Pros and Cons of Long-Chain Omega-3 Polyunsaturated Fatty Acids in Cardiovascular Health

Ivana Djuricic[1] and Philip C. Calder[2,3*]

[1]Department of Bromatology, Faculty of Pharmacy, University of Belgrade, 11221 Belgrade, Serbia;
[2]School of Human Development and Health, Faculty of Medicine, University of Southampton, Southampton SO16 6YD, United Kingdom;
[3]NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust and University of Southampton, Southampton SO16 6YD, United Kingdom.

*Author for correspondence: School of Human Development and Health, Faculty of Medicine, University of Southampton, IDS Building, MP887 Southampton General Hospital, Tremona Road, Southampton SO16 6YD, United Kingdom pcc@soton.ac.uk

Running title: Omega-3 fatty acids and cardiovascular health

Key words: Omega-3 fatty acid; Fish oil; Eicosapentaenoic acid; Docosahexaenoic acid; Heart disease; Risk factor

List of abbreviations: AF, atrial fibrillation; ALA, α-linolenic acid; AV, anisidine value; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FADS, fatty acid desaturase; GOED, Global Organization for EPA and DHA Omega-3s; HDL, high density lipoprotein; LDL, low density lipoprotein; MI, myocardial infarction; PUFA, polyunsaturated fatty acid; PV, peroxide value; RCT, randomized controlled trial; TOTOX, total oxidation value.

Abstract

The long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are found in seafood (especially fatty fish), supplements and concentrated pharmaceutical preparations. Long-term prospective cohort studies consistently demonstrate an association between higher intakes of EPA+DHA or higher levels of EPA and DHA in the body and lower risk of developing cardiovascular disease (CVD), especially coronary heart disease and myocardial infarction, and of cardiovascular mortality in the general population. The cardioprotective effect of EPA and DHA is likely due to the beneficial modulation of a number of known risk factors for CVD. Some large trials support the use of EPA+DHA (or EPA alone) in high-risk patients, as confirmed through recent meta-analyses, although the evidence is inconsistent. This review presents key studies that have investigated EPA and DHA in the primary and secondary prevention of CVD, briefly describes potential mechanisms of action, and discusses recently published RCTs and meta-analyses. Potential adverse aspects of long-chain omega-3 fatty acids in relation to CVD (peroxidation, increased bleeding, increased risk of atrial fibrillation) are discussed.

1. Introduction to omega-3 polyunsaturated fatty acids

Omega-3 fatty acids are a family of polyunsaturated fatty acids (PUFAs) characterized by the presence of the final double bond in the acyl chain being located three carbon atoms away from the terminal methyl group (omega or tail end). α-Linolenic acid (ALA, 18:3ω-3) is referred to as an essential fatty acid since humans, like other animals, do not express the delta-15 desaturase enzyme required for insertion of the "ω-3" double bond and so cannot synthesize ALA *de novo*. Because plants possess delta-15 desaturase, they can synthesize ALA from the omega-6 PUFA, linoleic acid (18:2ω-6); therefore, plant-derived foods are the main dietary sources of ALA. Although they cannot synthesize ALA, animals can metabolize it by further desaturation and elongation processes that are considered to mainly occur in the liver. Through this pathway, ALA is converted to eicosapentaenoic acid (EPA, 20:5ω-3) and then to docosahexaenoic acid (DHA, 22:6ω-3) (Figure 1). Here we refer to EPA and DHA as long-chain omega-3 PUFAs. The desaturase enzymes involved in this conversion are encoded by the fatty acid desaturase (FADS) genes: *FADS1* encodes delta-5 desaturase and *FADS2* encodes delta-6 desaturase. These enzymes are regulated by hormones, including insulin (1) and female sex hormones (2), and have a requirement for certain micronutrients, including zinc (3). Furthermore, there is evidence that genetic polymorphisms in both FADS genes affect the activity of the desaturase enzymes and could play a role in determining the activity of this conversion pathway (4). Studies in adult humans demonstrate that converting ALA to EPA and DHA is generally poor, with very limited conversion all the way to DHA being observed (5; 6). It is also thought that EPA can be synthesized from DHA by retro-conversion involving limited peroxisomal β-oxidation. However, a recent study using stable-isotopically-labelled DHA was interpreted to identify only very limited retro-conversion in humans (7).

ALA is found in significant amounts in several seeds, seed oils and nuts. Linseeds (also called flaxseeds) and their oil typically contains 45 to 55% of fatty acids as ALA. Soybean oil, rapeseed oil (also known as canola oil) and walnuts contain 5 to 10% of fatty acids as ALA. The most important dietary source of EPA and DHA is fatty fish or "oily fish" (e.g. salmon, tuna, herring, sardines, mackerel) providing approximately 1.5 to 3.0 g of these fatty acids per adult serving, as previously reviewed (8). Existing evidence of dietary habits suggests that typical intakes of ALA among adults in Western countries are between 0.5 and 2 g/day, while average (mean) intakes of long-chain omega-3 PUFAs (i.e. EPA and DHA) in some northern and eastern European, North American and Australasian countries are typically quoted to be about 0.1 to 0.2 g/day. Populations where oily fish consumption is more regular and in greater

amounts than in most Western populations (e.g. the Japanese) have a higher average intake of long-chain omega-3 fatty acids. Intake of EPA and DHA is closely reflected in blood levels of these fatty acids (9). Stark et al. (10) gathered data from 298 studies reporting long-chain omega-3 PUFAs in blood fractions in healthy humans. They assigned these data to one of four categories (high, moderate, low, very low) based upon EPA+DHA content. Very low blood levels of EPA+DHA were observed in North America, Central and South America, much of Europe, the Middle East, Southeast Asia, and Africa. These findings are consistent with low dietary intakes of EPA and DHA in these regions, mainly as a result of low consumption of oily fish, and low endogenous synthesis from ALA. In contrast, regions with high EPA+DHA blood levels included Japan, Scandinavia, and areas with native-born populations or populations not fully adapted to Westernized food habits.

Besides fish and other seafood, supplements of different kinds are also sources of EPA and DHA. These include fish oils, cod liver oil, krill oil and some algal oils; typical contents of EPA and DHA in such supplements are shown in Table 1. Concentrated pharmaceutical grade preparations of long-chain omega-3 PUFAs in different forms are also available (Table 1) and are highly relevant to prevention and treatment of cardiovascular disease (CVD). Due to the generally accepted health benefits of long-chain omega-3 fatty acids (8; 11; 12), strategies to increase their intake need to be identified; in this context, both seafood and the use of the various dietary supplements providing EPA and DHA currently play roles. Seafood is, of course, the primary source of the oil used for long-chain omega-3 supplements. This presents two challenges. The first is the sustainability of seafood as a source of long-chain omega-3 PUFAs (13). The second is the accumulation of toxic substances through the food chain into fatty fish, which are the richest dietary source of long-chain omega-3 fatty acids, due to pollution of the seas (14). Neurotoxic methylmercury and carcinogenic organochlorine compounds, such as dioxins and polychlorinated biphenyls, are the main toxic substances that accumulate. Thus, it is recognized that some types of fish have the risk of containing such substances. In general, processes used in extracting, purifying and treating oils for use as supplements remove the toxic substances (15). Nevertheless, it is important to note that different supplements and pharma-grade preparations provide different amounts of EPA and DHA, in different ratios and in different chemical forms (Table 1). In a typical standard fish oil supplement, EPA and DHA comprise about 30% of the fatty acids present. Thus, a one gram capsule of a standard fish oil would provide about 0.3 g of EPA+DHA. However, because the absolute and relative amounts of EPA and DHA vary among fish, they vary among fish oils. Most standard fish oils contain

EPA and DHA in a ratio of 1.5 to 1. More concentrated oil preparations are available; these "fish oil concentrates" may provide 0.45 to 0.65 g EPA+DHA per g of oil. Different chemical formulations of long-chain omega-3 fatty acids are also available. In most fish oils, the fatty acids are present in the form of triacylglycerols (aka triglycerides). However, it is now possible to obtain supplements where the long-chain omega-3 fatty acids are partly present as phospholipids, such as krill oil. Furthermore, ethyl ester and free fatty acid preparations are available, such as in highly concentrated pharmaceuticals. Processing can be used to standardize the concentrations of EPA and DHA present, for example in pharmaceutical-grade preparations, and to control the ratio of these fatty acids, which might be advantageous depending on the intended use of the supplement. Higher content of EPA in relation to DHA is primarily intended to reduce the risk of CVD, and preparations with a higher amount of DHA are mainly intended for specific populations/purposes such as pregnant and lactating women, infants and nervous system health.

The aim of this article is to discuss the role of long-chain omega-3 PUFAs in prevention and treatment of CVD, reviewing the evidence to date and also highlighting some of possible adverse effects of these fatty acids. This article builds upon, but updates and broadens the coverage of, a previous review on long-chain omega-3 PUFAs and CVD (16).

2. Overview of long-chain omega-3 fatty acids in cardiovascular disease: from early epidemiologic studies to date

Since early observations made in the Greenland Inuit, many cross-sectional and prospective cohort studies and many trials reporting on risk factors for CVD and on primary and secondary prevention of coronary heart disease (CHD) or CVD have demonstrated that higher intake of fish, fatty fish, long-chain omega-3 PUFA supplements (e.g. fish oils), and individual long-chain omega-3 fatty acids represents an effective strategy to reduce risk, morbidity and mortality from CVD, especially CHD. The history of long-chain omega-3 PUFAs and CVD has been reviewed in detail elsewhere (16-20). In summary, native populations in Greenland, Northern Canada and Alaska consuming their traditional diet had much lower rates of death from CVD, especially CHD, than predicted, despite their high dietary fat intake. The protective component of the diet was proposed to be the long-chain omega-3 PUFAs consumed in very high amounts as a result of the regular intake of whale and seal meat, whale blubber and fatty fish. Low cardiovascular mortality is also seen in Japanese consuming a traditional diet and this diet is rich in seafood, including fatty fish and sometimes marine mammals, which contain

significant amounts of EPA and DHA. Much evidence has accumulated from prospective and case-control studies indicating that higher intake of EPA and DHA is related to lower risk of CVD outcomes in Western populations. These studies have been summarized numerous times in systematic reviews and meta-analyses and are discussed in detail elsewhere (16; 17; 19; 21). For example, in the prospective cohort Nurse's Health Study, with 16 years of follow-up data from 84,688 female nurses who had no CVD or cancer at baseline, there was an inverse association for developing CHD, having a non-fatal myocardial infarction (MI) or dying from CHD across quintiles of intake of fish and omega-3 fatty acids (including ALA, EPA and DHA) (22). A prospective analysis of ~420,000 participants from the National Institutes of Health AARP Diet and Health Study during 16 years of follow-up reported a significant inverse association between fish and EPA+DHA intake and different mortality outcomes (23). Across extreme quintiles, higher EPA+DHA intake was related to 15% and 18% lower CVD mortality in men and women, respectively. More recently, a large population-based cohort study evaluated the association of habitual use of fish oil supplements with CVD and mortality (24). Nearly half a million individuals, both men and women free from CVD or cancer at baseline, were enrolled between 2006 and 2010 and followed up to the end of 2018. Habitual fish oil supplementation was associated with a 7% lower risk of CVD events, a 16% lower risk of CVD mortality, and a 13% lower risk of all-cause mortality in the general population (24).

A 2012 meta-analysis of seven prospective cohort studies involving over 176,000 participants confirmed consistency between long-chain omega-3 fatty acid consumption and a reduced likelihood of heart failure (25). The investigators reported a 15% risk reduction of heart failure associated with the highest intake compared to lowest fish intake and a 14% lower risk of heart failure for those with the highest versus lowest dietary intake or plasma concentrations of EPA+DHA. The nature of these cohort studies does not enable the identified lower risk of heart failure to be ascribed as a secondary effect of reducing CHD risk or an effect on cardiac contractile function independent of CHD. Both are possible. However, the GISSI-HF trial, randomised patients with prevalent heart failure to long-chain omega-3 fatty acids or placebo and reported significantly reduced mortality in the omega-3 fatty acid group (26); this would suggest that omega-3 fatty acids act to improve cardiac function in patients with heart failure.

Chowdhury et al. (27) aggregated prospective studies investigating the association of dietary or circulating EPA and DHA with risk of coronary outcomes. Results from 16 studies, including over 422,000 subjects, found a 13% reduction in risk for those in the top third of dietary intake of long-chain omega-3 fatty acids than those in the lower third of intake. Data

from 13 studies with over 20,000 participants showed 22%, 21% and 25% reduction in risk of coronary outcomes for those in the top third of blood levels of EPA, DHA or EPA+DHA, respectively, compared to those in the lower third (27). Alexander et al. brought together 17 prospective cohort studies examining the relation of long-chain omega-3 fatty acids with the risk of various coronary outcomes, and showed an 18% lower risk for any CHD event for subjects with higher dietary intake of EPA+DHA than those with lower intake (28). There were also significant reductions of 19%, 23% and 47% in the risk for fatal coronary death, coronary events, and sudden cardiac death, respectively. Another study pooled data from nineteen trials investigating the association between EPA or DHA concentration in a body pool like serum, plasma, red blood cells or adipose tissue and risk of future CHD in adults who were healthy at study entry (29). Both EPA and DHA were associated with a reduction in risk of fatal CHD, with about a 10% reduced risk for each one standard deviation increase in either EPA or DHA. Harris et al. (30) gathered together 10 cohort studies and found a 15% lower risk of fatal CHD for each one standard deviation increase in the omega-3 index (i.e. the sum of EPA and DHA in red blood cells). A de novo pooled analysis of 17 prospective cohort studies with 42,466 individuals confirmed association between a lower risk for death from CVD in patients with the highest versus the lowest quintile of circulating long-chain omega-3 fatty acids (i.e. EPA, DHA, and EPA+DHA) (31). These analyses support a clear role for EPA and DHA in primary prevention of CHD, and perhaps, more widely, of CVD, as discussed elsewhere (16).

The recognition of the benefits of long-chain omega-3 fatty acids has resulted in recommendations for fish, and more specifically for EPA+DHA intake, by various government, non-government and professional bodies. For example, United Kingdom Scientific Advisory Committee on Nutrition/Committee on Toxicity established dietary recommendations for adults in the general population to intake at least two portions of fish per week, at least one of which is fatty fish, equated to 450 mg EPA+DHA per day (32). The International Society for the Study of Fatty Acids and Lipids set the target of 500-650 mg EPA+DHA/day for the general population (33). According to other governing bodies, 400-500 mg EPA+DHA per day with at least 100-120 mg DHA per day is set by French Agency for Food, Environmental and Occupational Health Safety (34), while a minimum of 250 mg EPA+DHA is the appropriate daily intake recommended by Food and Agricultural Organisation of the United Nations (35).

3. Risk factors for CVD that are targeted by EPA and DHA

Beneficial modification of a broad range of risk factors probably explains the protective effect of long-chain omega-3 fatty acids towards CVD. These risk factors include blood triglyceride concentrations, blood pressure, thrombosis, cardiac function, vascular function and inflammation, which are all improved by long-chain omega-3 fatty acids (see (16) for References). Concerning modulation of blood lipid concentrations, there is overwhelming evidence that both EPA and DHA have a triglyceride-lowering effect (36), apparently with a slightly higher impact for DHA (37; 38). Although EPA and DHA do not show a total cholesterol-lowering effect to any significant degree in humans, they do have an independent effect on different lipid subfractions, with EPA lowering high density lipoprotein $(HDL)_3$-cholesterol and DHA increasing the more cardioprotective HDL_2-cholesterol (39; 40). It has also been suggested that DHA increases low density lipoprotein (LDL)-cholesterol (LDL-C) more than EPA and increases LDL particle size, an effect which was not seen for EPA (38-40). Although DHA increases LDL-C, it does not change apolipoprotein B concentration which is consistent with shifting LDL-C particles to a larger, less atherogenic profile (41-43). Regarding inflammation, several meta-analyses have demonstrated that EPA and DHA decrease serum or plasma concentrations of pro-inflammatory eicosanoids (e.g., thromboxane B_2 and leukotriene B_4) (44), C-reactive protein, and pro-inflammatory cytokines such as interleukin-6 and tumor necrosis factor α (45; 46). However, this effect may be dependent on the health status of the individuals. In addition to the anti-inflammatory effects, EPA and DHA appear to have an antioxidant role and regulate antioxidant signalling pathways. A high DHA content is found in mitochondrial membranes suggesting that DHA is important for adenosine triphosphate synthesis by oxidative phosphorylation (47). DHA is reported to reduce mitochondrial oxidative stress and cytochrome C oxidase activity while increasing manganese-dependent superoxide dismutase activity (47). The anti-inflammatory properties of long-chain omega-3 fatty acids may also be important in this regard, since inflammation induces oxidative stress. Recent meta-analyses have found a significant reduction in platelet aggregation with long-chain omega-3 fatty acids, with a greater impact observed in non-healthy participants and considerable improvement of vascular endothelial function through increasing flow-mediated dilatation (48-50). EPA and DHA have been reported to lower systolic and diastolic blood pressure and heart rate (51; 52). Interestingly, DHA is reported to be more effective than EPA at lowering blood pressure and heart rate in normotensive individuals, whilst neither EPA nor DHA had any effect in hypertensive diabetic patients (39). Overall, there is strong evidence that both EPA and DHA beneficially modify a range of risk factors for CVD and this most

likely account for the reduced risk of developing and mortality from CVD reported in cohort studies, as described in Section 2.

4. Trials of prevention and treatment of CVD with long-chain omega-3 fatty acids

4.1 Trials published before 2010

Table 2 summarises the key randomized controlled trials (RCTs) of long-chain omega-3 fatty acids that report on hard cardiovascular endpoints. The Diet and Reinfarction Trial (DART) involved 2,033 recent MI survivors (mean: 41 days since MI), who were advised concerning fat, fish and fibre intake and followed up for 2 years (53). Those patients who were given advice to eat fatty fish (at least two portions per week) or to take supplements of fish oil showed a 29% reduction in total mortality and a decreased risk of death from CHD at 2 years than those patients given other dietary advice. The landmark GISSI-Prevenzione study enrolled 11,324 survivors with recent MI (≤ 3 months since MI) who were treated with 840 mg/d EPA+DHA, 300 mg/d vitamin E, both EPA+DHA and vitamin E, or nothing (control group) for 3.5 years (54). Treatment with EPA+DHA significantly reduced the composite primary outcomes (-15% and -20%, respectively) and several secondary outcomes, including cardiovascular death by 30%, sudden death by 45%, and total fatal events by 20%. There was no benefit for non-fatal MI or stroke. The effect of EPA+DHA on sudden cardiac death and total mortality was observed after 3 months and 4 months of treatment, respectively, and raised interest in the potential anti-arrhythmic benefit of EPA and DHA (55). In the GISSI-HF trial (26), 6,975 patients with chronic heart failure received 840 mg/d EPA+DHA or placebo throughout ~4 years and results showed a small (9%) but significant reduction in all-cause mortality. The randomized open-label, Japan EPA Lipid Intervention Study (JELIS) included 18,645 patients with hypercholesterolemia (total cholesterol ≥ 6.5 mmol/L) who were assigned to receive either a statin alone or a statin along with highly purified EPA (1.8 g/d EPA as an ethyl ester) with a 5-year follow-up (56). Among those patients who were receiving statin therapy, a number of participants were on conventional medication due to pre-existing CHD. The primary outcome was any major coronary event, including sudden cardiac death, fatal and non-fatal MI and other non-fatal events, including unstable angina pectoris, angioplasty, stenting, and coronary artery bypass grafting. Long-term use of EPA-ethyl ester as an addition to statin therapy had no effect over statin alone on the primary outcome in the primary prevention arm of the trial, but in the secondary prevention arm, EPA supplementation resulted in a 19% reduction in non-fatal coronary events versus statin alone group (56).

4.2 Trials published from 2010 to date

Three RCTs published in 2010 (57-59) did not replicate the findings of the earlier treatment studies. The OMEGA prospective RCT examined the effect of supplementation with 840 mg/d EPA+DHA over a period of 1 year in 3,851 survivors after acute MI, with the primary outcome of sudden cardiac death (57). Investigators reported a low rate of sudden cardiac death, total mortality, major adverse cardiovascular events or revascularisation during the following year. There was no significant difference between the omega-3 group and the control (olive oil) group. It is possible that the low rate of occurrence of the main outcomes precluded an effect of long-chain omega-3 fatty acids from being observed. The Supplémentation en Folates et Omega-3 (SU.FOL.OM3) trial investigated whether dietary supplementation with B vitamins and/or long-chain omega-3 fatty acids could prevent major cardiovascular events in patients with a documented history of cardiovascular disease (MI, unstable angina or ischaemic stroke) (58). Of the 2501 enrolled patients, around 50% received omega-3 fatty acids alone (600 mg/d EPA+DHA at a ratio 2:1) or B vitamins + omega-3 fatty acids vs placebo for 4.2 years. Long-chain omega-3 fatty acids (alone or with B vitamins) did not show any effect on the primary outcome (composite of cardiovascular death, stroke and non-fatal MI) or on the secondary outcomes. It is possible that the dose of EPA+DHA used was below the threshold required for an effect to occur. The Alpha Omega study recruited 4,837 post-MI patients who were assigned to receive one of three trial margarines fortified with a targeted additional daily intake of ~375 mg/d EPA+DHA, or 1.9 g/d ALA, or EPA+DHA+ALA or a placebo margarine, and followed up for ~3.4 years (59). None of the treated groups had a reduction in cardiovascular events. The low dose of EPA+DHA used in this trial, compared to doses used in the pre-2010 trials should be noted. Nevertheless, on further analysis, a reduction in fatal CHD and in arrhythmia-related events was experienced among patients with diabetes in EPA+DHA and ALA-fortified margarine groups (59).

The ORIGIN (Outcome Reduction with an Initial Glargine Intervention) trial, published in 2012, investigated whether supplementation with 840 mg/d EPA+DHA can reduce cardiovascular mortality in dysglycaemic patients with recent MI or heart failure, together with a long-acting insulin (glargine) or standard care. A total of 12,536 participants were enrolled into the study with a median follow-up of 6.2 years (60). Compared with placebo, no effect of long-chain omega-3 fatty acids was reported for death from cardiovascular causes or arrhythmia and total mortality. The Risk and Prevention Study, from 2013, assessed the effect of

supplementation with 840 mg/d EPA+DHA in 12,513 patients at high cardiovascular risk but with no MI for a median of 5 years and reported no effect on death from cardiovascular causes or hospitalisation compared to placebo (61). In a prespecified subgroup analysis, compared with placebo, long-chain omega-3 fatty acids resulted in an 18% lower incidence of the revised primary outcome among women (composite of the time to death from cardiovascular causes or first hospital admission for cardiovascular causes). Most of the secondary outcomes (e.g., sudden death, fatal or non-fatal MI, stroke or coronary event) did not differ between groups; however, admissions for heart failure were significantly lower in the long-chain omega-3 fatty acid group.

A Study of Cardiovascular Events iN Diabetes (ASCEND) trial, published in 2018, randomly assigned 15,480 patients with diabetes and no evidence of CVD to receive either long-chain omega-3 fatty acids (840 mg/d EPA+DHA) or olive oil as placebo (62). The primary outcome was the first serious vascular event; there was no difference in the primary outcome between the two groups after a mean follow-up of 7.4 years. In exploratory analyses, there were 19% fewer deaths from vascular events in the long-chain omega-3 fatty acid arm, as well as a trend towards reduced risk of death (21%) from CHD.

The Vitamin D and Omega-3 (VITAL) trial, published in 2019, was a randomized controlled trial conducted as a two-by-two factorial design of vitamin D3 (at a dose of 50 μg/d) and long-chain omega-3 PUFA capsules (at a dose of 1 g/d containing 480 mg EPA + 360 mg DHA) among 25,871 healthy participants aged over 50 years for the primary prevention of CVD and cancer (63). After a median follow-up of 5.3 years, there was no statistically significant difference between the supplemented group with omega-3 PUFAs and the placebo group in the primary outcome of major cardiovascular events (a composite of MI, stroke or death from cardiovascular causes). An analysis of the individual components of the composite showed a significant reduction in the long-chain omega-3 fatty acid arm for MI (28% reduction) and CHD (17% reduction). Correspondingly, there was also a lower risk of death from these two non-prespecified outcomes (50% for MI and 24% for CHD), although the effect on CHD was not significant. There was a significant reduction in major adverse cardiovascular events (19%) and risk of MI (40%) for those who consumed fewer than 1.5 fish meals/week and then supplemented with long-chain omega-3 PUFAs.

In Reduction of Cardiovascular Events with Icosapent Ethyl Intervention Trial (REDUCE-IT) (64), published in early 2019, among the 8,179 patients, 29% comprised a primary prevention cohort with cardiovascular risk factors and 71% comprised a secondary

prevention cohort with established CVD or with diabetes (58% of all subjects had type 2 diabetes mellitus). Baseline plasma LDL-C levels were well-controlled with statins (1.06 to 2.59 mmol/L), while triglyceride levels were borderline and moderately elevated (1.52 to 5.63 mmol/L). In the trial, participants received 2 g of a formulation rich in EPA ethyl ester (referred to as icosapent ethyl - this is the same preparation as used in JELIS) twice daily, providing a total of 3.6 g of EPA as a total daily dose versus mineral oil as placebo. With a median follow-up of 4.9 years, the primary outcome (a composite of cardiovascular death, non-fatal MI, non-fatal stroke, coronary revascularization or unstable angina) was reduced in patients who received EPA-ethyl ester compared to placebo (hazard ratio: 0.75; 95% CI: 0.68, 0.83; $p < 0.001$). The key pre-specified secondary outcome (a composite of cardiovascular death, non-fatal MI, or non-fatal stroke) was also significantly reduced in the EPA-ethyl ester group (hazard ratio: 0.80; 95% CI: 0.66, 0.98; $p = 0.03$) as well as a whole range of other clinical outcomes (64). Post-hoc analysis of REDUCE-IT trial found an association between attained serum EPA level and clinical outcomes. EPA-ethyl ester treatment resulted in a 3.6-fold increase in serum EPA from baseline over five years, whereas in the placebo group level did not change (65). Another positive EPA-ethyl ester trial was EVAPORATE (Effect of Vascepa on Improving Coronary Atherosclerosis in People with High Triglycerides Taking Statin Therapy) (66). This study involved 80 patients with known angiographic coronary artery disease taking statins and with no history of MI, stroke, or life-threatening arrhythmia within the prior six months. The same EPA-ethyl ester preparation and the same dose were used as in REDUCE-IT. EVAPORATE demonstrated that EPA might directly promote atherosclerotic plaque attenuation in hypertriglyceridemic individuals at 18 months (66).

The findings of REDUCE-IT and EVAPORATE differ from those of the more recently published STRENGTH trial (Long Term Outcomes Study to Assess Statin Residual Risk with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridemia) (67). In this study, patients with hypertriglyceridemia and high cardiovascular risk on statin therapy were treated with 4 g/day of an oil containing EPA and DHA (as free fatty acids); this provided about 2.2 g EPA + 0.8 g DHA daily. Corn oil was used as placebo. There was no significant difference in a composite outcome of major adverse cardiovascular events among patients who received additional omega-3 fatty acids to usual background therapies versus control, and the trial was stopped early (67). There has been significant discussion of why REDUCE-IT and STRENGTH, with similar study designs, target populations and outcomes, produced different results (68). REDUCE-IT used pure EPA whereas STRENGTH used EPA+DHA; REDUCE-

IT used EPA as an ethyl ester whereas STRENGTH used EPA and DHA as free fatty acids; REDUCE-IT used a higher overall dose of long-chain omega-3 fatty acids; and the two studies used different placebos.

The OMEMI (Omega-3 Fatty acids in Elderly with Myocardial Infarction) trial, published in 2021, randomized a total of 1027 patients with a recent MI (in the previous 2-8 weeks) to receive ~1.6 g/day long-chain omega-3 fatty acids (930 mg EPA + 660 mg DHA) or corn oil (placebo) as an addition to standard care (69). After two years of follow-up, there was no significant difference between supplemented and control groups in the primary composite cardiovascular outcome.

4.3 Meta-analyses of trials of treatment

Meta-analyses published up to 2010 confirmed findings seen in individual trials investigating the effects of long-chain omega-3 fatty acids on different cardiovascular outcomes. In 2002 Bucher et al. (70) meta-analysed results from 11 RCTs (2 dietary RCTs and 9 supplemental RCTs) involving 15,806 patients with CHD who received long-chain omega-3 fatty acids with a dose range of 0.3-6 g/d EPA and 0.6-3.7 g/d DHA. The analysis showed 30% reduced risk of fatal MI, 20% reduced risk of non-fatal MI, 30% reduced risk for sudden death and 20% reduced risk in overall mortality in marine omega-3 fatty acids group versus control. A 2005 meta-analysis of 14 RCTs with 20,260 participants in primary and secondary prevention settings found a 23% reduction in all-cause mortality and a 32% reduction in cardiovascular mortality for those patients who were taking supplemental long-chain omega-3 fatty acids (dose range not given) (71). A further meta-analysis from 2009 of 3 dietary RCTs and 5 supplemental RCTs in 20,997 patients with CVD found a 57% reduction in sudden death in patients with prior MI who were given long-chain omega-3 fatty acids compared to placebo (72). The identified reductions in cardiac death (29%) and overall mortality (23%) with long-chain omega-3 fatty acids were not significant. Dosage was in a range of 0.3–4.1 g/d EPA and 0.4–2.8 g/d DHA (72). Another 2009 meta-analysis of 11 RCTs in 39,044 subjects with all stages of CVD, including both low- and high-risk patients, reported a 13% reduction in both cardiovascular death and sudden death and an 8% reduction in overall mortality in high-risk patients who were given supplemental long-chain omega-3 fatty acids with a dose range of 0.7 to 4.8 g/d EPA + DHA (73).

Over the last ten years, several meta-analyses reported more mixed conclusions than earlier meta-analyses, as reviewed elsewhere recently (16). For example, a meta-analysis from

2012, representing 20 RCTs (3 dietary RCTs and 17 supplemental RCTs) of 62,851 patients in primary and secondary prevention settings, reported a 14% reduction in vascular death but no effect on cardiovascular events, total mortality, coronary or arrhythmia events with long-chain omega-3 fatty acids (74). Dosage of omega-3 fatty acids used in the included trials was in range of 0.8-3.4 g/d EPA+DHA and duration was 0.6-6 years (74). Published in 2014, a meta-analysis examined 17 RCTs involving 76,580 subjects with a broader range of supplemental omega-3 fatty acid dosages (0.3 g/d EPA to 6 g/d EPA+DHA) and durations (0.1-8 years) (27). The study found a 7% reduction in the cardiovascular outcomes with long-chain omega-3 fatty acids (27). A meta-analysis published in 2017 covered 18 RCTs with approximately 93,000 subjects who were taking supplemental long-chain omega-3 fatty acids with doses ranging from 0.4 g to 5 g/d EPA+DHA (28). The study reported 14%-16% reduction in CHD in high-risk subgroups i.e., those with elevated triglycerides and LDL-C (28). A 2020 meta-analysis conducted by Abdelhamid et al. (75) aggregated data of all RCTs published before August 2019 that reported CVD outcomes. The study numbered 162,796 subjects in primary and secondary prevention settings who were given dietary or supplemental omega-3 fatty acids in a dose range from 0.5 g per day to more than 5 g per day for a duration of 1-7 years. A slightly reduced risk in CHD mortality and events was noted, with little or no effect on all-cause mortality, cardiovascular mortality, cardiovascular events, stroke or arrhythmia (75). A further meta-analysis was based on the work of Abdelhamid et al., but differed in the selection of clinical trials, focusing on studies in which the intervention was supplementation with EPA and/or DHA and not dietary advice (76). The investigators suggested that different foods contain widely varying amounts of EPA and DHA, making it challenging to estimate dosage and monitor compliance. In this meta-analysis restricted to supplemental trials, the dosage used varied from 0.4 g/d to 5.5 g/d EPA+DHA (average dose received was 1221 mg/d EPA+DHA). Pooled results did not show an association between supplementation and reduction in the risk of CVD events which is in accordance with Abdelhamid et al. However, long-chain omega-3 fatty acids were associated with statistically significant reductions in the risk of MI (13%), CHD events (10%), fatal MI (35%) and CHD mortality (9%); the results of these random-effects meta-analyses are presented in Figure 2. For CVD and MI, the protective effects of long-chain omega-3 fatty acids increased significantly with dosage (76). Meta-regression analysis found that increasing intake of EPA and DHA by 1 g/d EPA+DHA was associated with a 5.8% reduction risk in the CVD events. In the case of MI, the risk reduction was dose dependent, and each additional 1 g/d of EPA+DHA was associated with a significant risk reduction of 9%. This analysis did not find a

441 statistically significant benefit of EPA alone compared with EPA+DHA or a significant
442 association between the year of publication and the overall impact of EPA and/or DHA on CVD
443 outcomes.

444 Another recent meta-analysis of 13 RCTs (e.g., GISSI-Prevenzione, JELIS, GISSI-HF,
445 SU.FOL.OM3, Alpha Omega, OMEGA, ORIGIN, VITAL, ASCEND and REDUCE-IT)
446 demonstrated that EPA and DHA lower the risk of MI, CHD death, total CHD, CVD death and
447 total CVD, without including REDUCE-IT in the analysis (77). Including REDUCE-IT in the
448 analysis resulted in an even stronger inverse association for these outcomes (77). The total
449 number of participants was 127,477, while the mean duration of follow-up ranged from 1.0 to
450 7.4 years and the mean dose of EPA+DHA ranged from 0.37 to 4.0 g/d.

451 Very recently Rizos et al. (78) published a new meta-analysis of 17 RCTs of
452 supplemental long-chain omega-3 PUFAs according to prior defined doses (i.e., ≤ 1, 2, ≥ 3 one
453 g capsules/d) and duration ≥ 1 year. For two capsules/d, a significant reduction was found for
454 cardiac death while for ≥3 capsules/d, a statistically significant reduction in cardiac death,
455 sudden death and stroke was observed. There was no association with any CVD outcome for
456 doses less than two capsules/d.

457

458 **5. Recommendations for use of long-chain omega-3 fatty acids in patient treatment**

459 American and European authorities have made recommendations for clinical use of EPA and
460 DHA with respect to CVD, as detailed elsewhere (16). In recognition of the ability of long-
461 chain omega-3 PUFAs to lower blood triglycerides, in 2019 the American Heart Association
462 updated its earlier recommendation for the use of 2 to 4 g/d EPA+DHA for triglyceride
463 lowering: "we conclude that prescription n-3 fatty acids, whether EPA+DHA or EPA-only, at
464 a dose of 4 g/d, are clinically useful for reducing triglycerides, after any underlying causes are
465 addressed and diet and lifestyle strategies are implemented, either as monotherapy or as an
466 adjunct to other triglyceride-lowering therapies" (40). On the basis of the positive outcomes of
467 REDUCE-IT, the European Society of Cardiology and the European Atherosclerosis Society
468 issued an update to the "Clinical Practice Guidelines for the Management of Dyslipidaemias"
469 specifically recommending that "in high-risk patients with [triglyceride] levels between 1.5 and
470 5.6 mmol/L (135–499 mg/dL) despite statin treatment, n-3 polyunsaturated fatty acids
471 (icosapent ethyl 2 x 2 g/day) should be considered with a statin" (79). A more recent statement
472 broadens this by indicating that, together with standard medical care, EPA as icosapent ethyl at
473 a dose of 4 g/d is considered for patients with hypertriglyceridemia alone or with diabetes,

atherosclerotic cardiovascular disease and other cardiac risk factors in reducing cardiovascular risk (79). The National Lipid Association statement is that "for patients aged ≥ 45 years with clinical [atherosclerotic cardiovascular disease] ASCVD, or aged ≥ 50 years with diabetes mellitus requiring medication plus ≥ 1 additional risk factor, with fasting triglycerides 135 to 499 mg/dL on high-intensity or maximally tolerated statin therapy (± ezetimibe), treatment with icosapent ethyl is recommended for ASCVD risk reduction" (80). In 2017, the AHA repeated its earlier support for EPA+DHA in people with CVD (19) and extended it by stating that "the recommendation for patients with prevalent CHD such as a recent MI remains essentially unchanged: treatment with n-3 fatty acid supplements is reasonable for these patients. Even a potential modest reduction in CHD mortality (10%) in this clinical population would justify treatment with a relatively safe therapy. We now recommend treatment for patients with prevalent heart failure without preserved left ventricular function to reduce mortality and hospitalizations (9%) on the basis of a single, large RCT. Although we do not recommend treatment for patients with diabetes mellitus and prediabetes to prevent CHD, there was a lack of consensus on the recommendation for patients at high CVD risk. Because there are no reported RCTs related to the primary prevention of CHD, heart failure, and atrial fibrillation, we were not able to make recommendations for these indications" (81).

6. Potential adverse aspects of long-chain omega-3 fatty acids in relation to CVD

6.1. Oxidation of fish oil supplements

Long-chain omega-3 fatty acids present in fish oil supplements are highly susceptible to complex oxidative degradation, producing primary lipid peroxides and secondary oxidation products such as aldehydes and ketones (82). Lipid peroxides are chemically different from unoxidized molecules and therefore may have distinct and diverse biological effects (83). Numerous factors can influence the oxidation rate in fish oil supplements, including light and oxygen exposure, temperature, antioxidant content and the presence of water or heavy metals (84). The degrees of primary and secondary oxidation are presented as peroxide value (PV) and anisidine value (AV), respectively. In combination, these measurements serve to estimate the total oxidation value (TOTOX). The Global Organization for EPA and DHA Omega-3s (GOED) set the limits related to these parameters as PV < 5 meq O_2/kg, AV < 20 meq/kg and TOTOX < 26 meq/kg for GOED members (85). Oxidative quality for encapsulated and liquid forms of EPA and DHA has been evaluated in many studies with different findings. Some studies confirmed compliance with regulation among tested products, but others reported that

a high percentage of products from various manufacturers exceed one or more of the quality parameter values (82; 83). In 2010, the European Food Safety Authority panel on biological hazards reported that "information on the level of oxidation of fish oil (as measured by peroxide and anisidine values) and related toxicological effects in humans is lacking" (86). One RCT investigated the effect of oxidized compared with non-oxidized fish oil on lipid peroxidation and antioxidant activity markers (87). There was no association between oxidized fish oil and acute oxidative toxicity. This study did not assess critical markers related to atherosclerosis, such as oxidized LDL or carotid artery intimal thickness. However, the findings of omega-3 supplement trials are highly inconsistent, which could be partly attributed to less-efficacy of oxidized oils (83). To date, there are no sufficient specific clinical trials addressing the effects of oxidation on the efficacy of long-chain omega-3 fatty acids.

6.2 Long-chain omega-3 fatty acids and bleeding

Long-chain omega-3 fatty acids may affect platelet function, reducing platelet count and reactivity, prolonging bleeding time and increasing the ratio of anticoagulant versus procoagulant metabolites (prostacyclins and thromboxanes, respectively) (88; 89). In addition to their incorporation into platelet membrane, omega-3 fatty acids (i.e., EPA and DHA) compete with arachidonic acid for the cyclooxygenase and lipoxygenase pathways of metabolic transformation, lowering thromboxane A_2 production (see (90) for References). Modulation of platelet function depends on the dose of EPA and DHA and occurs mainly at doses greater than 2 g/d (91). The effects appear to be primarily mediated by the action of EPA (92). Because of these effects, there has been some concern that long-chain omega-3 PUFAs, especially when used at a high dose, will adversely promote bleeding and prolong bleeding time. Regarding the potential for excess bleeding with omega-3 fatty acids, a paper published in 2007 aggregated evidence from 19 well-designed clinical trials with cardiovascular patients undergoing major surgery (i.e., coronary artery bypass, carotid endarterectomy and femoral artery catheterisation) (93). Based on these data it was concluded that long-chain omega-3 fatty acids did not increase the risk for clinically significant bleeding, either in patients treated with EPA/DHA alone or with antithrombotic/antiplatelet drugs. In the recent OPERA (Fish Oil and Perioperative Bleeding) trial, 1516 patients undergoing cardiac surgery were randomized to receive 8-10 g of EPA+DHA over 2-5 days in the preoperative period and then 2 g/d in the postoperative period, or placebo (94). Perioperative bleeding occurred in 6.1% of patients taking EPA+DHA. Comparing to placebo, in the omega-3 group, the odds ratio for perioperative bleeding was 0.81

(95% CI: 0.53, 1.24); absolute risk difference, 1.1% lower (95% CI: −3.0%, 1.8%). Thus, the study indicates less likelihood of perioperative bleeding with long-chain omega-3 fatty acids. Indeed, unexpectedly, higher levels of plasma omega-3 fatty acids achieved with supplementation were related to lower perioperative bleeding events (94). The longitudinal Multi-Ethnic Study of Atherosclerosis (MESA) recruited 6814 participants with no clinical CVD at baseline to investigate whether higher baseline levels of EPA, DHA and EPA+DHA would be linked with major bleeding events (95). An inverse association in incident major bleeding events was reported with higher baseline plasma levels of EPA (hazard ratio 0.69; CI: 0.53, 0.9; P=0.01) and EPA+DHA (hazard ratio 0.78; CI: 0.65, 0.94; P=0.01), but not DHA (hazard ratio 0.68; CI: 0.44, 1.05; P=0.08) over a median of 14 years of follow-up. A publication from 2018 (96) reported previously unpublished data from 8 clinical trials of enteral nutrition products that included fish oil as a source of long-chain omega-3 fatty acids; these trials in a variety of patient groups (n = 600 patients) provided a range of doses of EPA+DHA (1.5 to 10.2 g/d) for a variety of durations (8 d to 52 wk). There was no effect of long-chain omega-3 PUFAs on coagulation parameters and there was no difference between omega-3 and placebo groups in bleeding events. A very recent study reported no effect of an EPA-rich formulation providing 3 g/d EPA for 4 to 10 weeks prior to surgery for prostate cancer on perioperative bleeding (97). Increased bleeding with long-chain omega-3 PUFAs is a theoretical consideration; however the accumulated evidence from human trials suggests that this is not a matter for concern.

6.3 Long-chain omega-3 fatty acids and atrial fibrillation

Some clinical trials have suggested that long-chain omega-3 fatty acids may be associated with an increased likelihood of developing atrial fibrillation (AF), especially in people with high cardiovascular risk and/or elevated blood lipids. These trials provided data on omega-3 fatty acids at different doses and in different formulations (98). In the previously mentioned VITAL trial, participants without CVD, cancer or AF at baseline and randomly assigned to receive 840 mg/d omega-3 PUFAs (460 mg EPA + 380 mg DHA) or olive oil as placebo were studied (99). After a median of 5.3 years, there was no difference in incidence of AF events between the groups (3.7% versus 3.4%). However, trials using higher doses of long-chain omega-3 PUFAs have found increased risk of AF. For example, in the STRENGTH trial, treatment with 3.2 g/day EPA+DHA as free fatty acids resulted in a higher likelihood of developing AF after a median of 3.5 years compared to placebo (2.2% vs 1.3% P < 0.001) (67). In REDUCE-IT, patients who were randomized to receive almost 4 g/d of purified EPA-ethyl ester, had a

significant increase in the risk of AF after a median of 4.9 years compared to control (5.3% vs 3.9%; P = 0.003) (64). An intermediate dose of ~1.6 g/d omega-3 PUFAs (930 mg EPA + 660 mg DHA) in the OMEMI trial also resulted in more AF in the treatment group compared with placebo (hazard ratio 1.84; P = 0.06) (69). Recent meta-analyses summarized results from large-scale clinical trials to answer the question of whether long-chain omega-3 fatty acids are dose-related with an increased risk for AF (100; 101). A meta-analysis of 5 RCTs (REDUCE-IT, ASCEND, Risk and Prevention, STRENGTH, OMEMI) found increased risk of incident AF with long-chain omega-3 PUFAs compared with placebo (incident risk ratio 1.37; 95% CI 1.22, 1.54; P < 0.01) (100). When findings from VITAL Rhythm (99) were included, the findings were unchanged (incident risk ratio 1.29; 95% CI 1.13, 1.48; P = 0.0002) (100). The meta-analysis of Gencer et al. (101) included seven RCTs published between 2012 and 2020. Of 81,210 patients, 72.6% were enrolled in trials testing ≤ 1 g long-chain omega-3 PUFAs/day and 27.4% in trials testing > 1 g long-chain omega-3 PUFAs/day with a median follow-up of 4.9 years. The use of long-chain omega-3 fatty acids was associated with an increased risk of AF (hazard ratio 1.25; 95% CI: 1.07, 1.46; P=0.013). In analyses specified by dose, the hazard ratio was higher in the trials testing > 1 g/d (hazard ratio 1.49; 95% CI: 1.04, 2.15; P=0.042) compared with those testing ≤ 1 g/d (hazard ratio 1.12; 95% CI: 1.03, 1.22; P=0.024; P for interaction <0.001). In meta-regression, the hazard ratio for AF increased for each 1 g increase of omega-3 fatty acids (hazard ratio 1.11; 95% CI: 1.06, 1.15; P=0.001). Thus, individual trials and meta-analyses of mostly recent trials indicate that long-chain omega-3 PUFAs increase the risk of AF, especially when used at high doses. A recent commentary has argued that the effect of long-chain omega-3 fatty acids on AF is "U-shaped": they reduce risk of AF at moderate doses but increase risk at high doses (102). This is obviously a concern, although it is interesting to note that REDUCE-IT, while reporting increased risk of AF, also reported reduced risk of a range of hard cardiovascular outcomes.

7. Summary, discussion & conclusions

There is a large body of evidence from long-term prospective cohort studies that consistently demonstrates an association between higher intakes of fish, fatty fish and long-chain omega-3 fatty acids (i.e., EPA+DHA) or higher levels of EPA and DHA in the body and lower risk of developing CVD, especially CHD, having an MI and cardiovascular mortality in the general population. This cardioprotective effect of EPA and DHA is plausible considering the robust identification of mechanisms that show that EPA and DHA beneficially modulate a number of

606 known risk factors for CVD, such as blood lipids, blood pressure, heart rate and heart rate
607 variability, platelet aggregation, endothelial function and inflammation. Despite this, evidence
608 for primary prevention of CVD through RCTs is relatively weak. Data from RCTs of
609 EPA+DHA (or EPA alone) in high-risk patients, especially in the secondary prevention setting
610 (e.g., post-MI) are inconsistent. Older (i.e., pre-2010) RCTs and meta-analyses based mainly
611 on those RCTs suggested significant benefit of long-chain omega-3 PUFAs on hard
612 cardiovascular outcomes. RCTs conducted since 2010 have not consistently confirmed this
613 benefit. Two recent large trials with high doses of long-chain omega-3 PUFAs (REDUCE-IT
614 and STRENGTH) had different findings, with REDUCE-IT showing significant benefits from
615 EPA-ethyl ester and STRENGTH being stopped for futility. Nevertheless, recent meta-analyses
616 confirm benefit from long-chain omega-3 PUFAs (76-78) and that these benefits are dose-
617 dependent (76; 78). Reasons for inconsistencies amongst RCT findings may relate to dose of
618 long-chain omega-3 PUFAs used, the exact composition and formulation used, the duration of
619 follow-up, and the event rate in the population being studied. Studies of short duration or using
620 low doses of EPA+DHA or being conducted against a background of multiple pharmaceutical
621 interventions may be less likely to observe effects of long-chain omega-3 PUFAs. Another
622 point to consider is that, unlike for pharmaceuticals, all individuals have some intake of long-
623 chain omega-3 PUFAs and/or synthesise at least some EPA and DHA endogenously meaning
624 that the "EPA and DHA native" state cannot occur, although background intake can be very
625 low. Another point to consider is that the bioavailability of EPA and DHA can vary among
626 individuals (103), with physiological differences and timing of consumption of supplements in
627 relation to meal intake being important. Despite the inconsistencies in the literature, there are
628 recommendations supporting the use of long-chain omega-3 PUFAs to treat
629 hypertriglyceridemia (41; 79) and patients with CVD (81). Identifying more clearly the dose-
630 dependent effects of EPA and DHA, separately and together, on cardiovascular risk factors and
631 on clinical outcomes is important to further develop long-chain omega-3 PUFAs as effective
632 therapeutic agents for CVD. Furthermore, robust primary prevention trials are still needed. It is
633 important the long-chain omega-3 PUFAs preparations (e.g. softgel capsules) be protected from
634 oxidation which may a) reduce the amount of active long-chain omega-3 PUFAs present and
635 b) produce peroxidation products that are harmful. There has been some concern that long-
636 chain omega-3 PUFAs might increase bleeding. However recent reports indicate that this is not
637 an issue at the doses of EPA and DHA that are commonly used in trials or therapeutically.
638 However, one concern that has recently become apparent from long-chain omega-3 PUFA trials

is a small, but significant, increase in risk of AF especially when the fatty acids are used at high doses. This has been seen in several RCTs and becomes clear through meta-analysis (100; 101). This effect is obviously concerning, although REDUCE-IT, while reporting increased risk of AF, also reported reduced risk of a range of hard cardiovascular outcomes. The mechanism by which high dose long-chain omega-3 PUFAs increase risk of AF needs to be identified so that more can be understood about this effect, those who are susceptible to it and how to mitigate it.

Financial Support

None.

Author contributions

PCC: conceptualization, review and editing; ID: drafting, review and editing. Both authors read and agreed the final version of the manuscript.

Conflict of Interest

ID has no conflict of interest to declare. PCC has received research funding from BASF AS and has acted as a consultant/advisor to BASF AS, Smartfish, DSM, Cargill, Bunge and Fresenius-Kabi.

References

1. Brenner RR. 2003. Hormonal modulation of Δ6 and Δ5 desaturases: case of diabetes. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 68:151-62
2. Childs CE. 2020. Sex hormones and n-3 fatty acid metabolism. *Proceedings of the Nutrition Society* 79:219-24
3. Hernández MC, Rojas P, Carrasco F, Basfi-Fer K, Valenzuela R, et al. 2020. Fatty acid desaturation in red blood cell membranes of patients with type 2 diabetes is improved by zinc supplementation. *Journal of Trace Elements in Medicine and Biology* 62:126571
4. Koletzko B, Reischl E, Tanjung C, Gonzalez-Casanova I, Ramakrishnan U, et al. 2019. FADS1 and FADS2 polymorphisms modulate fatty acid metabolism and dietary impact on health. *Annual Review of Nutrition* 39:21-44

5. Arterburn LM, Hall EB, Oken H. 2006. Distribution, interconversion, and dose response of n− 3 fatty acids in humans. *The American Journal of Clinical Nutrition* 83:1467S-76S
6. Baker EJ, Miles EA, Burdge GC, Yaqoob P, Calder PC. 2016. Metabolism and functional effects of plant-derived omega-3 fatty acids in humans. *Progress in Lipid Research* 64:30-56
7. Metherel AH, Irfan M, Klingel SL, Mutch DM, Bazinet RP. 2019. Compound-specific isotope analysis reveals no retroconversion of DHA to EPA but substantial conversion of EPA to DHA following supplementation: a randomized control trial. *The American Journal of Clinical Nutrition* 110:823-31
8. Calder PC. 2018. Very long-chain n-3 fatty acids and human health: fact, fiction and the future. *Proceedings of the Nutrition Society* 77:52-72
9. Browning LM, Walker CG, Mander AP, West AL, Madden J, et al. 2012. Incorporation of eicosapentaenoic and docosahexaenoic acids into lipid pools when given as supplements providing doses equivalent to typical intakes of oily fish. *The American Journal of Clinical Nutrition* 96:748-58
10. Stark KD, Van Elswyk ME, Higgins MR, Weatherford CA, Salem Jr N. 2016. Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults. *Progress in Lipid Research* 63:132-52
11. Troesch B, Eggersdorfer M, Laviano A, Rolland Y, Smith AD, et al. 2020. Expert opinion on benefits of long-chain omega-3 fatty acids (DHA and EPA) in aging and clinical nutrition. *Nutrients* 12:2555
12. Djuricic I, Calder PC. 2021. Beneficial outcomes of omega-6 and omega-3 polyunsaturated fatty acids on human health: An update for 2021. *Nutrients* 13:2421
13. Salem Jr N, Eggersdorfer M. 2015. Is the world supply of omega-3 fatty acids adequate for optimal human nutrition? *Current Opinion in Clinical Nutrition and Metabolic Care* 18:147-54
14. Ho QT, Bank MS, Azad AM, Nilsen BM, Frantzen S, et al. 2021. Co-occurrence of contaminants in marine fish from the North East Atlantic Ocean: Implications for human risk assessment. *Environment International* 157:106858
15. Alfio VG, Manzo C, Micillo R. 2021. From fish waste to value: an overview of the sustainable recovery of omega-3 for food supplements. *Molecules* 26:1002

16. Innes JK, Calder PC. 2020. Marine omega-3 (N-3) fatty acids for cardiovascular health: an update for 2020. *International Journal of Molecular Sciences* 21:1362
17. Calder PC. 2004. n–3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clinical Science* 107:1-11
18. De Caterina R. 2011. n–3 fatty acids in cardiovascular disease. *New England Journal of Medicine* 364:2439-50
19. Kris-Etherton PM, Harris WS, Appel LJ. 2002. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* 106:2747-57
20. Saravanan P, Davidson NC, Schmidt EB, Calder PC. 2010. Cardiovascular effects of marine omega-3 fatty acids. *The Lancet* 376:540-50
21. London B, Albert C, Anderson ME, Giles WR, Van Wagoner DR, et al. 2007. Omega-3 fatty acids and cardiac arrhythmias: prior studies and recommendations for future research: a report from the National Heart, Lung, and Blood Institute and Office Of Dietary Supplements Omega-3 Fatty Acids and their Role in Cardiac Arrhythmogenesis Workshop. *Circulation* 116:e320-e35
22. Hu FB, Bronner L, Willett WC, Stampfer MJ, Rexrode KM, et al. 2002. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *JAMA* 287:1815-21
23. Zhang Y, Zhuang P, He W, Chen J, Wang W, et al. 2018. Association of fish and long-chain omega-3 fatty acids intakes with total and cause-specific mortality: prospective analysis of 421 309 individuals. *Journal of Internal Medicine* 284:399-417
24. Li Z-H, Zhong W-F, Liu S, Kraus VB, Zhang Y-J, et al. 2020. Associations of habitual fish oil supplementation with cardiovascular outcomes and all cause mortality: evidence from a large population based cohort study. *BMJ* 368:m456
25. Djoussé L, Akinkuolie AO, Wu JH, Ding EL, Gaziano JM. 2012. Fish consumption, omega-3 fatty acids and risk of heart failure: a meta-analysis. *Clinical Nutrition* 31:846-53
26. Tavazzi L, Maggioni AP, Marchioli R, Barlera S, Franzosi MG, et al. 2008. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. The Lancet 372:1223-30
27. Chowdhury R, Warnakula S, Kunutsor S, Crowe F, Ward HA, et al. 2014. Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. *Annals of Internal Medicine* 160:398-406

28. Alexander DD, Miller PE, Van Elswyk ME, Kuratko CN, Bylsma LC. A meta-analysis of randomized controlled trials and prospective cohort studies of eicosapentaenoic and docosahexaenoic long-chain omega-3 fatty acids and coronary heart disease risk. *Mayo Clinic Proceedings*, *2017*, 92:15-29
29. Del Gobbo LC, Imamura F, Aslibekyan S, Marklund M, Virtanen JK, et al. 2016. ω-3 polyunsaturated fatty acid biomarkers and coronary heart disease: pooling project of 19 cohort studies. *JAMA Internal Medicine* 176:1155-66
30. Harris WS, Del Gobbo L, Tintle NL. 2017. The Omega-3 Index and relative risk for coronary heart disease mortality: Estimation from 10 cohort studies. *Atherosclerosis* 262:51-4
31. Harris WS, Tintle NL, Imamura F, Qian F, Korat AVA, et al. 2021. Blood n-3 fatty acid levels and total and cause-specific mortality from 17 prospective studies. *Nature Communications* 12:1-9
32. Scientific Advisory Committee on Nutrition/Committee on Toxicity. 2004. "Advice on fish consumption: benefits and risks", TSO, London
33. International Society for the Study of Fatty Acids and Lipids. 2004. Recommendations for the intake of polyunsaturated fatty acids in healthy adults (ISSFAL Policy Statement 3)
34. French Agency for Food EaOHS. 2003. The omega-3 fatty acids and the cardiovascular system: nutritional benefits and claims
35. Food and Agricultural Organisation of the United Nations. 2010. Fat and Fatty Acids in Human Nutrition: Report of an Expert Consultation. Food and Agricultural Organisation of the United Nations, Rome
36. Harris W. 2001. Omega-3 long-chain PUFA and triglyceride lowering: minimum effective intakes. *European Heart Journal Supplements* 3:D59-D61
37. Innes JK, Calder PC. 2018. The differential effects of eicosapentaenoic acid and docosahexaenoic acid on cardiometabolic risk factors: a systematic review. *International Journal of Molecular Sciences* 19:532
38. Allaire J, Couture P, Leclerc M, Charest A, Marin J, et al. 2016. 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. *The American Journal of Clinical Nutrition* 104:280-7

39. Woodman RJ, Mori TA, Burke V, Puddey IB, Watts GF, Beilin LJ. 2002. Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension. *The American Journal of Clinical Nutrition* 76:1007-15
40. Mori TA, Burke V, Puddey IB, Watts GF, O'Neal DN, et al. 2000. Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. *The American Journal of Clinical Nutrition* 71:1085-94
41. Skulas-Ray AC, Wilson PW, Harris WS, Brinton EA, Kris-Etherton PM, et al. 2019. Omega-3 fatty acids for the management of hypertriglyceridemia: a science advisory from the American Heart Association. *Circulation* 140:e673-e91
42. Barter PJ, Ballantyne CM, Carmena R, Cabezas MC, Chapman MJ, et al. 2006. Apo B versus cholesterol in estimating cardiovascular risk and in guiding therapy: report of the thirty-person/ten-country panel. *Journal of Internal Medicine* 259:247-58
43. Sniderman AD. 2005. Apolipoprotein B versus non–high-density lipoprotein cholesterol: and the winner is. *Circulation* 112:3366-7
44. Jiang J, Li K, Wang F, Yang B, Fu Y, et al. 2016. Effect of marine-derived n-3 polyunsaturated fatty acids on major eicosanoids: a systematic review and meta-analysis from 18 randomized controlled trials. *PloS One* 11:e0147351
45. AbuMweis S, Jew S, Tayyem R, Agraib L. 2018. Eicosapentaenoic acid and docosahexaenoic acid containing supplements modulate risk factors for cardiovascular disease: a meta-analysis of randomised placebo-control human clinical trials. *Journal of Human Nutrition and Dietetics* 31:67-84
46. Li K, Huang T, Zheng J, Wu K, Li D. 2014. Effect of marine-derived n-3 polyunsaturated fatty acids on C-reactive protein, interleukin 6 and tumor necrosis factor α: a meta-analysis. *PloS One* 9:e88103
47. Oppedisano F, Macrì R, Gliozzi M, Musolino V, Carresi C, et al. 2020. The anti-inflammatory and antioxidant properties of n-3 PUFAs: Their role in cardiovascular protection. *Biomedicines* 8:306
48. Gao L-G, Cao J, Mao Q-X, Lu X-C, Zhou X-l, Fan L. 2013. Influence of omega-3 polyunsaturated fatty acid-supplementation on platelet aggregation in humans: a meta-analysis of randomized controlled trials. *Atherosclerosis* 226:328-34

800 49. Wang Q, Liang X, Wang L, Lu X, Huang J, et al. 2012. Effect of omega-3 fatty acids
801 supplementation on endothelial function: a meta-analysis of randomized controlled
802 trials. *Atherosclerosis* 221:536-43
803 50. Xin W, Wei W, Li X. 2012. Effect of fish oil supplementation on fasting vascular
804 endothelial function in humans: a meta-analysis of randomized controlled trials. *Plos*
805 *One* 7:e46028
806 51. Miller PE, Van Elswyk M, Alexander DD. 2014. Long-chain omega-3 fatty acids
807 eicosapentaenoic acid and docosahexaenoic acid and blood pressure: a meta-analysis of
808 randomized controlled trials. *American Journal of Hypertension* 27:885-96
809 52. Hidayat K, Yang J, Zhang Z, Chen GC, Qin LQ, et al. 2018. Effect of omega-3 long-
810 chain polyunsaturated fatty acid supplementation on heart rate: a meta-analysis of
811 randomized controlled trials. *European Journal of Clinical Nutrition* 72:805-17
812 53. Burr ML, Gilbert J, Holliday RA, Elwood P, Fehily A, et al. 1989. Effects of changes
813 in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction
814 trial (DART). *The Lancet* 334:757-61
815 54. GISSI-P Invesigators. 1999. Dietary supplementation with n-3 polyunsaturated fatty
816 acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial.
817 *The Lancet* 354:447-455
818 55. Marchioli R, Barzi F, Bomba E, Chieffo C, Di Gregorio D, et al. 2002. Early protection
819 against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction:
820 time-course analysis of the results of the Gruppo Italiano per lo Studio della
821 Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. *Circulation* 105:1897-
822 903
823 56. Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, et al. 2007. Effects of
824 eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients
825 (JELIS): a randomised open-label, blinded endpoint analysis. *The Lancet* 369:1090-8
826 57. Rauch B, Schiele R, Schneider S, Diller F, Victor N, et al. 2010. OMEGA, a
827 randomized, placebo-controlled trial to test the effect of highly purified omega-3 fatty
828 acids on top of modern guideline-adjusted therapy after myocardial infarction.
829 *Circulation* 122:2152-9
830 58. Galan P, Kesse-Guyot E, Czernichow S, Briancon S, Blacher J, Hercberg S. 2010.
831 Effects of B vitamins and omega 3 fatty acids on cardiovascular diseases: a randomised
832 placebo controlled trial. *BMJ* 341

59. Kromhout D, Giltay EJ, Geleijnse JM. 2010. N-3 Fatty acids and cardiovascular events after myocardial infarction. *New England Journal of Medicine* 363:2015-26
60. ORIGIN Trial Investigators. 2012. n-3 Fatty acids and cardiovascular outcomes in patients with dysglycemia. *New England Journal of Medicine* 367:309-318.
61. Risk and Prevention Study Colaborsative Group. 2013. n-3 fatty acids in patients with multiple cardiovascular risk factors. *New England Journal of Medicine* 368:1800-1808
62. ASCEND Study Collaborative Group. 2018. Effects of n-3 fatty acid supplements in diabetes mellitus. *New England Journal of Medicine* 379:1540-1550
63. Manson JE, Cook NR, Lee I-M, Christen W, Bassuk SS, et al. 2019. Marine n-3 fatty acids and prevention of cardiovascular disease and cancer. *New England Journal of Medicine* 380:23-32
64. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, et al. 2019. Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. *New England Journal of Medicine* 380:11-22
65. Jo S-H, Han SH, Kim S-H, Eckel RH, Koh KK. 2021. Cardiovascular effects of omega-3 fatty acids: Hope or hype? *Atherosclerosis* 322:15-23
66. Budoff MJ, Bhatt DL, Kinninger A, Lakshmanan S, Muhlestein JB, et al. 2020. Effect of icosapent ethyl on progression of coronary atherosclerosis in patients with elevated triglycerides on statin therapy: final results of the EVAPORATE trial. *European Heart Journal* 41:3925-32
67. Nicholls SJ, Lincoff AM, Garcia M, Bash D, Ballantyne CM, et al. 2020. 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* 324:2268-80
68. Calder PC, Deckelbaum RJ. 2021. Omega-3 fatty acids: new studies, new data, new questions. *Current Opinion in Clinical Nutrition and Metabolic Care* 24:109-13
69. Kalstad AA, Myhre PL, Laake K, Tveit SH, Schmidt EB, et al. 2021. Effects of n-3 fatty acid supplements in elderly patients after myocardial infarction: a randomized, controlled trial. *Circulation* 143:528-39
70. Bucher HC, Hengstler P, Schindler C, Meier G. 2002. N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. *The American Journal of Medicine* 112:298-304

71. Studer M, Briel M, Leimenstoll B, Glass TR, Bucher HC. 2005. Effect of different antilipidemic agents and diets on mortality: a systematic review. *Archives of Internal Medicine* 165:725-30
72. Zhao Y-T, Chen Q, Sun Y-X, Li X-B, Zhang P, et al. 2009. Prevention of sudden cardiac death with omega-3 fatty acids in patients with coronary heart disease: a meta-analysis of randomized controlled trials. *Annals of Medicine* 41:301-10
73. Marik PE, Varon J. 2009. Omega-3 dietary supplements and the risk of cardiovascular events: a systematic review. *Clinical Cardiology: An International Indexed and Peer-Reviewed Journal for Advances in the Treatment of Cardiovascular Disease* 32:365-72
74. Kotwal S, Jun M, Sullivan D, Perkovic V, Neal B. 2012. Omega 3 fatty acids and cardiovascular outcomes: systematic review and meta-analysis. *Circulation: Cardiovascular Quality and Outcomes* 5:808-18
75. Abdelhamid AS, Brown TJ, Brainard JS, Biswas P, Thorpe GC, et al. 2018. Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. *Cochrane Database of Systematic Reviews* 11:CD003177
76. Bernasconi AA, Wiest MM, Lavie CJ, Milani RV, Laukkanen JA. 2021. Effect of omega-3 dosage on cardiovascular outcomes: an updated meta-analysis and meta-regression of interventional trials. *Mayo Clinic Proceedings* 96:304-13
77. Hu Y, Hu FB, Manson JE. 2019. Marine omega-3 supplementation and cardiovascular disease: an updated meta-analysis of 13 randomized controlled trials involving 127 477 participants. *Journal of the American Heart Association* 8:e013543
78. Rizos EC, Markozannes G, Tsapas A, Mantzoros CS, Ntzani EE. 2021. Omega-3 supplementation and cardiovascular disease: formulation-based systematic review and meta-analysis with trial sequential analysis. *Heart* 107:150-8
79. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, et al. 2020. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *European Heart Journal* 41:111-88
80. Orringer CE, Jacobson TA, Maki KC. 2019. National Lipid Association Scientific Statement on the use of icosapent ethyl in statin-treated patients with elevated triglycerides and high or very-high ASCVD risk. *Journal of Clinical Lipidology* 13:860-72
81. Siscovick DS, Barringer TA, Fretts AM, Wu JH, Lichtenstein AH, et al. 2017. Omega-3 polyunsaturated fatty acid (fish oil) supplementation and the prevention of clinical

cardiovascular disease: a science advisory from the American Heart Association. *Circulation* 135:e867-e84

82. Jairoun AA, Shahwan M, Zyoud SeH. 2020. Fish oil supplements, oxidative status, and compliance behaviour: Regulatory challenges and opportunities. *Plos One* 15:e0244688

83. Albert BB, Cameron-Smith D, Hofman PL, Cutfield WS. 2013. Oxidation of marine omega-3 supplements and human health. *BioMed research international* 2013:464921

84. Shahidi F, Zhong Y. 2010. Lipid oxidation and improving the oxidative stability. *Chemical Society Reviews* 39:4067-79

85. GOED, 2019. GOED Voluntary Monograph - v.7. Global Organization for EPA and DHA Omega-3.

86. EFSA Panel on Biological Hazards (BIOHAZ). 2010. Scientific opinion on fish oil for human consumption. Food hygiene, including rancidity. *EFSA Journal* 8:1874

87. Ottestad I, Vogt G, Retterstøl K, Myhrstad MC, Haugen J-E, et al. 2012. Oxidised fish oil does not influence established markers of oxidative stress in healthy human subjects: a randomised controlled trial. *British Journal of Nutrition* 108:315-26

88. Goodnight SJ, Harris WS, Connor WE. 1981. The effects of dietary omega 3 fatty acids on platelet composition and function in man: a prospective, controlled study. *Blood* 58:880-5

89. Von Schacky C, Fischer S, Weber PC. 1985. Long-term effects of dietary marine omega-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. *The Journal of Clinical Investigation* 76:1626-31

90. DiNicolantonio JJ, OKeefe J. 2019. Importance of maintaining a low omega-6/omega-3 ratio for reducing platelet aggregation, coagulation and thrombosis. *Open Heart* 6:e001011

91. Woodman RJ, Mori TA, Burke V, Puddey IB, Barden A, et al. 2003. Effects of purified eicosapentaenoic acid and docosahexaenoic acid on platelet, fibrinolytic and vascular function in hypertensive type 2 diabetic patients. *Atherosclerosis* 166:85-93

92. Din JN, Harding SA, Valerio CJ, Sarma J, Lyall K, et al. 2008. Dietary intervention with oil rich fish reduces platelet-monocyte aggregation in man. *Atherosclerosis* 197:290-6

93. Harris WS. 2007. Expert opinion: omega-3 fatty acids and bleeding—cause for concern? *The American Journal of Cardiology* 99:S44-S6

94. Akintoye E, Sethi P, Harris WS, Thompson PA, Marchioli R, et al. 2018. Fish oil and perioperative bleeding: insights from the OPERA randomized trial. *Circulation: Cardiovascular Quality and Outcomes* 11:e004584
95. Kapoor K, Alfaddagh A, Al Rifai M, Bhatt DL, Budoff MJ, et al. 2021. Association between omega-3 fatty acid levels and risk for incident major bleeding events and atrial fibrillation: MESA. *Journal of the American Heart Association* 10:e021431
96. Jeansen S, Witkamp RF, Garthoff JA, van Helvoort A, Calder PC. 2018. Fish oil LC-PUFAs do not affect blood coagulation parameters and bleeding manifestations: Analysis of 8 clinical studies with selected patient groups on omega-3-enriched medical nutrition. *Clinical Nutrition* 37:948-57
97. Fradet S, Pelletier JF, Singbo N, Lacombe L, Toren P, et al. 2022. Effects of omega-3 fatty acids supplementation on perioperative blood loss and complications after radical prostatectomy. *Clinical Nutrition ESPEN* 47:221-6
98. Curfman G. 2021. Omega-3 fatty acids and atrial fibrillation. *JAMA* 325:1063
99. Albert CM, Cook NR, Pester J, Moorthy MV, Ridge C, et al. 2021. Effect of marine omega-3 fatty acid and vitamin D supplementation on incident atrial fibrillation: a randomized clinical trial. *JAMA* 325:1061-73
100. Lombardi M, Carbone S, Del Buono MG, Chiabrando JG, Vescovo GM, et al. 2021. Omega-3 fatty acids supplementation and risk of atrial fibrillation: an updated meta-analysis of randomized controlled trials. *European Heart Journal. Cardiovascular Pharmacotherapy* 7:e69-e70
101. Gencer B, Djousse L, Al-Ramady OT, Cook NR, Manson JE, Albert CM. 2021. Effect of long-term marine ω-3 fatty acids supplementation on the risk of atrial fibrillation in randomized controlled trials of cardiovascular outcomes: a systematic review and meta-analysis. *Circulation* 144:1981-90
102. Fatkin D, Cox CD, Martinac B. 2022. Fishing for links between omega-3 fatty acids and atrial fibrillation. *Circulation* 145:1037-9.
103. Schuchardt JP, Hahn A. 2013. Bioavailability of long-chain omega-3 fatty acids. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 89:1-8

962 **Figure captions**

963

964 Figure 1. Pathway of conversion of α-linolenic acid to eicosapentaenoic acid and
965 docosahexaenoic acid.

966

967 Figure 2. Pooled results from meta-analysis of RCTs of long-chain omega-3 PUFAs and
968 cardiovascular outcomes. The figure shows the pooled estimate of relative risk and 95%
969 confidence interval, as well as the number of studies and combined number of participants.
970 CHD, coronary heart disease; CVD, cardiovascular disease; MI, myocardial infarction.
971 Reprinted from: Bernasconi, A.A., Wiest, M.M., Lavie, C.J., Milani, R.V., Laukkanen, J.A.
972 Effect of omega-3 dosage on cardiovascular outcomes: an updated meta-analysis and meta-
973 regression of interventional trials. Mayo Clin. Proc. 2021; 96: 304-313 (Ref (76)).

976

977 Table 1. Content of EPA and DHA in supplements and pharmaceuticals.

Supplement type	**Typical EPA + DHA content per g of oil (mg)**	**Comments**
Cod liver oil	200	Usually more EPA than DHA; mainly in triglyceride form
Standard fish oil	300	Usually more EPA than DHA; mainly in triglyceride form
Fish oil concentrate	450-600	Usually more EPA than DHA; mainly in triglyceride form
Tuna oil	460	More DHA than EPA; mainly in triglyceride form
Krill oil	205	Usually more EPA than DHA; some in phospholipid form
Algal oil	400	Mainly DHA
Flaxseed oil	0	Contains ALA, not EPA and DHA
Pharmaceutical	**Typical EPA + DHA content per g of oil (mg)**	**Comments**
Omacor/Lovaza	460 + 380	In ethyl ester form
Epanova	550 + 200	In free fatty acid form
Vascepa/Icosapent ethyl	900 + 0	In ethyl ester form

978

979

980

981 Table 2. Summary of the main randomized controlled trials of long-chain omega-3 fatty acids reporting on hard cardiovascular outcomes.

Trial (Reference)	Patient group	EPA+DHA dose (mg/day)	Form of EPA and DHA	Control	Average duration of follow-up (yr)	Sample size	Main findings with long-chain omega-3 fatty acids
GISSI-Prevenzione (54, 55)	Recent MI	460 + 380	Ethyl ester	None	3.5	11334 across 4 arms (2 arms with EPA+DHA)	Reduced both primary outcomes (composite of death, non-fatal MI or non-fatal stroke (-15%) and composite of cardiovascular death, non-fatal MI or non-fatal stroke (-20%)) and secondary outcomes (all fatal events (-20%); cardiovascular death (-30%); cardiac death (-35%); coronary death (-35%); sudden death (-45%))
GISSI-HF (26)	Chronic heart failure	460 + 380	Ethyl ester	"Matching placebo"	3.9	6975 across 2 arms	Reduced primary outcome (mortality (-9%))
JELIS (56)	Hypercholesterolemia (primary prevention) or Hypercholesterolemia + pre-existing CHD (secondary prevention)	1800 + 0 (+ statin)	Ethyl ester	Statin alone	4.6	18645 across 2 arms	Primary prevention: No effect Secondary prevention: Fewer non-fatal coronary events (-19%)
OMEGA (57)	Recent MI	460 + 380	Ethyl ester	Olive oil	1.0	3851 across 2 arms	No effect on primary outcome (sudden death) or secondary outcomes (mortality; composite of mortality, MI or stroke; revascularization)
SU.FOL.OM3 (58)	History of CVD (previous MI, unstable angina or ischemic stroke)	400 + 200 (± B vitamins)	Not stated but most likely triglyceride	"Placebo"	4.2	2501 across 4 arms (2 arms with EPA+DHA)	No effect on primary outcome (composite of non-fatal MI, stroke or death from CVD) or secondary outcomes (mortality; non-fatal MI; stroke; all coronary events; revascularization; other cardiovascular events)
Alpha-Omega (59)	Previous MI	226 + 150 (in margarine)	Triglyceride	Standard margarine	3.4	4837 across 4 arms (2 arms with EPA+DHA)	No effect on primary outcome (composite of fatal or non-fatal CVD or need for cardiac intervention) or secondary outcomes (mortality; fatal CVD; fatal CHD; ventricular arrhythmia-related evets) but reduced fatal CHD and fewer arrhythmia-related events in patients with diabetes
ORIGIN (60)	Dysglycemia plus recent MI or heart failure	460 + 380 (± long-acting insulin)	Ethyl ester	Long-acting insulin or standard care alone	6.2	12536 across 4 arms (2 arms with EPA+DHA)	No effect on primary outcome (death from cardiovascular causes), secondary outcomes (mortality; death from arrhythmia; composite of death from cardiovascular causes, non-fatal MI or

							non-fatal stroke) or other outcomes (MI; stroke; revascularization; hospitalization for heart failure)
Risk and Prevention (61)	High CVD risk	460 + 380	Ethyl ester	Olive oil	5.0	12513 across 2 arms	No effect on primary outcome (composite of time to death from cardiovascular causes or hospitalization for cardiovascular causes) or secondary outcomes (composite of death, non-fatal MI or non-fatal strake; composite of time to death from cardiovascular causes, non-fatal MI or non-fatal stroke; death from CHD; sudden death)
ASCEND (62)	Diabetes	460 + 380	Ethyl ester	Olive oil	7.4	15480 across 2 arms	No effect on primary outcome (serious vascular events: composite of non-fatal MI, non-fatal stroke, transient ischemic attack or vascular death), secondary outcome (serious vascular events or revascularization) or components of primary outcome except fewer vascular deaths (-19%)
VITAL (63)	Healthy (> 50 yr old)	460 + 380 (± vitamin D)	Ethyl ester	"Matching placebo"	5.3	25871 across 4 arms (2 arms with EPA+DHA)	No effect primary outcome (composite of MI, stroke or death from cardiovascular causes), secondary outcome (primary outcome or revascularization) or some individual components of these outcomes (stroke; death from CHD; death from CVD; death from stroke) but reduction in MI (-28%), CHD (-17%) and death from MI (-50%)
REDUCE-IT (64)	CVD risk and raised triglycerides (primary prevention) or Established CVD and diabetes and raised triglycerides (secondary prevention); all on statins	3600 + 0	Ethyl ester	Mineral oil	4.9	8179 across 2 arms	Reduction in primary outcome (composite of cardiovascular death, non-fatal MI, non-fatal stroke, revascularization or unstable angina) (-25%)), secondary outcome (composite of cardiovascular death, non-fatal MI or non-fatal stroke) (-20%)) and multiple other outcomes (cardiovascular death or non-fatal MI (-25%); MI (-31%); revascularization (-35%); cardiovascular death (-20%); hospitalization for angina (-32%); stroke (-28%); death, non-fatal MI or non-fatal stroke (-23%))
STRENGTH (67)	CVD risk and raised triglycerides; all on statins	2200 + 800	Free fatty acid	Corn oil	3.5	13078 across 2 arms	No effect on primary outcome (composite of cardiovascular death, non-fatal MI, non-fatal stroke, revascularization or unstable angina) or

							any secondary outcome (composites of outcomes; cardiovascular death; mortality)
OMEMI (69)	Recent MI	930 + 660	Triglyceride	Corn oil	2	1027 across 2 arms	No effect on primary outcome (composite of non-fatal MI, stroke, mortality, revascularization or heart failure) or on any component of primary outcome; no effect on secondary outcome (new onset atrial fibrillation although that tended in be higher with omega-3)

982 Abbreviations: CHD, coronary heart disease; CVD, cardiovascular disease; MI, myocardial infarction.