ANNUAL REVIEWS

Annual Review of Pharmacology and Toxicology

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, Belgrade, Serbia

[2]School of Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, United Kingdom; email: pcc@soton.ac.uk

[3]NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust and University of Southampton, Southampton, United Kingdom

Annu. Rev. Pharmacol. Toxicol. 2023. 63:383–406

The *Annual Review of Pharmacology and Toxicology* is online at pharmtox.annualreviews.org

https://doi.org/10.1146/annurev-pharmtox-051921-090208

Keywords

omega-3 fatty acid, fish oil, eicosapentaenoic acid, docosahexaenoic acid, heart disease, risk factor

Abstract

The long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are found in seafood, supplements, and concentrated pharmaceutical preparations. Prospective cohort studies 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 due to the beneficial modulation of a number of risk factors for CVD. Some large trials support the use of EPA+DHA (or EPA alone) in high-risk patients, although the evidence is inconsistent. This review presents key studies of 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 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

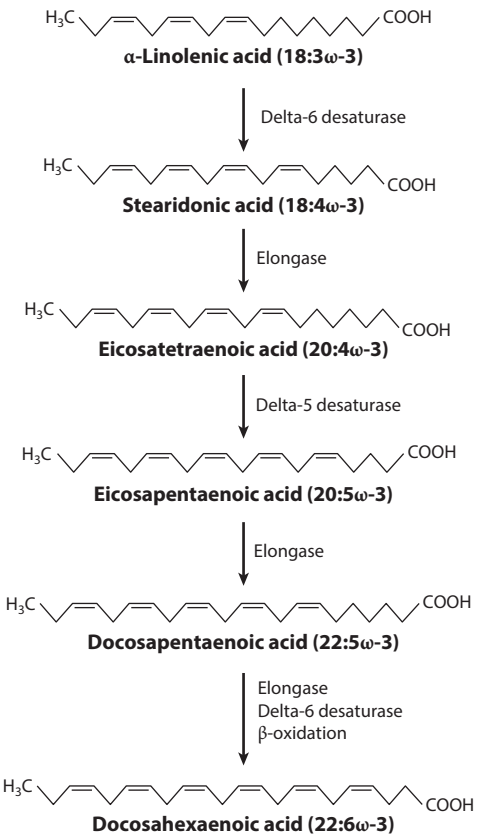

Figure 1

Pathway of conversion of α-linolenic acid to eicosapentaenoic acid and docosahexaenoic acid.

the activity of this conversion pathway (4). Studies in adult humans demonstrate that conversion of 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 retroconversion involving limited peroxisomal β-oxidation. However, a recent study using stable-isotopically-labeled DHA was interpreted to demonstrate only very limited retroconversion in humans (7).

ALA is found in significant amounts in several seeds, seed oils, and nuts. Linseeds (also called flaxseeds) and their oil typically contain 45–55% of fatty acids in the form of ALA. Soybean oil, rapeseed oil (also known as canola oil), and walnuts contain 5–10% of fatty acids in the form of ALA. The most important dietary source of EPA and DHA is fatty or oily fish (e.g., salmon, tuna, herring, sardines, mackerel), providing approximately 1.5–3.0 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 0.1–0.2 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 supplements 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 the prevention and treatment of cardiovascular disease (CVD). Due to

Table 1 Content of EPA and DHA in supplements and pharmaceuticals

Type	Typical EPA+DHA content per g of oil (mg)	Comments
Supplement		
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		
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

Abbreviations: ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

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 consumption 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, which 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 1-g 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 0.45–0.65 g EPA+DHA per gram 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 in which 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. A 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 review is to discuss the role of long-chain omega-3 PUFAs in the prevention and treatment of CVD, reviewing the evidence to date and also highlighting some of the possible adverse effects of these fatty acids. This review builds upon, but also 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 nonfatal 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 approximately 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 use of fish oil supplements 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 intake of fish 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 as an effect on cardiac contractile function independent of CHD. Both are possible. However, the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico heart failure (GISSI-HF) trial randomized 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. Data from 16 studies, including over 422,000 subjects, showed 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 a 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. (28) 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 for those with lower intake. 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 19 trials investigating the association between EPA or DHA concentration in a body pool, such as 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 the 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 the 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 the 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, nongovernment, and professional bodies. For example, the United Kingdom's 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 per day for the general population (33). Similarly, 400–500 mg EPA+DHA per day with at least 100–120 mg DHA per day was set by the French Agency for Food, Environmental, and Occupational Health and Safety (34), while a minimum of 250 mg EPA+DHA per day is the appropriate daily intake recommended by the Food and Agricultural Organization 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 toward 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 and references therein). Concerning the 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 significant total cholesterol-lowering effect 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 does EPA and increases LDL particle size, an effect that 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 proinflammatory eicosanoids (e.g., thromboxane B_2 and leukotriene B_4) (44), C-reactive protein, and proinflammatory 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 signaling 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 nonhealthy 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, while 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 accounts 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 summarizes the key randomized controlled trials (RCTs) of long-chain omega-3 fatty acids that report on hard cardiovascular end points. The Diet and Reinfarction Trial (DART) involved 2,033 recent MI survivors (mean: 41 days since MI) who were advised concerning fat, fish, and fiber 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 compared to 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/day EPA+DHA, 300 mg/day 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 nonfatal 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 antiarrhythmic benefit of EPA and DHA (55). In the GISSI-HF trial (26), 6,975 patients with chronic heart failure received 840 mg/day EPA+DHA or placebo for approximately 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/day EPA as an ethyl ester) with a 5-year follow-up (56). Among those patients who were receiving statin therapy, a number were on conventional medication due to pre-existing CHD. The primary outcome was any major coronary event, including sudden cardiac death, fatal and nonfatal MI, and other nonfatal 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 nonfatal coronary events versus statin alone (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/day 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 revascularization 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

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 per day)	Form of EPA and DHA	Control	Average duration of follow-up (years)	Sample size	Main findings with long-chain omega-3 fatty acids
GISSI-Prevenzione (54, 55)	Recent MI	460 + 380	Ethyl ester	None	3.5	11,334 across 4 arms (2 arms with EPA+DHA)	Reduced both primary outcomes [composite of death, nonfatal MI, or nonfatal stroke (−15%); and composite of cardiovascular death, nonfatal MI, or nonfatal stroke (−20%)] and secondary outcomes [all fatal events (−20%), cardiovascular death (−30%), cardiac death (−35%), coronary death (−35%), and sudden death (−45%)]
GISSI-HF (26)	Chronic heart failure	460 + 380	Ethyl ester	Matching placebo	3.9	6,975 across 2 arms	Reduced primary outcome [mortality (−9%)]
JELIS (56)	Hypercholesterolemia (primary prevention) OR Hypercholesterolemia + pre-existing CHD (secondary prevention)	1,800 + 0 (+ statin)	Ethyl ester	Statin alone	4.6	18,645 across 2 arms	Primary prevention: no effect Secondary prevention: fewer nonfatal coronary events (−19%)
OMEGA (57)	Recent MI	460 + 380	Ethyl ester	Olive oil	1.0	3,851 across 2 arms	No effect on primary outcome (sudden death) or secondary outcomes (mortality, composite of mortality, MI or stroke, and 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	2,501 across 4 arms (2 arms with EPA+DHA)	No effect on primary outcome (composite of nonfatal MI, stroke, or death from CVD) or secondary outcomes (mortality, nonfatal MI, stroke, all coronary events, revascularization, and other cardiovascular events)

(*Continued*)

Table 2 (*Continued*)

Trial (reference)	Patient group	EPA+DHA dose (mg per day)	Form of EPA and DHA	Control	Average duration of follow-up (years)	Sample size	Main findings with long-chain omega-3 fatty acids
Alpha Omega (59)	Previous MI	226 + 150 (in margarine)	Triglyceride	Standard margarine	3.4	4,837 across 4 arms (2 arms with EPA+DHA)	No effect on primary outcome (composite of fatal or nonfatal CVD or need for cardiac intervention) or secondary outcomes (mortality, fatal CVD, fatal CHD, and ventricular arrhythmia-related evets) but reduced fatal CHD and 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	12,536 across 4 arms (2 arms with EPA+DHA)	No effect on primary outcome (death from cardiovascular causes), secondary outcomes (mortality; death from arrhythmia; and composite of death from cardiovascular causes, nonfatal MI, or nonfatal stroke), or other outcomes (MI, stroke, revascularization, and hospitalization for heart failure)
Risk and Prevention (61)	High CVD risk	460 + 380	Ethyl ester	Olive oil	5.0	12,513 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, nonfatal MI or nonfatal stroke; composite of time to death from cardiovascular causes, nonfatal MI, or nonfatal stroke; death from CHD; and sudden death)

(*Continued*)

Table 2 (*Continued*)

Trial (reference)	Patient group	EPA+DHA dose (mg per day)	Form of EPA and DHA	Control	Average duration of follow-up (years)	Sample size	Main findings with long-chain omega-3 fatty acids
ASCEND (62)	Diabetes	460 + 380	Ethyl ester	Olive oil	7.4	15,480 across 2 arms	No effect on primary outcome (serious vascular events: composite of nonfatal MI, nonfatal 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 years old)	460 + 380 (± vitamin D)	Ethyl ester	Matching placebo	5.3	25,871 across 4 arms (2 arms with EPA+DHA)	No effect on 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, and 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	3,600 + 0	Ethyl ester	Mineral oil	4.9	8,179 across 2 arms	Reduction in primary outcome [composite of cardiovascular death, nonfatal MI, nonfatal stroke, revascularization, or unstable angina (−25%)], secondary outcome [composite of cardiovascular death, nonfatal MI, or nonfatal stroke (−20%)], and multiple other outcomes [cardiovascular death or nonfatal MI (−25%); MI (−31%); revascularization (−35%); cardiovascular death (−20%); hospitalization for angina (−32%); stroke (−28%); and death, nonfatal MI, or nonfatal stroke (−23%)]

(*Continued*)

Table 2 (*Continued*)

Trial (reference)	Patient group	EPA+DHA dose (mg per day)	Form of EPA and DHA	Control	Average duration of follow-up (years)	Sample size	Main findings with long-chain omega-3 fatty acids
STRENGTH (67)	CVD risk and raised triglycerides All on statins	2,200 + 800	Free fatty acid	Corn oil	3.5	13,078 across 2 arms	No effect on primary outcome (composite of cardiovascular death, nonfatal MI, nonfatal stroke, revascularization, or unstable angina) or any secondary outcome (composites of outcomes, cardiovascular death, and mortality)
OMEMI (69)	Recent MI	930 + 660	Triglyceride	Corn oil	2	1,027 across 2 arms	No effect on primary outcome (composite of nonfatal MI, stroke, mortality, revascularization, or heart failure) or on any component of primary outcome and no effect on secondary outcome (new-onset atrial fibrillation; although that tended in be higher with omega-3)

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

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 CVD (MI, unstable angina, or ischemic stroke) (58). Of the 2,501 enrolled patients, around 50% received omega-3 fatty acids alone (600 mg/day EPA+DHA at a ratio 2:1) or B vitamins and omega-3 fatty acids versus 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 nonfatal 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 approximately 375 mg/day EPA+DHA, 1.9 g/day ALA, EPA+DHA+ALA, or a placebo margarine and followed up for roughly 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 arrhythmia-related events was experienced among patients with diabetes in EPA+DHA- and ALA-fortified margarine groups (59).

The Outcome Reduction with an Initial Glargine Intervention (ORIGIN) trial, published in 2012, investigated whether supplementation with 840 mg/day EPA+DHA can reduce cardiovascular mortality in dysglycemic 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 or for total mortality. The Risk and Prevention Study, from 2013, assessed the effect of supplementation with 840 mg/day 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 hospitalization 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 nonfatal 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/day 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 toward reduced risk of death (21%) from CHD.

The Vitamin D and Omega-3 (VITAL) trial, published in 2019, was a RCT conducted as a two-by-two factorial design of vitamin D3 (at a dose of 50 μg/day) and long-chain omega-3 PUFA capsules (at a dose of 1 g/day containing 480 mg EPA and 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 group supplemented 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 per week and then supplemented with long-chain omega-3 PUFAs.

In the 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 diabetes (58% of all subjects had type 2 diabetes mellitus). Baseline plasma LDL-C levels were well controlled with statins (1.06–2.59 mmol/L), while triglyceride levels were borderline and moderately elevated (1.52–5.63 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, nonfatal MI, nonfatal stroke, coronary revascularization, or unstable angina) was reduced in patients who received EPA-ethyl ester compared to placebo [hazard ratio: 0.75; 95% confidence interval (CI): 0.68, 0.83; $p < 0.001$]. The key prespecified secondary outcome (a composite of cardiovascular death, nonfatal MI, or nonfatal 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 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 5 years, whereas in the placebo group, the serum level did not change (65). Another positive EPA-ethyl ester trial was Effect of Vascepa on Improving Coronary Atherosclerosis in People with High Triglycerides Taking Statin Therapy (EVAPORATE) (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 6 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 Long Term Outcomes Study to Assess Statin Residual Risk with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridemia (STRENGTH) trial (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 and 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 Omega-3 Fatty Acids in Elderly with Myocardial Infarction (OMEMI) trial, published in 2021, randomized a total of 1,027 patients with a recent MI (in the previous 2–8 weeks) to receive approximately 1.6 g/day of long-chain omega-3 fatty acids (930 mg EPA and 660 mg DHA) or corn oil (placebo) as an addition to standard care (69). After 2 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-analyzed results from 11 RCTs (two dietary RCTs and nine supplement RCTs) involving 15,806 patients with CHD who received long-chain omega-3 fatty acids with a dose range of 0.3–6 g/day EPA and 0.6–3.7 g/day DHA. The analysis showed a 30% reduced risk of fatal MI, a 20% reduced risk of nonfatal MI, a 30% reduced risk for sudden death, and a 20% reduced risk in overall mortality in the long-chain 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 three dietary RCTs and five supplement 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/day EPA and 0.4–2.8 g/day DHA (72). Another 2009 meta-analysis of 11 RCTs with 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/day EPA+DHA (73).

Over the last 10 years, several meta-analyses reported more mixed conclusions than did earlier meta-analyses, as recently reviewed elsewhere (16). For example, a meta-analysis from 2012, representing 20 RCTs (3 dietary RCTs and 17 supplement RCTs) with 62,851 patients in primary and secondary prevention settings, reported a 14% reduction in vascular death but no effect on cardiovascular events, total mortality, or coronary or arrhythmia events with long-chain omega-3 fatty acid administration (74). Dosages of omega-3 fatty acids used in the included trials were in range of 0.8–3.4 g/day EPA+DHA, and the 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 (ranging from 0.3 g/day EPA to 6 g/day 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 acid doses ranging from 0.4 to 5 g/day EPA+DHA (28). The study reported a 14–16% reduction in CHD in high-risk subgroups, that is, those with elevated triglycerides and LDL-C (28). A 2020 meta-analysis conducted by Abdelhamid et al. (75) aggregated data from all RCTs published before August 2019 that reported CVD outcomes. The study included 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/day to more than 5 g/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 supplement trials, the dosage used varied from 0.4 to 5.5 g/day EPA+DHA (average dose received was 1,221 mg/day EPA+DHA). Pooled results did not show an association between supplementation and reduction in the risk of CVD events, which is in accordance with the findings of Abdelhamid

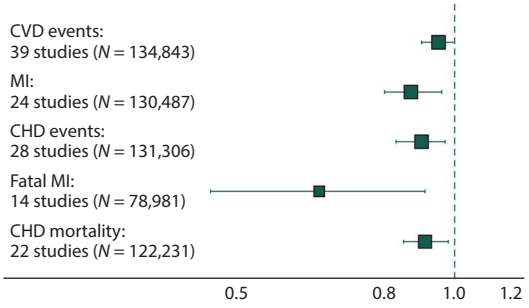

Figure 2

Pooled results from meta-analysis of randomized controlled trials of long-chain omega-3 polyunsaturated fatty acids and cardiovascular outcomes. The figure shows the pooled estimate of relative risk and 95% confidence interval, as well as the number of studies and combined number of participants. Abbreviations: CHD, coronary heart disease; CVD, cardiovascular disease; MI, myocardial infarction. Figure reprinted from Reference 76; copyright 2021 Mayo Foundation for Medical Education and Research, with permission from Elsevier.

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+DHA by 1 g/day was associated with a 5.8% reduction in the risk of CVD events. In the case of MI, the risk reduction was dose dependent, and each additional 1 g/day of EPA+DHA was associated with a significant risk reduction of 9%. This analysis did not find a statistically significant benefit of EPA alone compared with EPA+DHA or a significant association between the year of publication and the overall impact of EPA and/or DHA on CVD outcomes.

Another recent meta-analysis of 13 RCTs (e.g., GISSI-Prevenzione, JELIS, GISSI-HF, SU.FOL.OM3, Alpha Omega, OMEGA, ORIGIN, VITAL, ASCEND, and REDUCE-IT) demonstrated that EPA and DHA lower the risk of MI, CHD death, total CHD, CVD death, and total CVD (77). The total number of participants was 127,477, while the mean duration of follow-up ranged from 1.0 to 7.4 years and the mean dose of EPA+DHA ranged from 0.37 to 3.6 g/day. Excluding REDUCE-IT from the analysis resulted in a weaker, but still significant, inverse association for these outcomes (77).

Very recently, Rizos et al. (78) published a new meta-analysis of 17 RCTs of supplemental long-chain omega-3 PUFAs according to prior defined doses (i.e., $\leq$1, 2, $\geq$3 1-g capsules per day) and for a duration $\geq$1 year. For 2 capsules per day, a significant reduction was found for cardiac death, while for $\geq$3 capsules per day, a statistically significant reduction in cardiac death, sudden death, and stroke was observed. There was no association with any CVD outcome for doses less than two capsules per day.

5. RECOMMENDATIONS FOR USE OF LONG-CHAIN OMEGA-3 FATTY ACIDS IN PATIENT TREATMENT

American and European authorities have made recommendations for the clinical use of EPA and DHA with respect to CVD, as detailed elsewhere (16). In recognition of the ability of long-chain omega-3 PUFAs to lower blood triglycerides, in 2019, the American Heart Association (AHA) updated its earlier recommendation for the use of 2–4 g/day EPA+DHA for triglyceride lowering:

"we conclude that prescription n-3 fatty acids, whether EPA+DHA or EPA-only, at a dose of 4 g/d, are clinically useful for reducing triglycerides, after any underlying causes are addressed and diet and lifestyle strategies are implemented, either as monotherapy or as an adjunct to other triglyceride-lowering therapies" (41, p. e685). On the basis of the positive outcomes of REDUCE-IT, the European Society of Cardiology and the European Atherosclerosis Society issued an update to the "Clinical Practice Guidelines for the Management of Dyslipidaemias" specifically recommending that "in high-risk patients with [triglyceride] levels between 1.5 and 5.6 mmol/L (135–499 mg/dl) despite statin treatment, n-3 polyunsaturated fatty acids (icosapent ethyl 2 × 2 g/day) should be considered with a statin" (79, p. 148). A more recent statement from the American Diabetes Association (80) broadens this by indicating that, together with standard medical care, EPA as icosapent ethyl at a dose of 4 g/day is considered for patients with hypertriglyceridemia alone or with diabetes, atherosclerotic CVD, and other cardiac risk factors to reduce cardiovascular risk. 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" (81, p. 866). 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. (82, p. e880)

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 (83). Lipid peroxides are chemically different from unoxidized molecules and therefore may have distinct and diverse biological effects (84). 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 (85). 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 (86). 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 (83, 84). 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 (87). One RCT investigated the effect of oxidized compared with nonoxidized fish oil on lipid peroxidation and antioxidant activity markers (88). 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 the lessened efficacy of oxidized compared with unoxidized oils (84). To date, specific clinical trials addressing the effects of oxidation on the efficacy of long-chain omega-3 fatty acids are not sufficient.

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) (89, 90). In addition to their incorporation into platelet membranes, 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 91 and references therein). Modulation of platelet function depends on the dose of EPA and DHA and occurs mainly at doses greater than 2 g/day (92). The effects appear to be primarily mediated by the action of EPA (93). 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 study 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 catheterization) (94). 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 Fish Oil and Perioperative Bleeding (OPERA) trial, 1,516 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/day in the postoperative period, or placebo (95). 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); the absolute risk difference was 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 (95). The longitudinal Multi-Ethnic Study of Atherosclerosis (MESA) recruited 6,814 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 (96). An inverse association in incident major bleeding events was reported with higher baseline plasma levels of EPA (hazard ratio 0.69; 95% CI: 0.53, 0.90; $p = 0.01$) and EPA+DHA (hazard ratio 0.78; 95% CI: 0.65, 0.94; $p = 0.01$) but not DHA (hazard ratio 0.68; 95% CI: 0.44, 1.05; $p = 0.08$) over a median of 14 years of follow-up. A publication from 2018 (97) 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 (1.5–10.2 g/day) for a variety of durations (8 days to 52 weeks). 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/day EPA for 4–10 weeks prior to surgery for prostate cancer, on perioperative bleeding (98). 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 (99). In the previously mentioned VITAL trial, participants without CVD, cancer, or AF at baseline who were randomly assigned to receive 840 mg/day omega-3 PUFAs (460 mg EPA and 380 mg DHA) or olive oil as placebo were studied (100). 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 an 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% versus 1.3%; $p < 0.001$) (67). In REDUCE-IT, patients who were randomized to receive almost 4 g/day 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% versus 3.9%; $p = 0.003$) (64). An intermediate dose of approximately 1.6 g/day omega-3 PUFAs (930 mg EPA and 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 (101, 102). A meta-analysis of five RCTs (REDUCE-IT, ASCEND, Risk and Prevention, STRENGTH, OMEMI) found an 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; $p < 0.01$) (101). When findings from VITAL Rhythm (100) were included, the results were unchanged (incident risk ratio 1.29; 95% CI: 1.13, 1.48; $p = 0.0002$) (101). The meta-analysis of Gencer et al. (102) included seven RCTs published between 2012 and 2020. Of the 81,210 patients, 72.6% were enrolled in trials testing ≤1 g of long-chain omega-3 PUFAs per day and 27.4% in trials testing >1 g of long-chain omega-3 PUFAs per 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/day (hazard ratio 1.49; 95% CI: 1.04, 2.15; $p = 0.042$) compared with those testing ≤1 g/day (hazard ratio 1.12; 95% CI: 1.03, 1.22; $p = 0.024$; p for interaction < 0.001). In a 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 (103). This is obviously a concern, although it is interesting to note that REDUCE-IT, while reporting an increased risk of AF, also reported a reduced risk of a range of hard cardiovascular outcomes.

7. SUMMARY, DISCUSSION, AND 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 risks 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 known risk factors for CVD such as blood lipids, blood pressure, heart rate and heart rate variability, platelet aggregation, endothelial function, and inflammation. Despite this, evidence for primary prevention

of CVD from RCTs is relatively weak. Data from RCTs of EPA+DHA (or EPA alone) in high-risk patients, especially in the secondary prevention setting (e.g., post-MI), are inconsistent. Older (i.e., pre-2010) RCTs and meta-analyses based mainly on those RCTs suggested a significant benefit of long-chain omega-3 PUFAs on hard cardiovascular outcomes. RCTs conducted since 2010 have not consistently confirmed this benefit. Two recent large trials with high doses of long-chain omega-3 PUFAs (REDUCE-IT and STRENGTH) had different findings, with REDUCE-IT showing significant benefits from EPA-ethyl ester and STRENGTH being stopped for futility. Nevertheless, recent meta-analyses confirm the benefit from long-chain omega-3 PUFAs (76–78) and that these benefits are dose dependent (76, 78). Reasons for inconsistencies among RCT findings may relate to the dose of long-chain omega-3 PUFAs used, the exact composition and formulation used, the duration of follow-up, and the event rate in the population being studied. Studies of short duration, using low doses of EPA+DHA, or being conducted against a background of multiple pharmaceutical interventions may be less likely to observe effects of long-chain omega-3 PUFAs. Another point to consider is that, unlike for pharmaceuticals, all individuals have some intake of long-chain omega-3 PUFAs and/or synthesize at least some EPA and DHA endogenously, meaning that the EPA and DHA naive state cannot occur, although background intake can be very low. Another point to consider is that the bioavailability of EPA and DHA can vary among individuals (104), with physiological differences and the timing of consumption of supplements in relation to meal intake being important. Despite the inconsistencies in the literature, there are recommendations supporting the use of long-chain omega-3 PUFAs to treat hypertriglyceridemia (41, 79) and patients with CVD (82). Identifying more clearly the dose-dependent effects of EPA and DHA, separately and together, on cardiovascular risk factors and on clinical outcomes is important to further develop long-chain omega-3 PUFAs as effective therapeutic agents for CVD. Furthermore, robust primary prevention trials are still needed.

It is important that the long-chain omega-3 PUFA preparations (e.g., softgel capsules) be protected from oxidation, which may (*a*) reduce the amount of active long-chain omega-3 PUFAs present and (*b*) produce peroxidation products that are harmful. There has been some concern that long-chain omega-3 PUFAs might increase bleeding. However, recent reports indicate that this is not an issue at the doses of EPA and DHA that are commonly used in trials or therapeutically. 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 risk has been seen in several RCTs and becomes clear through meta-analysis (101, 102). This effect is obviously concerning, although REDUCE-IT, while reporting an increased risk of AF, also reported the reduced risk of a range of hard cardiovascular outcomes. The mechanism by which high-dose long-chain omega-3 PUFAs increase the 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.

DISCLOSURE STATEMENT

P.C.C. has received research funding from BASF AS and has acted as a consultant/advisor to BASF AS, Smartfish, DSM, Cargill, Bunge, and Fresenius Kabi. I.D. is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

AUTHOR CONTRIBUTIONS

P.C.C. conceptualized, reviewed, and edited this review. I.D. drafted, reviewed, and edited. Both authors read and agreed to the final version of the manuscript.

LITERATURE CITED

1. Brenner RR. 2003. Hormonal modulation of Δ6 and Δ5 desaturases: case of diabetes. *Prostaglandins Leukot. Essent. Fatty Acids* 68:151–62
2. Childs CE. 2020. Sex hormones and *n*-3 fatty acid metabolism. *Proc. Nutr. Soc.* 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. *J. Trace Elements Med. Biol.* 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. *Annu. Rev. Nutr.* 39:21–44
5. Arterburn LM, Hall EB, Oken H. 2006. Distribution, interconversion, and dose response of n−3 fatty acids in humans. *Am. J. Clin. Nutr.* 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 Lipid Res.* 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. *Am. J. Clin. Nutr.* 110:823–31
8. Calder PC. 2018. Very long-chain *n*-3 fatty acids and human health: fact, fiction and the future. *Proc. Nutr. Soc.* 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. *Am. J. Clin. Nutr.* 96:748–58
10. Stark KD, Van Elswyk ME, Higgins MR, Weatherford CA, Salem N Jr. 2016. Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults. *Progress Lipid Res.* 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 N Jr., Eggersdorfer M. 2015. Is the world supply of omega-3 fatty acids adequate for optimal human nutrition? *Curr. Opin. Clin. Nutr. Metab. 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. *Environ. Int.* 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. *Int. J. Mol. Sci.* 21:1362
17. Calder PC. 2004. *n*–3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clin. Sci.* 107:1–11
18. De Caterina R. 2011. n–3 Fatty acids in cardiovascular disease. *N. Engl. J. Med.* 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. *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:e320–35
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. *J. Intern. Med.* 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. *Clin. Nutr.* 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. *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. *Ann. Intern. Med.* 160:398–406
28. Alexander DD, Miller PE, Van Elswyk ME, Kuratko CN, Bylsma LC. 2017. 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 Clin. Proc.* 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 Intern. Med.* 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* 262:51–54
31. Harris WS, Tintle NL, Imamura F, Qian F, Ardisson Korat AV, et al. 2021. Blood n-3 fatty acid levels and total and cause-specific mortality from 17 prospective studies. *Nat. Commun.* 12:2329
32. Sci. Advis. Comm. Nutr., Comm. Toxic. 2004. *Advice on fish consumption: benefits and risks*. Rep., TSO, London
33. ISSFAL (Int. Soc. Study Fat. Acids Lipids). 2004. *Recommendations for intake of polyunsaturated fatty acids in healthy adults*. Policy Statement, ISSFAL, Washington, DC
34. French Agency Food Environ. Occup. Health Saf. 2003. *The omega-3 fatty acids and the cardiovascular system: nutritional benefits and claims*. Rep., AFSSA, Maisons-Alfort, France
35. FAO (Food Agric. Organ. United Nations). 2010. *Fat and fatty acids in human nutrition: report of an expert consultation*. Rep., FAO, Rome
36. Harris W. 2001. Omega-3 long-chain PUFA and triglyceride lowering: minimum effective intakes. *Eur. Heart J. Suppl.* 3:D59–61
37. Innes JK, Calder PC. 2018. The differential effects of eicosapentaenoic acid and docosahexaenoic acid on cardiometabolic risk factors: a systematic review. *Int. J. Mol. Sci.* 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. *Am. J. Clin. Nutr.* 104:280–87
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. *Am. J. Clin. Nutr.* 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. *Am. J. Clin. Nutr.* 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:e673–91
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. *J. Intern. Med.* 259:247–58

43. Sniderman AD. 2005. Apolipoprotein B versus non-high-density lipoprotein cholesterol: and the winner is... *Circulation* 112:3366–67
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. *J. Hum. Nutr. Diet.* 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, X-l Zhou, 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
49. Wang Q, Liang X, Wang L, Lu X, Huang J, et al. 2012. Effect of omega-3 fatty acids supplementation on endothelial function: a meta-analysis of randomized controlled trials. *Atherosclerosis* 221:536–43
50. Xin W, Wei W, Li X. 2012. Effect of fish oil supplementation on fasting vascular endothelial function in humans: a meta-analysis of randomized controlled trials. *PLOS ONE* 7:e46028
51. Miller PE, Van Elswyk M, Alexander DD. 2014. Long-chain omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and blood pressure: a meta-analysis of randomized controlled trials. *Am. J. Hypertens.* 27:885–96
52. Hidayat K, Yang J, Zhang Z, Chen GC, Qin LQ, et al. 2018. Effect of omega-3 long-chain polyunsaturated fatty acid supplementation on heart rate: a meta-analysis of randomized controlled trials. *Eur. J. Clin. Nutr.* 72:805–17
53. Burr ML, Gilbert J, Holliday RA, Elwood P, Fehily A, et al. 1989. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet* 334:757–61
54. GISSI-Prevenzione Investig. 1999. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* 354:447–55
55. Marchioli R, Barzi F, Bomba E, Chieffo C, Di Gregorio D, et al. 2002. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. *Circulation* 105:1897–903
56. Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, et al. 2007. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet* 369:1090–98
57. Rauch B, Schiele R, Schneider S, Diller F, Victor N, et al. 2010. OMEGA, a randomized, placebo-controlled trial to test the effect of highly purified omega-3 fatty acids on top of modern guideline-adjusted therapy after myocardial infarction. *Circulation* 122:2152–59
58. Galan P, Kesse-Guyot E, Czernichow S, Briancon S, Blacher J, Hercberg S. 2010. Effects of B vitamins and omega 3 fatty acids on cardiovascular diseases: a randomised placebo controlled trial. *BMJ* 341:c6273
59. Kromhout D, Giltay EJ, Geleijnse JM. 2010. n-3 Fatty acids and cardiovascular events after myocardial infarction. *N. Engl. J. Med.* 363:2015–26
60. ORIGIN Trial Investig. 2012. n-3 Fatty acids and cardiovascular outcomes in patients with dysglycemia. *N. Engl. J. Med.* 367:309–18
61. Risk Prev. Study Collab. Group. 2013. n-3 Fatty acids in patients with multiple cardiovascular risk factors. *N. Engl. J. Med.* 368:1800–8
62. ASCEND Study Collab. Group. 2018. Effects of n-3 fatty acid supplements in diabetes mellitus. *N. Engl. J. Med.* 379:1540–50
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. *N. Engl. J. Med.* 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. *N. Engl. J. Med.* 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. *Eur. Heart J.* 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 versus 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. *Curr. Opin. Clin. Nutr. Metab. 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. *Am. J. Med.* 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. *Arch. Intern. Med.* 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. *Ann. Med.* 41:301–10
73. Marik PE, Varon J. 2009. Omega-3 dietary supplements and the risk of cardiovascular events: a systematic review. *Clin. Cardiol.* 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. *Circ. Cardiovasc. Q. 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 Syst. Rev.* 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 Clin. Proc.* 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. *J. Am. Heart Assoc.* 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* 107:150–58
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. *Eur. Heart J.* 41:111–88
80. American Diabetes Association. 2020. 10. Cardiovascular disease and risk management: *Standards of Medical Care in Diabetes—2020*. *Diabetes Care* 43(Suppl. 1):S111–34
81. 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. *J. Clin. Lipidol.* 13:860–72
82. 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:e867–84
83. Jairoun AA, Shahwan M, Zyoud SH. 2020. Fish oil supplements, oxidative status, and compliance behaviour: regulatory challenges and opportunities. *PLOS ONE* 15:e0244688
84. Albert BB, Cameron-Smith D, Hofman PL, Cutfield WS. 2013. Oxidation of marine omega-3 supplements and human health. *BioMed. Res. Int.* 2013:464921
85. Shahidi F, Zhong Y. 2010. Lipid oxidation and improving the oxidative stability. *Chem. Soc. Rev.* 39:4067–79
86. GOED (Glob. Organ. EPA DHA Omega-3). 2019. *GOED voluntary monograph*, *version* 7. GOED, Salt Lake City, UT

87. EFSA Panel Biol. Hazards (BIOHAZ). 2010. Scientific opinion on fish oil for human consumption. Food hygiene, including rancidity. *EFSA J.* 8:1874
88. 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. *Br. J. Nutr.* 108:315–26
89. 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* 58:880–85
90. 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. *J. Clin. Investig.* 76:1626–31
91. 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
92. 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
93. 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* 197:290–96
94. Harris WS. 2007. Expert opinion: omega-3 fatty acids and bleeding—cause for concern? *Am. J. Cardiol.* 99:S44–46
95. Akintoye E, Sethi P, Harris WS, Thompson PA, Marchioli R, et al. 2018. Fish oil and perioperative bleeding: insights from the OPERA randomized trial. *Circ. Cardiovasc. Q. Outcomes* 11:e004584
96. 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. *J. Am. Heart Assoc.* 10:e021431
97. 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. *Clin. Nutr.* 37:948–57
98. 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. *Clin. Nutr. ESPEN* 47:221–26
99. Curfman G. 2021. Omega-3 fatty acids and atrial fibrillation. *JAMA* 325:1063
100. 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
101. 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. *Eur. Heart J. Cardiovasc. Pharmacother.* 7:e69–70
102. 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
103. Fatkin D, Cox CD, Martinac B. 2022. Fishing for links between omega-3 fatty acids and atrial fibrillation. *Circulation* 145:1037–39
104. Schuchardt JP, Hahn A. 2013. Bioavailability of long-chain omega-3 fatty acids. *Prostaglandins Leukot. Essent. Fatty Acids* 89:1–8

Annual Review of Pharmacology and Toxicology

Volume 63, 2023

Contents

Indexes

Errata

An online log of corrections to *Annual Review of Pharmacology and Toxicology* articles may be found at http://www.annualreviews.org/errata/pharmtox

Related Articles

From the ***Annual Review of Biochemistry***, Volume 91 (2022)

Better, Faster, Cheaper: Recent Advances in Cryo–Electron Microscopy
Eugene Y.D. Chua, Joshua H. Mendez, Micah Rapp, Serban L. Ilca, Yong Zi Tan, Kashyap Maruthi, Huihui Kuang, Christina M. Zimanyi, Anchi Cheng, Edward T. Eng, Alex J. Noble, Clinton S. Potter, and Bridget Carragher

Validating Small Molecule Chemical Probes for Biological Discovery
Victoria Vu, Magdalena M. Szewczyk, David Y. Nie, Cheryl H. Arrowsmith, and Dalia Barsyte-Lovejoy

Driving E3 Ligase Substrate Specificity for Targeted Protein Degradation: Lessons from Nature and the Laboratory
Angus D. Cowan and Alessio Ciulli

MAPK-Activated Protein Kinases: Servant or Partner?
Natalia Ronkina and Matthias Gaestel

The Wnt Pathway: From Signaling Mechanisms to Synthetic Modulators
Ellen Youngsoo Rim, Hans Clevers, and Roel Nusse

Sensory TRP Channels in Three Dimensions
Melinda M. Diver, John V. Lin King, David Julius, and Yifan Cheng

From the ***Annual Review of Cancer Biology***, Volume 6 (2022)

Targeting Solid Tumors with Bispecific T Cell Engager Immune Therapy
Tara Arvedson, Julie M. Bailis, Carolyn D. Britten, Matthias Klinger, Dirk Nagorsen, Angela Coxon, Jackson G. Egen, and Flavius Martin

Tracing and Targeting the Origins of Childhood Cancer
Tim H.H. Coorens and Sam Behjati

Targeting KRAS G12C with Covalent Inhibitors
Jonathan M.L. Ostrem and Kevan M. Shokat

Central Role of the Antigen-Presentation and Interferon-γ Pathways in Resistance to Immune Checkpoint Blockade
Annette Paschen, Ignacio Melero, and Antoni Ribas

TGFβ: Signaling Blockade for Cancer Immunotherapy
Szu-Ying Chen, Ons Mamai, and Rosemary J. Akhurst

Development of Tissue-Agnostic Treatments for Patients with Cancer
Steven Lemery, Lola Fashoyin-Aje, Leigh Marcus, Sandra Casak, Julie Schneider, Marc Theoret, Paul Kluetz, Richard Pazdur, and Julia A. Beaver

RB1, Cancer Lineage Plasticity, and Therapeutic Resistance
Letian Zhang and David W. Goodrich

Targeting BET Bromodomains in Cancer
Patrick Trojer

From the ***Annual Review of Genetics***, Volume 56 (2022)

Genome Maintenance in Mammalian Stem Cells
John C. Schimenti, Rui Huang, Liangdao Li, and Ryan James

From the ***Annual Review of Immunology***, Volume 40 (2022)

Resistance Mechanisms to Anti-PD Cancer Immunotherapy
Matthew D. Vesely, Tianxiang Zhang, and Lieping Chen

IL-6 Revisited: From Rheumatoid Arthritis to CAR T Cell Therapy and COVID-19
Tadamitsu Kishimoto and Sujin Kang

Human Antibodies for Viral Infections
James E. Crowe Jr.

From the ***Annual Review of Medicine***, Volume 73 (2022)

SARS-CoV-2 Neutralizing Antibodies for COVID-19 Prevention and Treatment
Dapeng Li, Gregory D. Sempowski, Kevin O. Saunders, Priyamvada Acharya, and Barton F. Haynes

mRNA Vaccines in the COVID-19 Pandemic and Beyond
Michael J. Hogan and Norbert Pardi

COVID-19 Vaccines: Adenoviral Vectors
Catherine Jacob-Dolan and Dan H. Barouch

Whole Inactivated Virus and Protein-Based COVID-19 Vaccines
Peter J. Hotez and Maria Elena Bottazzi

COVID-19 Critical Illness: A Data-Driven Review
Jennifer C. Ginestra, Oscar J.L. Mitchell, George L. Anesi, and Jason D. Christie

Diagnosis and Treatment of *Helicobacter pylori* Infection
Yi-Chia Lee, Maria Pina Dore, and David Y. Graham

Phage Therapy for Antibiotic-Resistant Bacterial Infections
Graham F. Hatfull, Rebekah M. Dedrick, and Robert T. Schooley

Progress in the Management of Pancreatic Neuroendocrine Tumors
Amy Chang, Scott K. Sherman, James R. Howe, and Vaibhav Sahai

A Tale of Two Checkpoints: ATR Inhibition and PD-(L)1 Blockade
Natalie Y.L. Ngoi, Guang Peng, and Timothy A. Yap

Hypoxia Reduction Sensitizes Refractory Cancers to Immunotherapy
Priyamvada Jayaprakash, Paolo Dario Angelo Vignali, Greg M. Delgoffe, and Michael A. Curran

Hepatocellular Carcinoma Immunotherapy
Rubens Copia Sperandio, Roberto Carmagnani Pestana, Beatriz Viesser Miyamura, and Ahmed O. Kaseb

New Approaches to Glioblastoma
Mustafa Khasraw, Yoko Fujita, Catalina Lee-Chang, Irina V. Balyasnikova, Hinda Najem, and Amy B. Heimberger

Heart Failure with Preserved Ejection Fraction: Mechanisms and Treatment Strategies
Kazunori Omote, Frederik H. Verbrugge, and Barry A. Borlaug

Hypertrophic Cardiomyopathy: New Concepts and Therapies
Barry J. Maron, Ethan J. Rowin, and Martin S. Maron

Cardiovascular Effects of Particulate Air Pollution
Aruni Bhatnagar

Treatment of Delirium During Critical Illness
Niall T. Prendergast, Perry J. Tiberio, and Timothy D. Girard

Contemporary Management of Thyroid Nodules
Kristen Kobaly, Caroline S. Kim, and Susan J. Mandel

From the ***Annual Review of Neuroscience***, Volume 45 (2022)

Signaling Pathways in Neurovascular Development
Amir Rattner, Yanshu Wang, and Jeremy Nathans

Fluorescence Imaging of Neural Activity, Neurochemical Dynamics, and Drug-Specific Receptor Conformation with Genetically Encoded Sensors
Chunyang Dong, Yu Zheng, Kiran Long-Iyer, Emily C. Wright, Yulong Li, and Lin Tian

Synaptic Mechanisms Regulating Mood State Transitions in Depression
Puja K. Parekh, Shane B. Johnson, and Conor Liston

From the ***Annual Review of Pathology: Mechanisms of Disease***, Volume 17 (2022)

Cutaneous Squamous Cell Carcinoma:The Frontier of Cancer Immunoprevention
Michael S. Chang, Marjan Azin, and Shadmehr Demehri

A Balancing Act: p53 Activity from Tumor Suppression to Pathology and Therapeutic Implications
Mengxiong Wang and Laura D. Attardi

Polycystic Liver Disease: Advances in Understanding and Treatment
Tatyana V. Masyuk, Antoliy I. Masyuk, and Nicholas F. LaRusso

Precision Medicine in Low- and Middle-Income Countries
Jerald P. Radich, Edward Briercheck, Daniel T. Chiu, Manoj P. Menon, Olga Sala Torra, Cecilia C.S. Yeung, and Edus H. Warren

Innate Immunity and Cancer Pathophysiology
Laura Maiorino, Juliane Daßler-Plenker, Lijuan Sun, and Mikala Egeblad

Lysophospholipid Mediators in Health and Disease
Kuniyuki Kano, Junken Aoki, and Timothy Hla

Engineering β Cell Replacement Therapies for Type 1 Diabetes: Biomaterial Advances and Considerations for Macroscale Constructs
Michelle J. Quizon and Andrés J. García

From the ***Annual Review of Physiology***, Volume 84 (2022)

β-Adrenergic Receptors and Adipose Tissue Metabolism: Evolution of an Old Story
Sheila Collins

β-Arrestins as Important Regulators of Glucose and Energy Homeostasis
Sai P. Pydi, Luiz F. Barella, Lu Zhu, Jaroslawna Meister, Mario Rossi, and Jürgen Wess

Running the Female Power Grid Across Lifespan Through Brain Estrogen Signaling
Holly A. Ingraham, Candace B. Herber, and William C. Krause

Alcohol-Associated Tissue Injury: Current Views on Pathophysiological Mechanisms
Liz Simon, Flavia M. Souza-Smith, and Patricia E. Molina

Adrenergic Regulation of Calcium Channels in the Heart
Arianne Papa, Jared Kushner, and Steven O. Marx

The Diverse Physiological Functions of Mechanically Activated Ion Channels in Mammals
Kate Poole

Mitochondria and Inflammatory Bowel Diseases: Toward a Stratified Therapeutic Intervention
Gwo-tzer Ho and Arianne L. Theiss

Roles of Mineralocorticoid Receptors in Cardiovascular and Cardiorenal Diseases
Jonatan Barrera-Chimal, Benjamin Bonnard, and Frederic Jaisser

Vaping and Lung Inflammation and Injury
Jin-Ah Park, Laura E. Crotty Alexander, and David C. Christiani