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REVIEW ARTICLE

Omega-3 Fatty Acid Therapy: A Review of Study Design Flaws, Quality, and Composition

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ABSTRACT

There is marked heterogeneity in the clinical response to omega-3 fatty acid therapy with many authors documenting futility in large-scale trials, secondary re-analysis, and meta-analysis. The question of failure in the context of omega-3 therapy is multifactorial and complicated by the observation that fish intake has been broadly linked to significant risk reductions across a range of conditions. The question that remains is how can we resolve the discrepancy between pre-clinical evidence and epidemiology, which dually emphasize the benefit of omega-3 therapy against the limited success of large-scale clinical trials and smaller scale clinical studies that do not consistently report benefit and may even report harm, especially as it pertains to atrial fibrillation. We present three primary considerations that may clarify the supposed failures of omega-3 therapy: 1) correction for omega-3:omega-6 ratio and competition, 2) variation in the fatty acid composition and quality of omega-3 products, and 3) fundamental concerns pertaining to the omega-3 vehicle and its impact on omega-3 metabolism. While the predominant source of omega-3 therapy is supplements, they are typically not regulated prior to market like drugs and have significant variability in fatty acid composition, vehicle, oxidation, and quality control. Further, the individual response to omega-3 therapy is likely variable and dependent on ambient dietary conditions and inherited differences in endogenous desaturase activity, which has infrequently been accounted for in large studies. The net effect of these concerns should engender pharmaceutical and consumer companies alike to consider 1) refining trial design and 2) consider the role of oxidation in the failure of omega-3 products.

Introduction

Large scale clinical trials of omega-3 Fatty Acid (ω-3 FA) therapies have achieved limited success, with the most recent trials documenting null or uncertain benefit ω-3 FA therapy across a range of indications, including but not limited to coronary heart disease, composite measures of primary cardiovascular events, and secondary revascularization. Secondary re-analysis of both STRENGTH and REDUCE-IT reported limited, if modest benefit[1-5]. Similarly, prominent meta-analyses have demonstrated conflicting findings, with a general trend of modest to neutral benefit reportedly associated with ω-3 FA therapy[6-10]. More striking is the relatively recent association linking dose-dependent intake of ω-3 FAs, particularly eicosapentaenoic acid (EPA) monotherapy, with coincident arrhythmia such as atrial fibrillation (AFib) – the net effect of these findings has been a gradual waning of confidence in ω-3 FA therapy as an adjunct in the prevention and treatment of age-related disease, including atherosclerotic cardiovascular disease (ASCVD)[11-15].

The question of failure in the context of ω-3 FA is multifactorial and complicated by the observation that fish intake has been broadly linked to significant risk reductions across a range of conditions, including ASCVD[16,17]. Moreover, serum and phospholipid ω-3 concentrations are associated with significant risk reductions in major adverse cardiovascular events, coronary heart disease risk and secondary revascularization[18-22]. In vitro evidence also continues to endorse the anti-thrombotic, atheroprotective, and pro-resolving effects of ω-3 FAs and their metabolites[23-26]. Thus, there exists a discrepancy in the theoretical, pre-clinical, and epidemiologic/historic benefit of ω-3 FA therapy and its actual success in the prevention and treatment of ASCVD and associated conditions in well-controlled, large-scale trials. We argue that this discrepancy may be better understood as a failure to control for baseline Omega-6:ω-3 (ω-6:ω-3) ratio, baseline serum ω-3 and related threshold effects, the vehicle and its propensity towards oxidation, and heterogeneity in ω-3 supplement quality and composition.

Omega-3:Omega-6 Ratios and the Composition Competition

The ramifications of ω-6 fatty acids (ω-6 FA), and their elevated intake in the modern era, have been extensively debated in the literature with multiple authors documenting mixed effects in meta-analysis, while several well-controlled clinical studies have shown clear, inverse, protective associations in those boasting the highest biological concentrations of certain ω-6 fats, namely linolenic acid (LA)[26-29]. The biological implications of ω-6:ω-3 ratio, however, are less contested with those boasting the highest ratio (or the highest serum concentrations of ω-6 versus ω-3) at significantly greater risk for a raft of conditions, including cardiovascular disease (CVD)[30-32].

The biological underpinnings for this relationship lie in the direct "competition" between ω-6 and ω-3 for incorporation into phospholipid membranes and the divergent cyclo-oxygenase (COX) and lipo-oxygenase (LOX) dependent pathways, giving rise to the crude understanding of ω-3 FA as "anti-inflammatory" and ω-6 as "pro-

inflammatory." Yet, in multiple trials evaluating supplemental ω-6 intake, there is a marked failure to show consistent elevations in inflammatory species nor that its biological substrates, such as arachidonic acid (AA), are elevated even by increasing intake of its direct precursors, including LA. Of note, even when AA has been directly administered, there are inconsistent changes in the inflammatory status of participants, seeming to contradict the simplistic assumption that ω-6 necessarily generates inflammatory states[22,24]. As argued by Harris, this framework, while perhaps conceptually useful, is deceptive, with many ω-6 metabolites actually performing anti-inflammatory effects and pro-resolution functions[35]. Further, the immune system is not a simple go or no-go inflammation signaling pathway. A myriad of inputs and outputs are weighed before a response (or no response) is triggered, meaning simply elevating AA may not be sufficient to stimulate an inflammatory immune response. Thus, it cannot be confidently asserted that ω-6 intakes are flatly contraindicated.

Yet, ω-6 status and its relationship to ω-3 status is complex. Both in vitro and in vivo evidence have demonstrated the ability of LA to blunt the conversion of α-linolenic acid (ALA) to EPA. While the conversion of ALA to EPA has been shown to be limited (≤ 10%), it may be important for some individuals (i.e. with limited seafood intake) and at certain times when EPA demand is greatest, potentially including obesity, making the blunting of conversion by LA problematic for some[36-39]. Further, LA directly competes for incorporation into cell membranes, thus actively reducing EPA and docosahexaenoic acid (DHA) tissue concentration. Blasbalg et al. elaborate on this effect noting competition with endogenous desaturase activity coupled with sn2 dependent competition for incorporation into phospholipid membranes leads to a significant reduction in ω-3 FA with the introduction of ω-6 polyunsaturated fatty acids (PUFA)[40]. Indeed, studies in which ω-6 FA enrichment is plotted against ω-3 FA, such as the Los Angeles Veterans Study, find that as LA increases, ω-3 may markedly decline[41,42]. In infants fed high LA formulas, authors noted significant declines in serum ω-3 FA[43]. That ω-6 intake, therefore, may mediate ω-3 status is a plausible concern that warrants further exploration.

Similarly, certain genetic variants, such as fatty acid desaturases (FADS1 and 2), have been reportedly associated with significantly limited EPA and DHA concentrations and increased conversion of highly unsaturated fats like ω-6 to AA, such as LA[44-47]. As Chilton et al. notes, the effects of FADS cannot be disregarded owing to the profound pre-clinical and clinical data supporting the role of FADS in mediating the effect of ω-3 and ω-6 intake and susceptibility to CVD, with the latter intake corresponding to greater serum AA and pro-inflammatory species. It is perhaps of interest to note that FADS1 represents an ancestral variant hypothesized to increase endogenous production of highly unsaturated fatty acids including AA. As humans migrated away from coastal areas rich in ω-3, the FADS allele would likely become necessary to support the necessary role of ω-6 related FA in facilitating various biological functions. Thus, certain populations, such as African Americans, Native, and Hispanics, retain a highly efficient LA to AA converting variant[47]. In these populations, low intakes of

ω-3 FA containing sources in combination with significantly enriched dietary consumption of ω-6 FA represents a plausibly atherogenic risk profile. Accordingly, it is both predictable and fascinating to note that even within weakly successful trials of ω-3, such as the VITAL Trial, African American populations appeared to derive outsized benefit from ω-3 FA therapy. In these populations, frank ω-3 deficiency and enhanced ω-6 intake may be more likely to demonstrate adverse effects and may be contributing to health disparities in these populations.

The implications of these findings are that the individual response to ω-3 therapy is likely variable and dependent on ambient dietary conditions and inherited differences in endogenous desaturase activity. This is common in nutrition, which is why organizations such as the National Institutes of Health are focusing research efforts on personalized and precision nutrition efforts. However, this is still a long way away from implementation in clinical practice.

Threshold Dependent Effects, Baseline ω-3 FA Intake, Concomitant Medication Use

Another question which emerges in re-analysis of large-scale studies is of baseline ω-3 FA status. It is of note that there may exist threshold, or dose-dependent, effects for ω-3 FA whereby low plasma concentrations and incorporated EPA + DHA content significantly predispose subjects to greater cardiovascular risk, while plasma and RBC phospholipid content above this threshold are protective[7,48-50]. Thus, baseline ω-3 concentrations and the degree to which supplementation may alter the ω-3 Index (the EPA + DHA content of erythrocytes as a percent of total FAs) is a factor that warrants examination. In fact, the ω-3 Index has also been linked to type 2 diabetes, and again with heterogeneity[51]. A recent scoping review of the effect of dietary and supplemental ω-3 intake on the ω-3 Index found that doses > 1 g/day for at least 12 weeks were necessary to show improvement in the ω-3 Index with triglyceride supplements having greater bioavailability and therefore efficacy.

Neither VITAL, nor ASCEND reported serum baseline ω-3 FA, nor did they track changes in phospholipid ω-3 FA from baseline to the end of the trial period. More importantly, both trials utilized relatively modest doses ≤1 g/day of minimally bioavailable ethyl ester – doses that are unlikely to generate plasma ω-3 FA or ω-3 Index RBC membrane phospholipid values in excess of 8% or "sufficiency". That subjects in the highest tertiles of achieved ω-3 FA derive the greatest cardioprotective benefit from ω-3 FA has been repeatedly demonstrated[48,52-55]. Moreover, certain genetic variants, including FADS1 and 2 and dietary patterns related to ω-6 consumption, as evidenced in the Sydney Heart Study, necessitate caution in interpreting the results of trials without reported baseline ω-3 and change in ω-3 FA status at the end of the intervention.

Yet, while such parameters were met in OMEMI and STRENGTH, there was an absence of benefit in each trial even when stratifying by achieved ω-3 FA status. The explanations for this may be complex and likely owe to individual errors in trial design and interpretation. For example, in the STRENGTH Trial, the mean ω-3 Index was

5.6% (5% RBC DHA) prior to therapy. A 5.6% ω-3 Index is not considered a "frank" deficiency and may actually be 25% higher than the average ω-3 Index in the United States (US)[56]. In fact, to achieve an ω-3 Index of 5.6%, most of it as DHA, it is likely that patients consume fatty fish in near recommended doses and, therefore, may be unlikely to derive substantial benefit from ω-3 FA therapy.

Similarly, while those patients in OMEMI were instructed to curtail their ω-3 intake beyond a child sized spoon of cod liver oil, they likely consumed a classically rich ω-3 diet prior to the start of therapy. In fact, baseline DHA ω-3 FA was > 5% with EPA at 1-2%, corresponding to a median ω-3 Index in excess of 6-7% or approaching optimality. Thus, the baseline participant in the OMEMI Trial was already replete with ω-3 FA (not by any standard considered deficient) and, therefore, unlikely to benefit from supplemental ω-3. It should also be noted that the OMEMI Trial was specifically designed to test the effect of ω-3 FA in patients 2-8 weeks post myocardial infarction (MI). This is an extremely vulnerable period that should not be considered representative of the ideal initiation of ω-3 FA therapy. As Nicolantonio and O'Keefe argue, much of the remodeling post MI likely took place well before the initiation of therapy[57]. Moreover, such patients are at greater risk of new-onset arrhythmia and therefore putatively weak arrhythmogenic therapies like ω-3 FA appear as a suboptimal adjunctive therapy.

ω-3 FA are fundamentally adjunctive in the treatment of ASCVD – not primary. The percentage of patients in all 5 trials – VITAL, STRENGTH, OMEMI, REDUCE-IT, ASCEND – treated with 1 or more primary lipid-lowering therapies, blood-pressure lowering agents, or anti-coagulants approached >85% or 100% in the case of REDUCE-IT and STRENGTH. Each of these agents alone, and particularly in combination, exceed the primary benefit of therapy with ω-3 FA with respect to CVD risk reduction. Therefore, the incremental benefit associated with ω-3 FA therapy may not be detectable in such populations. As Elagizi 2018 et al. argue, these findings may parsimoniously explain the relative success of early trials including GISSI-P and JELIS – both of which boasted fewer participants on one or more lipid-lowering or blood pressure-lowering therapies[58].

Supplement Quality and Heterogeneity

ω-3 FA supplementation is among the most prominent forms of intake. Among supplement-users, nearly 50% report having routinely consumed ω-3 FA products. However, supplements are not regulated prior to going to market like drugs in the domestic US; instead, the US Food and Drug Administration (FDA) relies heavily on reactive regulatory actions such as recalls. While the FDA has requirements for safety, labeling, health-related claims, and manufacturing these are not actively monitored prior to sale like they are with drugs. Accordingly, there exists broad heterogeneity in supplement quality and composition.

While modern ω-3 FA supplements are more likely to contain higher concentrations of ω-3, there still exists significant variability in minor and major fatty acid composition and oxidative quality with several authors documenting near equal concentrations of

atherogenic saturated FAs such as palmitic (16:0) and myristic acid (14:0) to ω-3 FA in consumer ω-3 FA supplements. Furthermore, the percentage of supplements that actually meet their stated ω-3 claim appears to be a slim minority, with reports that < 10% of manufacturers actually adhered to their stated ω-3 FA in a New Zealand study[59]. Consonantly, our lab recently reported the results of a multi-year rancidity analysis of 72 popular US ω-3 FA products finding that more than 50% exceeded GOED thresholds for total oxidation, and specific sub-parameters, such as peroxide and para-anisidine[60]. To reiterate, while modern analyses suggest that compliance with stated label claim has improved, concentration still remains relatively poor and few are comparable with pharmaceutical ω-3 FA formulations such as Lovaza, which demonstrate 80-90% ω-3 FA Importantly, Lovaza is regulated as a drug by the FDA[61].

The biological ramifications should be carefully considered when trying to account for inconsistencies in benefit attributable to supplementary ω-3 FA. For example, the finding that low-density lipoprotein (LDL) enhancing, atherogenic, saturated FAs are present in significant concentrations may undermine the benefit attributable to ω-3 FA and should, thus, warrant caution with routine consumption. Saturated FAs, especially palmitic and myristic acid, can promote an inflammatory character in myocytes and upregulate synthesis of ApoB, ceramides, branched chain fatty acids (BCFAs), and total cholesterol. The net effect of these findings may explain epidemiologic research that has consistently documented increasing atherogenic risk associated with increasing circulating saturated FA, which is reduced by replacing saturated FAs with PUFA and MUFA[62-67].

Oxidation of ω-3 FA is a similar confounder with significant implications. As ω-3 FAs are oxidized to their primary product, hydroperoxides, and secondary aldehydes, their biological character is changed such that their consumption may be inadvisable. Multiple authors have demonstrated pre-clinical evidence of harm associated with the consumption of oxidized ω-3 FA; though, this effect is contested[68-70]. Oxidized ω-3 FA metabolites have been repeatedly documented in pre-clinical models to exhibit pro-thrombotic, atherosclerotic, genotoxic, and cytotoxic properties that may accelerate underlying ASCVD, retinal deterioration, and prostatic toxicity[71-72]. What complicates this fact is that the main agent implicated in oxidative stress derived from oxidized ω-3 FA consumption, 4-Hydroxy-2-Hexenal (4-HHE), exhibits complex and even protective properties[73]. Intriguingly, Nakagawa et al. argue that this discrepancy is explained by the protective Nrf2 induction of heme-oxygenase HO-1 predominantly by DHA, which can result in protective benefits in a dose-dependent fashion. As DHA dose escalates, a hormetic like mechanism reaches a saturation point whereby it becomes pro-oxidant, and cytotoxic to tissues, especially the heart. Such provocative pre-clinical findings have impressive implications for large scale trials of DHA containing oils, like STRENGTH, which reported null or adverse secondary outcomes such as AFib.

In humans, the ingestion of oxidized ω-3 FA is less clear, with several authors reporting contradictory results. Further, the long-term effects of this consumption are even less

clear[70]. As native LDL is not atherogenic, the question of oxidation susceptibility is increasingly important. Several authors, particularly Turner et al., have documented that the dietary intake of oxidized fatty acids in human subjects does indeed lead to greater susceptibility of LDL to oxidation and, perhaps more striking, that oxidized cholesterols, in this case derived from soybean oil, consumed in the course of the diet were incorporated into chylomicrons, posing greater atherogenic risk as demonstrated by enhanced macrophage uptake[74-76]. Similarly, reported changes in inflammatory markers and the function of antioxidant transcriptional regulation of high-density lipoprotein (HDL) associated paraoxonase function, an important enzyme in protection from LDL oxidation, has been demonstrated to be negatively altered in the course of oxidized fat intake[74]. More fascinating is the suggestion that diabetics – heavily included in trials like STRENGTH (and by definition ASCEND) – report lower ambient paraoxonase function and do not appear to see the same elevations in paraoxonase following oxidized fat intake likely owing to increased background oxidative stress[77-78]. The contribution of endogenous inflammatory settings, like type 2 diabetes, is therefore a potential confounder that may actually hinder the benefit of ω-3 FA therapy.

Importantly, the oxidation of ω-3 FA in pharmaceutical products receives little attention in the literature. There is in fact no clearly published data on the total oxidation value for any FDA-approved ω-3 FA product. One study indirectly reported the oxidative status of a pharmaceutical ω-3 FA reported that it fell well below TOTOX<26[79]. Yet, in a similar study by Rupp et al. reported peroxide for Omacor was 3.84, which would mean that TOTOX may exceeds 10; however, this cannot be ascertained because anisidine was not reported[80]. That being said, alkenals, which correlate significantly with para-anisidine value (p-AV), were reported and would suggest our postulation to be correct. Oxidative parameters should be included in the provision for manufacture of any pharmaceutical ω-3.

Vehicle and Oxidation

The oxidation of ω-3 FA may indeed be a factor in the failure of recent trials. However, oxidation, while typically attributed to exogenous manufacturing and manipulation, may also be a function of the vehicle delivered leading to endogenous oxidation.

Secondary analyses of ω-3 fatty acids as carboxylic acids (CA) and ethyl esters (EE) have been disappointing. The sole exception being REDUCE-IT, which was ultimately confounded by the mineral oil control. In short, mineral oil is potentially detrimental to outcomes of interest and may have artificially expanded the effect size between the intervention (EE) and the control (mineral oil) in REDUCE-IT, perhaps resulting in spurious relationships. Despite this, the FDA has maintained approval of icosapent ethyl (ethyl eicosapentaenoic acid, an EE) demonstrating that the benefit associated could not simply be a function of harm derived by the mineral oil control[81, 82].

It may be the case that the variability in response to EE and CA ω-3 FA, and the signals pertaining to its arrhythmogenic risk, are better explained by the vehicles and dose,

as opposed to a triglyceride or phospholipid vehicle.

Fish oils are naturally present as predominantly triglyceride and phospholipids, likely in the ratio of 60:40[83,84]. The production of ω-3 fatty acids involves primary esterification of the natural triglycerides to EEs during manufacturing. Absorption of EEs is reduced in comparison with triglycerides and phospholipids[85].

Absorption of EEs necessarily requires hydrolysis by pancreatic lipase where it is then emulsified into micelles and packaged as chylomicrons, which enter into circulation through the thoracic duct, the largest lymphatic vessel. As Yang reported the hydrolysis of EEs is 10-50-fold slower than natural triglyceride[86,87]. The residence time of EE containing preparations is therefore longer than that of triglyceride formulations, which do not have to undergo such lengthy processing. The net effect is that ω-3 FA derived from EEs necessarily spend more time in the acidic gastrointestinal environment than triglycerides or phospholipids and, thus, may be subjected to greater oxidation prior to eventual incorporation. Such oxidation also may expose subjects to elevated short chain fatty acids (SCFA) products, such as butyric acid / butyrate[87]. It is of note that while dyspepsia is understood as a common symptom of FA therapy, there are relatively few articles exploring its appearance in EE trials. Excess production of oxidized SCFA is an interesting theory that would be consonant with such observations.

While free-fatty acid preparations, such as CAs, do not have to undergo hydrolysis for absorption, they are far more unstable and thus more prone to oxidation[88]. Residence time in the gut, therefore, is more likely to expose CA-based vehicles to enhanced oxidative stress thereby generating a potentially greater number of oxidized metabolites. Gastric and pancreas lipases exert significantly pro-oxidant effects on ω-3 containing food sources. Indeed, in vitro studies have reported dramatic elevations in hydroperoxides and secondary aldehydes following the gastric and intestinal phases of digestion. Of interest, astaxanthin (a compound present in natural fatty fish sources like salmon and shellfish) may be protective against oxidation[89,90]. Thus, exposure of exogenous, free ω-3 FA preparations to the gastrointestinal acidic milieu may generate oxidized metabolites introducing potentially harmful species into circulation.

Prior to the incorporation of agents combating oxidation of ω-3 FAs, such as mixed tocopherols and rosemary extract, elevations in malondialdheyde (MDA), the final product of ω-3 membrane peroxidation associated with free-radical-induced damage, were reported; though, this finding may be incidental[91]. It is also noteworthy that preclinical evidence has demonstrated significant anti-arrhythmic potential associated with fish oil and that no such association between AFib and ω-3 has been revealed among those that consume fish/seafood[92-95]. Many authors, in fact, report the opposite in retrospective studies; though, differences in trial design certainly contribute to this discrepancy and limit our interpretation. Fish consumption has been demonstrated replicated and uncontested reductions in coronary heart disease, major adverse cardiovascular events, and all cause decline[96,97].

The implication is that refined fish oils presented in less "natural" vehicles, such as CAs and EEs, might be acting, in-part and at higher doses, to increase the residence time of ω-3 fatty acids in the gastrointestinal system in the case of EEs or subject free FAs to greater oxidation than under basal conditions. This is especially important without the benefit of coincident antioxidants present in natural food sources. This, thereby, sets the stage for the incorporation of oxidized and, thus, potentially diminished or adverse ω-3 FAs into various tissues, including myocytes and pacemaker cells. This presents a potential mechanism for some of the observed side effects including AFib.

Notable questions persist, however, including the relatively increased incidence of AFib and micro-episodic fibrillation, likely related to plasma EPA, in the OMEMI Trial in which 1.8 g/day of a highly purified triglyceride-based supplement was selected. In the absence of formal evidence, these questions remain hypothetical, but demand consideration due to strong mechanistic plausibility coupled with differing results from supplemental versus dietary ω-3 fatty acids.

EPA Monotherapy vs. EPA-DHA Combination Therapy

Despite EPA and DHA having distinct chemical structures and, therefore, almost certainly different functions in the body, formulations of EPA alone or combined with DHA have typically been treated as similar if not the same in the literature. However, there is a divide in the results of clinical trials using EPA with those using EPA and DHA in combination. Those using EPA (JELIS, REDUCE-IT, RESPECT-EPA) showing benefit and those using EPA-DHA (ASCEND, VITAL, STRENGTH, OMEMI) lacking in benefit, supporting the idea that EPA monotherapy may exhibit pronounced cardiovascular benefit. This was comprehensively covered by Ty E. Sweeney, Sean P. Gaine, and Erin D. Michos in 2022 and serves as another point of concern in research design in this area[1, 2, 3, 5, 98, 99, 100, 101].

Conclusion

We have argued that the failure of recent ω-3 FA trials and observational studies to demonstrate benefit owes to fundamental errors in correcting for serum ω-6:ω3 and baseline serum ω-3 as well as concerns pertaining to the composition and quality of consumer fish oil products and emerging concerns pertaining to the oxidation and vehicle-specific ω-3 preparation. The net effect of these concerns should engender pharmaceutical and consumer companies alike to consider 1) refining trial design and 2) consider the role of oxidation in the failure of ω-3 products.

Conflict of Interest:

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Table:

Trial	Sample Size	Dose (g/day)	Interven-tion	Enroll. Criteria	Outcome	Vehicle	Oxidation	Control	Year	Follow-up (Years)	Arrythmia	Fish Intake Control	Comment
ASCEND	15,480	0.84	EPA + DHA (Lovaza)	Men/women ≥40 years with a diagnosis of diabetes mellitus (any type) but not evidence of cardiovascular disease	Serious vascular events: 689 (8.9%) intervention vs. 712 (9.2%) control (rate ratio [RR], 0.97; 95% confidence interval [CI], 0.87-1.08; P=0.55) Serious vascular event or revascularization: 882 (11.4%) vs. 887 (11.5%), respectively (RR, 1.00; 95% CI, 0.91-1.09)	Ethyl Ester	Not stated	Olive Oil	2018		NA	No	<1 g/day fish oil acceptable
REDUCE-IT	8179	4	Icosapent Ethyl (EPA)	≥50 years with diabetes mellitus and 1+ addition-al risk factor, fasting triglyceride of 150-499 mg/ dL (1.69-5.63 mmol/L) and LDL of 41-100 mg/dL (1.06-2.59 mmol/ L) using a stable statin dose ≥4 weeks	Composite MACE: 17.2% intervention vs. 22.0% control (hazard ratio [HR], 0.75; 95% CI, 0.68-0.83; P<0.001) Additional ischemic end points (prespecified hierarchical schema) including cardiovascular (CVD) death: lower in intervention vs. control (4.3% vs. 5.2%; HR, 0.80; 95% CI, 0.66-0.98; P=0.03) Hospitalization (atrial fibrillation / flutter): more in intervention vs. control (3.1% vs. 2.1%, P=0.004)	Ethyl Ester	Not stated	Miner al oil	2019	4.9	Yes (Afib)	No	
STRENGTH	13,078	4	EPA + DHA	Statin-treated, hypertriglyceridem ia, low HDL-C, high CVD risk*	Trial prematurely halted (1384 primary end point event vs. 1600 planned events) based on interim analysis indicating low probability of clinical benefit	Carbo xylic Acid	Not stated	Corn oil	2020	3	Yes (Afib)	No	

Trial	Sample Size	Dose (g/day)	Interven-tion	Enroll. Criteria	Outcome	Vehicle	Oxidation	Control	Year	Follow-up (Years)	Arrythmia	Fish Intake Control	Comment
VITAL	25871	0.84	EPA + DHA (Lovaza)	Men ≥50 years, women ≥55 years in the United States	Major CVD events: 386 intervention vs. 419 control (HR, 0.92; 95% CI, 0.80-1.06; P=0.24). Invasive cancer: 820 intervention vs. 797 control (HR, 1.03; 95% CI, 0.93-1.13; P=0.56). Key Secondary End Points HRs: • Expanded composite CVD events, 0.93 (95% CI, 0.82-1.04) • Total myocardial infarction, 0.72 (95% CI, 0.59-0.90) • Total stroke, 1.04 (95% CI, 0.83-1.31) • CVD death, 0.96 (95% CI, 0.76 to 1.21) • Cancer death (n=341), 0.97 (95% CI, 0.79-1.20)	Ethyl Ester	Not stated	Vitamin D (2000 IU)	2019	5.3	No	Matched for 1 serving of dark meat, fatty fish per week	Significant benefit in African American subgroup
OMEMI	1014	1.8	EPA + DHA (Pikasol)	Daily to standard of care, 70-82 years with recent (2-8 weeks) AMI	Primary endpoints (composite): 108 (21.4%) intervention vs. 102 (20.0%) control (HR, 1.08 [95% CI, 0.82-1.41]; P=0.60). Secondary endpoint: 28 (7.2%) intervention vs. 15 (4.0%) control (1.84 [0.98-3.45]; P=0.06).	Triglyceride	Not stated	Corn oil	2020	2	Yes (Afib)	No	Placebo (56% linoleic acid, 32% oleic acid, 10% palmitic acid)

*High CVD risk was defined as 1) the presence of established atherosclerotic CVD involving the coronary, peripheral, carotid, or aortic territories (secondary prevention); 2) type 1/2 diabetes, ≥40 years for men and ≥50 years for women, 1+ additional risk factor including chronic smoking, hypertension, high-sensitivity C-reactive protein (hs-CRP) ≥2 mg/L, or moderately increased albuminuria; or 3) high-risk primary prevention patients ≥50 years for men or ≥60 years for women with 1+ additional risk factor, including a family history of premature coronary artery disease, chronic smoking, hs-CRP ≥2 mg/L, impaired kidney function, or coronary calcium score >300 Agatston units