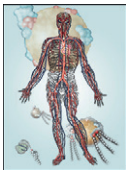

Thematic Review Series: New Lipid and Lipoprotein Targets for the Treatment of Cardiometabolic Diseases

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 [$C_{20–22}$ ω3 polyunsaturated fatty acids (PUFA)], blood levels of $C_{20–22}$ ω3 PUFA, and mortality associated with cardiovascular disease (CVD). $C_{20–22}$ ω3 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 ω3 PUFA supplements to prevent CVD. In addition to fatty fish, sources of ω3 PUFA are available from plants, algae, and yeast. A key question examined in this review is whether nonfish sources of ω3 PUFA are as effective as fatty fish-derived $C_{20–22}$ ω3 PUFA at managing risk factors linked to CVD. We focused on ω3 PUFA metabolism and the capacity of ω3 PUFA supplements to regulate key cellular events linked to CVD. The outcome of our analysis reveals that nonfish sources of ω3 PUFA vary in their capacity to regulate blood levels of $C_{20–22}$ ω3 PUFA and CVD risk factors.**—Jump, D. B., C. M. Depner, and S. Tripathy. **Omega-3 fatty acid supplementation and cardiovascular disease.** ***J. Lipid Res.*** **2012.** 53: **2525–2545.**

Supplementary key words dyslipidemia • inflammation • endothelial cell • cardiomyocyte • PUFA metabolism • single nucleotide polymorphism

Omega-3 (ω3) polyunsaturated fatty acids (PUFA) represent one of two major classes of long chain highly unsaturated fatty acids encountered in the diet. Dietary ω3 PUFA include α-linolenic acid (ALA, 18:3,ω3); stearidonic acid (SDA, 18:4,ω3); eicosapentaenoic acid (EPA, 20:5,ω3); docosapentaenoic acid (DPA, 22:5,ω3); and docosahexaenoic acid (DHA, 22:6,ω3) (**Fig. 1**). The major dietary ω6 PUFA is linoleic acid (LA, 18:2,ω6). 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 $C_{20–22}$ ω3 and ω6 PUFA found throughout the body.

Considerable interest in the health benefits of very long chain $C_{20–22}$ ω3 PUFA arose in the 1970s when epidemiological studies on Greenland Inuits established that this population had reduced rates of myocardial infarction (MI) compared with individuals in Western countries (2–7). These observations were linked to the high dietary intake of $C_{20–22}$ ω3 PUFA, enrichment of blood lipids with $C_{20–22}$ ω3 PUFA, and reduced fasting triglycerides (2, 4, 8). The potential health benefits of ω3 PUFA stimulated considerable research interest, resulting in over 500 clinical trials on ω3 PUFA (**Table 1**). Over 250 clinical studies have examined the impact of ω3 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 ω3 PUFA

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Abbreviations: ALA, α-linolenic acid; ARA, arachidonic acid; CHD, coronary heart disease; ChREBP, carbohydrate regulatory element binding protein; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DNL, de novo lipogenesis; DPA, docosapentaenoic acid; Elovl, fatty acid elongase; EPA, eicosapentaenoic acid; FADS, fatty acid desaturase; GPR, G-protein receptor; HDL-C, HDL-cholesterol; HNF4α, hepatic nuclear protein 4α; ICD, implantable cardioverter defibrillator; LA, linoleic acid; LDL-C, LDL-cholesterol; MetS, metabolic syndrome; MI, myocardial infarction; MLX, max-like factor X; NFκB, nuclear factor κB; PC, prospective cohort; PPARα, peroxisome proliferator activated receptor α; RBC, red blood cell; RCT, randomized clinical trial; SCD, sudden cardiac death; SDA, stearidonic acid; SREBP-1, sterol regulatory element binding protein-1; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

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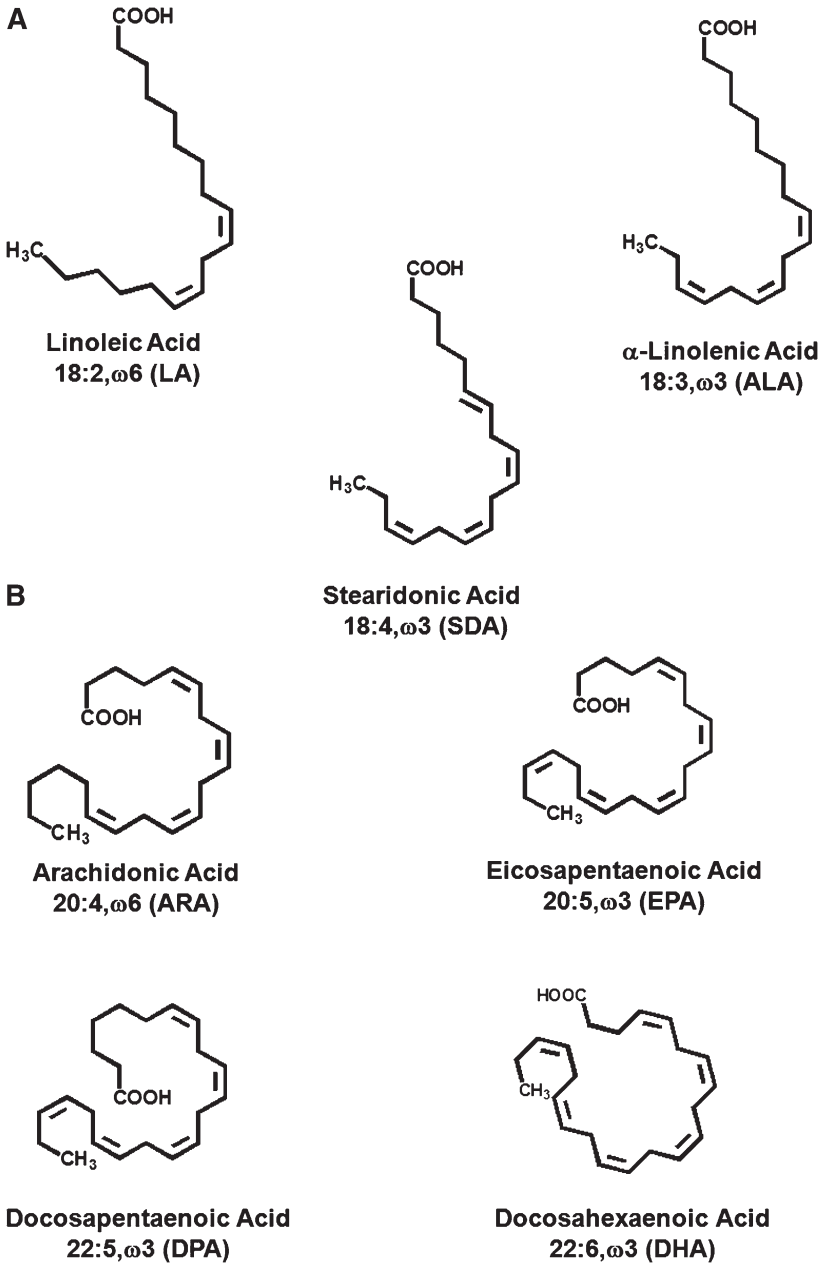

Fig. 1. Structures of dietary ω3 and ω6 polyunsaturated fatty acids. A: C_{18} ω3 and ω6 PUFA. B: $C_{20–22}$ ω3 and ω6 PUFA.

human research include visual acuity, cognitive development and decline, cancer prevention, and total mortality (7).

This review examines current recommendations for ω3 PUFA intake, ω3 PUFA metabolism, and their effects on physiological processes relevant to CVD. We also examine several prospective cohort (PC) studies (**Table 2**) and randomized clinical trials (RCT) (**Table 3**) that report on the benefit or lack of benefit of ω3 PUFA on cardiovascular health. Finally, we examine various sources of ω3 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 ω3 PUFA in the management of cardiovascular health.

CURRENT RECOMMENDATIONS FOR ESSENTIAL FATTY ACID CONSUMPTION

ALA and LA are precursors to ω3 and ω6 $C_{20–22}$ PUFA, i.e., DHA (22:6,ω3) (**Fig. 2**) and arachidonic acid (ARA, 20:4,ω6), 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, as nonesterified 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 ω6 PUFA (9, 10). Replacing

TABLE 1. Clinical trials on ω3 fatty acids

	Number of Trials
Cardiovascular disease and stroke	2340
Omega 3 fatty acids	534
Omega 3 fatty acids and:	
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 and stroke; omega 3 fatty acids.

saturated fat with PUFA (ω3 and ω6) 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 United States is estimated to be ≤10% and ≤1% total energy, respectively (14). Up to 10% of the dietary ω3 PUFA requirement can be provided by EPA or DHA (7, 15, 16). Food sources of ALA include flaxseed, walnuts, canola oil, and 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 ω3-PUFA consumption should be increased to 1 g/day if CVD is present (7, 15, 17–22).

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 percentage of whole-blood fatty acids that are the sum of EPA (20:5,ω3), DPA (22:5,ω3), and DHA (22:6,ω3). An omega-3 index of less than 4% is associated with low cardioprotection, whereas an index of 8% or more is associated with high cardioprotection. The rationale for this measure is that fatty acid composition in whole-blood and red blood cell (RBC) phospholipids parallels 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 mol% of ω3 and ω6 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 mol% 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 nontransplantation 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 $C_{20–22}$ ω3 PUFA content paralleled $C_{20–22}$ ω3 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 $C_{20–22}$ ω3 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 ($C_{20–22}$ ω3 PUFA), but not dietary flaxseed (ALA), significantly increases cardiac muscle $C_{20–22}$ ω3 PUFA content.

Such studies suggest that the omega-3 index may be a reasonable approach to assess cardiac ω3 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, such as baseline values for the omega-3 index and health status, affect the capacity of dietary ω3 PUFA to alter the omega-3 index and cardiac myocardial phospholipid content. 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 greater than 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: ω3 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, 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

TABLE 2. Prospective cohort studies on ω3 PUFA and clinical cardiovascular events

Year (Ref)	Population	Design	Follow-up Years	Key Outcome
2008 (182)	Atherosclerosis Risk in Communities (ARIC) Study USA (Minneapolis); 3,592 white men and women (ages 45–64)	Association of plasma cholesterol ester fatty acids with incident HF	14.3	110 men and 87 women developed HF 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] *ARA and DHA may reduce risk of HF, particularly in women.*
2009 (183)	Kuopio Ischemic Heart Disease Risk Factor Study Finland; 2,174 men (ages 42–60)	Association of serum ω3 PUFA with incident atrial fibrillation	17.7	240 men developed atrial fibrillation (AF) eduction 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 *Serum $C_{20–22}$ ω3 PUFA, but not ALA, was inversely associated with AF.*
2010 (184)	Heart and Soul Study USA (San Francisco); 956 patients (ages 65–69) with a history of CHD	Association of CHD with blood levels of EPA + DHA; Median blood level of EPA + DHA = 3.6%	5.9	237 deaths among 956 patients Below median: <3.6% EPA + DHA (N = 478) Above median: >3.6% EPA + DHA (N = 478) Below median versus above median (Hazard Ratio; 95% CI) 27% reduced risk of CHD [HR = 0.73; 95% CI = 0.56–0.94] *Blood EPA + DHA levels were inversely associated with total mortality in patients with stable CHD.*
2011 (185)	The Netherlands; 20,069 generally healthy men and women (ages 20–65)	Association of ALA intake (FFQ) with incident CHD (CVD and stroke)	8–13	280 CHD events (19% fatal); 221 strokes (4% fatal) Intake of energy-adjusted ALA in quintiles ALA intake range Q1: 1.0 g/day to Q5: 1.9 g/day 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 *ALA was not associated with incident CHD.*
2011 (187)	Cardiovascular Health Study USA (4 communities); 2,735 adults (age > 65) free of prevalent heart disease	Plasma phospholipid profiles and CHF	14	555 cases of CHF during 26,490 person-years Highest versus lowest quartile hazard ratio (95% CI) EPA: 0EPA: 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 ω3 PUFA: 0.7 (0.49 – 0.99; *P*-trend = 0.062) *Circulating individual and total ω3 PUFA were associated with lower incidence of CHF in older (>65 yrs) adults.*
2010 (186)	Rotterdam Study 1,570 men and women (ages 62–85) asymptomatic for CVD	Dietary ω3 fatty acid via FFQ and coronary calcification	7	Prevalence ratio (PR) for coronary calcification versus fish intake For fish intake > 19 g/day versus no fish intake Mild/moderate calcification: PR, 0.87 (95% CI = 0.78) Severe calcification: PR: 0.88 (95% CI = 0.74) *Weak inverse association between fish intake and coronary calcification.*

CHF, congestive heart failure; CI, confidence interval; FFQ, food frequency questionnaire; HF, heart failure; HR, hazard ratio; PR, prevalence ratio; Q, quintile.

TABLE 3. Randomized clinical trials of ω3 PUFA and clinical cardiovascular events

Trial [Year] (Ref)	Groups	Intervention	Years of Follow-up	Blood EPA and/or DHA ω3 PUFA versus Placebo	Events ω3 PUFA versus Placebo
DART-1 [1989] (171, 188)	1,015 males, fish advice	Advice: Increase fatty fish consumption or take fish oil supplement versus usual diet	2	EPA: 0.59 versus 0.46 mol%	All deaths: N = 94 (9.3%) versus N = 130 (12.8%); $P < 0.05$
	1,018 males, no fish advice				IHD deaths: N = 78 (7.7%) versus N = 116 (11.4%), $P < 0.01$
	Recovered from MI				Nonfatal MI: N = 49 (4.8%) versus N = 33 (3.2%), P = NS
	100% male, mean age <56.5				
DART-2 [2003] (188, 189)	1,571 males, fish advice	Advice: Increase fatty fish consumption or take fish oil supplement (3 g max EPA/day) versus usual diet	3–9	EPA: 45.8 versus 30.3 μg/ml	All deaths: N = 283 (18.0%) versus N = 242 (15.7%); P = 0.8
	1,543 males, no fish advice				Cardiac deaths: N = 180 (11.5%) versus N = 139 (9.0%); P = 0.02
	Recovered from MI				Sudden cardiac deaths: N = 73 (4.6%) versus N = 47 (3.0%); P = 0.02
	100% male, age <70 yr				
GISSI [1999] (172)	5,666 in ω3 PUFA group (85% male)	EPA + DHA ethyl ester	3.5	ND	Cardiovascular deaths [RR; 95% CI)]: N = 291 (5.1%) versus N = 348 (6.2%) [0.83; 0.71–0.097]
	5,668 in placebo group (85% male)	EPA + DHA (1:2)			Coronary deaths: N = 214 (3.8%) versus N = 265 (4.7%), [0.80; 0.67–0.96]
	Prior MI ⩽3 months	850–882 mg/day versus placebo (±300 mg/day Vitamin E)			Sudden cardiac deaths: N = 122 (2.2%) versus N = 164 (2.9%), [0.74; 0.58–0.93]
JELIS [2007] (191, 192)	9,326 in EPA + statin group (mean age 61; 32% male)	EPA ethyl ester (1.8 g/day) ± statin	4.6	EPA: 170 versus 95 μg/ml	Major coronary events: N = 262 (2.8%) versus N = 324 (3.5%); (P = 0.011)
	9,319 in statin-only group (mean age 61; 31% male)	Blood cholesterol ⩾ 6.5 mmol/l		DHA: 154 versus 165 μg/ml	Unstable angina: N = 147 (1.6%) versus N = 193 (2.1%); (P = 0.014)
					Nonfatal coronary events: N = 240 (2.6%) versus N = 297 (3.2%); (P = 0.015)
GISSI-HF [2008] (190)	3,494 in ω3 PUFA group (85% male)	EPA + DHA ethyl ester	3-9	ND	Cardiovascular deaths [HR; 95% CI]: N = 712 (20.4%) versus N = 765 (22.0%) [0.90; 0.81–0.99]
	3,481 in placebo group (85% male)	EPA + DHA (1:2)			Sudden cardiac deaths: N = 307 (8.8%) versus N = 325 (9.3%) [0.93; 0.79–1.08]
		850-882 mg/day versus placebo			Fatal and nonfatal stroke: N = 122 (3.5%) versus N = 103 (3.0%) [1.16; 0.89–1.51]
Omega [2010] (193)	1,919 in Omega group (mean age 64; 75% male)	EPA + DHA (1:1.2)	1	ND	Total mortality: N = 88 (4.6%) versus N = 70 (3.7%); (P = 0.18)
	1,885 in control group (mean age 64; 74% male)	850 mg/day of ethyl ester versus 1 g/day of olive oil			Sudden cardiac deaths: N = 28 (1.5%) versus N = 29 (1.5%); (P = 0.84)
	Recent MI ⩽ 2 weeks prior				Major adverse cerebro- or cardiovascular events: N = 182 (10.4%) versus N = 149 (8.8%); (P = 0.1)
					ICD-terminated ventricular tachycardia or fibrillation in survivor: N = 9 (0.5%) versus N = 2 (0.1%); (P = 0.06)
Alpha-Omega [2010] (194)	2,404 in EPA-DHA group (mean age 69; 78% male)	EPA-DHA group: margarine supplemented with EPA + DHA; targeted daily intake of ∼400 mg EPA + DHA	3.3	ND	EPA-DHA group versus placebo or ALA group
	2,433, ALA or placebo group (mean age 69; 78% male)	Placebo group: ±2 g ALA per day			Total mortality: N = 186 (7.7%) versus N = 184 (7.6%); (P = 0.92)

TABLE 3. Continued.

Trial [Year] (Ref)	Groups	Intervention	Years of Follow-up	Blood EPA and/or DHA ω3 PUFA versus Placebo	Events ω3 PUFA versus Placebo
	MI within prior 10 years	Patients were also on state-of-the-art antihypertensive, antithrombotic, lipid-lowering therapy			Major cardiovascular events: N = 336 (14%) versus N = 335 (13.8%); (P = 0.93) Death from cardiovascular events: N = 80 (3.3%) versus N = 82 (3.4%); (P = 0.75) Death from CHD: N = 67 (2.8%) versus N = 71 (2.9%); (P = 0.75) Incident CVD: N = 67 (2.8%) versus N = 71 (2.9%); (P = 0.75) Ventricular-arrhythmia-related events: N = 67 (2.8%) versus N = 74 (3.0%); (P = 0.55)
Su.Fol.Om3 [2010] (195)	1,253 in EPA + DHA group (mean age 60; 79% male)	EPA + DHA (2:1)	4.7	EPA (2.1 versus 1.2% of total)	Total mortality: N = 58 (4.7%) versus N = 59 (4.7%); (P = 0.33)
	Recent history of prior coronary or cerebral ischemic event	600 mg/day versus Vitamin B group or placebo		DHA (3.1 versus 2.7% of total)	Major cardiovascular events: N = 81 (6.5%) versus N = 76 (6.1%); (P = 0.64)

DART, Diet and Reinfarction Trial; GISSI, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardio; HR, hazard ratio; IHD, ischemic heart disease; JELIS, Japanese EPA Lipid Intervention Study; ND, not determined; OM3FA, omega-3 fatty acid; RR, relative risk; 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).

in certain pathophysiological states may not accurately predict cardiac ω3 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 and hormonal and health status (42). Although 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 C_{18} ESSENTIAL FATTY ACIDS TO $C_{20–22}$ PUFA

In addition to dietary sources, heart and blood levels of fatty acids depend on essential fatty acid metabolism. The pathway for conversion of the essential fatty acids LA and ALA to $C_{20–22}$ ω3 and ω6 PUFA involves two fatty acid desaturases (FADS1 and FADS2) and two fatty acid elongases (Elovl2 and 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 Fig. 2; intermediates in the ω3 PUFA pathway include SDA, EPA, DPA, and C_{24} 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

Fig. 2. Pathways for C_{18} PUFA conversion to $C_{20–22}$ PUFA synthesis. The pathway illustrates the conversion of the essential fatty acid ALA (18:3,ω3) to the end product DHA (22:6,ω3). The enzymes involved in this pathway include two fatty acid desaturases (FADS1 and FADS2), two fatty acid elongases (Elovl2 and Elovl5), and peroxisomal β-oxidation (p-βOx). Nonesterified fatty acids are converted to fatty acyl-CoAs by fatty acyl CoA 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 ω3 PUFA [ALA, SDA (18:4,ω3), EPA (20:5,ω3), and DHA] include plants, fish, yeast, and algae. Details of the pathway are presented in the text.

and other ω3 PUFA are converted to bioactive fatty acids that affect 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 they enter metabolic pathways involved in fatty acid oxidation, elongation, or desaturation. The desaturases and elongases involved in converting C_{18} PUFA-CoAs to $C_{20–22}$ fatty acyl-CoAs are associated with the endoplasmic reticulum (microsome) (Fig. 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 $C_{20–22}$ PUFA synthesis, and FADS2 is considered the rate-limiting enzyme in the pathway (44, 45). Fatty acid desaturation requires additional factors, including *i*) fatty acyl CoA substrate, *ii*) NADH, *iii*) cytochrome B5, and *iv*) cytochrome B5 reductase. Ablation of FADS1 or FADS2 expression in mice decreases ARA and DHA formation, but it increases the formation of C_{20} 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 C_{18} ω3 and ω6 PUFA to the corresponding $C_{20–22}$ ω3 and ω6 PUFA (Fig. 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 *i*) 3-keto acyl CoA reductase (Elovl), *ii*) 3-ketoacyl CoA reductase, *iii*) 3-hydroxy acyl CoA dehydratase, and *iv*) trans-2,3-enoyl CoA reductase. Fatty acid elongation is similar to the synthesis of fatty acids carried out by fatty acid synthase 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 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 C_{20} PUFA to form the C_{22} and C_{24} PUFA, whereas Elovl5 condenses malonyl CoA and C_{18} PUFA or C_{20} PUFA to form C_{20} and C_{22} PUFA. Elovl5 does not convert C_{22} PUFA to C_{24} 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 compared with other tissues like the liver (56).

Elovl5 participates not only in PUFA synthesis but also 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 of other CVD events (58). These fatty acids are in low abundance in the diet, but they 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 $C_{20–22}$ 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 PPARα activity, i.e., fibrates and fatty acids.

RETROCONVERSION OF C_{22} TO C_{20} PUFA

C_{22} PUFA are retroconverted to C_{20} PUFA (61). Primary rat hepatocytes treated with EPA accumulate 22:5,ω3 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 retroconversion 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 (Fig. 2) (61). This reaction is not trivial: it increases plasma and cellular levels of EPA in animals fed DHA (63), DHA retroconversion to EPA is sufficient to activate PPARα in primary hepatocytes (62). DPA is formed after retroconverted EPA is elongated by either Elovl2 or Elovl5. The retroconversion of DHA to EPA and 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

Although 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, and Elovl5 are regulated by SREBP1 and PPARα (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 ($C_{20–22}$ PUFA) formation (71). In addition to fatty acyl-CoAs, factors controlling cellular malonyl CoA affect 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 (ACC1 and ACC2) 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}~5 nM) by lowering cellular malonyl CoA. Soraphen A, however, does not block fatty acid desaturation (47). The ratio of 20:4,ω6 to 18:2,ω6 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,ω6 to 18:2,ω6 ratio and an increase in the 18:3,ω6 to 18:2,ω6 ratio. Thus, changes in 20:4,ω6 to 18:2,ω6 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 comes from human and animal studies. In 301 Swedish men (60 years of age), for example, FADS1 products (20:4,ω6/20:3,ω6) in adipose tissue and plasma phospholipids were inversely correlated with obesity and insulin resistance, whereas FADS2 products (18:3,ω6/18:2,ω6) showed a positive association (74). A study involving 97 Japanese men (~50 years of age) showed reduced 20:4,ω6/20:3,ω6 (FADS1 product), but higher levels of 18:3,ω6/18:2,ω6 (FADS2 product) in obese and MetS patients versus control (75). Feeding C57BL/6J mice a high-fat diet (60% energy as fat) induces obesity, insulin resistance, and hepatosteatosis (fatty liver) and suppresses the plasma and hepatic 20:4,ω6 to 18:2,ω6 ratio by ~60% compared with lean euglycemic mice fed a low-fat diet (10% energy as fat). The decline in the 20:4,ω6/18:2,ω6 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 $C_{20–22}$ 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 $C_{20–22}$ ω3 PUFA are feedback inhibitors of endogenous $C_{20–22}$ 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 PPARα-regulated mechanism, and increased fatty acid synthesis and triglyceride accumulation, SREBP1- and ChREBP/MLX-regulated mechanisms. PPARα, SREBP1, and the ChREBP/MLX heterodimer are well-established targets of $C_{20–22}$ 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). Although 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 $C_{18–22}$ 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; and 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 C_{20} to C_{22} PUFA, only Elovl2 converts C_{22} to C_{24} PUFA, a requirement for DHA synthesis (53, 86) (Fig. 2). Although 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 $C_{20–22}$ 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 $C_{20–22}$ 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 $C_{20–22}$ 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 $C_{20–22}$ ω3 PUFA content regulates membrane composition, cell signaling pathways originating from cell membranes, and gene expression (**Fig. 3**) (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 has 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.

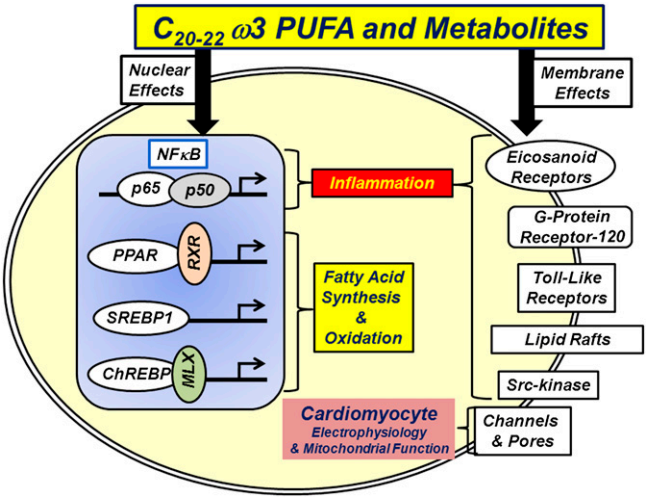

Fig. 3. Overview of $C_{20–22}$ ω3 PUFA regulation of cell function.

One receptor that has gained 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 macrophages and fat cells; its activation inhibits inflammatory cascades in macrophages, including the control of NFκB and JNK, and reverses insulin resistance in obese mice (103, 104, 107, 108).

Oxidation of ω3 PUFA

Although $C_{20–22}$ ω3 PUFA are β-oxidized in peroxisomes and mitochondria (61), other oxidation mechanisms convert these fatty acids to bioactive mediators of cell function. $C_{20–22}$ ω3 PUFA compete with ARA for assimilation into the sn2 position of membrane phospholipids. Dietary $C_{20–22}$ 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)], lipoxygenases (LOX-5, LOX-12, LOX-15), and cytochrome P450 enzymes.

Eicosanoids derived from the COX and LOX pathways (109) 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 compared with the ω6 PUFA products of COX and LOX (109–111). Resolvins and protectins have attracted considerable attention because of their capacity to protect against prolonged inflammation in animals models (112). The E-series resolvins (from EPA) and D-series resolvins (from DHA) are formed by the action of COX2 plus aspirin, whereas neuroprotectin-D1 is formed by the action of 5-LOX. Resolvins and neuroprotectins regulate inflammatory mechanisms (113, 114).

Nonesterified $C_{20–22}$ 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, synthesizes regioisomeric epoxides of ARA, EPA, and DHA, i.e., 14,15-epoxyeicosatrienoic acid, 17,18-epoxyeicosatetraenoic acid, and 19,20-epoxydocosapentaenoic acid, respectively (115, 116). 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 (EC_{50}∼1–2 nM) protects neonatal cardiomyocytes against Ca^{+2} overload (117). CYP4A/4F generates ω- and ω-1 hydroxylation products of ARA, EPA, and DHA (115, 118). In this case, inhibition of 20-hydroxyeicosatrienoic acid (20-HETE) synthesis protects the kidney from ischemia/reperfusion damage (119). These CYP450-dependent metabolites of EPA and DHA are found in the heart, and they may play a role in $C_{20–22}$ ω3 PUFA-linked cardioprotection (116).

ARA, EPA, and DHA are oxidized to F2-isoprostanes, F3-isoprostanes, and F4-neuroprostanes, respectively, by nonenzymatic processes (120–123). $C_{20–22}$ 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-, alkoxyl-, peroxyl-radicals or peroxynitrate) lipid peroxidation chain reaction generates oxidized fatty acids found in membrane lipids and as 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 a high-fat, high-cholesterol diet promotes hepatic oxidative stress. Including fish oil in this high-fat, high-cholesterol diet stimulates the production of F2-isoprostanes, F3-isoprostanes, and F4 neuroprostanes that appear in liver and urine (68, 121, 122). F2-isoprostanes activate thromboxane and 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 (123, 124). Whether the F3-isoprostanes and F4-neuroprostanes have anti-inflammatory properties like series 3 and series 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, 125). Of these, fatty-acid regulation of the PPAR family has been most extensively studied. In primary rat hepatocytes, EPA significantly induces PPARα-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 nonesterified EPA through retroconversion (62). Cocrystals of PPARβ/δ-EPA, but not PPAR-DHA, have been described (126). In vitro, EPA binds PPARα (K_d∼1 μM); in primary rat hepatocytes, EPA is the most potent ω3 PUFA activator of PPARα.

PUFA suppress the nuclear abundance of several transcription factors involved in carbohydrate and lipid metabolism, including SREBP1, ChREBP, and MLX (66, 92, 127, 128). PUFA (both ω3 and ω6 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 (129). Molecular mechanisms for PUFA control of ChREBP and MLX nuclear abundance remain undefined. $C_{20–22}$ ω3 PUFA control of these transcription factors represents a mechanism for $C_{20–22}$ ω3 PUFA suppression of DNL and triglyceride synthesis.

NFκB is a major transcription factor regulating expression of genes encoding proteins involved in inflammation (130); some target genes include COX2, cytokines (e.g., TNFα), and chemokines (e.g., MCP1). Omega-3 PUFA suppress the nuclear levels of NFκB in several model systems (99, 131). NFκB nuclear abundance is typically regulated by controlling the interaction of NFκB subunits (p50 and/or p65) with IκB subtypes (α, β, ε, ζ). IκB sequesters p50/p65 in the cytosol; phosphorylation of IκB by IκB-kinase promotes IκB dissociation from p50/p65, IκB is degraded in the proteasome, and p50/p65 accumulates in nuclei. IκB-kinase activity is regulated by its phosphorylation status; two kinases controlling IκB-kinase phosphorylation status include Akt and TAK1. The NFκB subunits bind promoters as heterodimers of p50/p65 and homodimers of p50; p65 can heterodimerize with other transcription factors, e.g., c/EBPα. Recent studies using the *Ldlr*$^{-/-}$ mouse model of high-fat, high-cholesterol diet-induced nonalcoholic fatty liver disease established that NFκB-p50 is more vulnerable to ω3 PUFA control than NFκB-p65 (68). Although the mechanism for this selective control remains to be established, this outcome suggests that ω3 PUFA controls a subset of NFκB-regulated genes.

TARGETS OF ω3 PUFA REGULATION IN CVD

The pleiotropic effects of ω3 PUFA on cell function are well established (92). In the cardiovascular system, the type and quantity of ω3 fatty acid ingested and cellular ω3 and ω6 PUFA content affect blood lipids, inflammation, and endothelial cell and cardiomyocyte function (**Fig. 4**). The underlying mechanisms for these effects are described below.

Dyslipidemia

Fasting and nonfasting triglycerides have long been associated with CVD (132–134). Monitoring fasting blood triglycerides represents a well-established marker for ω3-PUFA action. The association between blood triglycerides with CVD, however, has been the subject of debate (135). Miller and colleagues (136) 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 and LDL-cholesterol with low HDL-cholesterol. Triglycerides are associated with atherogenic remnant

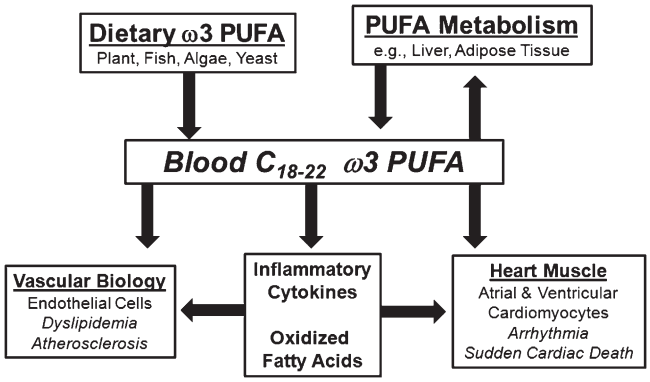

Fig. 4. Overview of dietary C_{18-22} ω3 PUFA effects on cardiovascular disease.

particles derived from chylomicrons and VLDL. Of these, fasting triglycerides are the most responsive to changes in blood and tissue levels of $C_{20–22}$ ω3 PUFA. Fish oil has little or no effect on total blood cholesterol, but it affects LDL-C, LDL particle size, and HDL-C; these effects are variable and depend on dose and population studied (137, 138). When tested alone, DHA supplements increased HDL-C and LDL particle size, whereas EPA decreased HDL_3 (137–139).

Both C_{18} ω3 and $C_{20–22}$ ω3 PUFA lower triglycerides. However, the effect seen with ALA was equivalent to the effect seen with dietary LA. ALA, however, is not equivalent to $C_{20–22}$ ω3 PUFA in controlling serum lipoproteins in humans (140). Accordingly, pharmaceutical control of blood triglycerides uses $C_{20–22}$ ω3 PUFA, not ALA. A 1 g capsule of Lovaza™ (GlaxoSmithKline) contains 465 mg of EPA and 375 mg of DHA as fatty acid ethyl esters (141). Lovaza™ is prescribed at ~4 g/day for patients with hypertriglyceridemia (142). Amarin Pharma 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-C, apolipoprotein B, lipoprotein-associated phospholipase A2, VLDL-C, and total cholesterol in patients with hypertriglyceridemia (143). EPA and DHA appear to be equally effective at reducing blood triglyceride levels (137).

The hypolipemic effect of $C_{20–22}$ ω3 PUFA is complex and involves control of hepatic VLDL production and clearance of triglyceride-rich lipoprotein (chylomicrons and VLDL) (144–149). 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, as DNL is suppressed (150). Dietary $C_{20–22}$ ω3 PUFA lower fasting blood levels of NEFA; dietary $C_{20–22}$ ω3 PUFA also reduce adipose tissue lipolysis (151). The mechanism for this effect is linked to activation of PPARγ and suppression of inflammation; it may also involve the GPR120 receptors mentioned earlier. In rodent liver and primary hepatocytes, $C_{20–22}$ 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 (148).

ApoCIII is a key apolipoprotein involved in controlling VLDL-triglyceride clearance. ApoCIII inhibits lipoprotein lipase, an enzyme involved in triglyceride hydrolysis (152, 153). Animal studies established that hepatic ApoCIII expression was suppressed by PPARα; PPARα is activated by fibrates and EPA (62, 154, 155). Human studies have established that fatty fish consumption or increased RBC $C_{20–22}$ ω3 PUFA content is linked to lower triglycerides and plasma ApoCIII (156–158). Interestingly, patients homozygous for the T-455C variant in the ApoCIII promoter are poorly responsive to the ApoCIII-lowering effect of ω3 PUFA (158).

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 (TXA_2), and prostaglandins (PGH_2)] and relaxation [prostacyclins (PGI_2) and nitric oxide (NO)] (159). Dysfunctional coronary and peripheral endothelial cells are linked to CVD, myocardial infarction, stroke, and diabetic retinopathy (160–164). Atherosclerosis is an inflammatory disease of the vascular system (162, 163). Cytokine-mediated induction of adhesion molecules in endothelial cells is an early step in monocyte binding to endothelial cell membranes that enable monocytes to move into vascular intima where they differentiate to macrophages and promote inflammation, culminating in plaque formation (162, 165, 166). Controlling endothelial cell expression of adhesion molecules, like ICAM and VCAM, is central to controlling the onset of the inflammatory events linked to atherosclerosis. As noted earlier, $C_{20–22}$ ω3 PUFA have anti-inflammatory capacity through effects on membrane lipid raft structure, NFκB, and COX2- and CYP450-generated eicosanoids and docosanoids (22, 167).

In human coronary endothelial cells, DHA regulates eNOS function through Akt activation (164). In mouse coronary and human retinal endothelial cells, $C_{20–22}$ ω3 PUFA suppresses ICAM1 and VCAM1 expression by interfering with NFκB signaling (99, 100, 168, 169). In freshly isolated human retinal endothelial cells, phospholipid DHA levels are very low (~1 mol%). 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 and cYes) with lipid rafts. The net effect of elevated membrane DHA content is the disruption of NFκB signaling and the downregulation acid sphingomyelinase. The suppression of sphingomyelinase activity is associated with a decline in ceramide, an inflammatory and pro-apoptotic lipid (170).

Although 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 (160). 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 ω3 PUFA control of endothelial cell function is the capacity of dietary ω3 PUFA to increase endothelial cell EPA + DHA content. Although the omega-3 index parallels changes in $C_{20–22}$ ω3 PUFA in cardiac muscle, it is unclear if the omega-3 index parallels changes in coronary and vascular endothelial cells.

ω3 PUFA regulation of cardiomyocyte function

Human studies have established that SCD is reduced in patients consuming fish oil (171–173). The consumption of EPA and DHA from fish oil increases atrial and ventricular EPA and DHA in membrane phospholipids (24, 25, 174). Several clinical trials have reported that ω3 PUFA were able to *i*) prevent SCD, *ii*) prevent ventricular arrhythmia in patients with implantable cardioverter, *iii*) lower occurrences of premature ventricular contractions, *iv*) reduce heart rate, and *v*) prevent atrial fibrillation (174, 175). Studies in dogs showed anti-arrhythmic effects of EPA or DHA when administered during ischemia (176).

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 (174, 175, 177, 178). As such, studies with cardiomyocytes have focused on ω3 PUFA regulation of Na^{+}-, Ca^{+2}-, K^{+}-channels, ion pumps, and the Na^{+}/Ca^{+2} exchangers (173, 175). The impact on intracellular calcium handling is particularly noteworthy. Stanley et al. (177, 179, 180) recently identified the mitochondrial permeability transition pore (MPTP) as a target for ω3 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 (177, 181). 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 ω3 PUFA, like ALA and EPA, modify mitochondrial membrane phospholipid composition, they do so to a lesser extent and have less effect on mitochondrial function (177). Thus, DHA control of MPTP may be one mechanism for ω3 PUFA-mediated protection against heart failure. Other mechanisms include the control of oxidized lipids and inflammatory processes mentioned previously.

PROSPECTIVE COHORT STUDIES AND RANDOMIZED CLINICAL TRIALS ON DIETARY ω3 PUFA AND CHD

Several PCs and RCTs have examined the effect of dietary ω3 PUFA on cardiovascular health. Representative PCs 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 ω3 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 consist of an overall patient population of 31,096 men and women, ranging from 20 to 85 years of age; the follow-up period ranged from 5.9 to 17.7 years (182–187). Most patients in these studies were free of CVD. The outcome of these studies was that elevated blood levels of $C_{20–22}$ ω3 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 agree favorably with several epidemiological studies (2–7) and suggest that dietary $C_{20–22}$ ω3 PUFA are useful in primary prevention of CVD.

The RCTs listed in Table 3 are secondary prevention trials (171, 172, 188–195). Each trial includes at least 1,000 patients (mostly males; age >50 years) with prior CVD, e.g., MI. The first Diet and Reinfarction Trial (DART) included patients (2,033 males <56.6 years of age) who had prior MI; these patients were advised to eat or not to eat at least two portions of fatty fish/week or take dietary EPA + DHA supplements (171). 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 they had a nonsignificant increase in nonfatal MI. The second DART trial used a similar design, but it 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 ω3-PUFA group (188, 189).

The Gruppo Italiano per la Sperimentazione della Streptochinasi nell'Infarto Miocardio Prevenzione (GISSI) trial (172) 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 ⩽ 3 months). Patients assigned to the EPA + DHA supplement group had a significant reduction in cardiovascular death (18%), coronary death (20%), and sudden cardiac death (24%) compared with the placebo group. These outcomes support the use of $C_{20–22}$ ω3 PUFA to reduce the incidence of cardiovascular-related deaths, including SCD and arrhythmias (173).

The Japanese EPA Lipid Intervention Study (JELIS) was the first large clinical study to examine the impact of EPA on CVD (191). 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%, and 19% reduction in major coronary events, unstable angina, and nonfatal coronary events, respectively (196). 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% (192). The decline in AA and DHA likely reflects the feedback inhibition of PUFA synthesis by elevated EPA ingestion.

The Omega (193), Alpha-Omega (194), and Su.Fol. OM3 (195) RCTs involved 3,704, 4,837, and 2,606 patients, respectively, with recent cardiovascular events, e.g., MI, coronary, or cerebral ischemic events. The Omega trial had patients consume 850 mg/day 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 $C_{20–22}$ ω3 PUFA consumption provided no significant benefit to cardiovascular health.

The outcome of these RCTs has lead several investigators to question whether increasing dietary $C_{20–22}$ ω3 PUFA significantly benefits cardiovascular health in secondary prevention (197–201). 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 (201). This report, however, was criticized for including small short-term analyses that were not designed to detect end points of CVD (202).

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 preexisting CVD. Second, the background levels blood EPA and/or DHA were high before beginning the trial; this was particularly the case in the JELIS trial. The Japanese population had 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 trials, 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 trial 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 who received ω3 PUFA supplementation might have reduced major cardiovascular events. Statin treatment appears to modify effects of ω3 PUFA on the incidence of major cardiovascular events (203). 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 Vitamin D and Omega-3 Fatty Acid (VITAL) trial is an ongoing RCT enrolling 20,000 participants (men $\geq$ 50 years of age and women $\geq$ 55 years of age). This double-blind, placebo-controlled primary prevention trial will be randomized to receive vitamin D (2000 IU/day), $C_{20–22}$ ω3 PUFA (840 mg/day), both supplements, or neither supplement. The study is powered to examine major cardiovascular events, as well as CVD and stroke individually (204). The outcome of the VITAL trial may clarify the utility of $C_{20–22}$ ω3 PUFA in primary prevention of CVD.

CAPACITY OF DIETARY C_{18-22} ω3 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 ω3 fatty acid supplements (EPA + DHA, 200–2,000 mg/day) to lower the risk for CVD (7, 15, 17–22, 138). A recent meta-analysis suggests that a daily intake of combined EPA + DHA (200–300 mg/day) is sufficient to provide cardioprotection (205). Dietary sources of ω3 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 ω3-PUFA consumption and CVD (147, 171, 172, 193, 206, 207). Only one large-scale clinical trial, i.e., JELIS, assessed the capacity of EPA in cardioprotection (191, 208). This section examines various sources of dietary ω3 PUFA for their capacity to control CVD risk factors, including the omega-3 index and inflammatory cytokines.

ω3 PUFA from farm-raised and wild-caught fish

The original observations by Dyerberg and colleagues on the cardioprotective effects of ω3 PUFA were based on Greenland Inuits ingesting wild-caught fish enriched in $C_{20–22}$ ω3 PUFA (2–7). Fish, fish oil supplements, or EPA + DHA ethyl esters have been used in most CVD prevention trials (Tables 2 and 3).

Although farm-raised fish provide an alternative source of $C_{20–22}$ ω3 PUFA, levels of these fatty acids in farm-raised fish are not as high as in wild-caught fish (209, 210). Efforts to increase $C_{20–22}$ ω3 PUFA in farm-raised Atlantic Salmon (*Salmo salar L.*) involve feeding fish ω3-PUFA supplements (210). 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 SDA (18:4,ω3). Compared with 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.

Whereas ω3 PUFA in fish or plants are supplied as triglycerides, ω3 PUFA in supplements are supplied as free fatty acids, fatty acids-ethyl esters, triglycerides, or reesterified triglycerides, most commonly in gel capsules (211, 212). The US 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 percentage 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 the 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 flax 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/day

are important for human health (15). Humans, however, convert 0.2–9% of ingested ALA to DHA (56, 213–215). The conversion of ALA to DHA in men is lower (~0.1%) than in women (~9%). This may relate to the decreased β-oxidation of ALA in women or increased expression of FADS2 (216). 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 (213, 217).

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, or 2.3 g/day of ALA, EPA, or DHA, respectively (218). 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. (219) 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 nonfatal 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 (220, 221). 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 and 65. ALA intake was not associated with the incidence of coronary heart disease, but low ALA intake may be a risk factor for stroke (185). The Alpha-Omega trial (194) (Table 3) reported that ALA supplementation at 2 g/day did not significantly reduce the rate of major cardiovascular events in patients with prior MI 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 (194, 219).

Stearidonic acid (18:4,ω3)

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 SDA (18:4,ω3) (Fig. 2). To overcome this metabolic bottleneck, investigators have examined the capacity of dietary SDA to be converted to $C_{20–22}$ PUFA in humans (222). Generally healthy humans who consumed 0.75 g SDA/day for 3 weeks followed by 1.5 g SDA/day for 3 weeks had significantly higher levels of EPA and DPA (22:5,ω3) in blood phospholipids and lowered blood levels of two cytokines (TNFα and IL1β). Dietary SDA, however, did not increase blood levels of DHA.

Soyamega™, developed by Monsanto and Solae (223), is a SDA-enriched oil derived from genetically modified soybeans. Several studies have examined the capacity of Soyamega™ to increase blood $C_{20–22}$ ω3 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 (222, 224–229). FADS2 not only desaturates ALA to form SDA, it also desaturates 24:5,ω3 to form 24:6,ω3; then 24:6,ω3 is β-oxidized in the peroxisome to form DHA (22:6,ω3) (Fig. 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. Findings that dietary SDA lowers some inflammatory markers suggest this dietary source of ω3 PUFA will be useful in modulating inflammatory factors linked to CVD. DHA, however, has a greater capacity to regulate cardiac mitochondrial MPTP than other ω3 PUFA (177, 179, 180). This is important for ventricular fibrillation and SCD (177, 180, 230–232). Whether SDA supplementation can affect SCD remains to be determined through more investigation.

Eicosapentaenoic acid (20:5,ω3)

The JELIS trial was the first large clinical study to examine the impact of EPA on CVD (191). The EPA group had a 19% risk reduction in major coronary events, including SCD, unstable angina, and coronary revascularization (196) (Table 3). Patients receiving EPA-ethyl esters showed an increase in plasma EPA from ~95 μg/ml to 170 μg/ml, while both ARA and DHA decreased ~9% (192). The reduction in nonfatal coronary events was achieved without significant increase in blood DHA. As noted earlier, the Japanese population has high fish consumption and high baseline blood levels of EPA and DHA and lower rates of SCD than do Western societies (34).

Amarin Pharma (Mystic, CT) has developed a highly enriched form of EPA-ethyl esters (AMR101, >96% EPA and no DHA) for clinical use. A multicenter, placebo-controlled, randomized, double-blind, 12-week study examined the effects of AMR101 in 229 diet-stable patients with fasting triglycerides greater than 500 mg/dl and less than 2,000 mg/dl (with or without statin therapy). Patients received AMR101 at 4 g/day, 2 g/day, or placebo for 12 weeks. At the 4 g/day dose, AMR101 significantly reduced plasma triglycerides 33% (n = 76); at the 2 g/day 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 (143).

Nonprescription EPA is available as a genetically engineered product derived from yeast; EPA in this product is a triglyceride (Newharvest-EPA™; DuPont, Wilmington, DE) (212). EPA represents ~60% of all fatty acyls in a 1 g

capsule. This product contains little or no ALA, SDA, DPA, or DHA. On the basis of 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)

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 in which ~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/day of DHA for 45 days (233). 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 ω3 PUFA supplementation to increase cardiac muscle DHA may be important in the prevention of ventricular fibrillation and SCD (179, 230, 234). Fatty fish, fish oil containing EPA + DHA, or the DHA-only supplements (e.g., DHASCO and 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 and meta-analyses, as well as several expert reviews and commentaries generally support the use of dietary sources of ω3 $C_{20–22}$ PUFA in primary and secondary prevention of CVD (Tables 2 and 3) (20, 138, 197–202, 205, 235). The RCTs, however, suggest there may be limitations to the use of supplementary $C_{20–22}$ ω3 PUFA in prevention of future cardiovascular events. The patient's health status, populations with high fish consumption, or the concurrent use of state-of-the-art therapies in CVD treatment, e.g., statin therapy, influence outcomes in $C_{20–22}$ ω3 PUFA therapy (203). At a minimum, individuals should consume sufficient $C_{20–22}$ ω3 PUFA to meet the current recommendations for primary prevention of CVD, i.e., 200–300 mg/day of combined EPA + DHA (205, 235), either as fatty fish or as $C_{20–22}$ ω3 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. Nonfish sources of ω3 PUFA from plants (for SDA), yeast (for EPA), and algae (for DHA) are available to increase blood and tissue levels of EPA and DHA. Although ALA is poorly converted to DHA, both dietary SDA (222, 224–229) and EPA (192) increase blood EPA levels. Increased blood EPA is associated with lower inflammatory cytokines (TNFα, IL1β), improved blood lipid biomarkers (236), 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: *i*) the intrinsic biochemical properties of FADS2 limit the conversion of ALA (18:3,ω3) to SDA (18:4,ω3) and the conversion of 24:5,ω3 to 24:6,ω3 (Fig. 2); and *ii*) $C_{20–22}$ ω3 PUFA suppress expression of the FADS1, FADS2, and Elovl5 genes, limiting $C_{20–22}$ ω3 and ω6 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 (177, 179, 180). In endothelial cells, DHA suppresses expression of adhesion molecules, a key early event in atherosclerosis (99, 100, 168, 169). The limited capacity of ALA, SDA, and EPA supplements to significantly increase blood DHA suggests 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 factors that may affect cellular levels of DHA. Because 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, and 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™ (GlaxoSmithKline)] will enable investigators to establish which formulation of ω3 PUFA confers the greatest cardioprotection with the fewest off-target effects.

Finally, the cardiovascular system is not the only site of ω3 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 ω3 PUFA leads to the replacement of DHA with 22:5,ω6. This minor change in fatty acid structure (22:6,ω3 to 22:5,ω6) in ω3 PUFA deficiency is sufficient to alter visual acuity and cognitive function (237–239).

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