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ω 3-Polyunsaturated Fatty Acids for Heart Failure: Effects of Dose on Efficacy and Novel Signaling through Free Fatty Acid Receptor 4

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Abstract

Heart failure (HF) affects 5.7 million in the U.S., and despite well-established pharmacologic therapy, the 5-year mortality rate remains near 50%. Furthermore, the mortality rate for HF has not declined in years, highlighting the need for new therapeutic options. Omega-3 polyunsaturated fatty acids (ω 3-PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are important regulators of cardiovascular health. However, questions of efficacy and mechanism of action have made the use of ω 3-PUFAs in all cardiovascular disease (CVD) controversial. Here, we review recent studies in animal models of HF indicating ω 3-PUFAs, particularly EPA, are cardioprotective, with the results indicating a threshold for efficacy. We also examine clinical studies suggesting that ω 3-PUFAs improve outcomes in patients with HF. Due to the relatively small number of clinical studies of ω 3-PUFAs in HF, we discuss EPA concentration-dependency on outcomes in clinical trials of CVD to gain insight into the perceived questionable efficacy of ω 3-PUFAs clinically, with the results again indicating a threshold for efficacy. Ultimately, we suggest that the main failing of ω 3-PUFAs in clinical trials might be a failure to reach a therapeutically effective concentration. We also examine mechanistic studies suggesting ω 3-PUFAs signal through free fatty acid receptor 4 (Ffar4), a G-protein coupled receptor (GPR) for long-chain fatty acids (FA), thereby identifying an entirely novel mechanism of action for ω 3-PUFA mediated cardioprotection. Finally, based on mechanistic animal studies suggesting EPA

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None

prevents interstitial fibrosis and diastolic dysfunction, we speculate about a potential benefit for EPA-Ffar4 signaling in heart failure preserved with ejection fraction.

Graphical Abstract

Keywords

Eicosapentaenoic acid (EPA); heart failure with preserved ejection fraction (HFpEF); free-fatty acid receptor 4 (Ffar4); GPR120; cardiac fibrosis; microvascular rarefaction

1. Introduction

Heart failure (HF) affects approximately 5.7 million people in the U.S. at an annual cost of nearly 30 billion dollars, and this is estimated to increase to nearly 9 million people at a cost of nearly 80 billion dollars by 2030 [1]. Despite a well-defined pharmacological therapeutic regimen that includes β -adrenergic receptor blockers, angiotensin converting enzyme inhibitor/angiotensin receptor blockers, diuretics, and aldosterone antagonists, the five-year mortality rate is still greater than 50% [1]. Furthermore, the mortality rate for HF has not declined in years, highlighting the unmet need for new therapeutic options.

Omega-3 polyunsaturated fatty acids (ω 3-PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are important regulators of cardiovascular health [2, 3]. Several clinical trials have demonstrated that ω 3-PUFAs confer a survival benefit in coronary heart disease (CHD) by preventing sudden death [4–8], and more recently clinical trials have indicated that ω 3-PUFAs might improve outcomes in HF [9–13]. Despite these potential benefits, the use of ω 3-PUFAs in CHD and HF remains controversial.

Here, we review evidence from basic studies in animal models of HF and clinical trials with ω 3-PUFAs in HF that suggest ω 3-PUFAs, particularly EPA, improve HF outcomes. Further, we examine data from animal models of HF suggesting a relationship between EPA concentration and cardioprotection, observed as prevention of interstitial fibrosis and diastolic dysfunction. Due to the relatively small number of clinical studies of ω 3-PUFAs in HF, we review the relationship between EPA concentration and cardiovascular disease (CVD) outcomes to gain insight into the perceived questionable efficacy of ω 3-PUFAs clinically. Ultimately, we suggest that the main failing of ω 3-PUFAs in clinical trials might

be a failure to reach a therapeutically effective concentration. Additionally, we review data suggesting that ω 3-PUFAs signal through free fatty acid receptor 4 (Ffar4), a G-protein coupled receptor (GPR) for long-chain fatty acids (FA). We suggest that Ffar4 might mediate the cardioprotective benefits of EPA in the heart, proposing an entirely novel mechanism of action for ω 3-PUFA mediated cardioprotection. Finally, based on data indicating EPA prevents interstitial fibrosis and diastolic dysfunction in an animal model of HF, we speculate about a potential benefit for EPA-Ffar4 signaling in heart failure preserved with ejection fraction (HFpEF).

2. ω 3-PUFAs in HF, a potential new therapeutic target

2.1. ω 3-PUFAs in dietary advice, dietary supplementation, and pharmacotherapy

ω 3-PUFAs, EPA and DHA, in health and disease are addressed via dietary advice, dietary supplementation, and pharmaceutical applications. They are prolifically studied in all three settings and guidelines exist for each in many nations. Most generally, dietary advice is aimed at good overall health, dietary supplementation is meant to manage present or latent risk, and pharmaceutical application is directed toward clinically indicated risk markers. Global recommendations are cataloged by the International Society for the Study of Fatty Acids and Lipids (ISSFAL) on behalf of the Global Organization for EPA and DHA (GOED), and include general health recommendations by national authoritative bodies as well as recommendations for supplementation by expert societies; Brownawell *et al* provide a detailed assessment of advice and the regulatory environment [14]. For prevention of cardiovascular disease, the National Heart Lung and Blood Institute (NHLBI) recommends increasing ω 3-PUFAs through a general increase of seafood intake.¹ Currently, both ISSFAL² and the American Heart Association (AHA) recommend ω 3-supplementation (0.5 g/d and 1 g/d respectively) for patients with CHD, citing benefits including lowering of triglycerides, prevention of arrhythmias, and prevention of atherosclerosis. Here, we will review current basic and clinical research suggesting the potential for ω 3-PUFAs in HF.

2.2. ω 3-PUFAs in animal models of HF

Few studies have examined ω 3-PUFAs in HF, particularly from a mechanistic standpoint in cultured cells or animal models of HF, although a handful of studies have demonstrated various positive effects of ω 3-PUFA-supplementation [15–19]. Yet, very few studies have examined the cellular and molecular mechanisms whereby ω 3-PUFAs are cardioprotective.

Recently, we reported that dietary supplementation with an ω 3-PUFA-rich diet prevented cardiac dysfunction and interstitial fibrosis induced by surgical constriction of the transverse aorta (TAC) in mice [20]. TAC is a common HF model in which ventricular remodeling is characterized by hypertrophy, systolic and diastolic dysfunction, and interstitial cardiac fibrosis. We found that 12 weeks of dietary supplementation with an ω 3-rich diet significantly increased ω 3-levels in blood and heart tissue to levels slightly higher than

¹<http://www.nhlbi.nih.gov/health-pro/guidelines/current/cardiovascular-health-pediatric-guidelines/full-report-chapter-5>

²International Society for the Study of Fatty Acids and Lipids (June 2004). Report of the Sub-Committee on Recommendations for Intake of Polyunsaturated Fatty Acids in Healthy Adults. [online] Available at: <http://www.issfal.org/news-links/resources/publications/PUFAIntakeRecomdFinalReport.pdf>

normally achieved in treated patients in the US (ω 3-index = 15.2%, defined as ([%DHA + %EPA]/total FA) in erythrocytes) [20]. Functionally, ω 3-PUFA supplementation prevented TAC-induced systolic and diastolic dysfunction. At the tissue level, ω 3-PUFAs prevented TAC-induced interstitial fibrosis, resulting in 63% less fibrosis in the left ventricle [20]. Furthermore, ω 3-PUFAs prevented collagen I and III expression, fibroblast proliferation, and myofibroblast transformation, all markers of a pro-fibrotic response [20]. In primary cultures of cardiac fibroblasts, EPA and DHA prevented transforming growth factor β 1 (TGF β 1) pro-fibrotic signaling by inhibiting fibroblast proliferation, collagen expression, and myofibroblast transformation, demonstrating a direct effect of ω 3-PUFAs in cardiac fibroblasts [20].

However, these findings raised several additional questions including: **1)** Which ω 3-PUFA (EPA, DHA, or both) mediates prevention of fibrosis *in vivo* (both EPA and DHA were sufficient to prevent fibrosis in cultured fibroblasts)? **2)** Whether the protective effects were due to the supra-physiologic ω 3-index produced by 12 weeks of dietary supplementation with an ω 3-diet (ω 3-index = 15.2% [20])? **3)** Whether prevention of cardiac dysfunction was due solely to prevention of fibrosis, or if ω 3-PUFAs have a protective effect independent of prevention of fibrosis? To address these questions, we performed a second study using the TAC model in mice fed diets supplemented with only EPA *or* DHA, and control mice fed the standard ω 3-diet or control diet from our previous study [21]. To achieve a more clinically relevant ω 3-index, we reduced the pre-TAC diet regimen to 2 weeks and continued the diet regimen for 6 weeks post TAC. Using the ω 3-specific diets and shorter diet regimen, we found that 8 weeks of dietary supplementation with an ω 3-diet (same ω 3-diet as [20]) increased the ω 3-index to 10.2% (control diet: 5.1%), 50% of the increase achieved previously [20], and closer to values that are achieved in U.S. patients. As expected, TAC induced fibrosis in mice fed the control diet. Interestingly, erythrocyte levels of EPA, but not DHA, were inversely correlated to ventricular fibrosis [21], replicating and expanding our earlier work [20]. TAC induced both diastolic and systolic dysfunction, but this was not reversed by any ω 3-dietary supplementation, perhaps due to the overall lower levels of ω 3-uptake in this experiment compared to our prior study [20]. Finally, 8 weeks of supplementation with either EPA *or* DHA increased the levels of each ω 3-PUFA in erythrocytes. Surprisingly, EPA was not enriched in cardiac myocyte or fibroblast membranes, the traditionally accepted mechanism of action for ω 3-PUFAs, thereby implying another mechanism of action [21].

2.3. ω 3-PUFAs in clinical trials examining HF

Few studies have examined ω 3-PUFAs in human HF (summarized in Table 1), but the most prominent is GISSI-HF [9]. In GISSI-HF, ω 3-PUFAs (1 g/d) reduced total mortality by 9%, and cardiovascular mortality and hospitalizations were reduced by 8% [9]. Although the effect was small (56 patients treated 4 years to prevent 1 death), the effects were additive on top of standard care, whereas statins (rosuvastatin, 10 mg/d) in the same context did not improve survival. Subsequently, other smaller clinical trials have examined the effects of dietary supplementation with ω 3-PUFAs on HF. In one small trial, investigators examined the effects of ω 3-PUFAs (2 g/d) on left ventricular function in 133 patients with stable class II–IV NYHA HF secondary to non-ischemic dilated cardiomyopathy. After 1 year, ω 3-

PUFAs significantly improved left ventricular ejection fraction (LVEF), peak VO_2 , exercise duration, and mean NYHA functional class [10]. More recently, in another small trial, 205 patients with Class I–III NYHA HF were randomized to ω 3-PUFAs (1 g/d) or vehicle on top of standard care. After 6 months, ω 3-PUFAs improved LV diastolic function and decreased brain natriuretic peptide in patients with non-ischemic dilated HF [11]. Interestingly, in a trial involving 139 HF patients with mild left ventricular systolic dysfunction (LVEF $37.6 \pm 8.0\%$ percent), EPA (1.8 g/d) improved LVEF, and decreased monocyte chemoattractant protein-1 (MCP-1) and asymmetric dimethylarginine (ADMA) levels suggesting reduced inflammation and improved endothelial function [12]. Another small study involving 43 patients indicated a dose dependent effect of ω 3-PUFAs (1 g/d or 4 g/d) to improve LVEF and decrease interleukin-6 and tumor necrosis factor- α in HF patients [13]. Finally, while not directly testing ω 3-PUFAs in HF, the results of the OMEGA-REMODEL trial indicated that in 358 patients with acute myocardial infarction randomized to high dose ω 3-PUFAs ($4 \times 1\text{g}$ Lovaza pills/d, EPA and DHA) or placebo on top of standard care, ω 3-PUFAs decreased left ventricular systolic volume index and non-infarct myocardial fibrosis, and patients with the highest quartile of ω 3-index had the largest effect [22]. In total, the results to date suggest that ω 3-PUFAs might provide some benefit in HF by preventing ventricular remodeling and a small survival benefit, but ultimately, whether ω 3-PUFAs improve HF outcomes is complex and will require further study of ω 3-PUFA-mediated cardioprotective mechanisms [23]. Accordingly, regulating and advisory bodies (NHLBI, the AHA, ISSFAL, etc.) do not have recommendations regarding the use of ω 3-PUFAs for HF.

3. ω 3-PUFAs and the case for an EPA-therapeutic index

3.1. ω 3-PUFA blood levels and the therapeutic effect in HF

The results of our most recent study in mice indicate that EPA, but not DHA, prevents interstitial fibrosis induced by TAC [21]. However, a deeper analysis of the combined data from our mouse studies suggests a concentration-dependent threshold for cardioprotection, or a therapeutic index, for EPA-mediated prevention of fibrosis and diastolic dysfunction (Figure 1). For this analysis, EPA levels were plotted against TAC-induced pathology (fibrosis and diastolic dysfunction). EPA levels were measured in both experiments and are plotted on the x-axis (e.g. EPA-diet/2 wk. indicates that mice were fed an EPA diet for 2 weeks prior to TAC, and the erythrocyte concentration of EPA at completion is plotted on the x-axis). EPA-mediated prevention of fibrosis or diastolic dysfunction was assessed by calculating the percent inhibition (e.g. %Fibrosis in TAC EPA/(%fibrosis TAC control diet - % fibrosis sham control diet)) and is plotted on the y-axis. This analysis indicates that **1)** Diet composition and time affect EPA uptake and **2)** EPA prevents TAC-induced fibrosis (green line, Figure 1) and diastolic dysfunction (maroon line, Figure 1) in a concentration-dependent manner, supporting our hypothesis for a therapeutic index. The higher level of EPA required to prevent diastolic dysfunction could suggest that prevention of fibrosis is not solely responsible for prevention of dysfunction, and it is important to note that other mechanisms, such as effects on microvascular density, Ca^{2+} handling, or another ω 3-PUFA could play a role.

3.2. ω 3-PUFAs in clinical trials examining CVD, and specifically CHD

Unfortunately, the modest number of clinical trials that have examined ω 3-PUFAs in HF precludes an analysis of ω 3-PUFA concentration and therapeutic effect in human HF. However, ω 3-PUFAs have been extensively studied in CHD and other CVD, and here, we will briefly review the trials examining ω 3-PUFAs in these trials to understand how EPA concentration might be related to outcome.

To date, several small and large clinical trials have examined ω 3-PUFAs for CHD (reviews: [3, 24, 25]), but based on some conflicting results, controversy remains as to whether ω 3-PUFAs confer a benefit. Three large randomized clinical trials demonstrated that increased blood levels of ω 3-PUFAs correlate with reduced mortality and hospitalizations in CHD (GISSI-Prevenzione [4–6], DART [7], and Cardiovascular Health Study [8]). This is supported by several meta-analyses indicating that ω 3-PUFAs reduce the risk of CHD by preventing sudden death [26–30], and observational cohort studies that show benefits of ω 3-PUFAs [31].

Conversely, some trials have failed to show a benefit of ω 3-PUFAs in CHD, including JELIS [32], DART2 [33], Alpha-Omega [34], Omega [35], and SU.FOL.OM3 [36]. However, among these trials, only DART2 was sufficiently powered to detect a reduction in CHD mortality, but DART2 was limited by lack of patient blinding, interruption of recruitment over 7 years, and lack of follow-up [3]. Another recent meta-analysis suggested no benefit of ω 3-PUFAs in CHD [37], but failure to consider ω 3-PUFA blood levels and statistical issues raised doubts about the conclusions [38–41]. A different systematic review and meta-analysis demonstrated that circulating fatty acid composition of EPA, DHA, and their combination, in prospective cohort studies (adjusting for non-blood based vascular disease risk factors) are negatively associated with coronary outcomes [42]. In short, despite some trials showing a benefit of ω 3-PUFAs in CHD, there remains some controversy as to whether the clinical data support a survival benefit of ω 3-PUFAs in CHD.

3.3. ω 3-PUFA blood levels and therapeutic effect in CVD: The case for a therapeutic index

A recent meta-analysis demonstrated that patients given a fixed ω 3-dose had significant individual variation in ω 3-blood levels and found that risk reduction in CHD correlated with higher ω 3-blood levels [43]. For example, in the JELIS trial, an EPA/AA (EPA/arachidonic acid) ratio of greater than 0.75 was associated with significantly lower risk of events in CHD patients [32]. In this study, although 28 days of EPA supplementation increased the EPA/AA ratio from 0.12 to 0.9, only 68% of the patients achieved a ratio of 0.78 to 1.02. More importantly, several trials that measured uptake of ω 3-PUFAs never achieved an EPA/AA ratio of 0.75 [44–46]. The investigators behind this analysis suggested three practical implications: **1)** There is a physiologic and genetic basis for the variability in blood levels of ω 3-PUFAs in patients receiving a fixed dose. **2)** Blood levels of ω 3-PUFAs are a better predictor of outcome than dose given (all randomized trials have given a fixed dose). **3)** Patients that, for whatever reason, have lower blood levels of ω 3-PUFAs might negatively affect trial results [43]. This suggests that best outcomes would be achieved by titrating the dose of ω 3-PUFAs to reach an optimal plasma or red blood cell concentration.

To support the argument of an EPA-specific therapeutic index, we analyzed the effect of 1) EPA dose and 2) the estimated final enrichment in tissue EPA using the approach of Flock *et al.* [47] to evaluate EPA in prevention of CVD from 18 clinical trials [4, 7, 9, 32–36, 48–57] (Trial details in Table 2). We used the work of Stark *et al.* [58] to estimate population ω 3-PUFA where it was not reported. This analysis demonstrates that: **1)** Outcomes are dependent on the dose given as well as the estimated tissue enrichment of EPA after treatment (% of total RBC FAs); and **2)** Surprisingly few studies have been implemented that treat subjects lacking EPA at baseline with doses large enough to substantially alter tissue EPA composition (Figure 2).

In total, a careful assessment of ω 3-PUFAs in CVD suggests that **1)** It is likely that patients are not receiving enough EPA to produce a therapeutic benefit; and **2)** We are not accounting for variability in basal EPA uptake. In total, we suggest that ω 3-PUFA concentration is a critical variable that has been overlooked (Figures 2) [43]. Furthermore, while there is variation in baseline EPA levels in the general population, based on current evidence, we suggest that high levels of EPA can be achieved in humans [59], up to 3.5% in the US population [60]. Finally, the analysis presented here suggests that we should reconsider how patients with CVD are treated with ω 3-PUFAs, and rather than focus on dose (again current recommendations suggest 0.5 to 1.0 g/d for patients with CHD), we should titrate ω 3-PUFA dose to achieve therapeutic blood levels. Whether this will translate to patients with HF remains to be seen, but we suggest that it is likely given what we have observed with EPA in animal models of HF (Figure 1).

4. The potential of Ffar4 signaling as a novel mechanism of ω 3-PUFA cardioprotective signaling

A major impediment to establishing a benefit for ω 3-PUFAs in HF or any other CVD is the lack of a clearly defined molecular mechanism that delineates how increased ω 3-PUFA levels confer cardioprotection. In that regard, our prior findings indicate that EPA prevents fibrosis in a mouse model of pressure overload-induced HF, but is not accumulated in cardiac myocyte or fibroblast membranes. Although incorporation into cellular membranes is considered the classic mechanism of action for ω 3-PUFAs, the failure of cardiac myocytes and fibroblasts to accumulate EPA suggests an alternate mechanism. In the last 10 years, a family of orphan G-protein coupled receptors (GPR) have been classified as receptors for free fatty acids (FFA). GPR41 and GPR43, now Ffar3 and Ffar2, were identified as receptors for short-chain FA (<8 carbons) [61], GPR84, which is unclassified, was identified as a receptor for medium chain FA (9–14 carbons) [62], and GPR40 and GPR120, now Ffar1 and Ffar4, were identified as receptors for long-chain FA [63, 64]. The identification of the Ffar family uncovers an entirely novel mechanism of action for FA signaling.

4.1. Ffar4 expression and signaling

Ffar4 expression is detected in several tissues, with high levels of expression in lung and colon, and lower levels in other tissues including brain, small intestine, adipose, taste buds, muscle, heart, and liver [63, 65, 66]. Ffar4 signals through G α q to induce release of intracellular Ca²⁺ and activation of ERK [63]. Alternatively, Ffar4 can recruit β -arrestin-2

(not β -arrestin-1) leading to internalization of the receptor and activation of downstream signaling [67]. Following on these studies, we detected Ffar4 expression in whole mouse heart at levels 20-fold higher than Ffar1, 2, or 3 [21]. Further, we detected Ffar4 expression in both isolated mouse cardiac myocytes and fibroblasts [21]. Interestingly, Ffar4 expression is increased in hearts from rats fed a high-fat diet [66]. Whether Ffar4 is expressed in human heart is unclear, and others have suggested that there are species-specific differences in Ffar4 expression [68]. In addition, human Ffar4 has two splice variants [69, 70], a short isoform (analogous to rodent, Ffar4S) and long isoform (Ffar4L) differing in a 16 amino acid insertion in the third intracellular loop, that signal differently with the Ffar4S activating calcium and recruiting β -arrestin, whereas the Ffar4L only recruits β -arrestin [70]. However, Ffar4L expression appears rather limited, and it has been suggested that it might be functionally irrelevant [71, 72]. Regardless, the expression profiles of Ffar4 in human heart, cardiac myocytes, or fibroblasts as well as the functional implications of splice form-specific expression in human heart are unclear at this time.

4.2. Ffar4 prevents fibrosis

Previously, we found that both EPA and DHA inhibited TGF β 1-induced fibrosis in primary mouse cardiac fibroblasts [20]. However, only EPA prevented fibrosis *in vivo*, but was not incorporated into cardiac fibroblast membranes *in vivo* [21]. Because both EPA and DHA are readily incorporated into fibroblast membranes in culture, we could not rule out an effect of membrane incorporation as a mechanism for ω 3-PUFA-mediated prevention of fibrosis *in vitro* [20]. Interestingly, we found that GW9508, a small molecule agonist for Ffar1/4 (more potent at Ffar1, but IC₅₀ at Ffar4 ~3 μ M, and we failed to detect significant Ffar1 expression in heart), prevented TGF β 1 pro-fibrotic signaling in a concentration dependent manner in primary cardiac fibroblasts [21]. As a small molecule with several benzyl ring structures, GW9508 is not likely incorporated into fibroblast membranes. This suggests that activation of Ffar4 is sufficient to prevent TGF β 1-induced fibrosis. To address the role of Ffar4 more directly, we used shRNAi to knockdown receptor expression and test the requirement for Ffar4 to prevent TGF β 1-induced fibrosis. We found that in primary cardiac fibroblasts treated with TGF β 1, knockdown of Ffar4 blocked the anti-fibrotic effects of GW9508, indicating that Ffar4 is required for this effect [21]. In total, our data suggest that Ffar4 mediates anti-fibrotic signaling in primary cardiac fibroblasts. Ffar4 is a receptor for ω 3-PUFAs, and combined with our data indicating that membrane incorporation of EPA is not required for prevention of fibrosis, Ffar4 is a likely candidate to explain EPA mediated prevention of fibrosis.

Our finding that EPA is cardioprotective but not incorporated into fibroblast membranes *in vivo* indicates that membrane incorporation is not required for EPA-mediated cardioprotection. Incorporation of ω 3-PUFAs, particularly DHA, into lipid membranes can alter membrane fluidity and affect signaling. DHA appears to be unique compared to other ω 3-PUFAs by virtue of its ability to alter membrane properties such as membrane fluidity, elasticity, phase behavior, ion permeability, fusion, and protein function [73–76]. More importantly, DHA can form unique non-caveolar membrane domains, while simultaneously disrupting caveolar membrane domains based on incompatibility with cholesterol and sphingolipid, which are enriched on caveolar lipid rafts [73–76]. In our prior report, we

found that DHA was readily incorporated into cardiac myocyte and fibroblasts membranes, but showed no benefit and even tended to worsen fibrosis *in vivo* [21]. Alternatively, we found that Ffar4 prevented fibrosis in cultured adult cardiac fibroblasts, suggesting that targeting Ffar4 in HF could be a safer approach, without the confounding effects of incorporation of ω 3-PUFAs into lipid membranes.

4.3. Ffar4 anti-fibrotic signaling mechanisms

cGMP/protein kinase G (PKG) signaling plays a counter-regulatory role against TGF β 1-induced cardiac fibrosis [77, 78]. Further, nitric oxide (NO) increases cGMP production through activation of soluble guanylyl cyclase [79, 80], and ω -3 PUFAs increase NO synthesis in humans [81]. We found that in primary mouse cardiac fibroblasts, EPA and DHA increased total intracellular nitrite (NO₂) and nitrate (NO₃), an index of total NO production, and increased eNOS phosphorylation at S1177 [20]. We also found that EPA and DHA increased cGMP and blocked TGF β 1-induced collagen synthesis, which was blocked by the PKG inhibitor DT-3 [20]. Finally, although EPA and DHA did not alter Smad 2/3 phosphorylation, both EPA and DHA inhibited TGF β 1-induced Smad 2/3 nuclear translocation, which is required for TGF β 1 pro-fibrotic signaling [20]. In total, these studies indicate ω 3-PUFAs induce an eNOS/cGMP/PKG-signaling pathway to inhibit Smad nuclear translocation, but how ω 3-PUFAs activate eNOS is unclear. Our finding that EPA signals through Ffar4 suggests a novel mechanism whereby Ffar4-Gq-PKC signaling induces eNOS phosphorylation, thereby activating the anti-fibrotic eNOS/cGMP/PKG-signaling pathway (Figure 3).

4.4. Other potential beneficial effects of Ffar4

In addition to preventing fibrosis, activation of Ffar4 might have other beneficial effects in HF. Microvascular rarefaction is a prominent finding in HF patients [82]. Interestingly, ω 3-PUFA-supplementation prevents microvascular rarefaction in a hind-limb ischemia model [83], while EPA induces VEGF-A expression through activation of Ffar4 in adipocytes [84]. Together, these findings suggest that activation of Ffar4 might induce angiogenesis offsetting microvascular rarefaction observed in HF. Furthermore, Ffar4 is anti-inflammatory, reducing macrophage infiltration into adipose tissue in mice fed a high-fat diet and inducing macrophage phenotype switching from pro-inflammatory M1 to anti-inflammatory M2 [67]. In short, Ffar4 mediated anti-inflammatory signaling and macrophage phenotype switching might reduce inflammation and fibrosis in HF [85].

Another potential benefit of ω 3-PUFAs that might involve Ffar4, although it remains to be proven, is signaling of anti-inflammatory PUFA metabolites through the receptor. Among PUFA metabolites, anti-inflammatory signaling is mediated by epoxide derivative of cytochrome P450 epoxygenases. This is illustrated by the benefit observed with inhibitors of soluble epoxide hydrolase inhibitors, the enzyme that metabolizes PUFA epoxides, which include induction of angiogenesis [86, 87], and broad anti-inflammatory actions, including promotion of M2 macrophage polarization in the heart [88], which would all benefit HF. Furthermore, EPA-epoxides are reportedly up to 100 times more potent than those made from arachadonic acid [89], and the EPA epoxide, 17,18-epoxy eicosatetraenoic acid, suppresses both pulmonary [90] and gut inflammation [91].

5. The potential of EPA-Ffar4 signaling as a novel intervention for HFpEF

5.1. HFpEF

Heart failure (HF) is a complex and heterogeneous clinical syndrome, and in the last 10 years, two broadly defined phenotypes have emerged: HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF). Currently, HF affects approximately 5.7 million in the U.S. [1], and half of all HF diagnoses are HFpEF, which is increasing at roughly 1% annually [92]. More importantly, standard HF therapies for HFrEF fail to show efficacy in HFpEF [93, 94].

HFpEF is defined by symptoms of HF but with a 'normal' left ventricular ejection fraction (EF). Generally, patients with HFpEF are older, female, and more likely to have hypertension, renal disease, atrial fibrillation, and/or pulmonary disease [95]. The survival rate for HFpEF is marginally higher than HFrEF, but still well below 50% at 5-years [93, 94]. Most importantly, standard HF therapies show no efficacy in HFpEF, including β -blockers (OPTIMIZE-HF [96]), angiotensin receptor blockers (I-Preserve [97], CHARM-Preserved [98]), angiotensin receptor-neprilysin inhibitors (PARAMOUNT [99]), phosphodiesterase inhibitors (RELAX [100]), and aldosterone antagonists (Aldo-DHF [101], TOPCAT [102]). Potential reasons for these failures might be: **1)** HFpEF lacks a standard diagnosis. The European Study Group on Diastolic HF [103], Framingham [104], the European Society of Cardiology [105], and the current ACCF/AHA guidelines [106], have different definitions of HFpEF. **2)** HFpEF is a multifactorial syndrome characterized by multiple cardiac and vascular pathologies ranging from calcium handling abnormalities, fibrosis, or renal failure, with HF symptoms and preserved EF. **3)** HFpEF patients have significant phenotypic variation and might present with diastolic dysfunction, fibrosis, fluid retention and/or pulmonary hypertension.

5.2. Ventricular remodeling in HFpEF

Diastolic dysfunction is a defining feature of HFpEF. The hallmarks of diastolic dysfunction are impaired ventricular relaxation and filling caused by: **1)** remodeling that increases ventricular passive stiffness (increased extracellular matrix deposition (ECM), microvascular rarefaction) [82, 107]; **2)** structural changes that increase passive stiffness (e.g. titin isoform expression) [108]; or **3)** altered calcium handling [109]. Here, we focus on interstitial fibrosis and microvascular rarefaction observed in HFpEF, which increase passive stiffness and contribute to diastolic dysfunction.

5.2.1. Interstitial fibrosis—Interstitial fibrosis is a common feature of hypertensive heart disease (HHD) and prevalent comorbidity in HFpEF that contributes to diastolic passive stiffness, impaired filling, and exercise intolerance [107, 110–112]. Recently, the relative role of collagen and titin in myocardial passive stiffness was examined in HFpEF patients and indicated that collagen contributes ~2/3 of the passive stiffness [112]. Further, increased passive stiffness in HFpEF patients was associated with increased pro-inflammatory and pro-fibrotic plasma biomarkers [112]. Resident fibroblasts are responsible for the production of ECM, and activated myofibroblasts increase proliferation, ECM production, and alter the balance of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases

(TIMPs) to favor fibrosis [113]. Inhibition of myofibroblast transformation is a significant, novel therapeutic target for cardiac fibrosis [114].

5.2.2. Microvascular rarefaction—Microvascular rarefaction, or loss of capillary vessels, was recently detected in HFpEF patients at autopsy (along with myocardial fibrosis) [82], which is the first report of this observation. Microvascular rarefaction can result in impaired oxygen delivery, reducing systolic and diastolic reserve, and exacerbating exercise intolerance [115].

5.3. Inflammation and HFpEF

Systemic inflammation leading to coronary microvascular injury is an emerging hypothesis to explain the unique phenotypic ventricular remodeling observed in HFpEF (interstitial fibrosis, microvascular rarefaction, cardiac myocyte stiffness) [116, 117]. In this scenario, comorbidities associated with HFpEF, including obesity, diabetes mellitus, and hypertension result in systemic inflammation, inducing multi-organ inflammatory responses [117]. In the heart, this inflammation induces microvascular endothelial injury and oxidative stress with the recruitment of infiltrating immune cells, including macrophages, leading to: **1)** activation and transformation of resident fibroblasts to myofibroblasts resulting in increased interstitial fibrosis [118], **2)** reduced nitric oxide (NO) production in endothelial cells resulting in increased cardiac myocyte stiffness [119, 120], and **3)** endothelial cell death and coronary microvascular rarefaction.

5.4. EPA-Ffar4 cardioprotection in HFpEF

Our findings suggest that in a mouse model of pressure overload induced HF, EPA prevents interstitial fibrosis and diastolic dysfunction, key components of ventricular remodeling in HFpEF [20, 21]. Furthermore, we found that EPA-Ffar4 signaling prevents profibrotic signaling in cardiac fibroblasts, identifying an entirely novel mechanism of action for EPA-mediated cardioprotective signaling [21]. While EPA-Ffar4 signaling might generally improve HF, it is interesting to note the particular overlap with HFpEF for which standard HF therapies show no efficacy. As discussed above, ventricular remodeling in HFpEF is characterized by interstitial fibrosis and microvascular rarefaction, and we have suggested that EPA-Ffar4 signaling might prevent both. Furthermore, ω 3-PUFAs might inhibit systemic inflammation through activation of Ffar4 in macrophages [67]. This further suggests a benefit of EPA-Ffar4 signaling through inhibition of inflammation produced by comorbidities in HFpEF including obesity, diabetes, and hypertension that result in systemic inflammation [117].

6. Conclusions and future directions

We suggest that the main impediments to wider and more effective use of ω 3-PUFAs in all CVD are: **1)** Understanding why some clinical trials fail to show a benefit of ω 3-PUFAs and **2)** Identification of a defined molecular mechanism to explain ω 3-mediated cardioprotection. In that regard, we have examined existing data from animal studies and clinical trials that suggest a dose dependency, or therapeutic index, for ω 3-PUFA/EPA-mediated cardioprotection. Further, we reviewed data from our group and others suggesting

that ω 3-PUFAs/EPA signal through Ffar4, a G-protein coupled receptor (GPR) for long-chain fatty acids (FA), thereby identifying an entirely novel molecular mechanism to explain ω 3-PUFA-mediated cardioprotection. Finally, we have suggested that EPA-Ffar4 signaling could be a novel therapeutic target for HFpEF. While EPA-Ffar4-cardioprotective signaling could fundamentally change how we understand ω 3-PUFAs mechanistically in heart and suggest Ffar4 as a novel target for HFpEF, many future directions remain including:

Optimization of ω 3-PUFA/EPA-therapy based upon titration to achieve therapeutic levels

A pressing clinical challenge is to continue to define the benefit of ω 3-PUFA/EPA-therapy. Recent analyses suggest that current clinical failures might be attributable to failure to achieve a therapeutic concentration of ω 3-PUFAs [43]. This is the case with triglycerides where efficacy is a function of both baseline ω 3-PUFAs and response to treatment [121]. This suggests that ω 3-PUFA/EPA-therapy should be dictated by targeting therapeutic levels of ω 3-PUFAs in patients rather than dictated by dosage given. However, most trials are performed with a fixed dose (intention-to-treat study design), leading to variability in patient blood levels of ω 3-PUFA, with most failing to achieve a therapeutic level.

Understanding the variability in basal cardiac fatty acid profiles and how this impacts basal Ffar4 signaling

Ffar4 binds to and is activated by numerous long-chain FA [63, 67], and at this time it is unclear how basal activity of Ffar4 in cardiac fibroblasts, or any other cell expressing Ffar4, is affected by variations in tissue-specific and/or blood FA composition.

How a shift away from thinking of free fatty acids as an energy source to how their effects on Ffar4 activity in cardiac fibroblasts and myocytes may be a promising future direction of research and therapeutics for HF.

Developing reliable small animal models of HFpEF

The lack of an animal model for HFpEF is one of the most significant challenges to understanding and developing novel therapies for HFpEF [94]. Several small animal hypertensive models have been proposed that resemble aspects of HFpEF including Dahl salt-sensitive rats, DOCA mice, angiotensin II infusion, spontaneously hypertensive rat, and TAC, but all have limitations (Review: [122]). At this time, there is no small animal HF model that fully recapitulates human HFpEF.

Development of novel Ffar4 ligands and testing in preclinical models of HFpEF and human HFpEF

Although there is research into developing novel Ffar4 ligands (Review: [72]), no current work is directed toward developing Ffar4 ligands specifically for HF.

Finally, based on the criteria reviewed here and the challenges outlined notwithstanding, we suggest that EPA-Ffar4 cardioprotective signaling, particularly in fibroblasts, could fundamentally change how we understand ω 3-PUFAs mechanistically in the heart and suggest Ffar4 as a novel target for HFpEF.

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Abbreviations

CVD	cardiovascular disease
CHD	coronary heart disease
HHD	hypertensive heart disease
HF	heart failure
HFpEF	heart failure with preserved ejection fraction
HFrfEF	heart failure with reduced ejection fraction
EF	ejection fraction
ω3-PUFA	omega 3 polyunsaturated fatty acid
EPA	eicosapentaenoic acid
DHA	docosahexaenoic acid
AA	arachadonic acid
FA	fatty acid
GPR	g-protein coupled receptor
Ffar	free fatty acid receptor
ECM	extracellular matrix
MMP	matrix metalloproteinase
TIMP	tissue inhibitors of metalloproteinases
NO	nitric oxide
eNOS	endothelial nitric oxide synthase
cGMP	Cyclic guanosine monophosphate
Smad	mothers against decapentaplegic, drosophila, homolog of
PKG	protein kinase G
MCP-1	monocyte chemoattractant protein-1

ADMA	asymmetric dimethylarginine
TAC	transverse aortic constriction
αSMA	α -smooth muscle actin
TGFβ1	transforming growth factor β 1

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Highlights

1. ω 3-PUFA cardioprotection is concentration dependent
2. EPA prevents remodeling associated with HF
3. EPA signals through Ffar4 to prevent cardiac fibrosis
4. EPA-Ffar4 signaling could be a novel therapeutic approach for HFpEF

MOUSE

TAC-induced pathology:

HUMAN

Figure 1. EPA Therapeutic index in mice: Erythrocyte EPA abundance (EPA-index) and prevention of pathologic phenotypes following TAC in mice compared to EPA levels in humans
 Upper section (Mouse). EPA levels were plotted against TAC-induced pathology (interstitial fibrosis or diastolic dysfunction). EPA levels from our prior reports [20, 21] are plotted on the x-axis (e.g. EPA-diet/2 wk: mice were fed an EPA supplemented diet for 2 weeks before TAC, and the erythrocyte EPA levels at termination are plotted on the x-axis). EPA-mediated prevention of fibrosis or diastolic dysfunction was assessed by calculating the percent inhibition (e.g. %Fibrosis in TAC EPA/(%fibrosis TAC control diet - % fibrosis sham control diet)) from our prior reports [20, 21], and are plotted on the y-axis. Lower section (Human).

In FONIA, baseline erythrocyte EPA levels increased from 0.4% to 2.2% in US patient (4 months 3.4 g/d ω -3-PUFAs unreported data), versus untreated Japanese (2.8%, JELIS) [123].

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Figure 2. EPA therapeutic index in humans

Results from 18 RCTs using ω 3-PUFA-based interventions to prevent CVD-endpoints were used to estimate the impact of ω 3-PUFA on CVD risk [4, 7, 9, 32–36, 48–57]. Primary outcome risk ratio is plotted against (A) study dose and (B) expected enrichment of in tissue EPA. The relative risk outcome is for the primary outcome except where the primary outcome was not event-related (e.g. regression of carotid intimal thickening); in such cases event-related endpoints were used (diamonds). Only 6 of 18 studies reported %EPA in tissue, thus the expected baseline ω 3 index of study populations was obtained from Stark et

al [58], and the change in omega-3 index was calculated using the method of Flock et al [47]. Since $\%EPA_{\text{final}}/\%EPA_{\text{baseline}}$ is not a strict function of $\%\omega 3_{\text{final}}/\%\omega 3_{\text{baseline}}$, we corrected for this using an algorithm as reported [121]. Transnational trials are indicated by open circles; for the ORIGIN trial, a nation from the lowest (USA) and highest (Norway) quartiles are represented to demonstrate the potential for differences in study dose. Two trials were not included: the first [124] due to population (hemodialysis subjects), and for the second [125] no national data was available from Stark et al [58] or the study itself to estimate treatment effects. The regression lines are unweighted across studies.

Figure 3. Ffar4 anti-fibrotic signaling mechanisms

TGFβ1 binding to the TGFβ1-receptor induces autophosphorylation of the receptor leading to phosphorylation of Smad2/3. Phosphorylated Smad2/3 binds to Smad4 facilitating nuclear translocation of the complex leading to induction of pro-fibrotic gene expression. Previously, we demonstrated that ω3-PUFAs induce phosphorylation of endothelial nitric oxide synthase (eNOS) and increase production of NO leading to activation of soluble guanylyl cyclase (sGC) and protein kinase G (PKG) [20]. Activation of cGMP/PKG signaling prevented Smad2/3-Smad4 nuclear translocation through a proposed phosphorylation of Smad2/3 on a

non-canonical site [20]. Recently, we found that Ffar4 prevents TGF β 1-induced pro-fibrotic signaling in cardiac fibroblasts [21], and we hypothesize (indicated by the brackets) that Ffar4 signaling through G α q activates protein kinase C (PKC) to induce phosphorylation of eNOS at S1177.

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ω3-PUFA HF Trials

Table 1

Trial (reference)	Treatment Groups	Inclusion/Exclusion Criteria	Outcomes	Reported Findings	Reported Interpretation
GISSI-HF (Tavazzi et al)	Groups: ω3-PUFA: n=3481 1 g/d, 850–882 mg EPA/DHA ethyl esters, ratio 1.1:2. Placebo: n=3481 Median follow-up: 3.9 yr. Patients 93%: ARB/ACE inhibitor 65%: β-blocker 90%: diuretic	Inclusion Criteria HF all cause, defined by ESC as NYHA I–IV LVEF measured <3 months prior LVEF >40% with one HF admission in last year Exclusion Criteria Contraindication to ω3-PUFA, Non-cardiac comorbidity (e.g. cancer), Acute coronary syndrome or revascularization within last month	1° Outcomes: Time to mortality or hospital admission for CV reasons 2° Outcomes: CV mortality CV mortality or admission for CV reasons Sudden death, any admission, CV admission, HF admission, MI, Stroke	All cause mortality: HR 0.91, $P=0.041$ All cause mortality or admission for CV reasons HR 0.92, $P=0.009$	For HF patients, ω3-PUFAs provide a small benefit to survival or hospital admission for CV reasons in the context of standard care.
ω3-PUFA in Non-ischemic cardiomyopathy (Nodari et al.)	Groups: ω3-PUFA: n=67 1 g/d: 850–882 mg EPA/DHA ethyl esters, ratio 0.9:1.5 5 g/d first month, 2 g/d duration Placebo: n=66 Duration 12 mo.	Inclusion Criteria 18–75 yr. old Non-ischemic cardiomyopathy LVEF 45% Stable w/ no or min symptoms for 3 mo. On evidence based therapy @ max dose for 6 mo. Exclusion Criteria Coronary artery disease, congenital or 1° valvular disease, atrial fibrillation, NYHA IV, chronic lung disease, advanced kidney or liver disease, disease that limits lifespan to <1 yr.	1° Outcomes: Change in EF over 12 mo. 2° Outcomes: Change in diastolic function Change in Peak V _{O2} Change in Exercise duration Change in NYHA class	ω3-levels enrollment: Placebo: 1.68±0.43% ω3-PUFA: 1.76±0.50% 1 yr.: Placebo: 1.66±0.41% ω3-PUFA: 4.31±0.83% LVEF change Placebo: -5.0% ω3-PUFA: +10.4% Peak V_{O2} change Placebo: -4.5% ω3-PUFA: +6.2% Exercise duration change Placebo: -4.8% ω3-PUFA: +7.5% NYHA Class change Placebo: 1.83 to 2.14 ω3-PUFA: 1.88 to 1.61 Improvements in LVEF, Peak V _{O2} , exercise duration and NYHA Classification	For patients with non-ischemic cardiomyopathy and minimal symptoms on evidenced based therapy, ω3-PUFAs increase LV systolic function and functional capacity and may reduce HF hospitalizations.
ω3-PUFA in CHF (Chrysoschoou et al)	Groups: ω3-PUFA: n=104 1 g/d (source not specified) Placebo: n=99 Duration 6 mo.	Inclusion Criteria Chronic compensated HF due to ICM or DCM NYHA I–III Optimal HF treatment LVEF <40% Exclusion Criteria Atrial Fibrillation	1° Outcomes: LV and RV systolic function LA function Biventricular filling pressures by pulse wave and TDI Plasma BNP	ω3-PUFA intervention group: BNP -34.6% EDD -2.5% ESD -3.7% L_A diam -8.4% LAEF +6.0% TDI E/A -6.3% Reductions in BNP levels, ventricular volumes Improved LA function and diastolic function	In CHF patients, ω3-PUFA supplementation was associated with improved LV diastolic function and decreased BNP levels
EPA in CHF w/ dyslipidemia (Kohasi et al)	Groups: EPA: n=71 1.8 g/d (source not specified) Placebo: n=68 139 total patients DCM 25 ICM 114 Median F/U: 28 mo.	Inclusion Criteria DCM: normal coronary angiogram, LV hypokinesis by echo ICM: history of MI w/ >70% occlusion in at least 2 arteries Optimal HF treatment Mean LVEF <37.6±8.0% Only patients with dyslipidemia treated w/EPA (TC 220 mg/dL; TG 150 mg/dL; LDL 140 mg/dL; HDL 40 mg/dL) Exclusion Criteria Acute infection, Autoimmune disorder, kidney or liver disease, cancer	Outcomes not specified Measured cardiac function by echocardiography, serum levels of EPA, AA, as well as markers of inflammation including TNFα, MCP-1, and plasma levels of ADMA	EPA Levels EPA 37.4 to 53.0 μg/ml Placebo 36.9 to 35.3 μg/ml EPA/AA EPA 0.30 to 0.52 Placebo 0.24 to 0.21 LVEF EPA: +14.1% Placebo: -9.3% NYHA Class I–IV EPA: Pre: 0/62/9/0 End: 14/48/7/2 Placebo Pre: 0/65/3/0 End: 16/19/31/2 Serum TNFα, MCP-1, AA, and plasma ADMA level negatively correlated w/LVEF Serum EPA	In CHF patients with dyslipidemia, EPA might improve cardiac function and long-term prognosis partly due to a reduction in inflammation and improved endothelial function.

Trial (reference)	Treatment Groups	Inclusion/Exclusion Criteria	Outcomes	Reported Findings	Reported Interpretation
Dose dependency of ω-PUFAs in CHF (Moertl et al)	Groups: ω3 4 g/d: n=13 Onacor 4 pills/d (900 mg of ω 3 ethyl esters with at ~465 g DHA and ~375 g EPA) ω3 1 g/d: n=14 Onacor 1 pill/d Placebo: n=16 Duration 12 wk.	Inclusion Criteria Age 18 yr, CHF, non-ischemic NYHA II-IV LVEF 35% On evidence based therapy @ max dose for 6 mo. Plasma NTproBNP 2000 pg/ml Exclusion Criteria Uncorrected valvular heart disease, congenital heart disease, restrictive cardiomyopathy, alcoholic heart disease, acute myocarditis	1° Outcomes: LVEF Flow mediated vasodilation (FMD, measure of endothelial function) Peak V0 ₂ Plasma IL-6, TNF α .	and EPA/AA positively correlated to LVEF LVEF ω 3 4 g/d: $+5.5 \pm 5.9\%$ ω 3 1 g/d: $+2.5 \pm 3.7\%$ Placebo: $+1.0 \pm 3.8\%$ Flow-mediated vasodilation ω 3 4 g/d significantly improved FMD Peak V0₂ No changes IL-6, TNFα ω 3 4 g/d significantly reduced IL-6	In CHF patients (non-ischemic), ω 3-PUFA increase LVEF in a dose dependent manner, and high doses improve endothelial function and reduce inflammation (IL-6).

Abbreviations: ω 3-PUFA, ω 3-polysaturated fatty acid; EPA, eicosapentaenoic acid; AA, arachadonic acid; GISSI-HF, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Heart Failure; ESC, European Society of Cardiology; NYHA, New York Heart Association; HF, heart failure; CHF, congestive heart failure; LV, left ventricular; EF, ejection fraction; LVEF, left ventricular ejection fraction; CV, cardiovascular; MI, myocardial infarction; BNP, brain natriuretic peptide; EDD, end diastolic dimension; ESD, end systolic dimension; LAdiam, left atrial diameter; TDI, tissue Doppler imaging; TNF α , tumor necrosis factor α ; MCP-1, monocyte chemoattractant protein-1; ADMA, Asymmetric dimethylarginine

Table 2

ω3-PUFA CHD Trials

Trial (reference)	Treatment Groups	Inclusion/Exclusion Criteria	Outcomes	Reported Findings	Reported Interpretation
JELIS (Yokoyama et al)	Groups: ω3-PUFA: n=9326 1.8 g/d ethyl esters, 300 mg EPA ethyl esters Control: n=9319 Median follow up: 4.6 yr. All Patients: 10 mg of pravastatin or 5 mg of simvastatin	Inclusion Criteria 40–75 yr. old with hypercholesterolemia (TC 6.5 mmol/l or LDL 4.4 mmol/l) Exclusion Criteria Acute MI, cardiovascular reconstruction, cerebrovascular disorders within the past 6 mo., unstable angina pectoris, a history or complications of serious heart disease, complications of serious hepatic or renal disease, malignant disease, uncontrollable diabetes, hyperlipidemia due to other disorders	1° Outcomes: Any major coronary events: sudden cardiac death, fatal and non-fatal myocardial infarction, unstable angina, CABG or PTCA 2° Outcomes: All-cause mortality, mortality and morbidity of coronary artery disease, stroke, peripheral artery disease and cancer	Plasma EPA concentration (mg/l) at baseline: Control: 93 ω3: 97 5 yr: Control: 93 ω3: 169 Major coronary events: Control: 324 (3.5%) ω3: 262 (2.8%) HR 0.81, <i>P</i> =0.011	EPA is a promising treatment for preventing major coronary events, and especially for non-fatal coronary events.
Alpha Omega Trial (Kromhout et al.)	Groups: ω3-PUFA and ALA: n=1212 margarine with 240 mg/d EPA, and 160 mg/d DHA and 2 g/d of ALA ω3-PUFA and ALA placebo: n=1192 margarine with 240 mg/d EPA, and 160 mg/d DHA ω3-PUFA placebo and ALA: n=1197 margarine with 2 g/d ALA ω3-PUFA placebo and ALA placebo: n=1236 margarine without ω3 fatty acids or ALA Median follow up: 3.4 yr.	Inclusion Criteria 60–80 yr. old Clinically diagnosed myocardial infarction up to 10 yr. Exclusion Criteria Daily consumption of less than 10g of margarine, use of ω3 fatty-acid supplements, unintended weight loss of more than 5 kg in the previous year, a diagnosis of cancer with life expectancy less than 1 yr.	1° Outcomes: Major cardiovascular events, include fatal and nonfatal CVD and the cardiac intervention PCI and CABG 2° Outcomes: Incident CVD, fatal CVD, fatal CHD, ventricular-arrhythmia-related events, and death from any cause	Major CVD events: Placebo or ALA only: 335 (13.8%) ω3: 336 (14.0%) HR 0.90, <i>P</i> =0.55	Low-dose supplementation with EPA-DHA or ALA did not significantly reduce the rate of major CVD events among patients with MI history and who were receiving antihypertensive, antithrombotic, and lipid-modifying therapy
DART (Burr et al)	Groups: ω3-PUFA: n=1015 Fish consumption advice: 200–400 g fatty fish Controls: n=1018 Either fat advice: Reduce fat intake to 30% of total energy, and to increase P/S ratio to 1.0 Or fiber advice: Increase intake	Inclusion Criteria Men under 70 yr., diagnosed of acute MI Exclusion Criteria Diabetic patients, men awaiting cardiac surgery, and men who already intended to eat one of the intervention diets	1° Outcomes: Total mortality and IHD events (IHD deaths plus non-fatal MI)	All deaths: Fish advice group: 94 (9.3%) No fish advice: 130 (12.8%) RR 0.71, <i>P</i> <0.05 IHD events Fish advice group: 127 (12.5%) No fish advice: 149 (14.6%) RR 0.84, <i>P</i> >0.05	A modest intake of fatty fish may reduce mortality in men who have recovered from MI.

Trial (reference)	Treatment Groups	Inclusion/Exclusion Criteria	Outcomes	Reported Findings	Reported Interpretation
SU.FOL.OM3 trial (Galan et al)	of cereal fiber to 18 g daily Duration: 2 yr.				
	Groups: ω 3-PUEFA: n=633 400 mg/d EPA and 200 mg/d DHA ω 3-PUEFA and vitamin B: n=620 400 mg/d EPA and 200 mg/d DHA, and a supplement containing 560 μ g 5-MTHF, 3 mg vitamin B-6 and 20 μ g B-12 Vitamin B: n=622 560 μ g 5-MTHF, 3 mg vit B-6 and 20 μ g B-12 Placebo: n=626 Content not reported Median follow up: 4.7 yr.	Inclusion Criteria Age 45–80 yr., who had an acute coronary or cerebral ischemic event within the 12 months Exclusion Criteria Age <45 yr., or >80 yr., ill-defined diagnosis of CVD, treatment or disease that interfere homocysteine or omega 3 metabolism	1° Outcomes: First major cardiovascular event 2° Outcomes: acute coronary syndrome without myocardial infarction, resuscitation from sudden death, coronary artery bypass surgery, coronary angioplasty, cardiac failure, ventricular arrhythmia, supraventricular arrhythmia, cardiac surgery of any kind, transient ischemic attack, deep vein thrombosis, etc.	Plasma EPA Levels (%) at baseline: ω 3 and Vitamin B: 1.15 ω 3: 1.14 Vitamin B: 1.12 Placebo: 1.26 1 yr: ω 3 and Vitamin B: 2.08 ω 3: 2.15 Vitamin B: 1.16 Placebo: 1.20 Plasma EPA+DHA levels (%) at baseline: ω 3 and Vitamin B: 3.68 ω 3: 3.73 Vitamin B: 3.64 Placebo: 4.04 1yr: ω 3 and Vitamin B: 5.13 ω 3: 5.34 Vitamin B: 3.66 Placebo: 3.92 Primary end point: major cardiovascular events ω 3: 81/1253 (6.5%) Placebo: 76/1248 (6.1%) HR 1.08, $P=0.64$	Routine use of dietary supplements containing B vitamins or ω 3 fatty acids does not prevent cardiovascular disease in people with a history of IHD or ischemic stroke.
OMEGA trial (Rauch et al)	Groups: ω 3-PUEFA: n=1919 1 g of ω 3 ethyl esters with 380 mg DHA and 460 mg EPA Placebo: n=1885 1 g olive oil Duration: 1 yr. All patients were given guideline-adjusted treatment	Inclusion Criteria Age 18 yr. old Acute STEMI or non-STEMI, Within 3–14 days after acute myocardial infarction; No early revascularization, ejection fraction <40%, presence of DM, age >70 Exclusion Criteria Not specified	1° Outcomes: Sudden cardiac death 2° Outcomes: total mortality, major adverse cerebrovascular and cardiovascular events, and revascularization; Implantable cardioverter-defibrillator-terminated VT/fibrillation	Sudden cardiac death: ω 3: 28/1919 (1.5%) Placebo: 29/1885 (1.5%) HR 0.95, $P=0.84$ Total mortality: ω 3: 88/1919 (4.6%) Placebo: 70/1885 (3.7%) HR 1.25, $P=0.18$	Guideline-adjusted treatment of acute myocardial infarction results in a low rate of sudden cardiac death, but application of ω 3-PUFAs could not further reduce it.
Fish oil on Coronary Atherosclerosis (Sacks et al)	Groups: ω 3-PUEFA: n=29 6 g/d 12 1-g capsules, 500mg of ω 3 ethyl esters with 160 mg DHA and 240 mg EPA Placebo: n=28 1g olive oil Duration: 2 yr.	Inclusion Criteria Narrowing of 30% lumen diameter of a major coronary artery, Total cholesterol <250 mg/dl, Triglycerides <350 mg/dl Exclusion Criteria Congestive heart failure; liver, renal or serious gastrointestinal disease; insulin dependent diabetes mellitus; current cigarette smoking; or alcohol intake >14 drinks/week	1° Outcomes: Change in minimal diameter of coronary artery lesions 2° Outcomes: Clinical coronary events	Minimal diameter of atherosclerotic coronary arteries: ω 3: -0.104 Placebo: -0.138 $P=0.6$ Clinical coronary events: ω 3: 8/29 Placebo: 11/28 EPA in adipose tissue (%) after 2.4 years: ω 3: 0.91 Placebo: 0.20 ω3 fatty acids in adipose tissue (%)	Fish oil treatment for 2 years does not promote major favorable changes in the diameter of atherosclerotic coronary arteries.

Trial (reference)	Treatment Groups	Inclusion/Exclusion Criteria	Outcomes	Reported Findings	Reported Interpretation
SCIMO (von Schacky et al)	<p>Groups: ω3-PUFA: n=89 6 g/d fatty acid mixture (2100 mg EPA and 1260 mg DHA) for 3 months, 3g/d fatty acid mixture (1050 mg EPA and 630 mg DHA) for 21 months</p> <p>Placebo: n=86 1g fatty acid mixture</p> <p>Duration: 2 yr.</p>	<p>Inclusion Criteria Age 18–75 yr. old, hospitalized for diagnostic coronary angiography; stenosis greater than 20% in at least one vessel and revascularization planned or performed in the previous 6 months in no more than one vessel</p> <p>Exclusion Criteria History of cardiac transplantation, hemodynamically relevant left main stenosis or proximal stenosis in all three main vessels, biplane left ventricular ejection fraction less than 35%, VT</p>	<p>1° Outcomes: coronary angiography changes on one film assessed using scores, global for each patient and each segment</p> <p>2° Outcomes: Quantitative coronary angiography and cardiovascular events: sudden death, fatal or nonfatal myocardial infarction, congestive heart failure, and neurologic deficits, coronary bypass surgery</p>	<p>after 2.4 years: ω3: 2.37 Placebo: 0.76</p> <p>Score for coronary segments: ω3: 16/55 regressed, 39/55 progressed Placebo: 7/48 regressed, 41/48 progressed</p> <p>luminal diameter: ω3: $P=0.041$ Minimal $P=0.041$ Placebo: $P=0.05$ -0.38 ± 0.8mm $P=0.05$ between groups</p> <p>Clinical CV events: ω3: 2/111 Placebo: 7/112 $P=0.10$</p> <p>EPA/DHA concentrations (%) in erythrocyte at baseline: to month 24: Placebo: not reported Fish oil group: 0.49/2.86 2 yr.: Placebo: reported as no change Fish oil group: 2.89/6.00</p>	<p>Dietary intake of ω3 fatty acids modestly mitigates the course of coronary atherosclerosis in humans.</p>
DART 2 (Burr et al)	<p>Groups: Fish: n=764 At least two portions of oily fish each week, or to take up to 3 g of fish oil</p> <p>Fruit: n=779 Four to five portions of fruit and vegetables and drink at least one glass of natural orange juice daily, and increase intake oats</p> <p>Fish + Fruit: n=807 A combination of above</p> <p>Sensible eating: n=764 Non-specific advice</p> <p>Duration: 3–9 yr.</p>	<p>Inclusion Criteria Men under 70 yr. old, treated for angina</p> <p>Exclusion Criteria Men who denied ever having exertional chest pain or discomfort; men awaiting coronary artery by-pass surgery; men who already ate oily fish twice a week; men who could not tolerate oily fish or fish oil; men who appeared to be unsuitable on other grounds</p>	<p>1° Outcomes: Coronary mortality and morbidity, all deaths, cardiac deaths, and sudden death</p>	<p>Total deaths: Fish: 283/1571 (18.0%) No fish: 242/1543 (15.7%) HR 1.15, $P=0.13$</p> <p>Cardiac deaths: Fish: 180/1571 (11.5%) No fish: 139/1543 (9.0%) HR 1.26, $P=0.047$</p> <p>Sudden deaths: Fish: 73/1571 (4.6%) No fish: 47/1573 (3.0%) HR 1.54, $P=0.025$</p> <p>Plasma EPA(mg/dl): Baseline: Fish: 3.35 No Fish: 3.19 6 months: Fish: 4.58 No Fish: 3.03</p>	<p>Men advised to eat oily fish, and particularly those supplied with fish oil capsules, had a higher risk of cardiac death. It is unexplained; it may arise from risk compensation or some other effect on patients' or doctors' behavior.</p>
Fish oil on VT/VF in ID (Raitt et al)	<p>Groups: ω3-PUFA: n=100 1.8 g/d fish oil, 756mgEPA and 540mg DHA</p> <p>Placebo: n=100 1.8 g olive oil, 73% oleic acid, 12% palmitic acid, 0% EPA/DHA</p> <p>Median follow up: 2 yr.</p>	<p>Inclusion Criteria Receiving an ICD for an electrocardiogram documented episode of sustained VT or VF; had ICD and ICD therapy</p> <p>Exclusion Criteria Taking class I or class III antiarrhythmic medication; who ate more than 1 fatty fish meal per week or who had taken</p>	<p>1° Outcomes: Time to first episode of ICD treatment for VT/VF</p> <p>2° Outcomes: Time to days with recurrent VT/VF events; Time to first use of antiarrhythmic medication; Change</p>	<p>ICD therapy for VT/VF: ω3: 46%, 51%, and 65% at 6,12, and 24 months Placebo: 36%, 41%, and 59% at 6, 12, and 24 months</p> <p>Subset of VT patients: ω3: 61%, 66%, and 79% at 6,12, and 24 months Placebo: 66%, and 79% at 6,12, and 24 months</p>	<p>Among patients with a recent episode of sustained ventricular arrhythmia and an ICD, fish oil supplementation does not reduce the risk of VT/VF and may be proarrhythmic in some patients</p>

Trial (reference)	Treatment Groups	Inclusion/Exclusion Criteria	Outcomes	Reported Findings	Reported Interpretation
N-3 on CV events in chronic hemodialysis (Sevensson et al)	Groups: ω 3-PUFA: n=103 1.7 g/d 2 capsules Omacor (omega-3-acid ethyl esters 90, 927mg EPA, 773mg DHA) Placebo: n=103 1.7 g olive oil Median follow-up: 1.5 yr.	Inclusion Criteria Established CVD and had been treated with stable HD for at least 6 mo. Exclusion Criteria Participating in other clinical trials Active malignant disease known poor compliance	1° Outcomes: Cardiovascular event or death (a composite of acute MI, angina pectoris, stroke, TIA, peripheral vascular disease and death) 2° Outcomes: acute MI, angina pectoris, stroke, TIA, peripheral vascular disease, and major coronary events	37%, 43%, and 65% at 6, 12, and 24 months <i>P</i> =0.007 Time to recurrent VT/VF events: ω 3: 3.5 days Placebo: 2.2 days <i>P</i> <0.001 ω3 fatty acids RBC concentrations (%): Baseline: ω 3: 1.9 Placebo: not reported 2 yr: ω 3: 8.3 Placebo: reported as no change	Treatment with ω 3-PUFA did not reduce the total number of cardiovascular events and death in high-risk population, but it reduced the number of MI.
				Cardiovascular event or death: ω 3: 62/103 (60.2%) Placebo: 59/103 (57.3%) HR 1.04, <i>P</i> =0.85 Secondary outcomes: MI: ω 3: 4/103 (3.9%) Placebo: 13/103 (12.6%) HR 0.3, <i>P</i> =0.036 No significance in coronary intervention, stroke, TIA, peripheral vascular disease and total death EPA/DHA concentrations (%) in serum at baseline: ω 3: 1.4/4.0 Placebo: 1.6/4.0 2 yr: ω 3: 3.8/5.5 Placebo: 1.6/3.8	
SOFA (Brouwer et al)	Groups: ω 3-PUFA: n=273 2 g/d purified fish oil, 961 mg of omega-3 PUFAs (464 mg EPA, 335 mg DHA and 162 mg other omega-3 PUFA) Placebo: n=273 2 g/d high-oleic acid flower oil Median follow up: 1 yr.	Inclusion Criteria >18 yr. old; At least 1 true, confirmed, spontaneous VT or VF in the preceding year, had an ICD or about to receive one Exclusion Criteria Receipt of an ICD for prophylactic reasons with no true VT or VF in the past year; ICD as a "bridge" to heart transplantation; Refractory supraventricular arrhythmias with rapid ventricular rates; A projected life span of less than 1 year; Use of supplemental ω 3-PUFAs during the past 3 months or consumption of more than 8 g of ω 3-PUFAs from fish or seafood per month	1° Outcomes: An appropriate ICD intervention, or death from any cause 2° Outcomes: Death from any cause ICD intervention for first event	ICD intervention and death from any cause: ω 3: 81/273 (30%) Placebo: 90/273 (33%) HR 0.86, <i>P</i> =0.33 Subgroup: In patients with solely VT: HR 0.91, <i>P</i> =0.17 In patients with prior MI: HR 0.76, <i>P</i> =0.13 EPA concentrations (%) in serum at baseline: ω 3: 1.1 Placebo: 1.09 1 yr: ω 3: 2.3 Placebo: 1.23	There is not strong evidence of a strong protective effect of intake of ω 3-PUFAs from fish oil against ventricular arrhythmia in patients with ICD.
High-dose N-3 on TG and HDL after acute MI (Nilsen et al)	Groups: ω 3-PUFA: n=150 4 g/d ω 3 PUFA ethyl esters, 588 mg	Inclusion Criteria Age>18 yr. old; Verified MI by WHO criteria; Discontinuation of other	1° Outcomes: first cardiac events, including fatal or	First fatal or nonfatal cardiac event: ω 3: 42/123 (28%) Placebo:	A high-dose concentrate of ω 3 fatty acid has no clinical benefit, except a

Trial (reference)	Treatment Groups	Inclusion/Exclusion Criteria	Outcomes	Reported Findings	Reported Interpretation
	EPA and 1176 mg DHA Placebo: n=150 4 g/d corn oil Median follow up: 1.5 yr.	fish-oil supplementation Exclusion Criteria Noncompliance, survival<2yr., gastrointestinal bleeding, thrombocytopenia, liver insufficiency, participation in other study, non-residence in the area	nonfatal cardiac event Cardiac death or resuscitation, Nonfatal cardiac events: recurrent MI, and unstable angina, revascularization, change in serum lipids	36/129 (24%) HR 1.19 NS No significant difference in prognosis between groups for single or combined cardiac events. Change in HDL cholesterol: $\omega 3$: +1.11% mo. Placebo: +0.55%/mo. Triglycerides: $\omega 3$: -1.30%/mo. Placebo: +0.35%/mo. $P<0.0001$	favorable effect on serum lipids.
GISSI-Prevenzione (GISSI-Prevenzione Investigators)	Groups: $\omega 3$-PUFA: n=2836 1 g/d PUFA ethyl esters, 403-510 mg EPA+ 340-447 mg DHA Vitamin E: n=2830 300 mg/d vitamin E $\omega 3$-PUFA + Vitamin E: n=2830 Combination of both Control: n=2828 none Median follow up: 3.5 yr.	Inclusion Criteria MI in the past 3 months, no contraindications to the dietary supplements, no unfavorable short-term outlook (e.g. overt congestive heart failure, cancer, &c). Exclusion Criteria Not specified	1° Outcomes: Cumulative rate of all-cause death, nonfatal myocardial infarction and nonfatal stroke Cumulative rate of CVD death, nonfatal MI and nonfatal stroke 2° Outcomes: Each component of the primary endpoints, The main causes of death	Death, non-fatal MI and non-fatal stroke: $\omega 3$: 715/5666 (12.6%) Non $\omega 3$: 785/5668 (13.9%) RR 0.90 non-fatal MI and non-fatal stroke: $\omega 3$: 547/5666 (9.7%) Non $\omega 3$: 608/5668 (10.8%) RR 0.89 $P=0.053$	Dietary supplementation with $\omega 3$ -PUFA led to a clinically important and statistically significant benefit.
Prevention of fatal arrhythmias by Fish oil (Leaf et al)	Groups: $\omega 3$: n=200 2.6 g/g gelatin capsule of $\omega 3$ -PUFA (EPA +DHA, ratio not reported) Placebo: n=202 Olive oil Median follow up: 1 yr.	Inclusion Criteria Implanted cardioverter/defibrillators (ICDs), sustained VT, or syncope with inducible, sustained VT or VF Exclusion Criteria Not reported	1° Outcomes: Time to first ICD event for VT or VF or death from any cause 2° Outcomes: "probable" ventricular arrhythmias	Time to first ICD event for VT or VF or death from any cause: $\omega 3$: 57/200 (28%) Placebo: 78/202 (39%) RR 0.72, $P=0.057$ Time to first ICD event and probable death from any cause: RR 0.69, $P=0.033$ EPA +DHA (%) in red blood cell at baseline $\omega 3$: 3.4 Placebo: 3.5 1 yr.; $\omega 3$: 7.6 Placebo: 3.5	For individuals at high risk of fatal ventricular arrhythmias, regular daily ingestion of fish oil fatty acids may significantly reduce potentially fatal ventricular arrhythmias.
DOIT (Einvik et al)	Groups: $\omega 3$-PUFA: n=140 2.4 g/d $\omega 3$ -PUFA, 1176 mg EPA + 840 mg DHA Diet: n=139 Dietary counseling and placebo (Corn oil) $\omega 3$-PUFA and Diet: n=142 Combination of $\omega 3$ -	Inclusion Criteria Men 64-76 y, without overt CVD Exclusion Criteria Total cholesterol>8 mmol/l, blood pressure levels >170/100 mmHg, specific disease states or practical causes thought to influence longevity, or compliance	1° Outcomes: All-cause mortality 2° Outcomes: CVD events: fatal or nonfatal sudden cardiac arrest, myocardial infarction, percutaneous	All-cause mortality: $\omega 3$: 14/282 Placebo: 24/281 HR 0.53, $P=0.063$ CVD events: $\omega 3$: 32/282 Placebo: 36/281 HR 0.89, $P=0.641$ Serum EPA/DHA (%) concentrations at	There was a tendency toward reduction in all-cause mortality in the $\omega 3$ -PUFA groups. This suggests a larger trial.

Trial (reference)	Treatment Groups	Inclusion/Exclusion Criteria	Outcomes	Reported Findings	Reported Interpretation
	PUFA and dietary counseling Placebo: n=142 No diet counseling Corn oil Median follow up: 3 yr.		coronary intervention, coronary artery bypass grafting, cerebral stroke, surgery on abdominal aortic aneurysm, or peripheral revascularization procedures	baseline: $\omega 3$: 1.3/1.1 Placebo: 1.6/1.2 3 yr: $\omega 3$: 4.6/4.2 Placebo: 1.7/3.0	
Origin (The ORIGIN Trial Investigators)	Groups: $\omega 3$: n=6281 1 g/d $\omega 3$ -PUFA ethyl esters, 465 mg EPA + 375 mg DHA Placebo: n=6255 Olive oil Median follow up: 6.2 yr.	Inclusion Criteria >50 y, high risk for CVD Impaired fasting glucose, impaired glucose tolerance, or diabetes History of MI, stroke or revascularization, angina with ischemia, ratio of urinary albumin/creatinine >30, left ventricular hypertrophy, >50% stenosis of a coronary, carotid, or lower-limb artery on angiography Exclusion Criteria HbA1c >9%; undergone coronary-artery bypass grafting within the previous 4 years with no intervening cardiovascular event, severe heart failure, or a cancer.	1° Outcomes: Death from CVD 2° Outcomes: Composite outcome of death from CVD, nonfatal MI, or nonfatal stroke death from any cause; and death from arrhythmia	Death from CVD: $\omega 3$: 574/6281 (9.1%) Placebo: 581/6255 (9.3%) HR 0.98, $P=0.72$ No significant effect on rates of major vascular events, death from any cause, or death from arrhythmia Hospitalization for heart failure: $\omega 3$: 331/6281 (5.3%) Placebo: 320/6255 (5.1%) HR 1.02, $P=0.76$	Daily supplementation with 1 g/d of $\omega 3$ fatty acids did not reduce the rate of cardiovascular events in patients at high risk for cardiovascular events.
n-3 PUFA in patients with multiple CVD risk factors (The risk and Prevention Study Collaborative Group)	Groups: $\omega 3$ -PUFA: n=6239 1 g/d PUFA ethyl esters, 403–510 mg EPA+ 340–447 mg DHA Placebo: n=6266 Olive oil Median follow up: 5 yr.	Inclusion Criteria Men and women with: Multiple cardiovascular risk factors (at least 4 of: age >65, male sex, hypertension, hypercholesterolemia, current smoker, obesity, family history of premature CVD disease), clinical evidence of atherosclerotic vascular disease, or any other condition putting patients at high risk Exclusion Criteria Previous myocardial infarction, hypersensitivity to n-3 fatty acids, pregnancy, clinical conditions with poor short-term prognosis	1° Outcomes: Time to death from cardiovascular causes or hospital admission for cardiovascular causes 2° Outcomes: Composite of time to death, nonfatal myocardial infarction, or nonfatal stroke; Composite of time to death from CV causes, nonfatal MI, or nonfatal stroke; death from CHD, and sudden death from cardiac causes	Primary end point: $\omega 3$: 733/6239 (11.7%) Placebo: 745/6266 (11.9%) HR 0.97, $P=0.58$ Same null results for all the secondary end points	Daily treatment with $\omega 3$ fatty acids did not reduce cardiovascular mortality and morbidity in patients with multiple cardiovascular risk factors.
N-3 on Coronary Artery bypass graft patency (Eritsland)	Groups: $\omega 3$ -PUFA: n=317 4 g/day of fish oil concentrate, 2040 mg EPA, 1280 DHA Control: n=293 4g/day olive oil	Inclusion Criteria Patients admitted for coronary artery bypass grafting without concomitant cardiac surgery Exclusion Criteria Medical contraindications to any of the	1° Outcomes: 1-year graft patency 2° Outcomes: Composite of time to death, nonfatal myocardial	Vein graft occlusion rates per distal anastomoses: $\omega 3$: 27% Control: 33% HR 0.77, $P=0.034$ 1 occluded vein graft $\omega 3$: 43%	In patients undergoing coronary artery bypass grafting, dietary supplementation with $\omega 3$ fatty acids reduced the

Trial (reference)	Treatment Groups	Inclusion/Exclusion Criteria	Outcomes	Reported Findings	Reported Interpretation
	patients received aspirin 300 mg/day or warfarin Median F/U: 1 yr.	treatment principles, early perioperative death or complications, presumed lack of compliance, indication for anticoagulation, and administrative reasons	infarction, or nonfatal stroke; Composite of time to death from CV causes, nonfatal MI, or nonfatal stroke; death from CHD, and sudden death from cardiac causes	Control: 51% HR 0.72, $P=0.05$ No significant difference in the occlusion rates of internal mammary artery grafts EPA/DHA concentrations in serum (mg/l): Before bypass operations: ω3: 38/113 Control: 33/112 After bypass operations: ω3: 92/129 Control: 34/107	incidence of vein graft occlusion.

Abbreviations: ω3-PUFA, ω3-polyunsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ALA, alpha linolenic acid; CABG, coronary artery bypass grafting; PTCA, percutaneous transluminal angioplasty; MI, myocardial infarction; LDL, low density lipoprotein; TC, total cholesterol; CVD, cardiovascular disease; PCI, percutaneous coronary intervention; IHD, ischemic heart disease; STEMI, ST-elevation myocardial infarction; VT, ventricular tachycardia; VF, ventricular fibrillation; CV, cardiovascular fibrillation; ICD, implantable cardioverter defibrillator; ID, implantable defibrillators; JELIS, Japan EPA Lipid Intervention Study; DART, Diet and Reinfarction Trial; SU.FOL.OM3, Supplementation en Folates et Omega-3; SCIMO, Study on Prevention of Coronary Atherosclerosis by Intervention with Marine Omega-3 fatty acids; GISSI-Prevenzione, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI); DOIIT, the Diet and Omega-3 Intervention Trial; Origin, the Outcome Reduction with an Initial Glargine Intervention trial.