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**Omega-3 Fatty Acid Supplementation
and Cardiovascular Disease.**

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ABSTRACT

Epidemiological studies on Greenland Inuits in the 1970s and subsequent human studies have established an inverse relationship between the ingestion of omega-3 fatty acids [C_{20-22} ω 3 polyunsaturated fatty acids (PUFA)], blood levels of C_{20-22} ω 3 PUFA and mortality associated with cardiovascular disease [CVD]. C_{20-22} ω 3 PUFA have pleiotropic effects on cell function and regulate multiple pathways controlling blood lipids, inflammatory factors and cellular events in cardiomyocytes and vascular endothelial cells. The hypolipemic, anti-inflammatory, anti-arrhythmic properties of these fatty acids confer cardioprotection. Accordingly, national heart associations and government agencies have recommended increased consumption of fatty fish or ω 3 PUFA supplements to prevent CVD. In addition to fatty fish, additional sources of ω 3 PUFA are available from plants, algae and yeast. A key question examined in this review is whether non-fish sources of ω 3 PUFA are as effective as fatty fish-derived C_{20-22} ω 3 PUFA at managing risk factors linked to CVD. We have focused on ω 3 PUFA metabolism and the capacity of ω 3 PUFA supplements to regulate key cellular events linked to CVD. The outcome of our analysis reveals that non-fish sources of ω 3 PUFA vary in their capacity to regulate blood levels of C_{20-22} ω 3 PUFA and CVD risk factors.

Key words: dyslipidemia, inflammation, endothelial cell, cardiomyocyte, PUFA metabolism, single nucleotide polymorphism.

Abbreviations

ALA, α -linolenic acid; arachidonic acid, ARA; apoB, apolipoprotein B; ChREBP, carbohydrate regulatory element binding protein; CHD, coronary heart disease; CVD, cardiovascular disease; DNL, de novo lipogenesis; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; Elovl, fatty acid elongase; FADS, fatty acid desaturase; FASN, fatty acid synthase; GPR, G-protein receptor; HDL-C, high density lipoprotein-cholesterol; HNF4 α , hepatic nuclear protein 4 α ; ICD, implantable cardioverter defibrillator; LA, linoleic acid; LDL-C, low density lipoprotein-cholesterol; MLX, max-like factor X; MetS, metabolic syndrome; MI, myocardial infarction; MUFA, monounsaturated fatty acid; NEFA, non-esterified fatty acids; NF κ B, nuclear factor κ B; PPAR α , peroxisome proliferator activated receptor α ; PUFA, polyunsaturated fatty acid; PC, prospective cohort studies; randomized clinical trial, RCT; SCD, sudden cardiac death; SDA, stearidonic acid; SREBP-1, sterol regulatory element binding protein-1; T1DM, type 1 diabetes; T2DM, type 2 diabetes; VLDL, very low density lipoprotein.

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Introduction.

Omega-3 (ω 3) polyunsaturated fatty acids (PUFA) represent one of 2 major classes of long chain highly unsaturated fatty acids encountered in the diet. Dietary ω 3 PUFA include α -linolenic acid (**ALA**, 18:3, ω 3); stearidonic acid (**SDA**, 18:4, ω 3), eicosapentaenoic acid (**EPA**, 20:5, ω 3), docosapentaenoic acid (**DPA**, 22:5, ω 3) and docosahexaenoic acid (**DHA**, 22:6, ω 3) (**Figure 1**). The major dietary ω 6 PUFA is linoleic acid (**LA**, 18:2, ω 6). LA and ALA are essential fatty acids; they cannot be synthesized *de novo* in humans and are required for good health (1). These two fatty acids are precursors for C₂₀₋₂₂ ω 3 and ω 6 PUFA found throughout the body.

Considerable interest in the health benefits of very long chain C₂₀₋₂₂ ω 3 PUFA arose in the 1970s when epidemiological studies on Greenland Inuits established that this population had reduced rates of myocardial infarction (**MI**) when compared to individuals in Western countries (2-7). These observations were linked to the high dietary intake of C₂₀₋₂₂ ω 3 PUFA, enrichment of blood lipids with C₂₀₋₂₂ ω 3 PUFA and reduced fasting triglycerides (2, 4, 8). The potential health benefits of ω 3 PUFA stimulated considerable research interest resulting in over 500 clinical trials on ω 3 PUFA (**Table 1**). Over 250 clinical studies have examined the impact of ω 3 PUFA on cardiovascular disease (**CVD**) or risk factors linked to CVD, such as metabolic syndrome (**MetS**), diabetes, obesity, inflammation, dyslipidemia and hypertension. Other equally important areas of ω 3 PUFA human research include visual acuity, cognitive development and decline, cancer prevention and total mortality (7).

This review examines current recommendations for ω 3 PUFA intake, ω 3 PUFA metabolism and their effects on physiological processes relevant to CVD. We also examine several prospective cohort studies (**PC**)[**Table 2**] and randomized clinical trials (**RCT**)[**Table 3**] which report on the benefit or lack of benefit of ω 3 PUFA on cardiovascular health. Finally, we examine various sources of ω 3 PUFA for their capacity to regulate risk factors relevant to CVD. Our goal is to provide up-to-date evidence-based

information on the benefits and limitations of dietary ω 3 PUFA in the management of cardiovascular health.

Current Recommendations for Essential Fatty Acid Consumption. ALA and LA are precursors to ω 3 and ω 6 C₂₀₋₂₂ PUFA, i.e., DHA (22:6, ω 3) (**Figure 2**) and arachidonic acid (**ARA**, 20:4, ω 6), respectively. These fatty acids play structural roles in cells and serve as substrates for β -oxidation and energy production. They also regulate many physiological processes impacting human health, either as non-esterified fatty acids, esterified (membrane-associated) fatty acids or oxidized fatty acids.

The American Heart Association has recommended the consumption of no more than 30% of total energy as fat and 5-10% of total energy as ω 6 PUFA (9, 10). Replacing saturated fat with PUFA (ω 3 & ω 6) has a strong health benefit (11, 12). The amount of ALA required to prevent deficiency symptoms is ~10% of LA, i.e., 0.6 – 1.2% of total energy [1.6 g for men and 1.1 g. for women per day (13)]. Consumption of LA and ALA in the US is estimated to be \leq 10% and \leq 1% total energy, respectively (14).

Up to 10% of the dietary ω 3 PUFA requirement can be provided by EPA or DHA (7, 15, 16). Food sources of ALA include flaxseed, walnuts, canola oil & chia seeds, while food sources of EPA and DHA include fatty fish, like salmon and anchovies or oils derived from fatty fish and krill. Consumption of ~500 mg/day of EPA and DHA (combined) is recommended to lower the risk for CVD. This level can be achieved by the consumption of two 3 ounce portions of fatty fish per week or the consumption of dietary fish or krill oil supplements. The level of ω 3-PUFA consumption should be increased to 1 g/day if CVD is present (7, 15, 17-22).

The Omega-3 Index. In 2004, Harris and Von Schacky (23) introduced a new risk factor for death from coronary heart disease (**CHD**), the omega-3 index. The omega-3 index is defined as the percent of whole blood fatty acids that are the sum of EPA (20:5, ω 3), DPA (22:5, ω 3) and DHA (22:6, ω 3). An omega-3 index of <4% is associated with low cardioprotection while an index of \geq 8% is associated with

high cardioprotection. The rationale for this measure is that fatty acid composition in whole blood and RBC phospholipids parallel fatty acid composition in cardiac muscle phospholipids (24).

The first evidence in support of the relationship between RBC and cardiac muscle fatty acid content was based on studies with heart transplant patients before and after fish oil supplementation. The mole% of ω 3 and ω 6 PUFA in cardiac muscle prior to fish oil supplementation was: LA (9.1%), ALA (0.3%), ARA (9.1%), EPA (0.18%), DPA (0.81%) and DHA (1.5%). After fish oil supplementation [1 g/day of EPA plus DHA for 6 months], the mole% of LA and ARA decreased by ~15%, while ALA, EPA and DHA increased by 33, 333 and 53%, respectively. Changes in cardiac fatty acid profiles paralleled changes in RBC and plasma fatty acid profiles. In a subsequent report, Metcalf, et al (25) examined the accumulation of fatty acids in heart tissue after non-transplant patients consumed 10 ml/day of flaxseed oil (~59% ALA), olive oil (~0.5% ALA), or fish oil (1.8% ALA; 32% EPA, 3.4% DPA and 31.2 % DHA). The time course studies (0-65 days) showed a significant curvilinear increase in EPA + DHA in atrial myocardial phospholipids, beginning as early as 7 days and continuing up to 30 days. Moreover, changes in RBC phospholipid C₂₀₋₂₂ ω 3 PUFA content paralleled C₂₀₋₂₂ ω 3 PUFA myocardial phospholipid. ARA declined in both myocardial and RBC phospholipids in patients receiving fish oil. Patients receiving the flaxseed or olive oil supplement had no significant increase in C₂₀₋₂₂ ω 3 PUFA in myocardial or RBC phospholipids. Both studies established that EPA shows the greatest fold increase in RBC and cardiac muscle phospholipids (24, 25) following fish oil supplementation. DHA, however, remains ~10-fold more abundant than EPA in RBC and myocardial phospholipids. Thus, dietary supplementation with fish oil (C₂₀₋₂₂ ω 3 PUFA), but not dietary flaxseed (ALA), significantly increases cardiac muscle C₂₀₋₂₂ ω 3 PUFA content.

Such studies suggest that the omega-3 index may be a reasonable approach to assess cardiac ω 3 PUFA content and predict future CVD events (26, 27). Sudden cardiac death (**SCD**), for example, is estimated to account for 50% of all deaths from **CHD** (28-30). An epidemiologic study reported that the

risk of SCD was lower in individuals with long-term fish consumption (31). This finding is consistent with studies reporting that a low omega-3 index increased the risk of ventricular fibrillation during acute ischemic phase of a MI and SCD (32, 33).

Several factors will affect the capacity of dietary ω 3 PUFA to alter the omega-3 index and cardiac myocardial phospholipid content; such as baseline values for the omega-3 index and health status. For example, the average omega-3 index in western countries is ~5% and the incidence of SCD is 150/100,000 person-years. In Japan, a country with a high fish consumption, the omega-3 index is >9% and the incidence of SCD is 7.8/100,000 person-years in the general population (34). Most sudden deaths are caused by ventricular arrhythmias in patients with structural heart disease and impaired left ventricular function (35). Primary prevention trials have established a survival benefit in high-risk patients who receive implantable cardioverter defibrillators (**ICD**)(29). Omega-3 PUFA supplementation trials in ICD patients, however, have produced conflicting results; ω 3 PUFA supplementation induced anti-arrhythmic, pro-arrhythmic or no response (29, 36-38). In patients with idiopathic cardiomyopathy, cardiac muscle fatty acid uptake and oxidation decrease, while glucose metabolism increases (39). Patients with Type 1 and type 2 diabetes, a major risk factors for CVD, have increased myocardial fatty acid uptake and oxidation and reduced glucose oxidation (40-42). Such changes in cardiac fatty acid uptake and metabolism reflect the plasticity of cardiac muscle metabolism for fuel utilization for energy production (42). Such findings also suggest that patients with chronic metabolic disease may have altered cardiac muscle fatty acid composition that may not be reflected in RBC phospholipids. As such, the omega-3 index in certain pathophysiological states may not accurately predict cardiac ω 3 PUFA content or health benefit. Factors governing the availability of fatty acids, glucose, lactate or ketone bodies for cardiac muscle energy metabolism (ATP generation) depend on the nutritional supply, hormonal and health status (42). While there has been considerable interest in cardiac lipid metabolism, lipid storage and lipotoxicity, much less attention has focused on cardiac muscle PUFA metabolism.

Conversion of C₁₈ Essential Fatty Acids to C₂₀₋₂₂ PUFA. In addition to dietary sources, heart and blood levels of fatty acids will also depend on essential fatty acid metabolism. The pathway for conversion of the essential fatty acids, LA and ALA to C₂₀₋₂₂ ω₃ and ω₆ PUFA involves two fatty acid desaturases (**FADS1 & FADS2**) and two fatty acid elongases (**Elovl2 & Elovl5**). The final step in DHA (22:6,ω₃) synthesis requires peroxisomal β-oxidation of 24:6,ω₃ (43). The conversion of ALA to DHA is illustrated in **Figure 2**; intermediates in the ω₃ PUFA pathway include SDA, EPA, DPA and C₂₄ ω₃ PUFA. DHA is the major product of this pathway and the major ω₃ PUFA accumulating in cells of all tissues, including cardiac muscle and vascular endothelial cells. The intermediates of the pathway are found in cells, but at levels well below DHA. The abundance of DHA in the heart is not the sole factor in cardioprotection; DHA and other ω₃ PUFA are converted to bioactive fatty acids that impact multiple signaling mechanisms controlling cardiac and vascular function.

Non-esterified fatty acids (**NEFA**) are transported into cells and rapidly converted to fatty acyl-CoAs by fatty acid CoA synthetases. These fatty acyls are either assimilated into neutral lipids (triglycerides and cholesterol esters) or phospholipids or enter metabolic pathways involved in fatty acid oxidation, elongation or desaturation. The desaturases and elongases involved in converting C₁₈ PUFA-CoAs to C₂₀₋₂₂ fatty acyl-CoAs are associated with the endoplasmic reticulum (microsome) (**Figure 2**). The products and some intermediates of the pathway are typically assimilated into complex lipids as phospholipids used in cell membranes. Both FADS1 and FADS2 are required for C₂₀₋₂₂ PUFA synthesis; and FADS2 is considered the rate limiting enzyme in the pathway (44, 45). Fatty acid desaturation requires additional factors; including 1) fatty acyl CoA substrate; 2) NADH; 3) cytochrome B5 and 4) cytochrome B5 reductase. Ablation of FADS1 or FADS2 expression in mice decreases ARA and DHA formation, but increases the formation of C₂₀ elongation products derived from LA or ALA, i.e., 20:2,ω₆ and 20:3,ω₃, (46-48). Eicosadienoic acid (20:2,ω₆) is a pro-inflammatory fatty acid (49).

Seven fatty acid elongase (**Elovl**) subtypes are expressed in humans and rodents (47, 50, 51). Expression of these enzymes is regulated by tissue-specific and developmental factors as well as diet and hormones (52-54). Two of these enzymes, Elovl2 and Elovl5, are involved in the conversion of C₁₈ ω3 and ω6 PUFA to the corresponding C₂₀₋₂₂ ω3 and ω6 PUFA (**Figure 2**). Like fatty acid desaturases, fatty acid elongation occurs in a multi-enzyme complex in the endoplasmic reticulum. The enzymes required for fatty acid elongation include: 1) 3-keto acyl CoA reductase (Elovl); 2) 3-hydroxy acyl CoA dehydratase; and, 3) trans-2,3-enoyl CoA reductase. Fatty acid elongation is similar to the synthesis of fatty acids carried out by fatty acid synthase (**FASN**) and involves the 2-carbon addition to the acyl chain. The key substrates include fatty acyl CoA, malonyl CoA and NADPH. Factors controlling cellular fatty acyl CoA, malonyl CoA or NADPH levels will affect the pace of fatty acid elongation (47). The elongase (Elovl, 3-keto acyl CoA synthase) establishes substrate specificity and is rate limiting. For example, Elovl2 condenses malonyl CoA and C₂₀ PUFA to form the C₂₂ and C₂₄ PUFA, while Elovl5 condenses malonyl CoA and C₁₈ PUFA or C₂₀ PUFA to form C₂₀ and C₂₂ PUFA. Elovl5 does not convert C₂₂ PUFA to C₂₄ PUFA (45, 50, 53). Elovl2 is a key enzyme in DHA synthesis and its cellular abundance determines the capacity of cells to generate DHA. In rodents, cardiac Elovl2 mRNA (52, 55) and the capacity of the heart to convert ALA to DHA is low when compared to other tissues like the liver (56).

Elovl5 not only participates in PUFA synthesis, it also participates in MUFA synthesis, e.g., conversion of 16:1,ω7 to 18:1,ω7 (53, 57). Recent studies suggest that fatty acids derived from the *de novo* lipogenesis (**DNL**), MUFA synthesis and β-oxidation of 18:1,ω9 and appearing in plasma phospholipids, i.e., 18:1ω7, 16:1ω9, are associated with the elevated risk of SCD, but not other CVD events (58). These fatty acids are in low abundance in the diet, but are generated from palmitate (16:0) by the action of fatty acid desaturases (stearoyl CoA desaturase) and elongases (Elovl5 and Elovl6). The expression of all three enzymes is regulated by C₂₀₋₂₂ ω3 PUFA through the control of two transcription factors, peroxisome proliferator activated receptor-α (**PPARα**) and sterol regulatory element binding protein-1 (**SREBP1**) in rodents (52, 53, 59, 60). As such, the expression of these

enzymes is sensitive to regulation by changes in blood insulin, a key regulator of SREBP1 nuclear abundance, and factors controlling PPAR α activity, i.e., fibrates and fatty acids.

Retro-conversion of C₂₂ to C₂₀ PUFA. C₂₂ PUFA are retroconverted to C₂₀ PUFA (61). Primary rat hepatocytes treated with EPA accumulate 22:5, ω 3 due to fatty acid elongation. These cells, however, do not accumulate DHA because of low FADS2 activity. In contrast, rat primary hepatocytes treated with DHA accumulate DHA, DPA and EPA (62). The accumulation of EPA is due to retro-conversion of DHA; a pathway that operates in rodents and humans (62-65). The reaction involves peroxisomal β -oxidation and the reduction of double bond to generate EPA from DHA (**Figure 2**) (61). This reaction is not trivial; it increases plasma and cellular levels of EPA in animals fed DHA (63); and DHA retroconversion to EPA is sufficient to activate PPAR α in primary hepatocytes (62). DPA is formed after retro-converted EPA is elongated by either Elovl2 or Elovl5. The retro-conversion of DHA to EPA & DPA in humans (65), rodents and cells (62) confounds efforts to establish cause and effect relationships between dietary EPA, DPA or DHA and specific physiological or biochemical outcomes.

Regulation of PUFA Synthesis. While DNL is a highly regulated pathway that is sensitive to changes in dietary carbohydrate and hormonal status (66), hepatic enzymes involved in PUFA synthesis are less sensitive to fasting-refeeding or dietary carbohydrate (52, 53, 66). Enzymes involved in DNL, MUFA and PUFA synthesis, however, are regulated by the type and quantity of dietary fat ingested (52-54, 67-69). In rodents the mRNAs encoding FADS1, FADS2, Elovl2, Elovl5 are regulated by SREBP1 and PPAR α (52, 53, 59, 66, 70); both transcription factors are well-established targets of regulation by dietary PUFA (66). Substrate availability also plays a major role in controlling product (C₂₀₋₂₂ PUFA) formation (71). In addition to fatty acyl-CoAs, factors controlling cellular malonyl CoA impacts both DNL and PUFA synthesis. Malonyl CoA is synthesized by acetyl CoA carboxylase (ACC) 1 and ACC2 and degraded by malonyl CoA decarboxylase (72). Malonyl CoA is utilized for DNL and fatty acid elongation; it also inhibits carnitine palmitoyl transferase-1. Enzymes that generate (ACC 1 & 2) and degrade

malonyl CoA (malonyl CoA decarboxylase) are regulated by insulin. Low levels of circulating insulin in Type 1 diabetes lowers ACC1 expression leading to decreased malonyl CoA for DNL and fatty acid elongation (72, 73). Moreover, the ACC inhibitor, soraphen A, inhibits DNL and fatty acid elongation ($IC_{50} \sim 5$ nM) by lowering cellular malonyl CoA. Soraphen A, however, does not block fatty acid desaturation (47). The ratio of 20:4, ω 6 to 18:2, ω 6 in plasma or RBC membrane lipids is often used as a surrogate marker for PUFA conversion *in vivo*. Suppressing fatty acid elongation in the prostate cancer cell line, LnCAP, inhibits LA conversion to ARA resulting in a decrease in the 20:4, ω 6 to 18:2, ω 6 ratio and an increase in the 18:3, ω 6 to 18:2, ω 6 ratio. Thus, changes in 20:4, ω 6 to 18:2, ω 6 ratio can be due to changes in activity of either the desaturases (FADS1, FADS2) or elongases (Elovl2, Elovl5).

Evidence that PUFA synthesis may be regulated in chronic disease come from human and animals studies. In 301 Swedish men (60 yrs), for example, FADS1 products (20:4, ω 6/20:3, ω 6) in adipose tissue and plasma phospholipids were inversely correlated with obesity and insulin resistance, while FADS2 products (18:3, ω 6/18:2, ω 6) showed a positive association (74). A study involving 97 Japanese men (~50 yrs) showed reduced 20:4, ω 6/20:3, ω 6 (FADS1 product), but higher levels of FADS2 product (18:3, ω 6/18:2, ω 6) in obese and MetS patients versus control (75). Feeding C57BL/6J mice a high fat diet (60% energy as fat) induces obesity, insulin resistance, hepatosteatosis (fatty liver) and suppresses of plasma and hepatic 20:4, ω 6 to 18:2, ω 6 ratio by ~60% when compared to lean-euglycemic mice fed a low fat diet (10% energy as fat). The decline in the 20:4, ω 6/18:2, ω 6 ratio in this mouse model was linked to suppressed hepatic expression and activity of Elovl5 and SREBP1 nuclear abundance. Other enzymes involved in PUFA synthesis, like Elovl2, FADS1 or FADS2 were not affected by the high fat diet (53). Changes in hepatic PUFA metabolism alter both hepatic and plasma PUFA composition. The conversion of ALA to DHA is low in the heart versus the liver (56); as such, the liver likely plays an important role in providing DHA to the heart.

In lean rats and obese mice, dietary C₂₀₋₂₂ ω₃ PUFA suppress the expression of Elovl5 and FADS1 (52, 68). The decline in expression of these enzymes correlates with a decrease (≥50%) ratio of 20:4,ω₆ to 18:2,ω₆ in liver and plasma. Dietary C₂₀₋₂₂ ω₃ PUFA are feedback inhibitors of endogenous C₂₀₋₂₂ ω₃ and ω₆ PUFA synthesis, as well as robust inhibitors of DNL (66). Recent studies by Pachikian et al (76) establish the impact of dietary ω₃ PUFA on whole body metabolism. In this study, all ω₃ PUFA were removed from the diet, but ω₆ PUFA were maintained at essential fatty acid sufficient levels. After 3 months on the ω₃ PUFA deficient diet, mice developed hepatosteatosis and insulin resistance. The mechanism was linked to a major decline in hepatic ALA, EPA and DHA, but no change in hepatic LA and ARA levels. Depletion of hepatic ω₃ PUFA lowered fatty acid oxidation, a PPAR α -regulated mechanism, and increased fatty acid synthesis and triglyceride accumulation, SREBP1- and ChREBP/MLX-regulated mechanisms. PPAR α , SREBP1 and the ChREBP/MLX heterodimer are well established targets of C₂₀₋₂₂ ω₃ PUFA control (66). These *in vivo* studies illustrate how ω₃ PUFA deficiency, alone, regulates a series of transcriptional regulatory mechanisms that alter hepatic and systemic lipid metabolism.

Gene Polymorphisms, Blood PUFA and CVD. Genes encoding fatty acid desaturases and elongases involved in PUFA synthesis display single nucleotide polymorphisms (**SNP**). The FADS gene cluster has been most extensively studied; it consists of FADS1, FADS2 and FADS3 on chromosome 11 (77). While the enzymatic functions of FADS1 and FADS2 are well described, FADS3 remains a gene without a clearly defined enzymatic function (78). More than 20 SNPs have been identified in the FADS gene cluster. One (rs174546) is found in the 3' UTR of FADS1, while 12 SNPs are found in or near the FADS2 gene. Much emphasis has been placed on associating specific SNPs with changes in blood C₁₈₋₂₂ ω₃ and ω₆ PUFA content (79, 80) (81-83). A meta-analysis of genome-wide associated studies (**GWAS**) from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium found that genetic variations in FADS1 and FADS2 were associated with high levels of ALA

versus EPA and DPA (84). Of the SNPs identified in the FADS gene cluster, all are found in introns, except rs968567; which is located in the 5' UTR of FADS2 (82). This SNP is linked to changes in FADS2 promoter activity through binding of Elk1, an ETS domain transcription factor (85).

In contrast to the FADS, fatty acid elongase genes are not clustered on a single chromosome; they are encoded from 5 different chromosomes: [Elovl1, chromosome (**Ch**) 1; Elovl2, Ch 6; Elovl3, Ch 10; Elovl4, Ch 6; Elovl5, Ch 5; Elovl6, Ch 4; Elovl7, Ch 5]. Genetic variation in the Elovl2 gene was associated with higher levels of EPA and DPA and lower levels of DHA (84). While Elovl2 and Elovl5 convert C₂₀ to C₂₂ PUFA, only Elovl2 converts C₂₂ to C₂₄ PUFA, a requirement for DHA synthesis (53, 86) (**Figure 2**). While these outcomes agree favorably with the previous INCHIANTI study (87), Aslibekyan et al (88) concluded that genetic variation in Elovl2 and Elovl5 was not associated with serum lipids, biomarkers of systemic inflammation or the risk of MI.

Studies on gene polymorphisms in desaturase and elongase genes are associated with changes in blood C₂₀₋₂₂ ω3 and ω6 PUFA. With the exception of studies on the polymorphism in the FADS2 promoter (85), few studies have established that changes in blood fatty acid profiles are due to an effect on the expression or activity of the enzymes involved in the pathway. Many of the FADS SNPs are in introns or 3' flanking regions. As such, cause and effect relationships between SNPs and activities of specific enzymes remain to be established.

Overview of C₂₀₋₂₂ ω3 PUFA regulation of cell function. Studies on essential fatty acid deficiency and null mutations in the FADS1, FADS2 and Elovl5 genes have established the requirement for this pathway to convert dietary LA and ALA to C₂₀₋₂₂ ω3 and ω6 PUFA and to regulate multiple physiological processes (46, 48, 89-91). FADS2^{-/-} mice or cells expressing low FADS2, do not desaturate LA or ALA. Instead, the elongation products, 20:2,ω6 and 20:3,ω3, are formed (44, 47). The decline in blood and hepatic ARA and DHA in Elovl5^{-/-} mice promotes hepatosteatosis, which correlates with increased nuclear content of SREBP1 (91). Health status, hormones, signaling pathways, diet,

drugs and possibly gene polymorphisms regulate the expression and/or activity of enzymes involved in hepatic PUFA synthesis. Mechanisms controlling cardiac PUFA metabolism are less well defined. Cellular C₂₀₋₂₂ ω3 PUFA content regulates membrane composition, cell signaling pathways originating from cell membranes as well as gene expression (**Figure 4**) (92). Below, is a brief overview of how changes in cellular content of these fatty acids control cell functions relevant to CVD.

Membrane effects of ω3 PUFA. Assimilation of ω3 PUFA into plasma membrane phospholipids have major effects on cell signaling by altering membrane fluidity, lipid raft structure and substrate availability for the synthesis of bioactive oxidized fatty acids (92). DHA plays a key structural role in membrane architecture; this highly unsaturated fatty acid alters membrane fluidity, membrane cholesterol content and lipid raft organization (93, 94). Some receptor systems affected by ω3 PUFA include the toll-like receptors [TRL2 and TRL4] (95-97) and Src-family kinases (Fyn, c-Yes)(98-100). Disruption of raft structure alters downstream signaling events, like NFκB, a key transcription factor controlling the expression of cyclooxygenase-2 (COX2) and multiple genes encoding cytokines and adhesion molecules. The attenuation of expression of these genes represents one mechanism for the anti-inflammatory actions of ω3 PUFA.

Fatty acids also bind G-protein coupled receptors (101, 102). These receptors control cellular levels of second messengers (cAMP and intracellular Ca⁺²) and signaling pathways and are expressed in a tissue-specific fashion. One receptor that has gain attention recently is GRP120; it binds ALA, EPA and DHA (103-106). GRP120 is expressed as a long and short form; its activity is regulated through agonist induced receptor phosphorylation. GRP120 is expressed in macrophage and fat cells; its activation inhibits inflammatory cascades in macrophage, including the control of NFκB and JNK, and reverses insulin resistance in obese mice (104, 107-109).

Oxidation of ω3 PUFA. While C₂₀₋₂₂ ω3 PUFA are β-oxidized in peroxisomes and mitochondria (61), other oxidation mechanisms convert these fatty acids to bio-active mediators of cell function. C₂₀₋₂₂ ω3

PUFA compete with ARA for assimilation into the sn2 position of membrane phospholipids. Dietary C₂₀₋₂₂ ω3 PUFA-mediated suppression of ARA synthesis also contributes to the decline in cellular phospholipid ARA levels. Fatty acids in the sn-2 position are excised from phospholipids by cellular phospholipase-A2 (cPLA2); the excised NEFA is a substrate for cyclooxygenases (COX1 [constitutive] and COX2 [inducible]) and lipoxygenases (LOX-5, -12, -15) and cytochrome P450 enzymes.

Eicosanoids derived from the COX and LOX pathways (110) regulate G-protein-coupled receptors that control signaling pathways in response to changes in intracellular second messengers (cAMP and Ca⁺²). Although EPA and DHA are poor substrates for COX and LOX, these enzymes generate series 3 and series 5 eicosanoids from EPA, respectively. These products have weak agonist activity when compared to the ω6 PUFA products of COX and LOX (110-112). Resolvins and protectins have attracted considerable attention because of their capacity to protect against prolonged inflammation in animals models (113). The E-series resolvins (from EPA) and D-series resolvins (from DHA) are formed by the action of COX2 plus aspirin, while neuroprotectin-D1 is formed by the action of 5-LOX. Resolvins and neuroprotectins regulate inflammatory mechanisms (114, 115).

Non-esterified C₂₀₋₂₂ ω3 and ω6 PUFA are substrates for cytochrome-P450 enzymes (e.g., CYP2C and CYP4A/4F). This is a major mechanism for generating oxidized fatty acids in cells lacking cyclooxygenase or lipoxygenase activity, like hepatic parenchymal cells. CYP2C/2J, for example, synthesize regioisomeric epoxides of ARA, EPA, and DHA, i.e., 14,15-epoxyeicosatrienoic acid, 17,18-epoxy-eicosatetraenoic acid and 19,20-epoxydocosapentaenoic acid, respectively (116, 117). These epoxides are converted to di-hydroxy fatty acids (diols) by epoxide hydrolases. The EPA-derived epoxide, 17(R),18(S)-epoxyeicosatetraenoic acid, has anti-arrhythmic effects; it rapidly and with high potency (EC₅₀~1-2 nM) protects neonatal cardiomyocytes against Ca⁺² overload (118). CYP4A/4F generates ω- and ω-1 hydroxylation products of ARA, EPA and DHA (116, 119). In this case, inhibition of 20-hydroxyeicosatrienoic acid (20-HETE) synthesis protects the kidney from ischemia/reperfusion

damage (120). These CYP450-dependent metabolites of EPA and DHA are found in the heart and they may play a role in C₂₀₋₂₂ ω3 PUFA-linked cardioprotection (117).

ARA, EPA and DHA are oxidized to F2-, F3-isoprostanes and F4-neuroprostanes, respectively, by non-enzymatic processes (121-124). C₂₀₋₂₂ PUFA are susceptible to free radical attack when oxidative stress is increased in cells. Lipid peroxidation is a hallmark of oxidative stress; excessive production of lipid peroxides has been implicated in the pathogenesis of human diseases. The free radical-mediated (hydroxy-, alkoxy-, peroxy- radicals or peroxy-nitrate) lipid peroxidation chain reaction generates products found in cells as membrane lipids and NEFA, in cells, blood and urine. Formation of these oxidized lipids in membranes likely affects membrane fluidity and the function of membrane associated proteins. Feeding *Ldlr*^{-/-} mice high fat-high cholesterol diets promotes hepatic oxidative stress. Including fish oil in this high fat-high cholesterol stimulates the production of F2-, F3-isoprostanes and F4 neuroprostanes that appear in liver and urine (68, 122, 123). F2-isoprostanes activate thromboxane & PGF₂α receptors and platelets; they also induce vasoconstriction in vascular smooth muscle cells. In contrast, F3-isoprostanes do not regulate these receptors, platelets or smooth muscles cells (124, 125). Whether the F3- isoprostanes and F4-neuroprostanes have anti-inflammatory properties like series 3 and 5 eicosanoids remains to be established.

Nuclear effects of ω3 PUFA. Non-esterified fatty acids bind to and regulate the activity of several nuclear receptors including, PPAR (α, β/δ, γ), LXR (α, β), RXR and HNF4α (43, 126). Of these, fatty-acid regulation of the PPAR family has been most extensively studied. In primary rat hepatocytes, EPA significantly induces PPAR_α-regulated target genes. While DHA modestly induces these same genes, ALA and DPA are ineffective. Analysis of hepatocyte NEFA following fatty acid treatment revealed that addition of DHA to cells increases esterified and non-esterified EPA through retro-conversion (62). Co-crystals of PPAR_{β/δ}-EPA, but not PPAR-DHA, have been described (127). In vitro, EPA binds PPAR_α (K_d ~1 μM); in primary rat hepatocytes EPA is the most potent ω3 PUFA activator of PPAR_α.

PUFA suppress the nuclear abundance of several transcription factors involved in carbohydrate and lipid metabolism, including SREBP1, ChREBP, MLX (66, 92, 128, 129). PUFA (both ω 3 and ω 6 PUFA) control the nuclear abundance of SREBP1 by regulating transcription of the SREBP1c gene and the turnover of the SREBP1 mRNA. DHA, however, is the only PUFA that induces proteasomal degradation of nuclear SREBP1 (130). Molecular mechanisms for PUFA control of ChREBP & MLX nuclear abundance remain undefined. C₂₀₋₂₂ ω 3 PUFA control of these transcription factors represents a mechanism for C₂₀₋₂₂ ω 3 PUFA suppression of DNL and triglyceride synthesis.

NF κ B is a major transcription factor regulating expression of genes encoding proteins involved in inflammation (131); some target genes include COX2, cytokines (e.g., TNF α) and chemokines (e.g., MCP1). Omega-3 PUFA suppress the nuclear levels of NF κ B in several model systems (99, 132). NF κ B nuclear abundance is typically regulated by controlling the interaction of NF κ B subunits (p50 and/or p65) with I κ B subtypes (α , β , ϵ , ζ). I κ B sequesters p50/p65 in the cytosol; phosphorylation of I κ B by I κ B-kinase promotes I κ B dissociation from p50/p65, I κ B is degraded in the proteasome and p50/p65 accumulates in nuclei. I κ B-kinase activity is regulated by its phosphorylation status; two kinases controlling I κ B-kinase phosphorylation status include Akt and TAK1. The NF κ B subunits bind promoters as heterodimers of p50/p65 and homodimers of p50; p65 can heterodimerize with other transcription factors, e.g., c/EBP α . Recent studies using the *Ldlr*^{-/-} mouse model of high fat-high cholesterol diet-induced non-alcoholic fatty liver disease established that NF κ B-p50 is more vulnerable to ω 3 PUFA control than NF κ B-p65 (68). While the mechanism for this selective control remains to be established, this outcome suggests that ω 3 PUFA controls a subset of NF κ B-regulated genes.

Targets of ω 3 PUFA regulation in CVD. The pleiotropic effects of ω 3 PUFA on cell function are well established (92). In the cardiovascular system, the type and quantity of ω 3 fatty acid ingested

and cellular ω 3 and ω 6 PUFA content affect blood lipids, inflammation, endothelial cell and cardiomyocyte function (**Figure 3**). The underlying mechanisms for these effects are described below.

Dyslipidemia. Fasting and non-fasting triglycerides have long been associated with CVD (133-135). Monitoring fasting blood triglycerides represents a well-established marker for ω 3-PUFA action. The association between blood triglycerides with CVD, however, has been the subject of debate (136). Miller and colleagues (137) recently reviewed the evidence linking triglycerides to CVD. While triglycerides are not directly atherogenic, they represent an important biomarker for CVD risk. Plasma triglycerides are part of the atherogenic triad consisting of elevated plasma triglycerides, LDL-cholesterol with low HDL-cholesterol. Triglycerides are associated with atherogenic remnant particles derived from chylomicrons and VLDL. Of these, fasting triglycerides are the most responsive to changes in blood and tissue levels of C₂₀₋₂₂ ω 3 PUFA. Fish oil has little or no effect on total blood cholesterol, but affects LDL-C, LDL particle size and HDL-C; these effects are variable and depend on dose and population studied (138, 139). When tested alone, DHA supplements increased HDL-C and LDL particle size, while EPA decreases HDL₃ (138-140).

Both C₁₈ ω 3 and C₂₀₋₂₂ ω 3 PUFA lower triglycerides. However, the effect seen with ALA was equivalent to the effect seen with dietary LA. ALA, however, is not equivalent to C₂₀₋₂₂ ω 3 PUFA in controlling serum lipoproteins in humans (141). Accordingly, pharmaceutical control of blood triglycerides uses C₂₀₋₂₂ ω 3 PUFA, and not ALA. A 1 gram capsule of Lovaza™ (GlaxoSmithKline) contains 465 mg of EPA and 375 mg of DHA, as fatty acid ethyl esters (142). Lovaza™ is prescribed at ~4 g/day for patients with hypertriglyceridemia (143). Amarin Pharma, Inc. recently developed Amr101™, a preparation of EPA as an ethyl ester. At a 4 g/day dose, AMR101 is well tolerated by patients; this dose significantly reduces non-HDL-cholesterol, apolipoprotein B, lipoprotein-associated phospholipase A2, VLDL-cholesterol, and total cholesterol in patients with hypertriglyceridemia (144). EPA and DHA appear to be equally effective at reducing blood triglyceride levels (138).

The hypolipemic effect of C₂₀₋₂₂ ω₃ PUFA is complex and involves control of hepatic VLDL production and clearance of triglyceride-rich lipoprotein [chylomicrons and VLDL] (145-150). A major driver for hepatic VLDL production during fasting is the availability of NEFA for esterification to form triglycerides. In humans and rodents, hepatic NEFA for triglyceride synthesis are derived from DNL, mobilization of adipose tissue lipid and the portal circulation. In fasting, when VLDL secretion is greatest, the major fraction of NEFA entering VLDL is derived from adipose tissue, since DNL is suppressed (151). Dietary C₂₀₋₂₂ ω₃ PUFA lower fasting blood levels of NEFA; dietary C₂₀₋₂₂ ω₃ PUFA also reduce adipose tissue lipolysis (152). The mechanism for this effect is linked to activation of PPAR_γ and suppression of inflammation; it may also involve the GPR120 receptors mentioned earlier. In rodent liver and primary hepatocytes, C₂₀₋₂₂ ω₃ PUFA inhibit DNL and induce fatty acid oxidation (66). In addition to effects of NEFA supply, elevated hepatic DHA induces oxidative stress mechanisms that promote ApoB degradation leading to a decline in VLDL assembly and secretion (149).

ApoCIII is a key apolipoprotein involved in controlling VLDL-triglyceride clearance. ApoCIII inhibits lipoprotein lipase, an enzyme involved in triglyceride hydrolysis (153, 154). Animal studies established that hepatic ApoCIII expression was suppressed by PPAR_α; PPAR_α is activated by fibrates and EPA (62, 155, 156). Human studies have established that fatty fish consumption or increased RBC C₂₀₋₂₂ ω₃ PUFA content is linked to lower triglycerides and plasma ApoCIII (157-159). Interestingly, patients homozygous for the T-455C variant in the ApoC3 promoter are poorly responsive to the ApoC3 lowering effect of ω₃ PUFA (159).

Effects of ω₃ PUFA on endothelial cells and inflammation. Vascular endothelial cells represent a barrier between the blood and the underlying intima and smooth muscle cells in coronary arteries and the peripheral arterial vascular system. Endothelial cells produce several vasoactive substances, including factors that promote vascular smooth cell contraction (endothelins [ET₁], thromboxanes [TXA₂], prostaglandins [PGH₂]) and relaxation (prostacyclins [PGI₂] and nitric oxide [NO]) (160).

Dysfunctional coronary and peripheral endothelial cells are linked to CVD, myocardial infarction, stroke and diabetic retinopathy (161-165). Atherosclerosis is an inflammatory disease of the vascular system (163, 164). Cytokine-mediated induction of adhesion molecules in endothelial cells is an early step in monocyte binding to endothelial cell membranes that enable monocyte to move into vascular intima where they differentiate to macrophages and promote inflammation culminating in plaque formation (163, 166, 167). Controlling endothelial cells expression of adhesion molecules, like ICAM and VCAM, is central to controlling the onset of the inflammatory events linked to atherosclerosis. As noted earlier, C₂₀₋₂₂ ω₃ PUFA have anti-inflammatory capacity through effects on membrane lipid raft structure, NFκB, COX2- and CYP450-generated eicosanoids and docosanoids (22, 168).

In human coronary endothelial cells, DHA regulates eNOS function through Akt activation (165). In mouse coronary and human retinal endothelial cells, C₂₀₋₂₂ ω₃ PUFA suppresses ICAM1 and VCAM1 expression by interfering with NFκB signaling (99, 100, 169, 170). In freshly isolated human retinal endothelial cells, phospholipid DHA levels are very low (~1 mole%). Treating these primary cells with DHA significantly increases phospholipid DHA levels, leading to changes in membrane cholesterol content, lipid raft organization and interaction of Src-kinases (Fyn & cYes) with lipid rafts. The net effect of elevated membrane DHA content is the disruption of NFκB signaling and the down regulation acid sphingomyelinase. The suppression of sphingomyelinase activity is associated with a decline in ceramide, an inflammatory and pro-apoptotic lipid (171).

While cell culture studies have established a strong case for DHA control of inflammatory events in vascular endothelial cells, human studies are less convincing. Egert and Stehle recently reviewed the evidence for ω₃ PUFA regulation of endothelial cells in humans (161). Their analysis covered 33 intervention trials on fasting and/or postprandial endothelial function and included individuals receiving EPA + DHA or ALA supplementation. While dietary ω₃ PUFA may improve endothelial function in individuals with CVD risk factors including overweight, dyslipidemia and type 2 diabetes, the strength of

the evidence for clinical efficacy was not sufficiently strong to make recommendations. A key feature for ω 3 PUFA control of endothelial cell function is the capacity of dietary ω 3 PUFA to increase endothelial cell EPA + DHA content. While the omega-3 index parallels changes in C₂₀₋₂₂ ω 3 PUFA in cardiac muscle, it is unclear if the omega-3 index parallels changes in coronary and vascular endothelial cells.

ω 3 PUFA regulation of cardiomyocyte function. Human studies have established that SCD is reduced in patients consuming fish oil (172-174). The consumption of EPA and DHA from fish oil increases atrial and ventricular EPA and DHA in membrane phospholipids (24, 175, 176). Several clinical trials have reported that ω 3 PUFA were able to: 1) prevent SCD; 2) prevent ventricular arrhythmia in patients with implantable cardioverter; 3) lower occurrences of premature ventricular contractions; 4) reduce heart rate and 5) prevent atrial fibrillation (176, 177). Studies in dogs showed anti-arrhythmic effects of EPA or DHA when administered during ischemia (178).

Approximately 80-90% of sudden cardiac deaths are linked to ventricular arrhythmias and arrhythmias are linked to control of heart contraction, a process involving electrophysiological mechanisms controlling muscle contraction (176, 177, 179, 180). As such, studies with cardiomyocytes have focused on ω 3 PUFA regulation of Na⁺-, Ca⁺²-, K⁺ -channels, ion pumps and the Na⁺/Ca⁺² exchangers (174, 177). The impact on intracellular calcium handling is particularly noteworthy. Stanley et al (179, 181, 182) recently identified the mitochondrial permeability transition pore (MPTP) as a target for ω 3 PUFA regulation. MPTP is a large diameter high conductance, voltage-dependent channel that allows passage of water, ions and molecules up to 1.5 kDa. Mitochondrial accumulation of Ca⁺² induces MPTP opening, whereas Ca⁺² chelation closes the channel. Opening of MPTP is linked to cell death and has been implicated in ischemic injury and heart failure (179, 183). Dietary supplementation with DHA, but not dietary EPA, dramatically increases cardiac mitochondrial phospholipid DHA levels. The accumulation of cardiac mitochondrial membrane DHA content correlates with the suppression of Ca⁺²-induced opening of MPTP. While other ω 3 PUFA, like ALA and EPA, modify mitochondrial membrane

phospholipid composition, they do so to a lesser extent and have less effect on mitochondrial function (179). Thus, DHA control of MPTP may be one mechanism for ω 3 PUFA-mediated protection against heart failure. Other mechanisms include control of oxidized lipids and inflammatory processes mentioned previously.

Prospective Cohort Studies and Randomized Clinical Trials on Dietary ω 3

PUFA and CHD. Several prospective cohort (PC) and randomized clinical trials (RCT) have examined the effect of dietary ω 3 PUFA on cardiovascular health. Representative PC and RCTs are presented in **Tables 2** and **3**. The PC studies examine associations between food consumption (data obtained by a food frequency questionnaire), blood levels of ω 3 PUFA and cardiovascular events, such as, heart failure, atrial fibrillation, CHD, stroke, and coronary calcification (**Table 2**). The six PC studies described in **Table 2** consists of an overall patient population of 31,096 men and women, ranging in age from 20 to 85 years of age; the follow-up period ranged from 5.9 to 17.7 years (184-189). Most patients in these studies were free of CVD. The outcome of these studies was that elevated blood levels of C₂₀₋₂₂ ω 3 PUFA were associated with reduced risk of cardiovascular events. In contrast, blood levels of ALA were not associated with a reduced risk of CVD. The PC studies agreed favorably with several epidemiological studies (2-7) and suggest that dietary C₂₀₋₂₂ ω 3 PUFA are useful in primary prevention of CVD.

The RCTs listed in **Table 3** are secondary prevention trials (172, 173, 190-197). Each trial includes at least 1000 patients (mostly male; age >50 years of age) with prior CVD, e.g., MI. The first Diet and Reinfarction Trial (DART) included patients (2033 males, <56.6 years of age) who had prior myocardial infarctions (MI); these patients were advised to eat or not to eat at least 2 portions of fatty fish/week or take dietary EPA + DHA supplements (172). Men assigned to the fish advice group had increased blood levels of EPA and a 27% and 32% reduction in total mortality and ischemic heart disease, but had a non-significant increase in non-fatal MI. The second DART trial used a similar design, but involved an

older male population (<70 years of age). As with the previous study, blood levels of EPA increased in the group advised to consume fish. In contrast to the previous study, all deaths were not significantly different between the two groups; but cardiac deaths and SCD increased significantly by 28% and 53%, respectively, in the ω 3-PUFA group (190, 191).

The Gruppo Italiano per la Sperimentazione della Streptochinasi nell'Infarto Miocardio Prevenzione (GISSI) Trial (173) was the first large scale RCT (11,334 patients) that had patients consume EPA + DHA supplements (850 – 882 mg/day, as ethyl esters) or a placebo. Like the DART trials, the GISSI patients had a history of heart disease, i.e., (MI, \leq 3 mos). Patients assigned to the EPA + DHA supplement group had a significant reduction in cardiovascular death (18%), coronary death (20%) and sudden cardiac death (24%) when compared to the placebo group. These outcomes support the use of C₂₀₋₂₂ ω 3 PUFA to reduce the incidence of cardiovascular related deaths, including SCD and arrhythmias (174).

The Japanese EPA Lipid Intervention Study (JELIS) trial was the first large clinical study to examine the impact of EPA on CVD (193). This trial enrolled 18,645 Japanese subjects with hypercholesterolemia; 14,981 had no history of CVD. The subjects received 1.8 g/day of purified EPA-ethyl esters in combination with statins or statins alone (control group). After 5 years, the EPA group had a 26%, 24%, 19% reduction in major coronary events, unstable angina and non-fatal coronary events, respectively (198). Patients receiving EPA-ethyl esters showed an increase in plasma EPA, from ~95 μ g/ml to 170 μ g/ml, while both AA and DHA decreased [~9%] (194). The decline in AA and DHA likely reflects the feedback inhibition of PUFA synthesis by elevated EPA ingestion.

The Omega, (195), Alpha-Omega (196) and Su.Fol.OM3 (197) RCTs involved 3704, 4837, 2606 patients with recent cardiovascular events, e.g., MI, coronary or cerebral ischemic events, respectively. The Omega trial had patients consume 850 mg/d EPA + DHA ethyl esters, the Alpha-omega trial had patients consumed margarine supplemented with EPA + DHA without and with ALA. The target

consumption was ~400 mg/day of EPA + DHA combined and 1.9 g ALA/day. The Su.Fol.OM3 trial had patients consume 600 mg/day EPA + DHA ethyl esters. The follow-up period for the Omega, Alpha-Omega and Su.Fol.Om3 trials was 1 year, 3.3 years and 4.7 years, respectively. The outcome of these trials indicated that C₂₀₋₂₂ ω3 PUFA consumption provided no significant benefit to cardiovascular health.

The outcome of these RCTs has lead several investigators to question whether increasing dietary C₂₀₋₂₂ ω3 PUFA significantly benefits cardiovascular health in secondary prevention (199-203). A meta-analysis of 14 randomized, double-blind, placebo-controlled trials involving 20,485 patients with a history of CVD indicated that supplementation with ω3 PUFA did not reduce the risk of cardiovascular events (203). This report, however, was criticized for including small short term analyses that were not designed to detect end points of CVD (204).

A number of factors may have a bearing on the outcome of RCTs in CVD treatment. First, each of the RCTs (**Table 2**) is a secondary prevention trial. As such, dietary EPA + DHA were used to treat or prevent recurrence of pre-existing CVD. Second, the background levels blood EPA and/or DHA are high before beginning the trial; this is particularly the case in the JELIS trail. The Japanese population has a higher fish consumption, higher blood levels of EPA and DHA and lower incidence of CVD than western societies (34, 36). Third, the follow-up for certain trails, like the Omega trial, may have been too short to measure significant effects. Fourth, several of the RCTs included state-of-the-art therapies for CVD in the design, e.g., statins in the JELIS trail and anti-hypertensive, anti-thrombotic and lipid-modifying therapy in the Alpha-Omega trial. In a recent follow-up analysis of the Alpha-Omega trial, the investigators reported that MI patients who were not treated with statins, but receive ω3 PUFA supplementation may have reduced major cardiovascular events. Statin treatment appears to modify effects of ω3 PUFA on the incidence of major cardiovascular events (205). Such studies suggest there may be limitations to the use of ω3 PUFA supplementation in secondary prevention of CVD, particularly in combination with state-of-the-art therapies.

Finally, the VITAL (Vitamin D and omega-3 fatty acid) trial is an ongoing RCT enrolling 20,000 participants [men older (≥ 50 years of age) and women (≥ 55 years of age)]. This double-blind placebo-controlled primary prevention trial will be randomized to receive vitamin D (2000 IU/d), C₂₀₋₂₂ ω 3 PUFA (840 mg/d), both supplements and neither supplement. The study is powered to examine major cardiovascular events, as well as CVD and stroke individually (206). The outcome of the VITAL trial may clarify the utility of C₂₀₋₂₂ ω 3 PUFA in primary prevention of CVD.

The capacity of dietary C₁₈₋₂₂ ω 3 PUFA to manage CVD risk factors. National health associations and government agencies recommend the consumption of fatty fish [two 3.5 oz portions/week] or ω 3 fatty acid supplements [EPA + DHA, 200-2000 mg/day] to lower the risk for CVD (7, 15, 17-22, 139). A recent meta-analysis suggest a daily intake of combined EPA + DHA (200-300 mg/day) is sufficient to provide cardioprotection (207). Dietary sources of ω 3 PUFA are in the form of whole foods or supplements and are derived from cold water seafood like salmon (wild and farm raised), anchovy, menhaden, or krill, as well as plants, yeast and algae. Most human studies have used fish, fish oil or a mix of EPA + DHA ethyl esters to examine associations between ω 3-PUFA consumption and CVD (148, 172, 173, 195, 208, 209). Only one large scale clinical trial, i.e., JELIS trial, has assessed the capacity of EPA in cardioprotection (193, 210). This section examines various sources of dietary ω 3 PUFA for their capacity to control CVD risk factors, including the omega-3 index and inflammatory cytokines.

ω 3 PUFA from farm-raised and wild-caught fish. The original observations by Dyerberg and colleagues on the cardioprotective effects of ω 3 PUFA were based on Greenland Inuits ingesting wild-caught fish enriched in C₂₀₋₂₂ ω 3 PUFA (2-7). Fish, fish oil supplements or EPA + DHA ethyl esters have been used in most CVD prevention trials (**Tables 2 and 3**).

While farm-raised fish provide an alternative source of C₂₀₋₂₂ ω₃ PUFA, levels of these fatty acids in farm-raised fish are not as high as in wild-caught fish (211, 212). Efforts to increase C₂₀₋₂₂ ω₃ PUFA in farm-raised Atlantic Salmon (*Salmo salar* L.) involve feeding fish ω₃-PUFA supplements (212). For example, farm-raised salmon were fed diets enriched in fish oil, canola oil (enriched in ALA) or echium oil (*Echium plantagineum*). *E. plantagineum* oil seeds are enriched in ALA (18:3,ω₃), γ-linolenic acid (18:3,ω₆) and stearidonic acid (SDA, 18:4,ω₃). When compared to farm-raised salmon fed fish oil diets, fish fed the diets enriched in either canola oil (enriched in ALA) or echium oil (enriched in SDA) did not accumulate comparable levels of DHA. Fish, like humans, require a dietary source of DHA to significantly increase cellular DHA.

While ω₃ PUFA in fish or plants are as triglycerides, ω₃ PUFA in supplements are supplied as free fatty acids, fatty acids-ethyl esters, triglycerides or re-esterified triglycerides, most commonly in gel capsules (213, 214). The Food and Drug Administration requires the dietary supplement industry to list the contents of the capsule and the source of the omega-3 fatty acid. The percent of ω₃ PUFA in supplements relative to other fatty acids and the ratio of EPA to DHA in these preparations are highly variable. The information contained on these labels is helpful in determining one's daily consumption of ω₃-PUFA.

ω₃ PUFA from plants, algae or yeast. ALA is an essential fatty acid and it is the major plant-based ω₃ PUFA. Dietary supplements of ALA are derived from flaxseed or chia seeds or oils produced from these sources. Chia seeds (*Salvia hispanica*) have high levels of ALA; ~50-60% of the plant oil is ALA. The amount of ALA required to prevent deficiency symptoms is ~10% of LA, i.e., 0.6 – 1.2% of total energy [1.6 g for men and 1.1 g. for women per day] (13). ALA intakes higher than 1.5 g/d are important for human health (15). Humans, however, convert 0.2 to 9% of ingested ALA to DHA (215-218). The conversion of ALA to DHA in men is lower in ~0.1% than women, ~9%. This may relate to the decreased β-oxidation of ALA in women or increased expression of FADS2 (219). Despite its low level of

conversion to DHA, ALA does not accumulate in blood or most cells; ALA is channeled to β -oxidization or adipose storage (215, 220).

ALA is added as a supplement to several foods, like margarine. A recent study compared the capacity of margarines supplemented with ALA, EPA or DHA ethyl esters to affect the omega-3 index. Seventy-four health men and women were randomly assigned to have a total intake of 4.4, 2.2 and 2.3 g/day of ALA, EPA or DHA, respectively (221). In contrast to the group consuming the EPA- and DHA-supplemented margarines, the group consuming the ALA-supplemented margarine diet did not have an increased omega-3 index. Earlier studies (25) established that dietary ALA supplementation (10 mg/day, flaxseed oil ~60% ALA) did not significantly affect the omega-3 index or myocardial EPA or DHA content.

Geleijnse et al (222) recently reviewed the clinical evidence on the capacity of dietary ALA supplementation to impact cardiovascular health. Short term trials (6-12 weeks) in healthy patients showed no consistent effects of dietary ALA supplementation on blood lipids, LDL protein oxidation, Lp(a), ApoA1 or ApoB. Observational studies provide evidence for a protective effect against non-fatal MI. However, no protective association was observed between ALA status and the risk of heart failure, atrial fibrillation or SCD. Effects of ALA on inflammatory markers linked to CVD were inconsistent (223, 224). A prospective cohort analysis (**Table 2**) examined the association of ALA intake with 10-year incidence of coronary heart disease and stroke in the Netherlands. The analysis involved over 20,000 men and women between the ages of 20-65. ALA intake was not associated with the incidence of coronary heart disease, but low ALA intake may be a risk factor for stroke (187). The Alpha-Omega trial (196) (**Table 3**) reported that ALA supplementation at 2 g/d did not significantly reduce the rate of major cardiovascular events in patients with prior MI and who were treated with state-of-the-art therapies. The large scale PC studies (**Table 2**) and RCTs (**Table 3**) provide little support for the use of dietary ALA supplementation, over that required to maintain essential fatty acid sufficiency, for cardioprotection (196, 222).

Stearidonic Acid (SDA, 18:4, ω 3). The poor conversion of ALA to DHA in humans is well established. The problem is metabolic; the rate limiting step in very long chain PUFA synthesis is the initial desaturation step catalyzed by FADS2; FADS2 desaturates ALA to form stearidonic acid (SDA, 18:4, ω 3) (**Figure 2**). To overcome this metabolic bottleneck, investigators have examined the capacity of dietary SDA to be converted to C₂₀₋₂₂ PUFA in humans (225). Generally healthy humans who consumed 0.75 g SDA/d for 3 weeks followed by 1.5 g SDA/d for 3 weeks had significantly higher levels of EPA and DPA (22:5, ω 3) in blood phospholipids and lowered blood levels of two cytokines (TNF α and IL1 β). Dietary SDA, however, did not increase blood levels of DHA.

Soyamega™, developed by Monsanto and Solae, LLC (226), is a SDA-enriched oil derived from genetically modified soybeans. Several studies have examined the capacity of Soyamega™ to increase blood C₂₀₋₂₂ ω 3 PUFA. Each study has yielded essentially the same outcome: SDA is converted to EPA and DPA, but it does not increase blood levels of DHA (225, 227-232). FADS2 not only desaturates ALA to form SDA, it also desaturates 24:5, ω 3 to form 24:6, ω 3; 24:6, ω 3 is subsequently β -oxidized in the peroxisome to form DHA (22:6, ω 3) (**Figure 2**). Based on our understanding of the omega-3 index, and the effect of SDA on blood lipids, dietary SDA is expected to increase cardiac and endothelial cell EPA and DPA, but not DHA. Thus, SDA ingestion increases the omega-3 index, as EPA and DPA, but not as DHA. Finding that dietary SDA lowers some inflammatory markers suggest this dietary source of ω 3 PUFA will be useful in modulating inflammatory factors linked to CVD. DHA, however, has a greater capacity to regulate cardiac mitochondrial MPTP than other ω 3 PUFA (179, 181, 182). This is important for ventricular fibrillation and SCD (179, 182, 233-235). Whether SDA supplementation can impact SCD remains to be determined through more investigation.

Eicosapentaenoic Acid (20:5, ω 3, EPA). The JELIS trial was the first large clinical study to examine the impact of EPA on CVD (193). The EPA group had a 19% risk reduction in major coronary events, including SCD, unstable angina, and coronary revascularization (198) (**Table 3**). Patients receiving EPA-

ethyl esters showed an increase in plasma EPA, from ~95 µg/ml to 170 µg/ml, while both AA and DHA decreased ~9% (194). The reduction in non-fatal coronary events was achieved without significant increase in blood DHA. As noted earlier, the Japanese population has a high fish consumption and high baseline blood levels of EPA and DHA and lower rates of SCD than western societies (34).

Amarin Pharma, Inc (Mystic, Conn.) has developed a highly enriched form of EPA-ethyl esters (AMR101, >96% EPA and no DHA) for clinical use. A multi-center, placebo-controlled, randomized, double blind 12-week study examined the effects of AMR101 in 229 diet-stable patients with fasting triglycerides >500 mg/dl and <2000 mg/dl (with or without statin therapy). Patients received AMR101 at 4 g/day, 2g/day or placebo for 12 weeks. At the 4 g/d dose, AMR101 significantly reduced plasma triglyceride 33% (n = 76); at the 2 g/d dose, fasting triglycerides were reduced by 19.7% (p < 0.001). AMR101 also significantly reduced non-HDL-cholesterol, apolipoprotein B, lipoprotein-associated phospholipase A(2), VLDL-cholesterol, and total cholesterol, and it did not increase LDL-C (144).

Non-prescription EPA is available as a genetically engineered product derived from yeast; EPA in this product is as triglyceride (Newharvest-EPA™, Dupont, Wilmington, DE) (236). EPA represents ~60% of all fatty acyls in a 1 g capsule. This product contains little or no ALA, SDA, DPA or DHA. Based on the results of the JELIS trial and the short term AMR101 study described above, EPA is likely to be useful in improving lipid profiles and lowering inflammation; two important clinical targets for improving cardiovascular health. Because EPA inhibits PUFA synthesis, the impact of AMR101 and Newharvest-EPA on blood and cellular levels of DHA needs to be assessed.

Docosahexaenoic Acid (22:6,ω3, DHA). Algae producing high levels of DHA as triglycerides were developed by Martek Biosciences (Columbia, MD) in the 1980s. In DHASCO™, approximately 40% of all fatty acyls are DHA with little or no ALA, SDA, EPA or DPA. MATK-90™, also produced by Martek Biosciences, contains DHA-ethyl esters where ~90% of the fatty acyls are DHA. DHASCO is added as a supplement to many commercially available foods. Despite the availability of DHASCO for many years,

few human studies on CVD risk factors have been reported. In one report, two CVD risk factors, i.e., fasting blood triglycerides and small dense LDL, were reduced in hypertriglyceridemic men consuming 3 g/d of DHA for 45 days (237). Unlike, dietary ALA, SDA or EPA, dietary DHA significantly increases blood and tissue levels of DHA. Dietary DHA also significantly increased blood and tissue levels of EPA and DPA in rodents and humans, likely through retroconversion (**Fig. 2**) (62-65).

As noted above the capacity of the dietary ω 3 PUFA supplementation to increase cardiac muscle DHA may be important in the prevention of ventricular fibrillation and SCD (181, 233, 238). Fatty fish, fish oil containing EPA + DHA or the DHA-only supplements (e.g., DHASCO, MATK-90) represent the only routes to significantly increase blood and tissue DHA content. Because dietary DHA is reconverted to EPA and DPA in humans (65), DHA supplementation represents an alternative to fish oil to increase blood and tissue EPA, DPA and DHA content.

Concluding Remarks. Epidemiological, PC and RCT studies, meta-analyses, as well as several expert reviews and commentaries generally support the use of dietary sources of ω 3 C₂₀₋₂₂ PUFA in primary and secondary prevention of CVD (**Table 2 and 3**) (20, 139, 199-204, 207, 239). The RCTs, however, suggest there may be limitations to the use of supplementary C₂₀₋₂₂ ω 3 PUFA in prevention of future cardiovascular events. The patient's health status, populations with a high fish consumption or the concurrent use of state-of-the-art therapies in CVD treatment, e.g., statin therapy, influence outcomes in C₂₀₋₂₂ ω 3 PUFA therapy (205). At a minimum, individuals should consume sufficient C₂₀₋₂₂ ω 3 PUFA to meet the current recommendations for primary prevention of CVD, i.e., 200-300 mg/day of combined EPA + DHA (207, 239), either as fatty fish or as C₂₀₋₂₂ ω 3 PUFA supplement.

Some individuals are unable or unwilling to eat fish or products derived from fish, e.g., vegetarians, vegans or individuals with seafood allergies. As such non-fish sources of ω 3 PUFA from plants (for SDA), yeast (for EPA) or algae (for DHA) are available to increase, blood and tissue levels of EPA and DHA. While ALA is poorly converted to DHA, both dietary SDA (225, 227-232) and EPA (194) increase

blood EPA levels. Increased blood EPA is associated with lower inflammatory cytokines (TNF α , IL1 β), improved blood lipid biomarkers (240) and reduced risk of some CVD events (**Table 3**).

As discussed above, increasing dietary ALA, over and above the essential daily requirement, or consumption of SDA or EPA, fails to significantly increase blood DHA. Two mechanisms account for this problem: 1) the intrinsic biochemical properties of FADS2 limit the conversion of ALA (18:3, ω 3) to SDA (18:4, ω 3) and the conversion of 24:5, ω 3 to 24:6, ω 3 (**Figure 2**); and 2) C₂₀₋₂₂ ω 3 PUFA suppress expression of the FADS1, FADS2 and Elovl5 genes limiting C₂₀₋₂₂ ω 3 and ω 6 PUFA synthesis (52, 68). DHA plays a major structural role in cell membranes and a regulatory role as a substrate for oxidized bioactive fatty acids. In cardiomyocytes, DHA prevents Ca⁺²- and MPTP-dependent arrhythmias and heart failure (179, 181, 182). In endothelial cells, DHA suppresses expression of adhesion molecules, a key early event in atherosclerosis (99, 100, 169, 170). The limited capacity of ALA, SDA and EPA supplements to significantly increase blood DHA suggest cardiac muscle and endothelial cell DHA may not increase with ALA, SDA or EPA supplementation. Moreover, the impact of SNPs and chronic diseases that affect PUFA metabolism are additional factors that may impact cellular levels of DHA. Since DHA is retroconverted to EPA and DPA in humans (65), DHA supplementation is a logical mechanism to increase blood and cellular levels of EPA, DPA & DHA. Over-the-counter sources of EPA (Newharvest-EPA [DuPont]) and DHA (DHASCO, [Martek Biosciences]) and pharmaceutical quality EPA (Amr101™, [Amarin Pharma]), DHA (MATK-90™, [Martek Biosciences]) and EPA + DHA (Lovaza™, GSK) will enable investigators to establish which formulation of ω 3 PUFA confers the greatest cardioprotection with the fewest off-target effects.

Finally, the cardiovascular system is not the only site of ω 3 PUFA action. The brain and retina have the highest levels of DHA of all cell types; ~50% of all acyl chains contain DHA. Depletion of the body of ω 3 PUFA leads to the replacement of DHA with 22:5, ω 6. This minor change in fatty acid structure

(22:6, ω 3 to 22:5, ω 6) in ω 3 PUFA deficiency is sufficient to alter visual acuity and cognitive function (241-243).

TABLES

Table 1: Clinical Trials on ω 3 Fatty Acids.

	<u># of Trials</u>
Cardiovascular Disease and Stroke	2340
Omega 3 Fatty Acids	534
<u>Omega 3 Fatty Acids and:</u>	
CVD or Stroke	110
Metabolic syndrome	16
Diabetes or Obesity	12
Inflammation	97
Dyslipidemia	55
Hypertension	10
Total	290

Source: www.clinicaltrials.gov.

Queries: Cardiovascular disease & Stroke; omega 3 fatty acids and:.

Table 2: Prospective Cohort Studies on ω 3 PUFA and Clinical Cardiovascular Events^a.

Study [Year] (Ref)	Population	Design	Follow-Up Years	Key Outcome
Yamagishi et al [2008] (184)	ARIC Study USA, Minneapolis 3592 white men and women from Age: 45-64	Association of plasma cholesterol ester fatty acids with incident heart failure (HF)	14.3	<u>110 men and 87 women developed heart failure (HF)</u> Hazard Ratio (95% CI); [P for trend]: LA status and HF: 0.54; [0.001] ALA status and HF: 0.99; [0.81] ARA status and HF: Men: 1.37; [0.28]; Women: 0.43 [0.02] EPA status and HF: 1.37; [0.26] DHA status and HF: Men: 1.3, [0.47]; Women: 0.21 [0.001] <i>ARA and DHA may reduce risk of HF, particularly in women.</i>
Virtanen et al [2009] (185)	Kuopio Ischemic Heart Disease Risk Factor Study Finland: 2174 men Age: 42-60	Association of serum ω 3 PUFA with incident atrial fibrillation	17.7	<u>240 men developed atrial fibrillation (AF)</u> Reduction of AF; [P for trend]: EPA + DPA + DHA: 35% [0.07] versus lowest quartile ALA: Serum ALA was not associated with the incidence of AF <i>Serum C_{20:22} ω3 PUFA, but not ALA, was inversely associated with AF.</i>
Pottala, et al [2010] (186)	Heart and Soul Study USA: San Francisco 956 Patients with a history of CHD Age: 65-69	Association of CHD with Blood levels of EPA + DHA Median blood level of EPA + DHA = 3.6%	5.9	237 deaths among 956 patients; <u>Below median: <3.6% EPA + DHA (N = 478)</u> <u>Above median: \geq3.6% EPA + DHA (N = 478)</u> <u>Below Median versus Above Median (Hazard Ratio; 95% CI)</u> 27% reduced risk of CHD [HR = 0.73; 95% CI = 0.56-0.94] <i>Blood EPA + DHA levels were inversely associated with total mortality in patients with stable CHD.</i>
De Goede, et al [2011] (187)	The Netherlands: 20,069 men and women Generally healthy Age: 20-65	Association of ALA Intake (FFQ) with incident CHD (CVD and stroke)	8-13	<u>280 CHD events (19% fatal); 221 strokes (4% fatal)</u> Intake of energy-adjusted ALA in quintiles ALA intake range Q1: 1.0 g/d to Q5: 1.9 g/d For incident CHD: HR 0.89 – 1.01 in Q2-Q5 versus Q1 For incident stroke: HR: 0.65, 0.49, 0.53 and 0.65 for Q2-Q5, respectively. <i>ALA was not associated with incident CHD.</i>
Mozaffarian, et al [2011] (189)	Cardiovascular Health Study; 4 US communities; 2735 adults free of prevalent heart disease. Age: >65	Plasma phospholipid profiles and congestive heart failure (CHF)	14	<u>555 cases of CHF during 26,490 person-years</u> Highest versus lowest quartile hazard ratio (95% CI) EPA: 0.52 (0.38 - 0.72; P-trend = 0.001) DPA: 0.76 (0.56 - 1.04; P-trend = 0.057) DHA: 0.84 (0.58 - 1.21; P-trend = 0.38) Total ω 3 PUFA: 0.7 (0.49 – 0.99; P-trend = 0.062) <i>Circulating individual and total ω3 PUFA were associated with lower incidence of CHF in older (>65 yrs) adults.</i>
Heine-Broring, et al [2010] (188)	Rotterdam Study 1570 Men and women Asymptomatic for CVD Age: 62-85	Dietary ω 3 fatty acid via FFQ and coronary calcification	7	Prevalence ratio (PR) for coronary calcification versus fish intake <u>For fish intake > 19 g/d versus no fish intake</u> Mild/moderate calcification: PR, 0.87 (95% CI = 0.78) Severe calcification: PR: 0.88 (95% CI = 0.74) <i>Weak inverse association between fish intake and coronary calcification.</i>

^aARIC, atherosclerosis risk in communities; Q, quintile; HR, hazard ratio; PR, prevalence ratio; CI, confidence interval; FFQ, food frequency questionnaire.

Table 3: Randomized Clinical Trials (RCT) of ω 3 PUFA and Clinical Cardiovascular Events.^a

Trial [Year] (Ref)	Groups	Intervention	Years of Follow-Up	Blood EPA and/or DHA		Events
				ω 3 PUFA versus Placebo		ω 3 PUFA versus Placebo
DART-1 [1989] (172, 190)	1015, Male, Fish advice 1018, Male, No fish advice Recovered from MI 100% Male, Mean Age <56.5 yr	Advice: Increase fatty fish consumption or take fish oil supplement versus usual diet	2	EPA: 0.59 vs 0.46 mol%		<u>All Deaths</u> N = 94 (9.3%) vs N = 130 (12.8%); P <0.05 <u>IHD Deaths</u> N = 78 (7.7%) vs N = 116 (11.4%), P < 0.01 <u>Non-Fatal MI</u> N = 49 (4.8%) vs N = 33 (3.2%), P = NS
DART-2 [2003] (190, 191)	1571 Male, Fish advice 1543 Male, No fish advice Recovered from MI 100% Male, Age <70 yr	Advice: Increase fatty fish consumption or take fish oil supplement (3 g MaxEPA/day) versus usual diet	3-9	EPA: 45.8 vs 30.3 μ g/ml		<u>All Deaths</u> N = 283 (18.0%) vs N = 242 (15.7%); P = 0.8 <u>Cardiac Deaths</u> N = 180 (11.5%) vs N = 139 (9.0%); P = 0.02 <u>Sudden Cardiac Deaths</u> N = 73 (4.6%) vs N = 47 (3.0%); P = 0.02
GISSI [1999] (173)	5666 ω 3 PUFA Group Male, 85% 5668 Placebo Group Male, 85% (Prior MI, \leq 3 mos)	EPA + DHA ethyl ester EPA + DHA (1:2) 850-882 mg/day versus placebo [-/+ Vit. E; 300 mg/day]	3.5	ND		<u>Cardiovascular Deaths</u> : [RR; 95% CI] N = 291 (5.1%) vs N = 348 (6.2%) [0.83; 0.71-0.097] <u>Coronary Deaths</u> N = 214 (3.8%) vs N = 265 (4.7%), [0.80; 0.67-0.96] <u>Sudden Cardiac Deaths</u> N = 122 (2.2%) vs N = 164 (2.9%), [0.74; 0.58-0.93]
JELIS [2007] (193, 194)	9326, EPA + Statin Group Mean age 61 Male, 32% 9319, Statin Only Group Mean age 61; Male, 31%	EPA ethyl ester (1.8 g/d) +/- statin Blood cholesterol \geq 6.5 mmol/l	4.6	EPA: 170 vs 95 μ g/ml DHA: 154 vs 165 μ g/ml		<u>Major Coronary Events</u> N = 262 (2.8%) vs N = 324 (3.5%); (P = 0.011) <u>Unstable Angina</u> N = 147 (1.6%) vs N = 193 (2.1%); (P = 0.014) <u>Non-fatal Coronary Events</u> N = 240 (2.6%) versus N = 297 (3.2%); (P = 0.015)
GISSI-HF [2008] (192)	3494, ω 3 PUFA Group Male, 85% 3481 Placebo Male, 85%	EPA + DHA ethyl ester EPA + DHA (1:2) 850-882 mg/day versus placebo	3-9	ND		<u>Cardiovascular Deaths</u> [HR; 95% CI] N = 712 (20.4%) vs N = 765 (22.0%)[0.90; 0.81-0.99] <u>Sudden Cardiac Deaths</u> N = 307 (8.8%) vs N = 325 (9.3%)[0.93; 0.79-1.08] <u>Fatal and non-fatal Stroke</u> N = 122 (3.5%) vs N = 103 (3.0%) [1.16; 0.89-1.51]
Omega (2010)	1919, Omega Group Mean age 64; Male, 75% 1885, Control Group Mean age: Male, 74% Recent MI (\leq 2wk prior)	EPA + DHA (1:1.2) 850 mg/d, ethyl ester versus olive oil (1g/d)	1	ND		<u>Total Mortality</u> N = 88 (4.6%) vs N = 70 (3.7%); (P = 0.18) <u>Sudden Cardiac Death</u> N = 28 (1.5%) vs N = 29 (1.5%); (P = 0.84) <u>Major adverse cerebro- or cardiovascular enents</u> N = 182 (10.4%) vs N = 149 (8.8%); (P = 0.1) ICD-terminated ventricular tachycardia or fibrillation in survivor N = 9 (0.5%) vs N = 2 (0.1%); (P = 0.06)
Alpha Omega [2010] (196)	2404, EPA-DHA Group Mean Age 69; Male, 78% 2433, ALA or placebo group Mean age: 69 Male, 78% (MI within prior 10 yrs)	Margarine supplemented with EPA + DHA. Targeted daily intake: ~ 400 mg EPA + DHA Placebo Group -/+ 2 g ALA per day Patients were also on state-of-the-art anti-hypertensive, anti-thrombotic and lipid lower therapy	3.3	ND		EPA-DHA group vs Placebo or ALA group <u>Total Mortality</u> N = 186 (7.7%) vs N = 184 (7.6%); (P = 0.92) <u>Major cardiovascular Events</u> N = 336 (14%) vs N = 335 (13.8%); (P = 0.93) <u>Death from cardiovascular events</u> N = 80 (3.3%) vs N = 82 (3.4%); (P = 0.75) <u>Death from coronary heart disease</u> N = 67 (2.8%) vs N = 71 (2.9%); (P = 0.75) <u>Incident cardiovascular disease</u> N = 67 (2.8%) vs N = 71 (2.9%); (P = 0.75) <u>Ventricular-arrhythmia-related events</u> N = 67 (2.8%) vs N = 74 (3.0%); (P = 0.55)

Su.Fol.Om3 [2010] (197)	1253, EPA + DHA group Mean age 60; 79% male Recent history of prior Coronary or cerebral ischemic event	EPA + DHA (2:1) 600 mg/day versus Vit.B group or Placebo	4.7	EPA 2.1 vs 1.2 % of total DHA 3.1 vs 2.7 % of total	<u>Total Mortality</u> N = 58 (4.7%) vs N = 59 (4.7%); (P = 0.33) <u>Major cardiovascular events</u> N = 81 (6.5%) vs N = 76 (6.1%); (P = 0.64)
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^aRCT, randomized clinical trial; OM3FA, omega-3 fatty acids; DART: diet and reinfarction trial; IHD: ischemic heart disease; MI: myocardial infarction; NC: no change; ND: not determined; RR, relative risk; HR, hazard ratio; 95% CI: 95% confidence interval); SCD: sudden cardiac death; CHD: coronary heart disease; GSSI: Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardio; JELIS: Japan Lipid Intervention Study; Su.Fol.Om3: supplementation with folate, vitamins (B6 and B12) and/or ω3 fatty acids [B vitamins: 5-methyltetrahydrofolate, 560 μg; vitamin B6, 3 mg; vitamin B12, 20 μg].

FIGURE LEGEND

Figure 1: Structures of dietary ω 3 and ω 6 polyunsaturated fatty acids.

Panel A: C₁₈ ω 3 and ω 6 PUFA; Panel B: C₂₀₋₂₂ ω 3 and ω 6 PUFA.

Figure 2: Pathways for C₁₈ PUFA conversion to C₂₀₋₂₂ polyunsaturated fatty acid (PUFA)

synthesis. The pathway illustrates the conversion of the essential fatty acid, α -linoleic acid (ALA, 18:3, ω 3) to the end product, docosahexaenoic acid (DHA, 22:6, ω 3). The enzymes involved in this pathway include two fatty acid desaturases (FADS1, FADS2), two fatty acid elongases (Elovl2, Elovl5) and peroxisomal β -oxidation (p - β Ox). Non-esterified fatty acids are converted to fatty acyl-CoAs by fatty acid synthetases; the fatty acids progress through the pathway as fatty acyl CoAs. Humans and rodents ingesting essential fatty acid sufficient diets accumulate DHA in blood and tissues. Sources of dietary ω 3 PUFA [ALA, stearidonic acid (SDA, 18:4, ω 3), eicosapentaenoic acid (EPA, 20:5, ω 3) and DHA] include plants, fish, yeast and algae. Details of the pathway are presented in the text.

Figure 3. Overview of dietary C₁₈₋₂₂ ω 3 PUFA effects on cardiovascular disease.

Figure 4: Overview of C₂₀₋₂₂ ω 3 PUFA regulation of cell function.

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