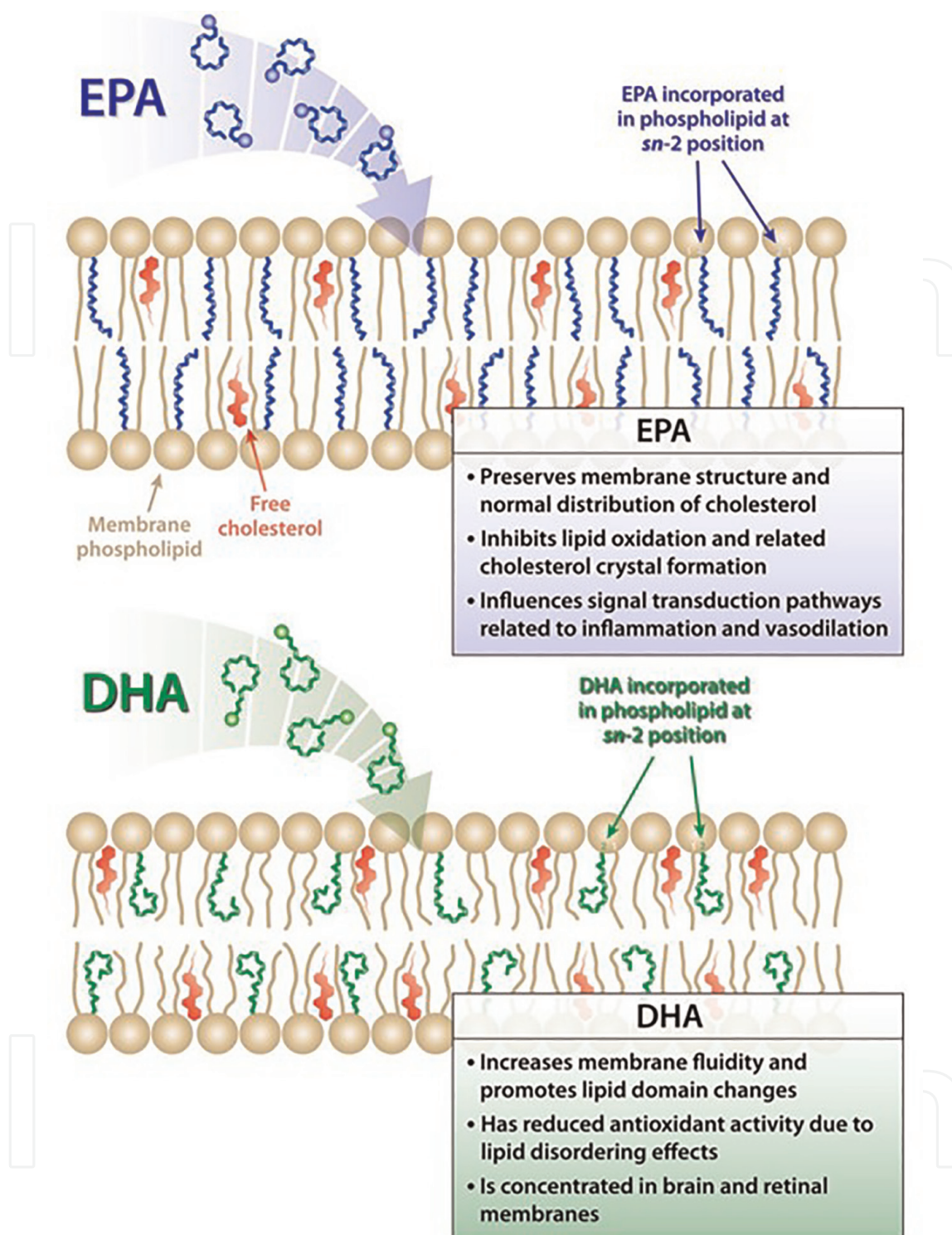
Figure 5.
Molecular membrane interactions of omega-3 fatty acids. Taken from Mason RP et al. [28].

5. Controlled clinical trials of different formulations of n-3 PUFAs. Efficacy on lipid profile and outcomes

The efficacy on lipid profile and outcomes of controlled clinical trials are summarized in **Figure 6** and **Table 3**, respectively. **GISSI-Prevention trial (1999)**. This multicenter [32] "open-label" study evaluated 11,324 patients with recent (<3 months) AMI randomized equally into four treatment groups: group 1, n-3

PUFAs (850–882 mg EPA and DHA as ethyl esters in EPA/DHA 1,2 ratio); group 2, vitamin E (300 mg, in 1 cps of synthetic α-tocopherol); group 3, n-3 PUFAs + vitamin E; control group 4 (no supplement). Combined primary efficacy endpoint represented by cumulative rate of all-cause mortality, nonfatal AMI, and nonfatal stroke; and cumulative rate of death from CV causes, nonfatal AMI, and nonfatal stroke. Secondary analyses were performed for each component of the primary endpoints and for major causes of death.

Treatment with n-3 PUFAs, but not vitamin E, significantly reduced the primary endpoint (RR reduction of 10% (95% CI 1–18) with two-way analysis of variance and 15% (2–26) with four-way analysis). Benefits were attributed to reduction in risk of death (14% (3–24) with two-way, 20% (6–33) four-way) and CV death (17% (3–29) two-way, 30% (13–44) four-way). No significant change in C values (total, HDL, LDL) nor fibrinogen was observed in all groups.

In comparison with the control group, the slight reduction in triglyceridemia was shown more frequently in patients treated with PUFA n-3. The effects of combined treatment were similar to those observed in treatment with n-3 PUFAs alone, for both the primary endpoint (14% (1–26)) and fatal events (20% (5–33)).

These results demonstrate the statistically significant clinical benefits of dietary supplementation with n-3 PUFAs, where vitamin E supplementation demonstrated no benefit.

JELIS (Japan EPA Lipid Intervention Study; 2007). Randomized "open label" trial with blinded endpoint analysis [33] evaluated the long-term prevention effects of EPA of major coronary events in patients with hypercholesterolemia.

Over a 3-year period, 18,645 patients (60% women; mean age 61 years) with coronary artery disease (established angina pectoris, coronary interventions, previous AMI; n = 3664) or free of coronary artery disease (n = 14,981), with baseline total C value >250 mg/dL (corresponding to C-LDL >170 mg/dL) were enrolled. First-line therapy for all patients consisted of pravastatin 10 mg or simvastatin 5 mg once daily (20 mg pravastatin and 10 mg simvastatin in severe hypercholesterolemia). Primary endpoints were major coronary events: sudden cardiac death, fatal and nonfatal AMI,
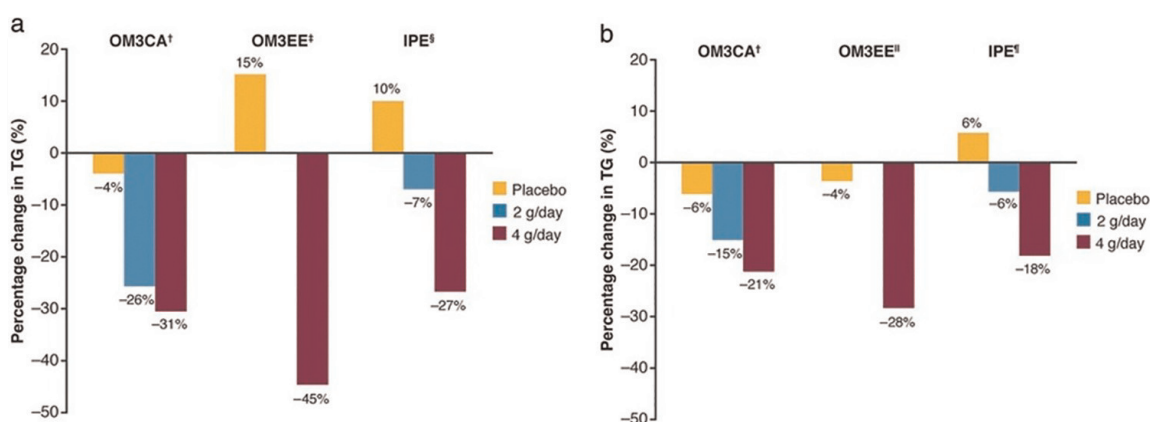

Figure 6.
TG reduction observed with varying doses of omega-3 carboxylic acids, omega-3 ethyl esters, and icosapent ethyl in (a) patients with severe hypertriglyceridemia (TG ≥ 500 mg/dL) and (b) statin-treated patients with high baseline TG levels (TG level ≥ 200 mg/dL and < 500 mg/dL). Taken from Backes J et al. [15]. OM3CA = percentage change from baseline expressed as least-squares geometric mean; OM3EE = percentage change from baseline expressed as geometric mean; IPE = percentage change from baseline expressed as median. [†]Placebo used = olive oil; [‡]placebo used = vegetable oil; [§]placebo used = mineral oil; [‖]placebo used = corn oil; [¶]Placebo not specified. IPE icosapent ethyl, OM3CA omega-3 carboxylic acids, OM3EE omega-3 ethyl esters, TG triglyceride.

Trial	Population	N	Follow-up, y	Interventions	Baseline Statin Use, %	Baseline LDL-C, mg/dL	Baseline TG, mg/dL	Primary Endpoint	Risk Ratio (95% CI)	Adverse Events[a]
Diet and/or supplement										
DART	Recent MI (men)	2033	2	Fatty fish 2 servings/week or supplement EPA + DHA 855 mg/d; total fat 30% of calories, PUFA/SFA ratio 1.0; cereal fiber 18 g/d; no diet advice	NR	TC 250	NR	Total mortality CHD mortality	RR0.71 (0.54–0.93) RR 0.84 (0.66–1.07)	NR
EPA + DHA (low dose)										
GISSI-P	Recent MI	11,324	3.5	EPA + DHA 850 mg/d; vitamin E 300 mg/d; n-3 + vitamin E; no supplement	Cholesterol-lowering agents: 5 (end of study: 46)	137	162	Death or nonfatal MI or nonfatal stroke	RR 0.90 (0.82–0.99) [2-way analysis]; RR 0.85 (0.74–0.98) [4-way analysis]	GI disturbance, nausea
GISSI-HF	Chronic HF	6975	3.9	EPA + DHA 850 mg; placebo (unspecified)	23	TC 188	126	Death Death or CV hosp	HR 0.91 (0.833–0.998) HR 0.92 (0.849–0.999)	GI disturbance
OMEGA	Recent MI	3851	1	DHA + EPA 840 mg; placebo	94–95	NR	NR	SCD	&$$$;DR 0.95 (0.56–1.60)	Neoplasms (19 vs. 8), cardiac device therapeutic procedures (16 vs. 2)
Alpha-omega-3	Prior MI	4837	3.3	400 mg EPA + DHA ± 2 g ALA; 2 g ALA; placebo	Baseline lipid-lowering agents: 85–87	99–102	144–150	Fatal/nonfatal CV events or revasc	HR: 1.01 (0.87–1.17)	GI symptoms, prostate cancer incidence, death

Trial	Population	N	Follow-up, y	Interventions	Baseline Statin Use, %	Baseline LDL-C, mg/dL	Baseline TG, mg/dL	Primary Endpoint	Risk Ratio (95% CI)	Adverse Events[a]
SU.FOL. OM3	Recent acute coronary or cerebral ischemic event	2501	4.7	EPA + DHA 600 mg; placebo + vitamin B	Baseline lipid-lowering agents: 83–87	101–104	108 (no coronary event); 125 (coronary event)	MACE	HR 1.08 (0.79–1.47)	GI disturbance, nausea, cutaneous reactions
ORIGIN	Dysglycemia or high-risk CVD	12,536	6.2	EPA + DHA 840 mg; placebo (olive oil)	53–54	112	140–142	CV death	HR 0.98 (0.87–1.10)	NR
ORIGINALE	ORIGIN participants	4771	8.9	EPA + DHA 840 mg; placebo (olive oil)	57–59	108–109	142	CV death	HR 0.98 (0.88–1.09)	NR
Risk & Prevention	High-risk CVD	12,513	5	EPA + DHA 850 mg; placebo	41	132	150	CV death or CV hosp	HR 0.97 (0.88–1.08)	GI symptoms
ASCEND	Diabetes without CVD	15,480	7.4	DHA + EPA 840 mg; placebo (olive oil)	75	TC 161	NR	Nonfatal MI, nonfatal stroke, TLA, or vascular death	RR 0.97 (0.87–1.08)	Similar between groups
VITAL	Healthy, no history of CVD	25,871	5.3	DHA + EPA 840 mg; placebo	Cholesterol-lowering agents: 37	NR	NR	MI, stroke, or CV death	HR 0.97 (0.85–1.12)	GI symptoms
EPA (hiah& $$$; dose)										
JELIS	TC >250 mg/dL	18,645	4.6	EPA 1800 mg + statin; statin only	97	182	151	Sudden cardiac death, fatal or nonfatal MI, unstable angina, or revasc	HR 0.81 (0.69–0.95)	GI, skin, hemorrhage (cerebral, fundal, epistaxis, subcutaneous) (0.6% vs. 1.1%, P = 0.0006)

Trial	Population	N	Follow-up, y	Interventions	Baseline Statin Use, %	Baseline LDL-C, mg/dL	Baseline TG, mg/dL	Primary Endpoint	Risk Ratio (95% CI)	Adverse Events[a]
REDUCE-IT	CVD or diabetes + additional CVD risk	8179	4.9	EPA 4000 mg: placebo (mineral oil)	100	75	216	CV death, nonfatal MI, nonfatal stroke, revasc, or unstable angina	HR 0.75 (0.68–0.83)	Hosp for atrial fibrillation and atrial flutter (3.1% vs. 2.1%, *P* = 0.004), serious bleeding events (2.7% vs. 2.1%, *P* = 0.06)

Modified from Wu H et al. [7]. A Not significantly different between treatment groups except as noted. ALA, alpha-linolenic acid; ASCEND, A Study of Cardiovascular Events in Diabetes; CHD, coronary heart disease; CI, confidence interval; CV, cardiovascular; CVD, cardiovascular disease; DART, Diet and Reinfarction Trial; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GI, gastrointestinal; GISSI-P, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico Prevenzione; GISSI-HF, GISSI–Heart Failure; HF, heart failure; hosp, hospitalization; HR, hazard ratio; JELIS, Japan EPA Lipid Intervention Study; LDL-C, low-density lipoprotein cholesterol; MACE, major adverse cardiac events; MI, myocardial infarction; NR, not reported; OR, odds ratio; ORIGIN, Outcome Reduction with an Initial Glargine Intervention; ORIGINALE, Outcome Reduction with an Initial Glargine Intervention Legacy Effects; PUFA, polyunsaturated fatty acid; REDUCE-IT, Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial; revasc, revascularization; RR, relative risk; SCD, sudden coronary death; SFA, saturated fatty acid; SU.FOL.OM3, Supplementation with Folate, Vitamin B6 and B12 and/or Omega-3 Fatty Acids trial; TC: total cholesterol; TG, triglyceride; TIA, tran-sient ischemic attack; VITAL, Vitamin D and Omega-3 Trial; y, years.

Table 3.
Reviewed outcomes trials of n-3 PUFAs.

and nonfatal events such as development of unstable angina, CABG, angioplasty, and/ or coronary stenting. Patients were randomized to treatment with EPA (EPA+ statin) or statin alone (controls).

After a mean follow-up of 4.6 years, the primary endpoint had occurred in 262 patients (2.8%) in the EPA group and 324 patients (3.5%) in the control group, with a relative 19% reduction in major coronary events (p = 0.011). The 25% reduction in C-LDL in both groups after treatment was not found to be a significant factor in reducing the risk of events. The risk of unstable angina and nonfatal coronary events was reduced by 19% in the EPA group, while sudden cardiac death and death from coronary causes were overlapping in the 2 groups.

In patients with coronary artery disease (secondary prevention), EPA reduced major coronary events by 19% compared with controls (8.7% vs. 10.7%; p = 0.048). In patients free of coronary artery disease, EPA did not significantly reduce major coronary events (−18%; 1.4% vs. 1.7% of controls; p = 0.132). The study results suggested that EPA was a promising treatment for primary and secondary prevention of major coronary events, especially nonfatal coronary events (**Figure** 7).

A sub-analysis of JELIS in 2012, showing 38% (p = 0.007) reduction in coronary events in patients who did not reach target values of C-LDL and non-C-HDL, concluded that EPA supplementation was also more effective in patients who did not reach target values of cholesterolemia.

REDUCE-IT (2019). Multicenter, randomized, placebo-controlled, double-blind study [34] enrolled and followed, on average for 4.9 years, 8179 patients diagnosed with CV disease (70.7%; secondary prevention) or with diabetes mellitus and other risk factors on statin therapy. Patients had to have triglyceridemia between 135 and 499 mg/dL and C-LDL between 41 and 100 mg/dL at baseline.

The aim of the study was to evaluate the potential benefits of EPA ethyl ester on CV outcomes in patients with elevated triglyceridemia levels. Patients were randomized to receive 2000 mg of the EPA ethyl ester twice daily (with a total dose of 4 g) or placebo (mineral oil).

The primary composite endpoint was the association of CV death, nonfatal AMI, nonfatal stroke, coronary revascularization, or unstable angina. Key secondary endpoints were the association of CV death, nonfatal AMI, and nonfatal stroke.

The primary endpoint occurred in 17.2% of patients in the EPA group compared with 22% of controls (HR 0.75; 95% CI, 0.68–0.83; p < 0.001). Secondary endpoints were observed in 11.2% of EPA and 14.8% of placebo patients, respectively (HR 0.74;
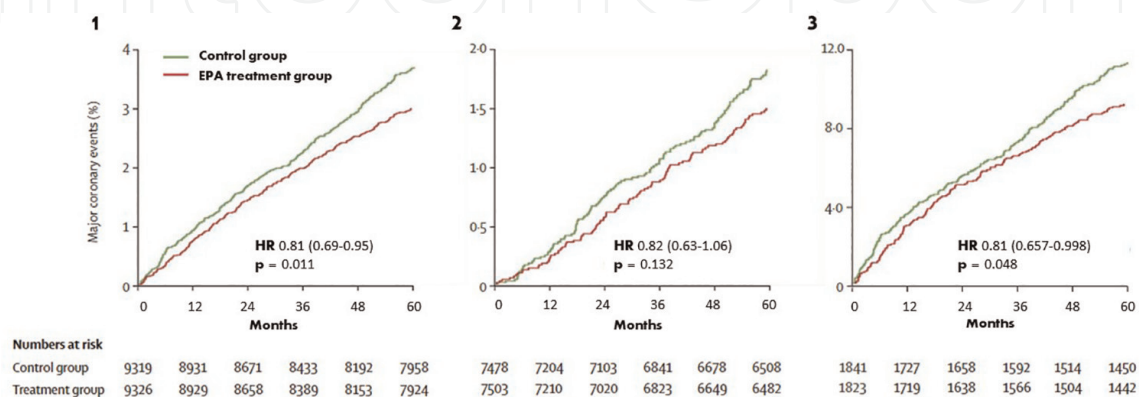

Figure 7.
Kaplan-Meier estimates of incidence of coronary events in the total study population (panel 1), the primary prevention arm (panel 2), and the secondary prevention arm (panel 3). Modified from Yokoyama et al. [33]. HR = Hazard ratio.

95% CI, 0.65–0.83; p < 0.001). The rate of ischemic events was significantly lower in the EPA group than in the placebo group, as was the CV mortality rate (4.3% vs. 5.2%; HR 0.80; 95% CI, 0.66–0.98; p = 0.03).

Regarding the safety profile of EPA, it was observed that hospitalization for atrial fibrillation or flutter occurred in a significant proportion of EPA-treated patients (3.1% vs. 2.1%; p = 0.004). Serious bleeding events occurred in 2.7% of EPA patients and 2.1% of controls (p = 0.06).

The study shows that in patients with elevated triglyceridemia, even with statin therapy, the risk of ischemic events and CV death is further reduced by EPA supplementation (**Figure 8**).

STRENGTH (2020). Multicenter, randomized, double-blind study [35], which evaluated the effects of carboxyl formulation (CA) of EPA and DHA on CV outcomes in adult patients on statin therapy for at least 4 weeks and with elevated CV risk, defined by the presence of:

1. Coronary, carotid, aortic, or peripheral atherosclerotic CVM (secondary prevention);

2. Diabetes mellitus type I or type II (age > 40 years for men and > 50 years for women) and at least one additional risk factor: smoking, hypertension, hsPCR >2 mg/L, or moderate albuminuria;

3. High-risk patients (age > 50 years for men and > 60 years for women) with at least one risk factor, including: family history of CAD, chronic smoking, hsPCR >2 mg/L, impaired renal function, or a coronary calcium score > 300 Agatston units (primary prevention).

A total of 13,078 patients were enrolled in 675 community and teaching hospitals and 22 states in North America, Europe, South America, Asia, Australia, New Zealand, and South Africa. All included patients had high TRI values (between 180 and 499 mg/dL) and low C-HDL values (<42 mg/dL in men, <47 mg/dL in women). More than 50% of the enrolled patients met the criteria for secondary CV prevention.
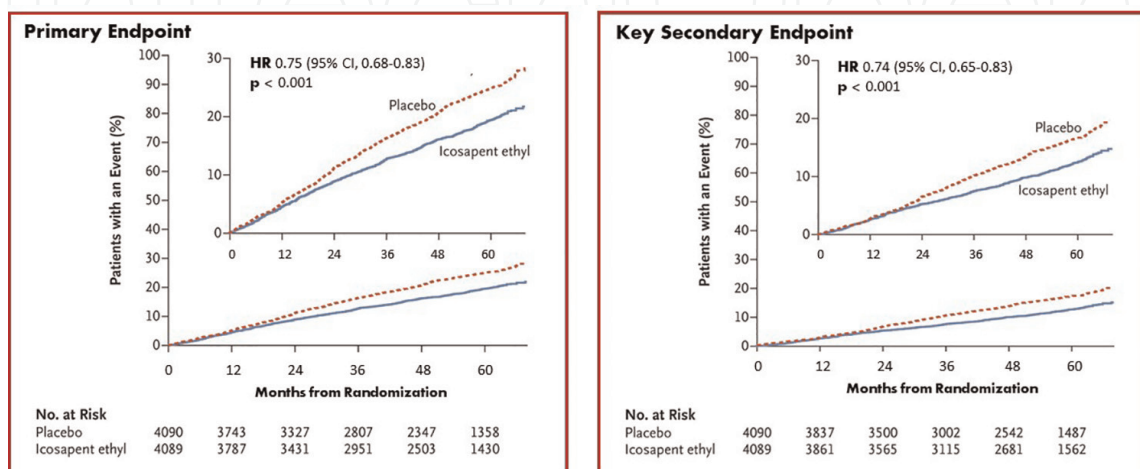

Figure 8.
Cumulative incidence of cardiovascular events in REDUCE-IT trial. Modified from Bhatt et al. [34]. HR = Hazard ratio.
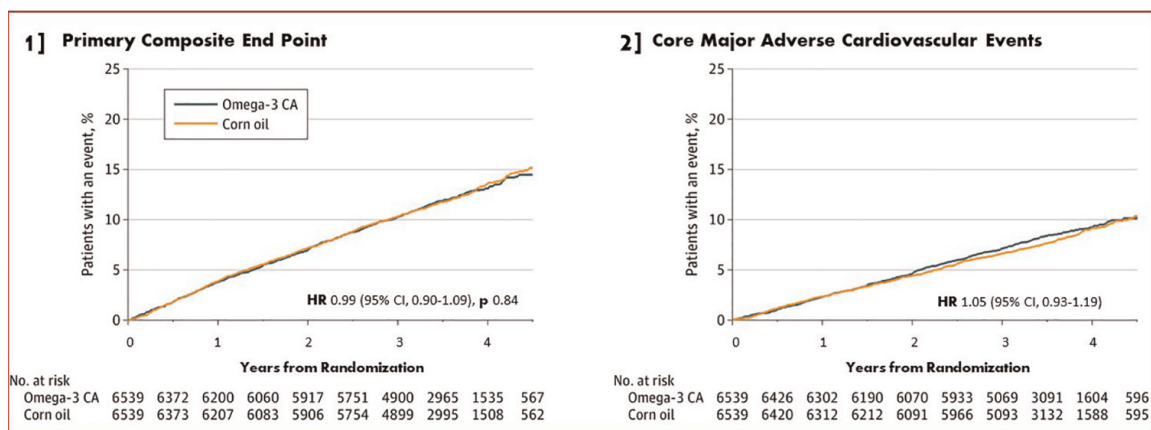
Figure 9.
Time to first incidence of any component of the primary composite end point (1) and time to Core major adverse cardiovascular events (2). Modified from Nicholls SJ et al. [35]. 1, the primary composite end point consisted of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization and hospitalization for unstable angina. 2, Core major adverse cardiovascular events included cardiovascular death, nonfatal myocardial infarction and nonfatal stroke.

Patients were randomized to receive 4 grams/day of CA n-3 PUFAs (n = 6539) or corn oil placebo (n = 6539), in combination with usual medical therapy.

The primary composite efficacy endpoint was an association of CV death, nonfatal AMI, nonfatal stroke, coronary revascularization, and hospitalization for unstable angina. Secondary endpoints included (1) the association of CV death, nonfatal IMA, nonfatal stroke, coronary revascularization, and hospitalization for unstable angina in patients with pre-existing CVD at baseline, (2) the association of CV death, nonfatal IMA, and nonfatal stroke in the whole cohort of patients and in patients with pre-existing CVD at baseline, (3) the association of cardiac death, nonfatal AMI, coronary revascularization, and hospitalization for unstable angina in the entire cohort of patients and in those with pre-existing CVD at baseline, (4) CV death in the entire cohort of patients and in those with pre-existing CVD at baseline, and (5) all-cause mortality in all patients and in those with pre-existing CVD at baseline.

When 1384 patients developed a primary endpoint event (compared with 1600 expected), the trial was stopped early on the basis of a biased analysis of the data, which showed a low likelihood of clinical benefit of n-3 PUFA CA compared with placebo. Study population baseline characteristics were as follows: mean age 62.5 + 9.0 years; 35% women; 70% with diabetes; median C-LDL 75.0 mg/dL; median triglyceride levels 240 mg/dL; median HDL-C levels 36 mg/dL; and median hsPCR 2.1 mg/L. Out of 12,633 patients (96.6%) who completed the trial, the primary end point occurred in 785 (12.0%) treated with n-3 PUFAs compared with 795 (12.2%) treated with corn oil (HR, 0.99 [95% CI, 0.90–1.09]; P = 0.84). There was, in addition, a high rate of gastrointestinal adverse events in the treatment group (24.7% vs. 14.7%).

Therefore, the results of this study do not support the use of PUFA n-3 formulations for improving clinical outcome in patients at high CV risk and on statin therapy (**Figure 9**).

5.1 REDUCE-IT and STRENGTH trials: Why conflicting results?

Several hypotheses have been developed to interpret the reasons that led REDUCE-IT and STRENGTH (two high-quality studies that employed full doses of n-3 PUFAs), to deeply conflicting results [36].

1. One possible explanation theorizes that in the REDUCE-IT study, EPA did not reduce the risk of CV events but, rather, its comparator (mineral oil) increased them. An increase in C-LDL, apoB, and hsPCR associated with mineral oil seems to support this interpretation. However, a U.S. FDA review of the REDUCE-IT results concluded that this hypothesis could explain only part of the highlighted difference between EPA and mineral oil.

2. A second consideration concerns the use in STRENGTH of CA's formulation of n-3 PUFAs composed of both EPA and DHA, in contrast to studies conducted with purified EPA. Although EPA levels in plasma and red blood cells were higher in REDUCE-IT than in STRENGTH, it is unclear whether these differences are sufficient to explain the observed results. This doubt is accentuated by the fact that in STRENGTH trial there was no significant reduction in the risk of CV events in patients with higher levels of EPA, compared with those with lower levels. Furthermore, the reduction in TRI after 12 months was 18 percent in both trials, suggesting a similar effect of the formulations used.

3. Another food for thought is the fact that STRENGTH was discontinued during the first phase of the COVID-19 pandemic and that the "end-of-treatment" visits were conducted *via* telephone contact in order to allow the trial to be terminated as early and orderly as possible. This may have compromised the integrity of the study itself.

To resolve the discrepancies between STRENGTH and REDUCE-IT, regulatory agencies such as the FDA could authorize a post-marketing clinical trial comparing high-dose EPA vs. corn oil in patients at high risk of developing CV events.

6. Real word data about n-3 PUFAs

TG-REAL (2020). Longitudinal, retrospective cohort study that used 3 administrative databases from 3 Italian local health authorities [37]. It included 158,042 individuals with at least one serum TG assay between January 1, 2010 and December 31, 2015. Individuals with normal TG values (<150 mg/dL; n = 142,289) were compared with patients with high values (150–500 mg/dL; n = 15,558) and those with very high values (>500 mg/dL; n = 195).

The outcomes were atherosclerotic CV events (ASCVD) and all-cause mortality. Overall, the incidences of ASCVD and all-cause mortality were 7.2 and 17.1 per 1000 individual-years, respectively. After multivariate correction for potential confounding factors, in individuals with high and very high TG values, there was a significant increased risk of all-cause mortality (HR = 1.49 (95 percent CI 1.36–1.63), $p < 0.001$, and HR = 3.08 (95 percent CI 1.46–6.50), $p < 0.01$, respectively), and of ASCVD (HR = 1.61 (95 percent CI 1.43–1.82), $p < 0.001$, and HR = 2.30 (95 percent CI 1.02–5.18), $p < 0.05$, respectively).

The TG-REAL study captured real-world data related to a large cohort of patients with low-to-moderate CV risk, demonstrating how moderate to severely elevated TG values are associated with a significant increased risk of ASCVD and all cause death.

7. Final considerations

Following the results of the REDUCE-IT trial, the regulatory agencies FDA and EMA have given their approval for the use of n-3 PUFAs in patients at high CV risk in order to reduce atherosclerotic-based events. The recommendations for the use of n-3 PUFAs in the more recent European and US guidelines are shown in **Table 4** [9].

An Update is expected from the European Scientific Societies. In fact, in the 2019 European Society of Cardiology (ESC) guidelines, the recommendation for the use of

Medical society	Date	Guidelines/standards/advisory statements
American Diabetes Association – ADA	March 2019	ADA's Standards of Medical Care updated Section 10, Treatment of Other Lipoprotein Fractions or Targets, states:
		In patients with ASCVD or other cardiac risk factors on a statin with controlled LDL-C but elevated TG (135–499 mg/dL), the addition of icosapent ethyl should be considered to reduce CV risk—Level A
		It should be noted that data are lacking with other omega-3 fatty acids, and the results of REDUCE-IT should not be extrapolated to other products
		Combination therapy (statin/fibrate) has not been shown to improve atherosclerotic CV disease and is generally not recommended—Level A
		Combination therapy (statin/niacin) has not been shown to provide additional CV benefit above statin therapy alone, may increase the risk of stroke with additional side effects, and is generally not recommended—Level A
American Heart Association – AHA Science Advisory	August 2019	The AHA Issued a Scientific Advisory that dietary supplements are not recommended and that positive outcomes results were demonstrated in REDUCE-IT:
		The use of omega-3 fatty acids (4 g/day) for improving ASCVD risk in patients with hypertriglyceridemia is supported by a 25% reduction in MACE in REDUCE-IT
		The potency, quality, and efficacy of dietary supplement are not initially reviewed or approved, nor are they subsequently monitored or assured by the FDA; thus, they are not indicated for the treatment of disease
		In the treatment of patients with very high TG levels with 4 g/day, EPA + DHA agents reduce TG by ≥30% with concurrent increases in LDL-C, whereas EPA-only does not raise LDL-C in patients with very high TG levels
European Atherosclerosis Society/ European Society of Cardiology – EAS/ESC	August 2019	The EAS and ESC jointly updated patient treatment guidelines to state: In high-risk (or above) patients with TG between 1.5 and 1.6 mmol/L (135–499 mg/dL) despite statin treatment, n-3 PUFAs (Icosapent ethyl 2 × 2 g/day) should be considered in combination with statins – Class IIa, Level B

Medical society	Date	Guidelines/standards/advisory statements
National Lipid Association – NLA	September 2019	NLA Position on the Use of Icosapent Ethyl in High- and Very High-Risk Patients states:
		For patients, ≥45 years with clinical ASCVD, or ≥ 50 years with type 2 diabetes requiring medication and ≥ 1 additional risk factor, and fasting TGs 135–499 mg/dL on a maximally tolerated statin, with or without ezetimibe, treatment with icosapent ethyl is recommended for ASCVD reduction – Class 1, Level B-R
American Heart Association Scientific Statement	April 2020	The AHA recommends consideration of icosapent ethyl in patients with type 2 diabetes for further CV risk reduction when TGs remain elevated despite maximally tolerated statin for management of coronary artery disease
New FDA-approved Indication:		
FDA-approved label expansion beyond TG lowering	December 2019	Icosapent ethyl is indicated as an adjunct to **maximally tolerated statin therapy** to reduce the risk of MI, stroke, coronary revascularization, and unstable angina requiring hospitalization in adult patients with elevated TG levels (≥ **150 mg/dL**) and established CV disease or diabetes mellitus and **2 or more additional risk factors** for CV disease[a]

[a]*Bold text highlights aspects of expanded label indication that notably differ from language in updated guidelines and REDUCE-IT entry criteria.*
Modified from Trivedi K et al. [11]. ADA, American Diabetes Association; AHA, American Heart Association; CV, cardiovascular; EAS, European Atherosclerosis Society; EPA, eicosapentaenoic acid; ESC, European Society of Cardiology; FDA, Food and Drug Administration; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; NLA, National Lipid Association; REDUCE-IT, Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial; TG, triglyceride.

Table 4.
Statements from medical societies and the US FDA regarding IPE (pure EPA) post-REDUCE-IT.

n-3 PUFAs in high-risk patients with hypertriglyceridemia is still class IIa, despite the positive results that have recently emerged from clinical trials.

Currently, the strategy for the management and prevention of CV diseases follows the so-called "ABCDEF" scheme.

In that strategy:

- **A** corresponds to CV risk assessment (Assessment of risk), the need to set antiplatelet therapy (Antiplatelet therapy), and the need to manage atrial fibrillation (Atrial fibrillation management).

- **B** corresponds to the management of Blood pressure.

- **C** corresponds to Cholesterol management and cessation of cigarette smoking.

- **D** corresponds to Diet and lifestyle modification, as well as diabetes management.

- **E** corresponds to Exercise.

- **F** to the management of heart Failure.

Based on current evidence, it is believed that under "E," along with exercise, the intake of EPA and IPE, rather than fish oil dietary supplements, should also be considered as a "new entry."

It is also important to note that previous studies on n-3 PUFAs, in which there was a goal of lowering TG levels, have not obtained concordant results regarding their efficacy in reducing CV events. These conflicting results seem attributable to several factors, including frequent use of inappropriate drug dosages, use of different formulations, and/or inadequate selection of the target population. Therefore, it is of great relevance to find answers to the outstanding questions and to overcome the biases highlighted in past clinical trials. Such doubts or questions could only be clarified by performing comparison studies between different available formulations, such as those used in the REDUCE-IT and STRENGTH clinical trials, in order to fully understand the real differences in terms of efficacy and safety of these drugs.

Finally, it is important to point out that IPE therapy has been shown to be closely related to a reduction in the residual risk of total ischemic events in patients with ASCVD or with type II diabetes mellitus and other CV risk factors, already on statin therapy. These evidences would therefore support a preferential indication for the use of IPE over currently marketed formulations.

Conflict of interest

The authors declare no conflict of interest.

Author details

Savina Nodari* and Francesco Fioretti
Cardiology Unit, Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University and Spedali Civili Hospital of Brescia, Brescia, Italy

*Address all correspondence to: savina.nodari@unibs.it

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