Atherosclerosis 322 (2021) 15–23

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Review article

# Cardiovascular effects of omega-3 fatty acids: Hope or hype?

Sang-Ho Jo [a], Seung Hwan Han [b,e], Sang-Hyun Kim [c], Robert H. Eckel [d], Kwang Kon Koh [b,*]

[a] Cardiovascular Center, Hallym University Sacred Heart Hospital, Anyang-si, South Korea
[b] Division of Cardiovascular Disease, Gachon University Gil Hospital, Incheon, South Korea
[c] Division of Cardiology, Seoul National University Boramae Hospital, Seoul National University College of Medicine, Seoul, South Korea
[d] Division of Endocrinology, Metabolism and Diabetes, Division of Cardiology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA
[e] Gachon Cardiovascular Research Institute, Incheon, South Korea

ARTICLE INFO

*Keywords:*
Omega-3 fatty acids
Residual cardiovascular risk
Atherosclerosis

ABSTRACT

Omega-3 fatty acids have emerged as a new option for controlling the residual risk for cardiovascular disease (CVD) in the statin era after a clinical trial (REDUCE-IT) reported positive results with icosapent ethyl (IPE) in patients receiving maximally tolerated statin therapy. However, another trial which used high dose eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) combination (STRENGTH) has failed. Together, these results raise clinically important questions. Are effects of omega-3 fatty acids neutral or beneficial in patients on statin therapy, or perhaps even harmful? The current contradictory results could be attributed to different types of omega-3 fatty acids (only EPA or combination of EPA + DHA), doses (higher *vs.* lower dose) of omega-3 fatty acids or different comparators (corn oil or mineral oil), as well as the underlying severity of the CVD risk or use of statins.

Together with these issues, we will discuss different biological and clinical effects of various types of omega-3 fatty acids and then interpret different results of past and current clinical studies and propose practical suggestions, which could be applied in patient management.

## 1. Introduction

Omega-3 fatty acids have emerged as another option for reducing the atherosclerotic cardiovascular disease (ASCVD) burden. This opportunity comes from positive results of the recent randomized controlled clinical trial using icosapent ethyl (IPE, highly purified eicosapentaenoic acid [EPA] ethyl ester) *vs.* placebo (Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial [REDUCE-IT]) [1]. In this clinical trial, patients with underlying cardiovascular disease or diabetes with additional risk factors gained benefit of IPE on top of statin therapy [1]. However, other recent trials, which tested low or high dose omega-3 fatty acids (EPA + docosahexaenoic acid [DHA]) including STRENGTH (A Long-Term Outcomes Study to Assess STatin Residual Risk Reduction With EpaNova in HiGh Cardiovascular Risk PatienTs With Hypertriglyceridemia) trial that used 4 g omega-3 carboxylic acids (EPA-NOVA), failed [2–5].

This discrepancy casts clinically important questions. Who is eligible and which omega-3 fatty acids and what dose will be beneficial to high-risk patients on statin therapy? There have been numerous studies showing the effects of omega-3 fatty acids on lipoprotein metabolism, cell membrane stabilization, plaque composition/progression/development, and anti-platelet/anti-oxidative/anti-inflammatory mechanisms of atherosclerosis [6–8]. However, it has been quite difficult to understand the results of clinical studies because of a number of reasons: the low *vs.* high doses of omega-3 fatty acids, EPA *vs.* combination of EPA + DHA, target populations including those with/without statin therapy and on various statin doses and very recently, placebos such as corn or mineral oil. Therefore, we will discuss different biological and clinical effects of various types of omega-3 fatty acids and how to interpret disparate results of clinical studies to provide insights related to clinical care.

## 2. Biological effects of omega-3 fatty acids

### 2.1. Effects on lipoprotein metabolism

Omega-3 fatty acids are known to lower plasma triglyceride levels by reducing very low-density lipoprotein production, augmenting very

* Corresponding author. Cardiometabolic Syndrome Unit, Division of Cardiology, Gachon University Gil Hospital, 774 Beongil 21, Namdongdaero, Namdong-Gu, Incheon, 21565, South Korea.
*E-mail address:* kwangk@gilhospital.com (K.K. Koh).

https://doi.org/10.1016/j.atherosclerosis.2021.02.014
Received 18 January 2020; Received in revised form 16 February 2021; Accepted 18 February 2021
Available online 23 February 2021

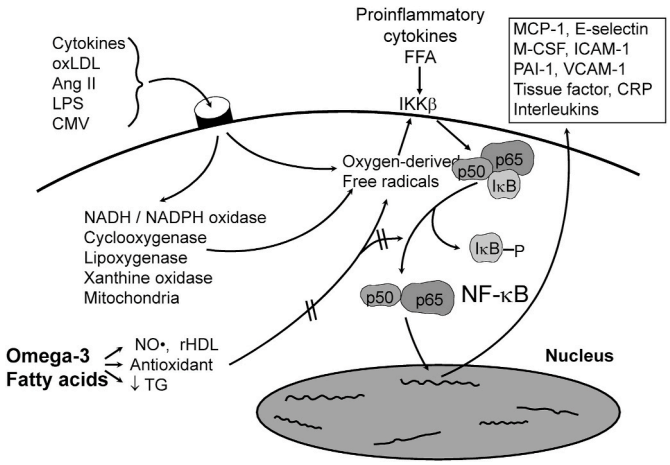


**Fig. 1.** In experimental studies with endothelial cells in culture, numerous molecules initiate transcription of genes that encode protein mediators of inflammation.
Omega-3 fatty acids may modulate this process by inhibiting the activation of nuclear transcription factors. rHDL = reconstituted HDL. Modified from Koh et al. [26,27].

low-density lipoprotein clearance and in some studies by stimulating lipoprotein lipase activity [7]. Highly purified EPA 2–4 g/d reduced remnant-like particle cholesterol, apolipoprotein C-III, and oxidized low-density lipoprotein (LDL) levels in patients with hypertriglyceridemia. EPA reduced triglyceride levels without raising LDL-C levels at 2–4 g/d, compared with DHA, which has potential to raise LDL-C levels modestly when used at similar doses in patients with hypertriglyceridemia [8,9]. Accordingly, some studies suggested the potential of EPA + DHA on LDL-C elevation [10–12], despite contradictory results that at lower triglyceride levels, the combination of EPA and DHA failed to raise LDL-C [13]. The mechanism in patients treated with EPA may relate to reduced production and faster clearance of triglyceride-rich lipoproteins in concert with more rapid clearance of LDL particles and slower production of very low-density lipoprotein particles [14]. To some extent, these differences relate to how levels of LDL-C are determined.

EPA and DHA combination did reduce apolipoprotein C-III levels as observed with EPA [13] and some head-to-head comparison trials demonstrated that DHA could reduce the apolipoprotein C-III levels like EPA [15]. A decrease in apolipoprotein C-III would lower triglycerides levels through several mechanisms, including reduced inhibition of lipoprotein lipase. In addition, EPA, or a combination of EPA + DHA, modestly reduced non-HDL-C and apolipoprotein B [14].

High dose DHA could also increase LDL particle size and LDL-C level and this explains the increase in LDL-C [16].

Some placebo-controlled randomized clinical trials showed that HDL-C increased in the DHA group [17–19], however, both in the EPA and DHA group, there was an increase in ratio of HDL-C to apolipoprotein A1 [17]. Other articles argue that HDL-C is not increased by EPA nor DHA [20,21]. Thus together, the impact of omega-3 fatty acids on HDL composition and metabolism still needs to be elucidated.

Apolipoprotein B has been decreased by EPA but not by DHA [17]. Of interest, both EPA and DHA have been shown to increase the fasting glucose level, presumably a modest effect because no changes in $HbA_1C$, fasting insulin and C-peptide level were observed in patients with diabetes [21].

### 2.2. Effects on vasomotor function

Several studies reported that omega-3 fatty acids improved flow-mediated dilation [22,23]. EPA induces $Ca^{2+}$-independent activation and translocation of endothelial nitric oxide synthase to the cytosol, which results in endothelium-dependent vasorelaxation [24].

This function also enhanced endothelial function in combination with a statin, an effect not seen with DHA [25]. However, we observed that EPA + DHA administered over 2 months to hypertriglyceridemic patients who were not on statins improved flow-mediated dilation [23].

### 2.3. Effects on inflammation

The increased flux of free fatty acids associated with insulin resistance, obesity, metabolic syndrome, and diabetes caused endothelial dysfunction in part by activating innate immune inflammatory pathways upstream of NF-κB. Activation of NF-κB also increases synthesis and release of proinflammatory cytokines, which activate inflammatory cells and enhance their attachment to the vessel wall (Fig. 1) [26,27]. Several mechanisms have been postulated. Although DHA decreased cytokine-induced expression of endothelial leukocyte adhesion molecules and secretion of interleukin (IL)-6 and IL-8 into the medium in cultured endothelial cells [28], EPA reduced high-sensitivity C-reactive protein (hsCRP) in patients with hypertriglyceridemia compared to DHA [29]. In contrast, the combination of EPA + DHA did not reduce hsCRP levels in patients with hypertriglyceridemia [22]. However, some researchers have shown that the efficacy of lowering hsCRP was similar between EPA and DHA [30,31].

A recent study revealed that purified EPA generates EPA-rich high-density lipoprotein (HDL) [32]. This reconstituted HDL (rHDL) contains EPA-phosphatidylcholine, a subspecies that had anti-atherogenic properties and reduced expression of cytokine-stimulated vascular cell adhesion molecule-1. In addition, rHDL increased cholesterol efflux and production of EPA-derived metabolites, anti-inflammatory resolving E3 and its intermediate metabolites 18-hydroxyeicosapentaenoic acid and particles with a variety of anti-inflammatory properties.

Interestingly, one head-to-head comparison in a small sized placebo-controlled trial showed that DHA was more effective than EPA in reducing inflammatory marker like interleukin-18 (IL-18) [18].

### 2.4. Effects on oxidation

Omega-3 fatty acids were reported to reduce oxidative stress [33]. Omega-3 fatty acids increase serum total antioxidant capacity, glutathione peroxidase activity and decrease malondialdehyde [34]. Oxidative stress estimated by urinary F2-isoprostanes measurement was reduced with both EPA and DHA as compared to olive oil [35].

EPA inhibits oxidation of apolipoprotein B-containing particles (like LDL, VLDL, small dense LDL) unlike DHA [36]. HDL isolated from EPA-only treated individuals exhibited enhanced cholesterol efflux from monocytes and augmented antioxidant and anti-inflammatory actions [37]. In addition, EPA partitions into the outer monolayer of the HDL particle and exhibits greater antioxidant function than DHA-loaded HDL [38].

### 2.5. Effects on thrombosis

Omega-3 fatty acids also affect thrombotic mechanisms including platelet aggregation. One mechanism may relate to the observation that EPA and DHA generate the cardioprotective and antithrombotic metabolites thromboxane A3 and prostacyclin. For platelet aggregation, males may benefit more from EPA supplementation while females are more responsive to DHA. This may suggest that interactions between sex hormones and omega-3 fatty acids exist to differentially reduce platelet aggregation in healthy individuals [39].

It should be noted, however, that DHA (highly purified) may be more anti-thrombotic than EPA [40].

High dose omega-3 fatty acids may lead to beneficial effects in patients without coronary ischemia such as more zealous lowering of triglycerides, improved vasomotor function, inflammation, and platelet function/hemostasis [41,42].

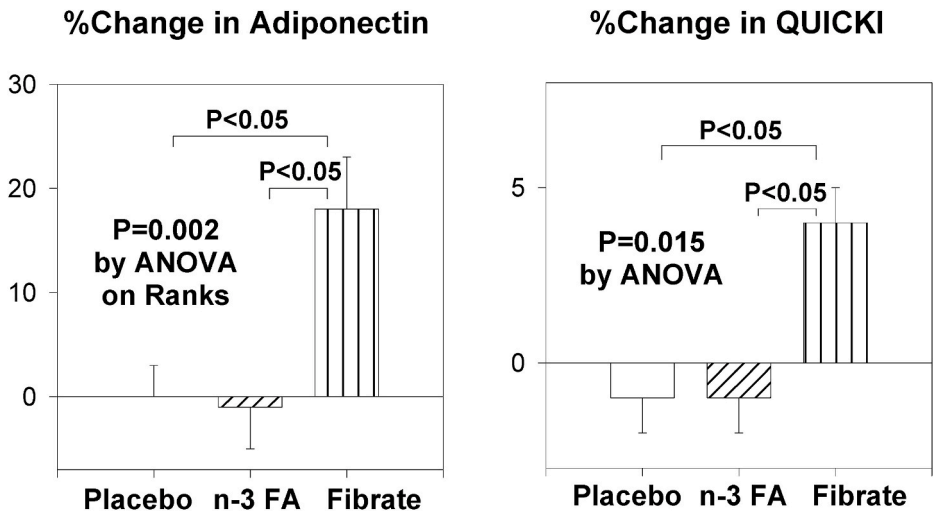


**Fig. 2.** Placebo and omega-3 fatty acids (n-3 FA) did not change plasma adiponectin levels and insulin sensitivity (determined by QUICKI) relative to baseline measurements.
Fenofibrate significantly increased plasma adiponectin levels ($p < 0.001$ by Wilcoxon Signed Rank test) and insulin sensitivity ($p = 0.003$ by paired *t*-test) when compared with baseline. Moreover, these effects of fenofibrate were significant when compared with either placebo or omega-3 fatty acids ($p$=0.002 for adiponectin by ANOVA on Ranks and $p = 0.015$ for QUICKI by ANOVA). Standard error of the mean is identified by the bars. Reprinted with permission from Koh et al. [6].

### *2.6. Effects on membrane fluidity, cholesterol domain, and crystal formation*

Excessive cholesterol accumulation in the membranes of vascular smooth muscle cells and macrophages can promote the formation of distinct lipid domains within the cell membrane consisting of bilayers of cholesterol monohydrate. Such cholesterol domains may facilitate the formation of extracellular cholesterol crystals, a hallmark of atherosclerotic plaques [14].

EPA has a hydrocarbon length and number of double bonds that foster preferential intercalation into the alkyl chain core of the membrane bilayer, where it inhibits cholesterol domain formation [43]. By contrast, DHA has a longer hydrocarbon length and thus promotes conformational changes in the membrane [44,45], while EPA preserves a more ordered membrane structure [45,46]. Therefore, these conformational differences cause DHA to change the normal distribution of cholesterol and even promote membrane cholesterol domains as compared to EPA. Thus, EPA and DHA could impose a different impact on the cell membrane [46–48].

### *2.7. Effects on adiponectin and insulin resistance*

Adiponectin is an adipose-derived secretory factor that couples regulation of insulin sensitivity with energy metabolism and augments both metabolic and vascular actions of insulin [49,50].

Dietary fish oil and omega-3 fatty acids increase total adiponectin levels [51,52]. However, omega-3 fatty acids or fish consumption has been associated with modestly higher incidence of type 2 diabetes [53,54] and large scale meta-analysis demonstrated that use of omega-3 fatty acids did not prevent nor treat patients with diabetes [55]. Of interest, EPA decreased adiponectin gene expression and protein secretion and reduced peroxisome proliferator-activated receptor-gamma mRNA levels in primary cultured rat adipocytes [56]. In contrast, one head-to-head comparison found that the DHA increased adiponectin more than EPA [9]. However, DHA did not change fasting or postprandial insulin and glucose concentrations and insulin sensitivity in hypertriglyceridemic men [57]. We demonstrated EPA + DHA treatment did not change plasma adiponectin levels and insulin sensitivity in patients with hypertriglyceridemia in contrast to fibrate therapy (Fig. 2) [6,23,58].

## 3. Randomized clinical trials

We will review and discuss the clinical study according to the type of formula, IPE, or EPA only or EPA + DHA used in clinical trials.

### *3.1. IPE or EPA alone studies*

Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial (REDUCE-IT) shed new light on omega-3 fatty acids and their benefit to patients with or at high risk for ASCVD [1]. The omega-3 fatty acids used in the active group was icosapent ethyl, a highly purified EPA which was different from omega-3 fatty acids used in previous studies (Table 1) and similar to EPA administered in the Japan Eicosapentaenoic acid Lipid Intervention Study (JELIS) study that also demonstrated a positive outcome [59].

In REDUCE-IT, among the 8179 participants with high CV risk of whom 71% had established cardiovascular disease, 29% comprised a primary prevention cohort, and 58% had type 2 diabetes mellitus (diabetes): the baseline LDL-C levels were well-controlled with statins (median value, 75.0 mg/dL), while triglyceride levels were moderately elevated (median value, 216.0 mg/dL). With a median follow-up of 4.9 years, the primary endpoint was reduced in patients in the IPE group at 17.2% compared with 22.0% in the placebo group (hazard ratio, 0.75; 95% CI 0.68–0.83). Notably in subgroup analysis, the benefit was observed irrespective of initial and attained levels of triglyceride or LDL-C and irrespective of statin use. The targeted level of triglyceride to be achieved, i.e., 150 mg/dL, failed to impact the primary or key secondary efficacy endpoint. However, if patients had both baseline triglyceride levels >200 mg/dL and HDL-C <35 mg/dL (~20% of all patients), the benefit of IPE was slightly more favorable with a hazard ratio of 0.62 than the other group, hazard ratio of 0.79 ($p = 0.04$ for interaction). The investigator of REDUCE-IT trial performed *post-hoc* analysis on the association with attained EPA serum level and clinical outcomes. They found IPE treatment increased the EPA level from 26.1 to 135.2 μg/mL (from baseline to daily average value over 5 years, 3.6-fold increase), whereas the placebo group level did not change (from 26.1 to 27.7 μg/mL) (Bhatt D. EPA levels and cardiovascular outcomes in the Reduction of Cardiovascular Events with Icosapent Ethyl Intervention Trial. Presented on: March 30, 2020. ACC 2020).

The other positive omega-3 fatty acids trial was JELIS [59]. In this study, 18,645 patients with or without coronary artery disease, defined as previous myocardial infarction, coronary interventions, or confirmed angina pectoris, were recruited. Underlying risk factors were smoking in 20%, diabetes in 16% and hypertension in 35%, respectively. Patients were randomly assigned to receive either EPA 1800 mg/d with statin or statin only. At mean follow-up of 4.6 years, they observed the primary endpoint in 2.8% patients in the EPA group and 3.5% in controls (hazard ratio, 0.81; 95% CI 0.69–0.95).

The omega-3 fatty acids used in both trials was only EPA or IPE albeit at different doses (1.8 g/d in JELIS *vs.* 4 g/d in REDUCE-IT). In the JELIS

**Table 1**
Comparison of major omega-3 fatty acids clinical trials.

| Study name | VITAL | ASCEND | ORIGIN | STRENGTH | REDUCE-IT | JELIS |
|---|---|---|---|---|---|---|
| Year, journal | 2019, NEJM [4] | 2018, NEJM [3] | 2012, NEJM [2] | 2020, JAMA [5] | 2019, NEJM [1] | 2007, Lancet [59] |
| Patients number, N | 25,871 | 15,480 | 12,536 | 13,078 | 8179 | 18,645 |
| Study characteristics | Primary prevention | Primary prevention | Primary prevention | Primary + Secondary prevention | Primary + Secondary prevention | Primary + Secondary prevention |
| Study drug/ omega-3 fatty acids formula and daily dose | EPA + DHA, 840 mg; 460 mg of EPA +380 mg/d of DHA | 840 mg of marine n−3 fatty acids, 460 mg of EPA +380 mg/d of DHA | Ethyl esters of n–3 fatty acids, 900 mg/ d (90% or more ethyl esters) | EPA + DHA (omega-3 carboxylic acid), 4 g/d | Icosapent-ethyl, 4 g/ d | EPA, 1.8 g/d |
| Patients inclusion | Healthy, no cancer, no CVD, men≥50, women≥55 | Men and women≥40 both. Diabetes but without evidence of CVD | At high risk for cardiovascular events and with impaired fasting glucose, impaired glucose tolerance, or diabetes | (1) Established CVD, (2) type 1 or 2 diabetes with ≥40 (men) and ≥50 (women) with ≥1 risk factor of chronic smoking, hypertension, hs-CRP level ≥2 mg/L, or moderately increased albuminuria, (3) primary prevention patients ≥50 (men) or ≥60 (women) with ≥1 risk factor of family history of premature CVD, chronic smoking, hs-CRP level ≥2 mg/L, impaired kidney function, or coronary calcium score >300 Agatston units | Diabetes or established CVD, on statin with high TG | Men aged 40–75 years and postmenopausal women aged up to 75 years, with or without coronary artery disease (previous MI, PCI or confirmed angina pectoris), MI history 5%, angina 15–16%, CABG, PTCA 5% |
| Follow-up period (years) | 5.3 | 7.4 | 6.2 | 3.5 | 4.9 | 4.6 |
| Primary outcome measures | Major cardiovascular events (a composite of myocardial infarction, stroke, or death from cardiovascular causes) | First serious vascular event (nonfatal myocardial infarction or stroke, transient ischemic attack, or vascular death, excluding confirmed intracranial hemorrhage) | Death from cardiovascular causes | Composite of CV death, nonfatal MI, nonfatal stroke, coronary revascularization, hospitalization for unstable angina | Composite of CV death, MI, revascularization, unstable angina | Composite of sudden cardiac death, fatal and non-fatal MI, and other non-fatal events including unstable angina pectoris, angioplasty, stenting, or CABG |
| Active drug group event rate (%, n/N) *vs.* placebo group event rate (%, n/N) | 2.98% (386/12,933) *vs.* 3.24% (419/12,938) | 8.9% (689/15,480) *vs.* 9.2% (712/15,480) | 9.1% (574/12,536) *vs.* 9.3% (581/12,536) | 12.0% (785/6539) *vs.* 12.2% (795/6539) | 17.2% (705/4089) *vs.* 22.0% (901/4090) | 2·8% (262/18,645) *vs.* 3·5%, (324/18,645) |
| HR, 95% CI, *p* for primary endpoints | 0.92; 0.80–1.06; $p = 0.24$ | 0.97; 0.87–1.08; $p = 0.55$ | 0.98; 0.87–1.10; $p = 0.72$ | 0.99; 0.90–1.09. $p = 0.84$ | 0.75; 0.68–0.83, $p < 0.001$ | 0.81, 0.69–0.95, $p = 0.011$ |
| Secondary outcome measures or major subgroup analysis | Individual components of the composite cardiovascular end point, the composite end point plus coronary revascularization | First serious vascular event or any arterial revascularization | Composite outcome of death from cardiovascular causes, nonfatal MI, or nonfatal stroke; death from any cause; death from arrhythmia | (1) Composite of CV death, nonfatal MI, nonfatal stroke, PCI, hospitalization for unstable angina in patients with established CVD,<br>(2) composite of CV death, nonfatal MI, and nonfatal stroke,<br>(3) composite of cardiac death, nonfatal MI, PCI, and hospitalization for unstable,<br>(4) CV death,<br>(5) all-cause death | Composite of cardiovascular death, nonfatal MI, or nonfatal stroke in a time-to-event analysis | All-cause mortality, stroke, peripheral artery disease, and cancer.<br>Primary prevention arm and secondary prevention arm |
| Major secondary outcome measure and results; rate, (HR, CI) | Total MI. 1.12% (145/12,933) *vs.* 1.55% (200/ 12,938); (HR 0.72, 95% CI, 0.59 to 0.90) death from MI; 0.1% *vs.* 0.2% (HR, 0.50; 95% CI, 0.26 to 0.97) PCI; 1.25% *vs.* 1.61% | Serious vascular events or revascularization: 11.4% *vs.* 11.5% (rate ratio, 1.00; 95% CI, 0.91 to 1.09).<br>Exploratory analysis: vascular death; 2.5% *vs.* 3.1% (RR 0.82, CI 0.68–0.98) | Major vascular events; 16.5% (1034) *vs.* 16.3% (1017) (HR, 1.01; 95% CI, 0.93–1.10; $p = 0.81$); death from any cause 15.1% (951) *vs.* 15.4% (964) (HR 0.98; 95% CI, 0.89 to 1.07; $p =$ | CV death, MI, or stroke occurred with 8.3% (541/6539) treated with omega-3 CA *vs.* 7.9% (517/6539) treated with corn oil (HR, 1.05, 95% CI, 0.93–1.19; $p = 0.40$). Cardiac death, MI, PCI, or hospitalization for | 11.2% *vs.*14.8%; HR 0.74 (95% CI 0.65 to 0.83, $p < 0.001$) | All-cause mortality; 2.8% *vs.* 3.1% (HR 1.09, 95% CI 0.92–1.28, $p = 0.333$).<br>Secondary prevention subgroup: 8·7% (158) in EPA group *vs* 10.7% (197) in control group; $p = 0.048$) |

(*continued on next page*)

**Table 1** (*continued*)

| Study name | VITAL | ASCEND | ORIGIN | STRENGTH | REDUCE-IT | JELIS |
|---|---|---|---|---|---|---|
| | (HR 0.78; 95% CI, 0.63 to 0.95); total coronary heart disease 2.4% *vs.*2.9% (HR, 0.83; 95% CI, 0.71 to 0.97) | | 0.63); death from arrhythmia, 4.6% (288) *vs.* 4.1% (259) (HR, 1.10, 95% CI, 0.93 to 1.30; $p = 0.26$) | unstable angina, occurred in 8.5% of cases (556/6539) treated with omega-3 CA and 9.4% (616/5539) of patients treated with corn oil (HR, 0.91; 95% CI, 0.81–1.02; $p = 0.09$). | | (HR 0.81, 95% CI 0.66–1.00, $p = 0.048$). Primary prevention: (1.4%) *vs.*1.7% (127) (HR 0.82, CI 0.63–1.06, $p = 0.132$) |

*NEJM, New England Journal of Medicine; JAMA, Journal of American Medical Association; MI, myocardial infarction; hs-CRP, high-sensitivity C-reactive protein; PCI, percutaneous coronary intervention; HR, hazard ratio; CI, confidence interval; CV, cardiovascular; CABG, coronary arterial bypass graft; PTCA, percutaneous transluminal coronary angioplasty; EPA, eicosapentaenoic acid.

study, 74% of patients were categorized as primary prevention and 26% as secondary prevention. Meanwhile, in REDUCE-IT, most of the patients (70.7%) were secondary prevention and 29.3% primary prevention. Even though most of the patients were primary prevention in JELIS, the study result was positive favoring EPA and IPE. This may be attributed to the unique EPA and IPE effects and to the size of the study population. Relevant here is that the size of JELIS population was like that from the VITAL study, which examined the impact of vitamin D and omega-3 fatty acids using EPA + DHA, wherein similar event rates were experienced in placebo groups, 3.24% in VITAL and 3.5% in JELIS. Recent randomized placebo-controlled clinical trial, Effect of Vascepa on Improving Coronary Atherosclerosis in People With High Triglycerides Taking Statin Therapy (EVAPORATE) to assess the atherosclerotic plaque characteristics change with IPE as compared to mineral oil control evaluated by coronary computed tomographic angiography (CT angiogram) provided some insight into the mechanism of benefit with IPE [60]. Patient with one or more angiographic stenosis≥ 20% on baseline statin were randomized to either IPE 4 g/d or placebo (mineral oil) for 18 months, and CT angiogram was performed to detect the change in low-attenuated plaque (LAP) volume, a strong predictor of future CVD. This trial demonstrated LAP volume was reduced by 17% in the IPE treatment group. Other plaques like fibro-fatty and fibrous were also decreased in the IPE group. By contrast, the placebo group showed progression. This effect on atherosclerotic plaque composition could, in part, explain the beneficial effect of IPE in REDUCE-IT study.

### 3.2. EPA plus DHA combination study

Recent clinical trials using EPA + DHA combination failed to demonstrate an effect in reducing CVD events. The VITAL study was one of them [4]. This was the largest randomized, placebo-controlled trial which tested the efficacy of EPA + DHA and vitamin D using a 2 by 2 factorial design. A major cardiovascular event occurred 2.98% in the EPA + DHA group and 3.24% in the placebo group (hazard ratio, 0.92; 95% CI 0.80–1.06) during a median 5.3-year follow-up. Despite the overall negative results for the primary outcome, among key secondary end points, total myocardial infarction (MI) risk and death from MI were substantially lower in the EPA + DHA group. The rate of performing percutaneous coronary intervention was also lower in the EPA + DHA group (hazard ratio, 0.78; 95% CI 0.63–0.95) as well as total coronary heart disease rate (hazard ratio, 0.83; 95% CI 0.71–0.97).

Another trial which failed to achieve the primary outcome was ASCEND, "A Study of Cardiovascular Events in Diabetes [3]. In VITAL and ASCEND, 460 mg of EPA and 380 mg of DHA were used. The ASCEND study was carried out as a primary prevention trial that included 15,480 patients with diabetes but without a history of CVD [3]. The outcome was negative (hazard ratio, 0.97; 95% CI 0.87–1.08) at 7.4 years of follow-up. Because all the patients had diabetes, the event rate in placebo was higher than those in VITAL, 9.2% *vs.* 3.25%, respectively (Table 1). However, the event rate in EPA + DHA group was not different from the placebo group. However, there was some benefit in an exploratory analysis of vascular death, 2.5% *vs.* 3.1% (rate ratio 0.82; 95% CI 0.67–0.99) over a longer follow-up duration of 7.4 years.

Another primary prevention outcome trial was Outcome Reduction with an Initial Glargine Intervention (ORIGIN) [2]. This trial enrolled 12,536 subjects at high risk with (or at risk for) diabetes without a CVD history. Participants were randomly allocated to either a 1 g EPA + DHA capsule or placebo daily. The primary outcome was cardiovascular death. During a median follow-up of 6.2 years, the incidence of the primary outcome was not different between patients receiving EPA + DHA and those receiving placebo, 9.1% *vs.* 9.3% (hazard ratio, 0.98; 95% CI 0.87–1.10). The patient population and underlying cardiovascular risk were like the participants in ASCEND. Indeed, the event rate of primary endpoint defined as cardiovascular death was around 9% in ORIGIN, which was like the total death rate in ASCEND, ~10% (Table 1). The failure to achieve the benefit in these two trials may have been formula and/or dose related.

The patients in VITAL and ASCEND were generally healthy and baseline risk was at most diabetes (ASCEND) with no evident ASCVD. In ORIGIN, the patients were at high risk of CVD. The relative risk reduction rate with EPA or IPE was similar between JELIS and REDUCE-IT studies, 19% in JELIS and 25% in REDUCE-IT despite absolute number differences: 2.8% *vs.* 3.5% in JELIS (active *vs.* control) and 17.2% *vs.* 22.0% (active *vs.* control) in REDUCE-IT study. Rather, in JELIS, the absolute event rate was close to that of VITAL with the absolute event rate being 2.98% and 3.24%, respectively (active group *vs.* placebo group), yet JELIS was comprised of 75% patients who had no prior history of CVD and VITAL was entirely designed for primary prevention. These major omega-3 fatty acids trials are summarized in Table 1.

The Alpha Omega trial investigated the effects of 400 mg of EPA + DHA or alpha-linolenic acid (ALA) in patients with previous MI on the rate of major cardiovascular events, which comprised fatal and nonfatal cardiovascular events and cardiac interventions. This study failed to show a reduction of cardiovascular endpoints in patients with EPA + DHA or ALA compared to placebo [61]. Like the Alpha Omega trial, another trial investigating the efficacy of supplementation with B vitamins or 600 mg of EPA + DHA as a secondary prevention of CVD strategy also failed [62].

These negative results may have been unsuccessful for several reasons: being underpowered; low doses of omega-3 fatty acids; EPA + DHA combination; or concomitant use of other guideline-adjusted therapy. In addition, recent meta-analyses, which included only randomized placebo-controlled trials, showed that omega-3 fatty acids did not reduce the risk of overall CVD events [63]. This is contradictory to previous positive results from other meta-analyses [64,65].

A Long-Term Outcomes Study to Assess STatin Residual Risk Reduction With EpaNova in HiGh Cardiovascular Risk PatienTs With Hypertriglyceridemia (STRENGTH) study was a randomized double-blind, placebo-controlled trial to test 4 g/d omega-3 carboxylic acid (EPANOVA) daily administration as an add-on to statin in high risk patients with triglycerides levels between 200 and 500 mg/dL and low HDL-C levels < 42 mg/dL (men) or < 47 mg/dL (women) [5,66]. The proportion of established atherosclerotic CVD patients was 56%. The comparator was corn oil. Included patients were those with CVD,

diabetes with CVD risk factors and elderly who were at high risk for CVD. The study design was quite like the REDUCE-IT study. Unexpectedly, in January 2020, this study was terminated earlier due to futility following recommendation of the independent data-monitoring committee [5].

The study results were published recently; among the 13,078 treated patients, the primary endpoint of composite of CV death, MI, stroke, coronary revascularization, or unstable angina requiring hospitalization occurred in 12.0% (785/6539) treated with omega-3 fatty acids and 12.2% (795/6539) treated with corn oil with HR, 0.99 (95% CI, 0.90–1.09, $p = 0.84$) [5]. Secondary and tertiary endpoints were also not different except for atrial fibrillation occurrence (also true for REDUCE-IT), more common in omega-3 fatty acids group as compared to corn oil group. (2.2% *vs.* 1.3%, HR 1.69 [95% CI, 1.29–2.21, $p < 0.001$]). Plasma EPA level in EPA + DHA users group increased from 21.0 to 89.6 μg/mL at 12-month follow-up (2.7-fold increase) as compared to no change in placebo group (from 21.3 to 19.0 μg/mL).

## 4. Plausible explanation for different outcomes using only EPA or IPE and EPA + DHA combination

Convincing clinical outcomes data have been lacking from prior randomized trials of various omega-3 preparations, which mostly included combination EPA + DHA, when added to a high-intensity statin [67]. The conflicting outcomes among studies may relate to the amount or dosage of EPA or IPE (low *vs.* high), baseline levels of EPA/DHA, levels achieved during the trials, and distinct effects of EPA *vs.* DHA on lipoproteins, inflammation, oxidation, insulin sensitivity, pro-thrombotic effects, membrane stability and cholesterol metabolism including intraarterial crystal formation as addressed earlier. Several studies, including primary and secondary prevention trials and meta-analyses of omega-3 fatty acids, have so far yielded inconsistent results on ASCVD outcomes.

One explanation for this apparent heterogeneity of treatment effect of omega-3 fatty acids preparations, which may also apply to many dietary fish oil supplements that are in widespread use is given below.

First, the low dose such as those present in dietary fish oil supplements are likely insufficient to lower triglycerides effectively. However, this speculation may be incorrect because in the REDUCE-IT study, the triglyceride-lowering effect failed to explain the benefit and in STRENGTH using a high dose combination failed to achieve the primary outcome despite triglycerides lowering. Also, it is important to differentiate prescription omega-3 fatty acids from dietary fish oil supplements. These supplements contain multiple long chain oils and oxidized omega-3 fatty acids that may render the preparations ineffective [14,68].

The STRENGTH trial enrolled patients with similar or even lower CVD risks than in REDUCE-IT (established ASCVD patients were 56% *vs.* 71%). The differences in STRENGTH compared to REDUCE-IT were (1) omega-3 fatty acids, DHA + EPA *vs.* IPE only (2) comparator, corn oil *vs.* mineral oil and (3) level of baseline risk.

As for the formula, the DHA could mitigate or negate the efficacy of EPA in DHA + EPA or EPA serum was not high enough to exert a favorable efficacy in DHA + EPA 4 g group, because naturally the absolute dose of EPA in EPA + DHA formula may be lower than 4 g of pure EPA (IPE). This could be postulated by the attained EPA serum level (135.2 μg/mL *vs.* 89.6 μg/mL) and change from baseline (3.6 *vs.* 2.7-fold), which was higher in REDUCE-IT than in STRENGTH trial, respectively.

The authors of STRENGTH argue that the positive results of IPE from REDUCE-IT trial could be attributed to an adverse effect of mineral oil, i.e., raising levels of LDL-C and differences in hsCRP resulting in poor clinical outcome. However, as mentioned earlier, IPE (4 g) showed plaque regression and stabilization in EVAPORATE compared to baseline plaque status [60]. In other words, this benefit was proven with the comparator, mineral oil.

Other plausible mechanisms also support the IPE 4 g/d in clinical trials. When we closely look at the STRENGTH and REDUCE-IT data, the established CVD patients were 56% in STRENGTH and 71% in REDUCE-IT, so the clinical event rate was higher in REDUCE-IT trial (17–20%) as compared to those from STRENGTH (12%). Thus, we may assume that the IPE could exert benefit in higher risk patients who were on statins. Nonetheless, the definite answer could be obtained after a similar randomized clinical trial is performed comparing IPE only 4 g *vs.* corn oil comparator, a trial that appears unlikely.

Distinct effects of EPA and DHA have been observed mostly in experimental and clinical studies with small numbers of participants. There have been no randomized trials on CVD outcomes that compare EPA and DHA directly. Nonetheless, JELIS and REDUCE-IT trials consistently reported positive results of EPA or IPE and by contrast, major trials such as ORIGIN, VITAL, ASCEND and now STRENGTH failed. Thus, distinct biological and clinical effects of EPA and DHA may result in different outcomes. EPA does not raise LDL-C [9], reduces hsCRP [11], enhances endothelial function [14], inhibits oxidation of apolipoprotein B particles [21], and enhances membrane stability and cholesterol metabolism in the plaque including crystal formation [28] unlike DHA.

EPA incorporated into phospholipid at the sn2 position helps preserve membrane structure and function and inhibits lipid oxidation and cholesterol crystal formation. It can also influence signal transduction pathways related to inflammation and vasodilation [14,45]. Very recent animal studies demonstrated that EPA administration can be incorporated into thin cap plaque rather than thick cap plaque, which might be related to plaque stability and lower myocardial infarct occurrence [69]. Additional evidence has shown that plaque was stabilized with EPA 1800 mg/day on top of rosuvastatin 10 mg/day in non-obstructive neo-atherosclerotic plaques [70].

A recent sub-study of REDUCE-IT asked whether the higher serum level of EPA was associated with reduced CVD outcomes. After adjusting for EPA levels, the risk reduction was minimal (HR 1.03; 95% CI 0.91–1.16). They argued that the EPA serum level could account for a large portion of the benefit of IPE (American college of Cardiology annual scientific meeting 2020. (https://www.acc.org/latest-in-cardiology/articles/2020/03/24/16/41/mon-1045-eicosapentaenoic-acid-levels-in-reduce-it-acc-2020) (Bhatt D. EPA levels and cardiovascular outcomes in the Reduction of Cardiovascular Events With Icosapent Ethyl Intervention Trial. Presented on: March 30, 2020. ACC 2020.).

This report suggests that the main contribution for risk reduction is EPA rather than the combination with DHA. If this is correct, patients who are deficient in omega-3 fatty acids could have benefit [71]. However, there is a disputing observation because high tertile serum level of EPA failed to reduce in the STRENGTH study.

Another issue to be considered is the formulation of omega-3 fatty acids preparations used. Use of non-purified mixtures of omega-3 fatty acids instead of prescription products could lead to the study failure because of relatively small amount of EPA.

Another issue to be considered is co-administration of statins. As many clinicians and researchers acknowledge, in the era of statin use, it is more difficult to prove the benefit of additional lipid-modifying drugs. Omega-3 fatty acids and other triglyceride-lowering drugs like fibrates or niacin were no exceptions [72–74]. Many clinical trials using those drugs have failed because these drugs fell short of sufficient power to exert their efficacy when statins are co-administered. Also, lowering triglycerides levels might have no effect on ASCVD.

Another issue is the comparator in REDUCE-IT study. Unlike previous trials, the REDUCE-IT study used mineral oil, which resulted in increase of LDL-C and hsCRP levels, compared to corn oil being neutral and thus, the benefit of IPE could be exaggerated. Concerns were also raised about whether mineral oil may have prevented absorption of statins, an effect that may have influenced the outcome. Of course, the differences of patient's characteristics (CVD and diabetes in REDUCE-IT trial *vs.* primary prevention in ASCEND and VITAL trials) may have

predicted and resulted in different outcomes. We compared these in Table 1.

Finally, regarding safety and adverse events, a larger percentage of patients in IPE group in the REDUCE-IT and EPA + DHA in STRENTH study than in the placebo group were hospitalized for atrial fibrillation or flutter (3.1% *vs.* 2.1%, $p = 0.004$), while serious bleeding events occurred in 2.7% of the patients in IPE group and in 2.1% in the placebo group ($p = 0.06$). The significance of these observations is uncertain, and further analyses are ongoing, however, the increased risk of bleeding, which is likely an effect on platelet aggregation, may be one important mechanism by which CVD risk is reduced by IPE.

## 5. Clinical implication and future perspective

Recent American and European guidelines have stated that prescription of omega-3 fatty acids (EPA + DHA or EPA-only) at a dose of 4 g/d (>3 g/d total EPA + DHA) is an effective and safe option for reducing triglycerides as monotherapy or as an adjunct to other lipid-lowering agents [75,76].

Previous clinical studies reported modest reductions in the rates of CVD events, however, recent randomized double-blind, placebo-controlled trials using EPA + DHA have failed. By contrast, others that used only EPA or IPE consistently demonstrated reductions. Furthermore, the STRENGTH trial was terminated early due to futility. Based on the study populations at high risk on statin treatment, high dose and only EPA seems to be effective in hypertriglyceridemic patients with diabetes, metabolic syndrome, or obesity who require other treatments to control residual risk beyond statins. Among patients with ASCVD, hypertriglyceridemia is common, and is associated with higher ASCVD risk across a range of triglycerides levels [77].

Nowadays, triglycerides *per se* or other components of triglycerides-rich lipoproteins containing apolipoprotein B molecule are also important risk factors. It is possible that as many as one in four patients with ASCVD may be candidates for omega-3 fatty acids. However, several aspects including the placebo issue should be resolved before convincing benefits are promoted. If then, we may recommend that the dose of EPA 1.8 g/d for East Asians such as Korean, Japanese and Chinese and IPE 4 g/d for Western people may be appropriate because a plasma EPA level (170 μg/mL in a Japanese population) is similar to that attained in a previous 12-week lipid study, in which a total daily dose of 4 g of IPE was used in a Western population (183 μg/mL) [78,79].

In conclusion, in hypertriglyceridemic patients with diabetes, metabolic syndrome, or obesity on statin treatment, EPA 1.8 g/d for East Asians such as Korean, Japanese, and Chinese and IPE 4 g/d for Western people could be a promising strategy to overcome residual risk in the statins era, if several aspects, including placebo issue, could be resolved (Fig. 3).

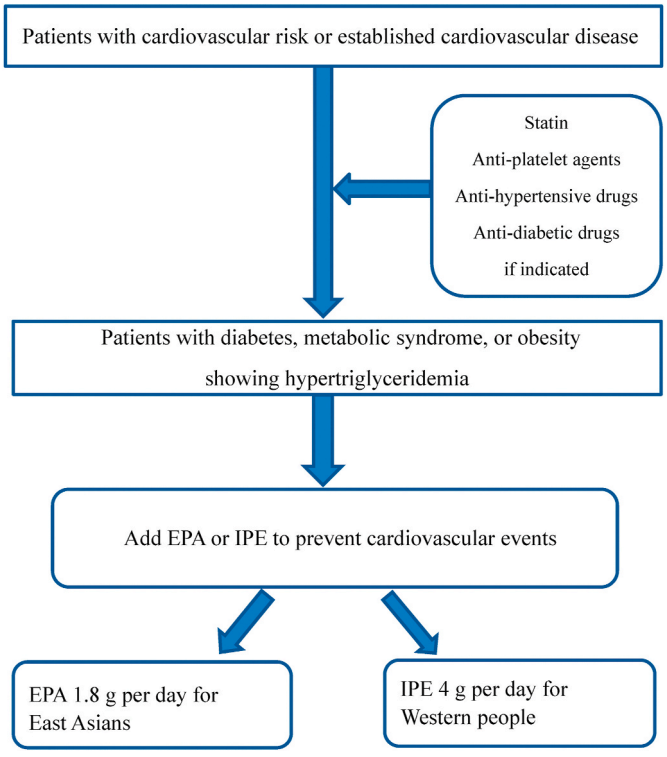


**Fig. 3.** Algorithm for omega-3 fatty acids in the treatment of patients with established atherosclerotic cardiovascular disease (ASCVD) or at high risk for ASCVD.
EPA = eicosapentaenoic acid, IPE = icosapent ethyl.

## Financial support

This work was supported by a grant from the Korean Society of CardioMetabolic Syndrome.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

[1] D.L. Bhatt, P.G. Steg, M. Miller, et al., Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia, N. Engl. J. Med. 380 (2019) 11–22.
[2] O.T. Investigators, J. Bosch, H.C. Gerstein, et al., n-3 fatty acids and cardiovascular outcomes in patients with dysglycemia, N. Engl. J. Med. 367 (2012) 309–318.
[3] L. Bowman, M. Mafham, K. Wallendszus, et al., Effects of n-3 fatty acid supplements in diabetes mellitus, N. Engl. J. Med. 379 (2018) 1540–1550.
[4] J.E. Manson, N.R. Cook, I.M. Lee, et al., Marine n-3 fatty acids and prevention of cardiovascular disease and cancer, N. Engl. J. Med. 380 (2019) 23–32.
[5] S.J. Nicholls, A.M. Lincoff, M. Garcia, et al., Effect of high-dose omega-3 fatty acids vs corn oil on major adverse cardiovascular events in patients at high cardiovascular risk: the STRENGTH randomized clinical trial, Jama (2020).
[6] K.K. Koh, M.J. Quon, K.C. Shin, et al., Significant differential effects of omega-3 fatty acids and fenofibrate in patients with hypertriglyceridemia, Atherosclerosis 220 (2012) 537–544.
[7] G.C. Shearer, O.V. Savinova, W.S. Harris, Fish oil – how does it reduce plasma triglycerides? Biochim. Biophys. Acta 1821 (2012) 843–851.
[8] T.A. Jacobson, S.B. Glickstein, J.D. Rowe, et al., Effects of eicosapentaenoic acid and docosahexaenoic acid on low-density lipoprotein cholesterol and other lipids: a review, J. Clin. Lipidol. 6 (2012) 5–18.
[9] J. Allaire, C. Vors, A.J. Tremblay, et al., High-dose DHA has more profound effects on LDL-related features than high-dose EPA: the ComparED study, J. Clin. Endocrinol. Metabol. 103 (2018) 2909–2917.
[10] I. Bairati, L. Roy, F. Meyer, Effects of a fish oil supplement on blood pressure and serum lipids in patients treated for coronary artery disease, Can. J. Cardiol. 8 (1992) 41–46.
[11] I. Bairati, L. Roy, F. Meyer, Double-blind, randomized, controlled trial of fish oil supplements in prevention of recurrence of stenosis after coronary angioplasty, Circulation 85 (1992) 950–956.
[12] A.M. Minihane, S. Khan, E.C. Leigh-Firbank, et al., ApoE polymorphism and fish oil supplementation in subjects with an atherogenic lipoprotein phenotype, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 1990–1997.
[13] C.M. Ballantyne, H.E. Bays, R.A. Braeckman, et al., Icosapent ethyl (eicosapentaenoic acid ethyl ester): effects on plasma apolipoprotein C-III levels in patients from the MARINE and ANCHOR studies, J. Clin. Lipidol. 10 (2016) 635–645.e631.
[14] R.P. Mason, P. Libby, D.L. Bhatt, Emerging mechanisms of cardiovascular protection for the omega-3 fatty acid eicosapentaenoic acid, Arterioscler. Thromb. Vasc. Biol. 40 (2020) 1135–1147.
[15] R. Buckley, B. Shewring, R. Turner, et al., Circulating triacylglycerol and apoE levels in response to EPA and docosahexaenoic acid supplementation in adult human subjects, Br. J. Nutr. 92 (2004) 477–483.
[16] J. Allaire, C. Vors, A.J. Tremblay, et al., High-dose DHA has more profound effects on LDL-related features than high-dose EPA: the ComparED study, J. Clin. Endocrinol. Metabol. 103 (2018) 2909–2917.
[17] S. Grimsgaard, K.H. Bonaa, J.B. Hansen, et al., Highly purified eicosapentaenoic acid and docosahexaenoic acid in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids, Am. J. Clin. Nutr. 66 (1997) 649–659.

[18] J. Allaire, P. Couture, M. Leclerc, et al., A randomized, crossover, head-to-head comparison of eicosapentaenoic acid and docosahexaenoic acid supplementation to reduce inflammation markers in men and women: the Comparing EPA to DHA (ComparED) Study, Am. J. Clin. Nutr. 104 (2016) 280–287.
[19] S. Egert, F. Kannenberg, V. Somoza, et al., Dietary alpha-linolenic acid, EPA, and DHA have differential effects on LDL fatty acid composition but similar effects on serum lipid profiles in normolipidemic humans, J. Nutr. 139 (2009) 861–868.
[20] T.A. Mori, V. Burke, I.B. Puddey, et al., Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men, Am. J. Clin. Nutr. 71 (2000) 1085–1094.
[21] R.J. Woodman, T.A. Mori, V. Burke, et al., Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension, Am. J. Clin. Nutr. 76 (2002) 1007–1015.
[22] F. Dangardt, W. Osika, Y. Chen, et al., Omega-3 fatty acid supplementation improves vascular function and reduces inflammation in obese adolescents, Atherosclerosis 212 (2010) 580–585.
[23] P.C. Oh, K.K. Koh, I. Sakuma, et al., Omega-3 fatty acid therapy dose-dependently and significantly decreased triglycerides and improved flow-mediated dilation, however, did not significantly improve insulin sensitivity in patients with hypertriglyceridemia, Int. J. Cardiol. 176 (2014) 696–702.
[24] M. Omura, S. Kobayashi, Y. Mizukami, et al., Eicosapentaenoic acid (EPA) induces Ca(2+)-independent activation and translocation of endothelial nitric oxide synthase and endothelium-dependent vasorelaxation, FEBS Lett. 487 (2001) 361–366.
[25] R.P. Mason, H. Dawoud, R.F. Jacob, et al., Eicosapentaenoic acid improves endothelial function and nitric oxide bioavailability in a manner that is enhanced in combination with a statin, Biomed. Pharmacother. 103 (2018) 1231–1237.
[26] K.K. Koh, S.H. Han, M.J. Quon, Inflammatory markers and the metabolic syndrome: insights from therapeutic interventions, J. Am. Coll. Cardiol. 46 (2005) 1978–1985.
[27] K.I. Cho, J. Yu, T. Hayashi, et al., Strategies to overcome residual risk during statins era, Circ. J. : Off. J. Jpn Circ. Soc. 83 (2019) 1973–1979.
[28] R. De Caterina, M.I. Cybulsky, S.K. Clinton, et al., The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells,, Arterioscler. Thromb. : J. Vasc. Biol. 14 (1994) 1829–1836.
[29] H.E. Bays, C.M. Ballantyne, J.J. Kastelein, et al., Eicosapentaenoic acid ethyl ester (AMR101) therapy in patients with very high triglyceride levels (from the Multi-center, plAcebo-controlled, Randomized, double-blINd, 12-week study with an open-label Extension [MARINE] trial), Am. J. Cardiol. 108 (2011) 682–690.
[30] J.K. Innes, P.C. Calder, The differential effects of eicosapentaenoic acid and docosahexaenoic acid on cardiometabolic risk factors: a systematic review, Int. J. Mol. Sci. 19 (2018).
[31] X.F. Guo, K.L. Li, J.M. Li, et al., Effects of EPA and DHA on blood pressure and inflammatory factors: a meta-analysis of randomized controlled trials, Crit. Rev. Food Sci. Nutr. 59 (2019) 3380–3393.
[32] N. Tanaka, Y. Irino, M. Shinohara, et al., Eicosapentaenoic acid-enriched high-density lipoproteins exhibit anti-atherogenic properties, Circ. J. : Off. J. Jpn Circ. Soc. 82 (2018) 596–601.
[33] P. Zhuang, Y. Zhang, W. He, et al., Dietary fats in relation to total and cause-specific mortality in a prospective cohort of 521 120 individuals with 16 Years of follow-up, Circ. Res. 124 (2019) 757–768.
[34] J. Heshmati, M. Morvaridzadeh, S. Maroufizadeh, et al., Omega-3 fatty acids supplementation and oxidative stress parameters: a systematic review and meta-analysis of clinical trials, Pharmacol. Res. (2019), 104462.
[35] T.A. Mori, R.J. Woodman, V. Burke, et al., Effect of eicosapentaenoic acid and docosahexaenoic acid on oxidative stress and inflammatory markers in treated-hypertensive type 2 diabetic subjects, Free Radical Biol. Med. 35 (2003) 772–781.
[36] R.P. Mason, S.C. Sherratt, R.F. Jacob, Eicosapentaenoic acid inhibits oxidation of ApoB-containing lipoprotein particles of different size in vitro when administered alone or in combination with atorvastatin active metabolite compared with other triglyceride-lowering agents, J. Cardiovasc. Pharmacol. 68 (2016) 33–40.
[37] N. Tanaka, T. Ishida, M. Nagao, et al., Administration of high dose eicosapentaenoic acid enhances anti-inflammatory properties of high-density lipoprotein in Japanese patients with dyslipidemia, Atherosclerosis 237 (2014) 577–583.
[38] S.C.R. Sherratt, R.P. Mason, Eicosapentaenoic acid inhibits oxidation of high density lipoprotein particles in a manner distinct from docosahexaenoic acid, Biochem. Biophys. Res. Commun. 496 (2018) 335–338.
[39] M. Phang, A.J. Sinclair, L.F. Lincz, et al., Gender-specific inhibition of platelet aggregation following omega-3 fatty acid supplementation, Nutr. Metabol. Cardiovasc. Dis. 22 (2012) 109–114.
[40] R.J. Woodman, T.A. Mori, V. Burke, et al., Effects of purified eicosapentaenoic acid and docosahexaenoic acid on platelet, fibrinolytic and vascular function in hypertensive type 2 diabetic patients, Atherosclerosis 166 (2003) 85–93.
[41] D. Moertl, R. Berger, A. Hammer, et al., Dose-dependent decrease of platelet activation and tissue factor by omega-3 polyunsaturated fatty acids in patients with advanced chronic heart failure, Thromb. Haemostasis 106 (2011) 457–465.
[42] D. Moertl, A. Hammer, S. Steiner, et al., Dose-dependent effects of omega-3-polyunsaturated fatty acids on systolic left ventricular function, endothelial function, and markers of inflammation in chronic heart failure of nonischemic origin: a double-blind, placebo-controlled, 3-arm study, Am. Heart J. 161 (2011) 915 e911–919.
[43] R. Preston Mason, New insights into mechanisms of action for omega-3 fatty acids in atherothrombotic cardiovascular disease, Curr. Atheroscleros Rep. 21 (2019) 2.
[44] O. Soubias, K. Gawrisch, Docosahexaenoyl chains isomerize on the sub-nanosecond time scale, J. Am. Chem. Soc. 129 (2007) 6678–6679.
[45] S.C.R. Sherratt, R.P. Mason, Eicosapentaenoic acid and docosahexaenoic acid have distinct membrane locations and lipid interactions as determined by X-ray diffraction, Chem. Phys. Lipids 212 (2018) 73–79.
[46] K.M. Borow, J.R. Nelson, R.P. Mason, Biologic plausibility, cellular effects, and molecular mechanisms of eicosapentaenoic acid (EPA) in atherosclerosis, Atherosclerosis 242 (2015) 357–366.
[47] R.P. Mason, R.F. Jacob, S. Shrivastava, et al., Eicosapentaenoic acid reduces membrane fluidity, inhibits cholesterol domain formation, and normalizes bilayer width in atherosclerotic-like model membranes, Biochim. Biophys. Acta 1858 (2016) 3131–3140.
[48] R.P. Mason, R.F. Jacob, Eicosapentaenoic acid inhibits glucose-induced membrane cholesterol crystalline domain formation through a potent antioxidant mechanism, Biochim. Biophys. Acta 1848 (2015) 502–509.
[49] S.H. Han, M.J. Quon, J.A. Kim, et al., Adiponectin and cardiovascular disease: response to therapeutic interventions, J. Am. Coll. Cardiol. 49 (2007) 531–538.
[50] S. Lim, M.J. Quon, K.K. Koh, Modulation of adiponectin as a potential therapeutic strategy, Atherosclerosis 233 (2014) 721–728.
[51] S. Neschen, K. Morino, J.C. Rossbacher, et al., Fish oil regulates adiponectin secretion by a peroxisome proliferator-activated receptor-gamma-dependent mechanism in mice, Diabetes 55 (2006) 924–928.
[52] M.K. Duda, K.M. O'Shea, B. Lei, et al., Dietary supplementation with omega-3 PUFA increases adiponectin and attenuates ventricular remodeling and dysfunction with pressure overload, Cardiovasc. Res. 76 (2007) 303–310.
[53] M. Kaushik, D. Mozaffarian, D. Spiegelman, et al., Long-chain omega-3 fatty acids, fish intake, and the risk of type 2 diabetes mellitus, Am. J. Clin. Nutr. 90 (2009) 613–620.
[54] L. Djoussé, J.M. Gaziano, J.E. Buring, et al., Dietary omega-3 fatty acids and fish consumption and risk of type 2 diabetes, Am. J. Clin. Nutr. 93 (2011) 143–150.
[55] T.J. Brown, J. Brainard, F. Song, et al., Omega-3, omega-6, and total dietary polyunsaturated fat for prevention and treatment of type 2 diabetes mellitus: systematic review and meta-analysis of randomised controlled trials, Br. Med. J. 366 (2019) l4697.
[56] S. Lorente-Cebrian, P. Perez-Matute, J.A. Martinez, et al., Effects of eicosapentaenoic acid (EPA) on adiponectin gene expression and secretion in primary cultured rat adipocytes, J. Physiol. Biochem. 62 (2006) 61–69.
[57] D.S. Kelley, Y. Adkins, L.R. Woodhouse, et al., Docosahexaenoic acid supplementation improved lipocentric but not glucocentric markers of insulin sensitivity in hypertriglyceridemic men, Metab. Syndr. Relat. Disord. 10 (2012) 32–38.
[58] S.H. Han, P.C. Oh, S. Lim, et al., Comparative cardiometabolic effects of fibrates and omega-3 fatty acids, Int. J. Cardiol. 167 (2013) 2404–2411.
[59] M. Yokoyama, H. Origasa, M. Matsuzaki, et al., Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis, Lancet 369 (2007) 1090–1098.
[60] M.J. Budoff, D.L. Bhatt, A. Kinninger, et al., Effect of icosapent ethyl on progression of coronary atherosclerosis in patients with elevated triglycerides on statin therapy: final results of the EVAPORATE trial, Eur. Heart J. 41 (2020) 3925–3932.
[61] D. Kromhout, E.J. Giltay, J.M. Geleijnse, et al., n-3 fatty acids and cardiovascular events after myocardial infarction, N. Engl. J. Med. 363 (2010) 2015–2026.
[62] P. Galan, E. Kesse-Guyot, S. Czernichow, et al., Effects of B vitamins and omega 3 fatty acids on cardiovascular diseases: a randomised placebo controlled trial, BMJ 341 (2010) c6273.
[63] S.M. Kwak, S.K. Myung, Y.J. Lee, et al., Efficacy of omega-3 fatty acid supplements (eicosapentaenoic acid and docosahexaenoic acid) in the secondary prevention of cardiovascular disease: a meta-analysis of randomized, double-blind, placebo-controlled trials, Arch. Intern. Med. 172 (2012) 686–694.
[64] K. He, Y. Song, M.L. Daviglus, et al., Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies, Circulation 109 (2004) 2705–2711.
[65] C. Wang, W.S. Harris, M. Chung, et al., n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review, Am. J. Clin. Nutr. 84 (2006) 5–17.
[66] S.J. Nicholls, A.M. Lincoff, D. Bash, et al., Assessment of omega-3 carboxylic acids in statin-treated patients with high levels of triglycerides and low levels of high-density lipoprotein cholesterol: rationale and design of the STRENGTH trial, Clin. Cardiol. 41 (2018) 1281–1288.
[67] N.A. Marston, R.P. Giugliano, K. Im, et al., Association between triglyceride lowering and reduction of cardiovascular risk across multiple lipid-lowering therapeutic classes: a systematic review and meta-regression analysis of randomized controlled trials, Circulation 140 (2019) 1308–1317.
[68] R.P. Mason, S.C.R. Sherratt, Omega-3 fatty acid fish oil dietary supplements contain saturated fats and oxidized lipids that may interfere with their intended biological benefits, Biochem. Biophys. Res. Commun. 483 (2017) 425–429.
[69] T. Sato, M. Horikawa, S. Takei, et al., Preferential incorporation of administered eicosapentaenoic acid into thin-cap atherosclerotic plaques, Arterioscler. Thromb. Vasc. Biol. (2019), Atvbaha119313093.
[70] K. Kuroda, H. Otake, M. Shinohara, et al., Effect of rosuvastatin and eicosapentaenoic acid on neoatherosclerosis: the LINK-IT trial, EuroIntervention, J. EuroPCR Collab. Working Group Intervent. Cardiol. Eur. Soc. Cardiol. (2019).

[71] P. Perez-Martinez, N. Katsiki, D.P. Mikhailidis, The role of n-3 fatty acids in cardiovascular disease: back to the future, Angiology 71 (2020) 10–16.
[72] T. Aung, J. Halsey, D. Kromhout, et al., Associations of omega-3 fatty acid supplement use with cardiovascular disease risks: meta-analysis of 10 trials involving 77 917 individuals, JAMA Cardiol. 3 (2018) 225–234.
[73] W.E. Boden, J.L. Probstfield, T. Anderson, et al., Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy, N. Engl. J. Med. 365 (2011) 2255–2267.
[74] M.J. Landray, R. Haynes, J.C. Hopewell, et al., Effects of extended-release niacin with laropiprant in high-risk patients, N. Engl. J. Med. 371 (2014) 203–212.
[75] A.C. Skulas-Ray, P.W.F. Wilson, W.S. Harris, et al., Omega-3 fatty acids for the management of hypertriglyceridemia: a science advisory from the American heart association, Circulation 140 (2019) e673–e691.
[76] F. Mach, C. Baigent, A.L. Catapano, et al., 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk, Eur. Heart J. 41 (2020) 111–188.
[77] P.R. Lawler, G. Kotrri, M. Koh, et al., Real-world risk of cardiovascular outcomes associated with hypertriglyceridaemia among individuals with atherosclerotic cardiovascular disease and potential eligibility for emerging therapies, Eur. Heart J. 41 (2020) 86–94.
[78] H. Itakura, M. Yokoyama, M. Matsuzaki, et al., Relationships between plasma fatty acid composition and coronary artery disease, J. Atherosclerosis Thromb. 18 (2011) 99–107.
[79] H.E. Bays, C.M. Ballantyne, R.T. Doyle Jr., et al., Icosapent ethyl: eicosapentaenoic acid concentration and triglyceride-lowering effects across clinical studies, Prostag. Other Lipid Mediat. 125 (2016) 57–64.