STATE-OF-THE-ART PAPER

Omega-3 Fatty Acids and Cardiovascular Disease

Effects on Risk Factors, Molecular Pathways, and Clinical Events

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We reviewed available evidence for cardiovascular effects of n-3 polyunsaturated fatty acid (PUFA) consumption, focusing on long chain (seafood) n-3 PUFA, including their principal dietary sources, effects on physiological risk factors, potential molecular pathways and bioactive metabolites, effects on specific clinical endpoints, and existing dietary guidelines. Major dietary sources include fatty fish and other seafood. n-3 PUFA consumption lowers plasma triglycerides, resting heart rate, and blood pressure and might also improve myocardial filling and efficiency, lower inflammation, and improve vascular function. Experimental studies demonstrate direct antiarrhythmic effects, which have been challenging to document in humans. n-3 PUFA affect a myriad of molecular pathways, including alteration of physical and chemical properties of cellular membranes, direct interaction with and modulation of membrane channels and proteins, regulation of gene expression via nuclear receptors and transcription factors, changes in eicosanoid profiles, and conversion of n-3 PUFA to bioactive metabolites. In prospective observational studies and adequately powered randomized clinical trials, benefits of n-3 PUFA seem most consistent for coronary heart disease mortality and sudden cardiac death. Potential effects on other cardiovascular outcomes are less-well-established, including conflicting evidence from observational studies and/or randomized trials for effects on nonfatal myocardial infarction, ischemic stroke, atrial fibrillation, recurrent ventricular arrhythmias, and heart failure. Research gaps include the relative importance of different physiological and molecular mechanisms, precise dose-responses of physiological and clinical effects, whether fish oil provides all the benefits of fish consumption, and clinical effects of plant-derived n-3 PUFA. Overall, current data provide strong concordant evidence that n-3 PUFA are bioactive compounds that reduce risk of cardiac death. National and international guidelines have converged on consistent recommendations for the general population to consume at least 250 mg/day of long-chain n-3 PUFA or at least 2 servings/week of oily fish.   (J Am Coll Cardiol 2011;58:2047–67) © 2011 by the American College of Cardiology Foundation

In vitro studies, animal experiments, observational studies, and randomized clinical trials (RCTs) have examined the

cardiovascular effects of seafood consumption and long-chain n-3 polyunsaturated fatty acids (PUFAs) (Fig. 1) (1,2). Although much has been learned, several questions remain, including the precise physiological effects and molecular mechanisms that account for the observed benefits, the magnitudes and dose-responses of effects on specific clinical outcomes, and the potential heterogeneity in different populations. Several recent clinical trials of n-3 PUFA have also had mixed findings, raising concern about the consistency of the evidence.

We reviewed the current evidence for cardiovascular disease (CVD) effects of seafood and n-3 PUFA consumption, including the principal dietary sources; effects on physiological risk factors; potential molecular pathways of effects; and scientific evidence, including conflicting evidence, for effects on specific clinical endpoints. We also considered various dietary guidelines for fish and n-3 PUFA consumption and, based on evidence reviewed herein, suggest potential dietary recommendations for patients and populations. We focused principally on long-chain (seafood-derived) n-3 PUFA; promising but more limited evidence for plant-derived n-3 fatty acids is briefly dis-

cussed. Finally, we highlight gaps in current knowledge and key areas for future research. The information presented in this review is intended to provide a useful framework for scientists, health practitioners, and policymakers to consider the contemporary evidence for effects of seafood and n-3 PUFA consumption on cardiovascular health.

Dietary Sources

Fish (used hereafter to refer to finfish and shellfish) is the major food source of long-chain n-3 PUFA, including eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3) (Table 1). Docosapentaenoic acid (DPA) (22:5n-3), a long-chain n-3 PUFA metabolite of EPA, is present in smaller amounts in fish (Table 1) (3). Circulating DPA levels correlate weakly with fish consumption (4), suggesting that DPA levels in humans are predominantly determined by endogenous metabolism rather than diet. Although DPA might have relevant physiological effects (3), relatively little is known about its clinical effects; a few studies have observed inverse associations between circulating DPA and risk of coronary events (4–6). In addition to long-chain n-3 PUFA, fish provide specific proteins, vitamin D, selenium, and other minerals and elements (7–9).

Alpha-linolenic acid (ALA) (18:3n-3) is the plant-derived n-3 fatty acid found in a relatively limited set of seeds, nuts, and their oils (Table 1). Alpha-linolenic acid cannot be synthesized in humans and is an essential dietary fatty acid. Biochemical pathways exist to convert ALA to EPA and EPA to DHA, but such endogenous conversion is limited in humans: between 0.2% and 8% of ALA is converted to EPA (with conversion generally higher in women) and 0% to 4% of ALA to DHA (10–14). Thus, tissue and circulating EPA and DHA levels are primarily determined by their direct dietary consumption. Some effects on physiological risk factors and observational studies of clinical endpoints suggest that ALA might have cardiovascular benefits, but overall evidence remains mixed and inconclusive (Fig. 2) (15–20). Thus, plant sources of n-3 fatty acids cannot currently be considered as a replacement for seafood-derived n-3 PUFA (15). Additional studies of
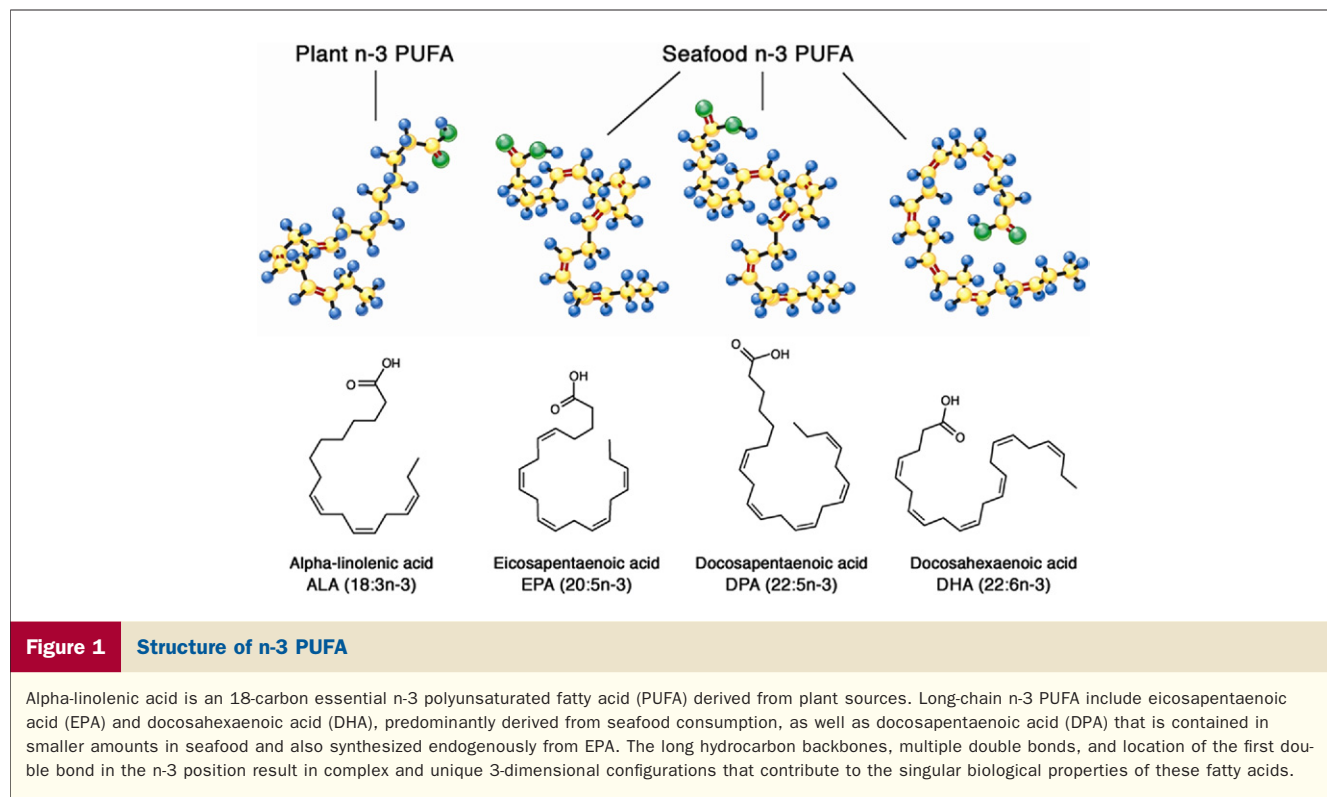
ALA's effects are urgently needed, because of the lower cost and greater potential global supply of ALA as opposed to EPA+DHA. The remainder of this report focuses on the much larger body of evidence for cardiovascular effects of EPA and DHA (referred to as simply n-3 PUFA hereafter).

In addition to potential cardiovascular benefits of fish consumption, concerns have been raised over potential harm from contaminants present in some fish species, such as methylmercury, dioxins, and polychlorinated biphenyls (PCBs) (21–28). In most fish species, mercury levels are quite low; selected few species contain moderate levels (e.g., albacore tuna, approximately 0.36 $\mu g/g$) or higher levels near the U.S. Food and Drug Administration action level of 1 $\mu g/g$ (e.g., tilefish [golden bass], swordfish, shark, Gulf of Mexico King mackerel) (29). At exposure levels common in the United States, mercury exposure from fish consumption has no relations with higher CVD risk (30). Most commercially sold fish contain low levels of PCBs and dioxins, and overall fish consumption contributes a minority of dietary exposure compared with other foods (in 1 U.S. analysis, approximately 9% of total dietary exposure) (31). In some local waters, recreationally caught sport fish might contain relatively higher levels of PCBs/dioxins. For the general population of adults, risk–benefit analyses conclude that the health benefits of modest fish consumption significantly outweigh the potential risks (1,15,32,33). Thus, this present review of cardiovascular risk does not further focus on contaminants. Specific guidance is available for sensitive subpopulations such as women of childbearing age and young children (15).

The environmental impact and long-term sustainability of aquaculture and commercial fishing are relevant (34–37). Such concerns are not unique to seafood but also exist for agricultural, forestry, freshwater, atmospheric, and energy resources (38,39). A review of the complex environmental considerations related to fish and fish oil consumption is beyond the scope of this report. Based on evidence for the importance of fish and n-3 PUFA consumption in health, environmental concerns must be addressed to ensure sustainable, environmentally sound, and financially viable commercial fishing and aquaculture practices into the future. However, environmental and health aspects of fish consumption should not be conflated: accurate and distinct information on each should be provided to consumers and policy makers to permit informed decision-making.

Cardiovascular Risk Factors

Plasma triglycerides. n-3 PUFA have multiple CVD-related physiological effects (Fig. 3). Lowering of plasma triglycerides is well recognized (40). Reduced hepatic very low-density lipoprotein synthesis contributes to this effect, with implicated mechanisms including reduced fatty acid availability for triglyceride synthesis due to decreased de novo lipogenesis (DNL) (the process of converting carbohydrates into fat), increased fatty acid beta-oxidation, and

Figure 1   Structure of n-3 PUFA

Alpha-linolenic acid is an 18-carbon essential n-3 polyunsaturated fatty acid (PUFA) derived from plant sources. Long-chain n-3 PUFA include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), predominantly derived from seafood consumption, as well as docosapentaenoic acid (DPA) that is contained in smaller amounts in seafood and also synthesized endogenously from EPA. The long hydrocarbon backbones, multiple double bonds, and location of the first double bond in the n-3 position result in complex and unique 3-dimensional configurations that contribute to the singular biological properties of these fatty acids.

reduced delivery of nonesterified fatty acids to the liver; reduced hepatic enzyme activity for triglyceride synthesis; and increased hepatic synthesis of phospholipids rather than triglycerides (40–45). In experimental models and human studies, reduced DNL appears to be particularly important (40,41,45–49). Triglyceride-lowering is linearly dose-dependent across a wide range of consumption but with variable individual responses, including greater absolute reductions among individuals with higher baseline levels (Fig. 4). At typical dietary doses, only modest triglyceride-lowering occurs and it is unlikely that this contributes appreciably to the reduced clinical risk seen with lower-dose fish oil supplements in randomized trials or habitual fish consumption in observational studies (see the following text). Conversely, accrued modest benefits of reduced hepatic DNL, sustained over time from habitual n-3 PUFA consumption, could partly contribute to lower cardiovascular risk, for example mitigating development of hepatic steatosis and hepatic insulin resistance (46–52).

Heart rate and blood pressure. n-3 PUFA consumption reduces resting heart rate (HR) and systolic and diastolic blood pressure (53,54). Experimental studies suggest that HR lowering could result from direct effects on cardiac electrophysiological pathways (55–57). n-3 PUFA might also lower HR by more indirect effects, such as by improving left ventricular diastolic filling (see the following text) or augmenting vagal tone (58). In short-term trials, n-3 PUFA consumption increases nitric oxide production, mitigates vasoconstrictive responses to norepinephrine and angiotensin II, enhances vasodilatory responses, and improves arterial compliance (59–70). Such effects could contribute to lowering of systemic vascular resistance and blood pressure.

Thrombosis. n-3 PUFA are commonly considered to have anti-thrombotic effects, based on increased bleeding times at very high doses (e.g., 15 g/day). Conversely, in human trials, n-3 PUFA consumption has no consistent effects on platelet aggregation or coagulation factors (71–73). Overall, at doses of at least up to 4 g/day (and perhaps higher), anti-thrombotic effects are unlikely to be a major pathway for lower CVD risk, although subtle effects cannot be excluded. No excess clinical bleeding risk has been seen in RCTs of fish or fish oil consumption, including among people undergoing surgery or percutaneous intervention and/or also taking aspirin or warfarin (74–76).

Endothelial and autonomic function. Several trials have demonstrated improved flow-mediated arterial dilation, a measure of endothelial function and health, after n-3 PUFA supplementation (62,64–66,77–80). Because endothelial health is strongly linked to endothelial nitric oxide synthesis (81), experimental effects of n-3 PUFA on related biomarkers provide plausible biological mechanisms for such effects (61,82–85). Several although not all trials have also found that n-3 PUFA consumption lowers circulating markers of endothelial dysfunction, such as E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 (86–88). Thus, normalization of endothelial function could partly mediate protective effects of n-3 PUFA against CVD. Observational studies and small trials of n-3 PUFA and HR variability—a marker of autonomic function, circadian rhythms, and underlying cardiac health—have produced

Table 1	Food Sources of Long-Chain n-3 PUFA					
Common Dietary Sources	EPA, mg/100 g	DPA, mg/100 g	DHA, mg/100 g	EPA+DHA, mg/100 g	Common Dietary Sources	ALA, g/100 g
Anchovy	763	41	1,292	2,055	Flaxseed (linseed) oil	53.3
Herring, Atlantic	909	71	1,105	2,014	Canola (rapeseed oil)	9.1
Salmon, farmed	862	393	1,104	1,966	Walnuts, English	9.1
Salmon, wild	411	368	1,429	1,840	Butternuts	8.7
Mackerel, Atlantic	504	106	699	1,203	Soybean oil, nonhydrogenated	6.8
Bluefish	323	79	665	988	Mustard oil	5.9
Sardines, Atlantic	473	0	509	982	Soybean oil, hydrogenated	2.6
Trout	259	235	677	936	Walnuts, black	2.0
Golden bass (tilefish)	172	143	733	905	Beechnuts	1.7
Swordfish	127	168	772	899	Pecans	1.0
Tuna, white (albacore)	233	18	629	862	Seaweed, Spirulina, dried	0.8
Mussels	276	44	506	782	Soybeans, boiled	0.6
Striped bass	169	0	585	754	Navy beans, boiled	0.2
Shark	258	89	431	689	Kale, raw	0.2
Pollock, Atlantic	91	28	451	542	Kidney beans, boiled	0.1
Oysters, wild	274	16	210	484		
King Mackerel	174	22	227	401		
Tuna, light (skipjack)	91	17	237	328		
Snapper	48	22	273	321		
Flounder and sole	168	34	132	300		
Clams	138	104	146	284		
Grouper	35	17	213	248		
Halibut	80	20	155	235		
Lobster	117	6	78	195		
Scallops	72	5	104	176		
Blue Crab	101	9	67	168		
Cod, Pacific	42	5	118	160		
Shrimp	50	5	52	102		
Catfish, farmed	20	18	69	89		
Eggs	0	7	58	58		
Chicken breast	10	10	20	30		
Beef	2	4	1	3		
Pork	0	10	2	2		

Data from the U.S. Department of Agriculture National Nutrition Database for Standard Reference Release 23, 2010 (274). These are average values that might vary due to methodological, geographic, temporal, and sample-to-sample differences.

ALA = alpha-linolenic acid; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; PUFA = polyunsaturated fatty acid.

mixed findings, perhaps owing to variable statistical power, n-3 PUFA dosing, durations of consumption, and methods for HR variability assessment (58,89–100). Overall, these studies suggest that n-3 PUFA might improve autonomic function, especially related to augmentation of vagal activity or tone, but further confirmation of such effects and their dose-responses is required.

Cardiac filling and myocardial efficiency. Animal experiments and growing evidence in human studies suggest that n-3 PUFA consumption improves cardiac filling and myocardial efficiency. In animal models, including among non-human primates; in observational studies of habitual fish consumption; and in short-term experimental trials of fish oil in healthy adults and in patients with chronic heart failure, n-3 PUFA consumption augments both early (energy-dependent) and late (compliance-dependent) left ventricular diastolic filling (101–105). Such effects could partly relate to long-term improvements in ventricular compliance due to reduced systemic vascular resistance. Conversely, the relatively rapid improvement in early diastolic filling in some studies suggests a degree of functional or metabolic rather than simply structural benefit. In animal experiments and at least 1 RCT in humans, fish oil consumption also improves myocardial efficiency, reducing workload-specific myocardial oxygen demand without reducing peak performance (106,107). In 2 recent placebo-controlled trials, n-3 PUFA consumption also improved left ventricular ejection fraction in patients with established heart failure (102,108).

Insulin resistance and diabetes. In some observational cohorts, estimated fish or n-3 PUFA consumption was associated with modestly higher incidence of type 2 diabetes (109,110). However, such positive associations were not seen in other observational studies (111–115) and protective associations were seen in a study utilizing objective circulating n-3 PUFA biomarkers (116). In trials, n-3 PUFA
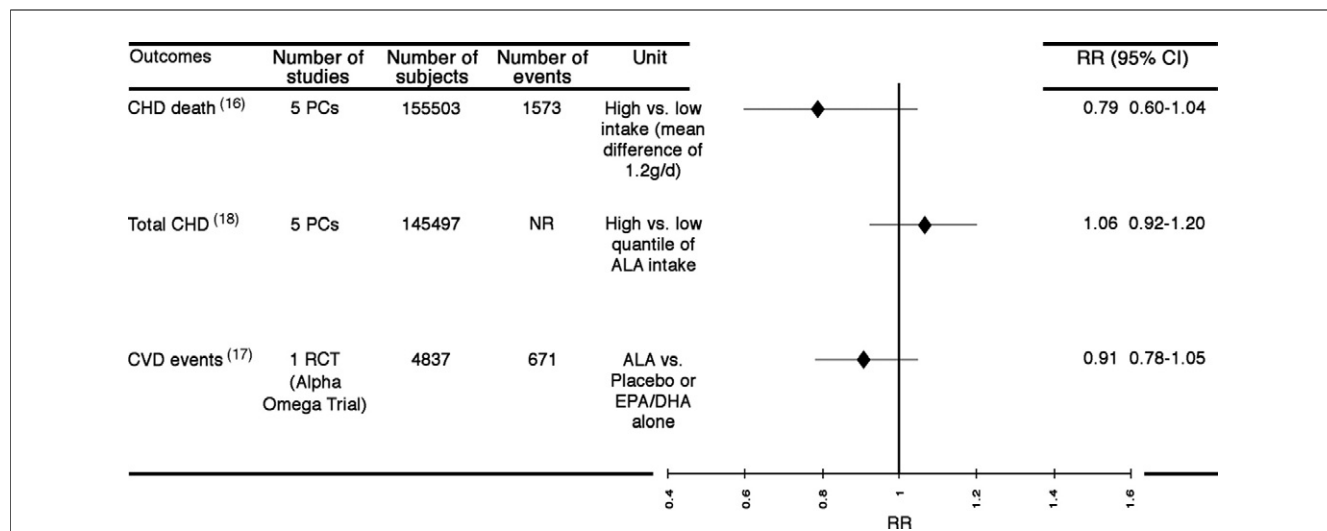
Outcomes	Number of studies	Number of subjects	Number of events	Unit		RR (95% CI)
CHD death [16]	5 PCs	155503	1573	High vs. low intake (mean difference of 1.2g/d)		0.79 0.60-1.04
Total CHD [18]	5 PCs	145497	NR	High vs. low quantile of ALA intake		1.06 0.92-1.20
CVD events [17]	1 RCT (Alpha Omega Trial)	4837	671	ALA vs. Placebo or EPA/DHA alone		0.91 0.78-1.05

Figure 2   Meta-Analyses of Observational Studies and Results From a Large RCT of ALA Consumption and Risk of CVD Outcomes

Relatively few prospective cohort studies (PCs) have evaluated the relationship between consumption of ALA and risk of coronary heart disease (CHD). Meta-analyses of these studies suggest no significant association with total CHD and a trend toward lower risk of CHD death (16,18). A recent randomized controlled trial (RCT) found no significant effect of ALA supplementation (1.9 g/day) in patients with history of myocardial infarction, although only one-half of the patients in the comparison group received placebo, with the other one-half receiving long-chain n-3 PUFA (EPA+DHA) supplements (17). CI = confidence interval; CVD = cardiovascular disease; NR = not reported; RR = relative risk; other abbreviations as in Figure 1.

consumption does not substantially alter biomarkers of glucose-insulin homeostasis. In a meta-analysis of 26 RCTs, fish oil supplementation (2 to 22 g/day) slightly raised fasting glucose in patients with non−insulin-dependent diabetes (+0.4 mmol/l, 95% CI: 0.0 to 0.9) and lowered fasting glucose in patients with insulin-dependent diabetes (−1.9 mmol/l, 95% CI: −0.6 to −3.1); hemoglobin A1c levels were not significantly affected (117). Two additional meta-analyses of 18 and 23 RCTs found no overall effects of fish oil (0.9 to 18 g/day) on fasting glucose or hemoglobin A1c in patients with non−insulin-dependent diabetes (118,119). n-3 PUFA directly regulate hepatic genes (see the following text), suppressing triglyceride production by means of decreased DNL as well as other possible effects (40,41,45,47−49,52). We wonder whether this decrease in triglyceride synthesis from carbohydrates as a substrate could in some individuals result in modestly increased shunting of carbohydrates and/or glycerol to glucose production, which could raise fasting plasma glucose levels but reduce hepatic steatosis and insulin resistance and not adversely affect peripheral insulin resistance or systemic metabolic dysfunction (50−52,120−122). Further investigation is needed, but at present it is unclear whether n-3 PUFA has clinically relevant effects on insulin resistance or diabetes risk in humans.

Inflammation. Although the biological effects of n-3 PUFA could alter several inflammatory pathways (see the following text), it remains unclear whether such anti-inflammatory effects are clinically meaningful, especially at usual dietary doses. In several trials, n-3 PUFA supplementation reduced plasma and urine levels of eicosanoids such as leukotriene E_4 (123−126). Findings for other circulating inflammatory biomarkers, such as interleukin-1−beta and tumor necrosis factor-alpha, are mixed (79,127−133). Fish oil is a proposed adjunctive therapy for inflammatory diseases such as rheumatoid arthritis (134), and meta-analyses of placebo-controlled trials found that high-dose n-3 PUFA supplementation (1.7 to 9.6 g/day) reduced morning stiffness and joint pain in patients with rheumatoid arthritis (135). Eicosapentaenoic acid and DHA could also have local anti-inflammatory effects that might be difficult to detect with circulating biomarkers. In particular, n-3 PUFA are precursors to resolvins, protectins, and other inflammation-resolving mediators that, based on emerging evidence, might have potent anti-inflammatory properties and assist in the resolution of inflammation (see the following text) (136). The influence of dietary fish consumption or usual fish oil supplement doses on levels of these inflammation-resolving mediators and the clinical relevance of such potential effects represent promising areas for further study.

Arrhythmia. Among the most intriguing potential physiological effects of n-3 PUFA and also among the most challenging to document in humans is antiarrhythmia. In vitro and animal experiments suggest that n-3 PUFA directly influence atrial and ventricular myocyte electrophysiology, potentially mediated by effects on membrane ion channels or cell−cell connexins (see the following text) (55,56,137−140). Confirmation of such effects in humans has been limited by absence of reliable physiological measures or biomarkers to quantify antiarrhythmic potential. In
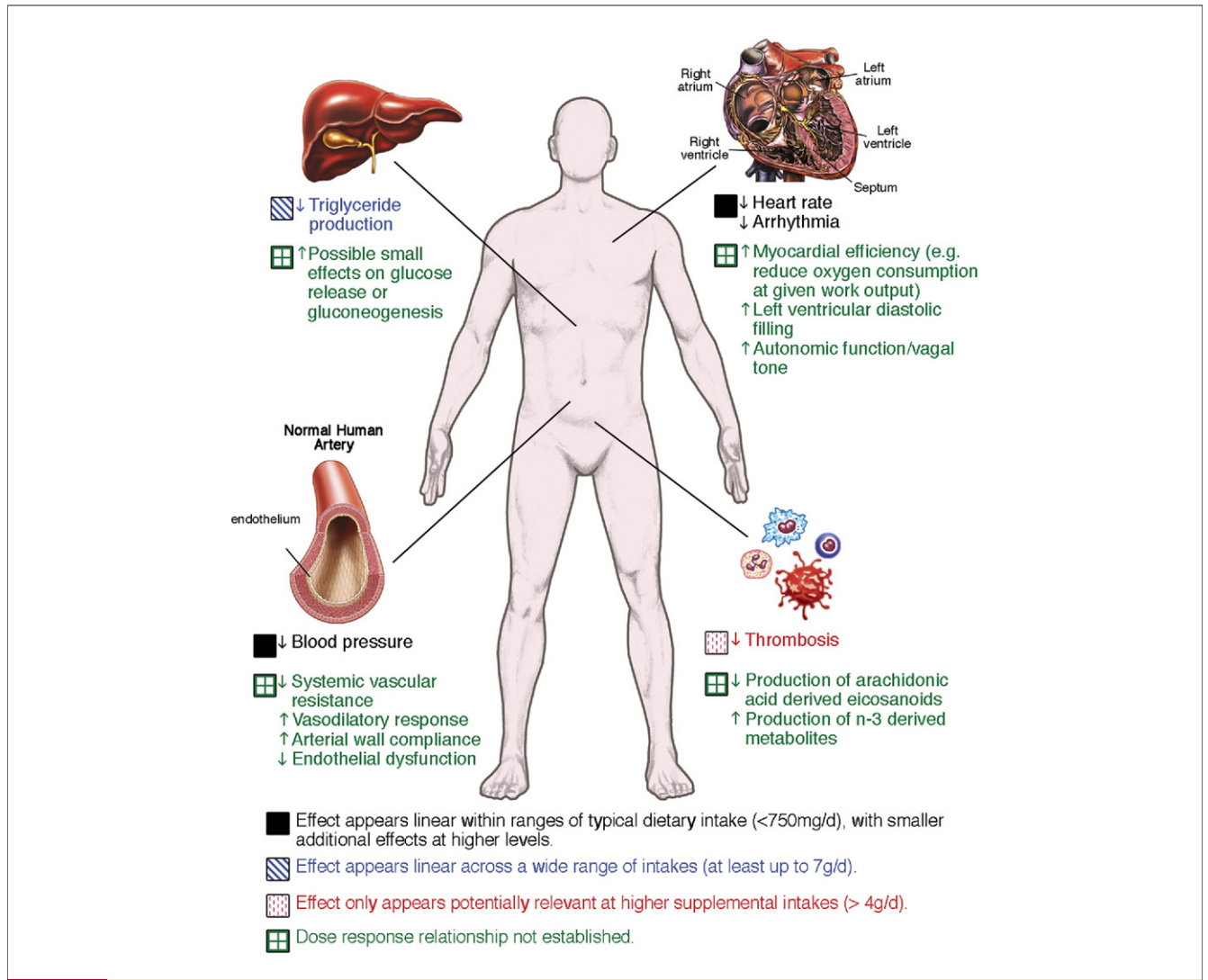
Figure 3   Physiological Effects of n-3 PUFA That Might Influence CVD Risk

n-3 polyunsaturated fatty acid (n-3 PUFA) affects a wide range of physiological functions in multiple tissues, including the heart, liver, vasculature, and circulating cells. Dose-responses of these effects seem to vary. In vitro and animal experiments show that n-3 PUFA directly modulate cardiac electrophysiology, which could contribute to reductions in heart rate and arrhythmic risk **(top right)**. Growing evidence suggests that n-3 PUFA might improve myocardial efficiency, left ventricular diastolic filling, and vagal tone. n-3 PUFA reduce plasma triglyceride levels in a dose-dependent fashion, which is at least partly due to reduced hepatic very low-density lipoprotein production rate. Several mechanisms have been implicated, including effects on hepatic gene expression that down-regulate de novo lipogenesis and possibly other effects such as increased fatty acid beta-oxidation **(top left)**. These hepatic effects might also lead to modest shunting of carbohydrates and/or glycerol to glucose production, which could raise plasma glucose levels but reduce hepatic steatosis and insulin resistance and not adversely affect peripheral insulin resistance or systemic metabolic dysfunction. In the vasculature, n-3 PUFA reduces systemic vascular resistance and improves endothelial dysfunction, arterial wall compliance, and vasodilatory responses **(bottom left)**. These changes together contribute to the established blood pressure-lowering effects of n-3 PUFA. n-3 PUFA supplementation alters ex vivo platelet function, but no clinical effects on bleeding or thrombosis have been seen except perhaps at very high doses (e.g., 15 g/day) **(bottom left)**. n-3 PUFA also reduce production of arachidonic acid-derived eicosanoids and increase synthesis of n-3 PUFA metabolites, although clinical effects of these alterations remain uncertain, particularly at typical dietary doses **(bottom right)**. CVD = cardiovascular disease.

observational studies and in 1 large open-label RCT, n-3 PUFA consumption reduced risk of sudden cardiac death (see the following text), suggesting that anti-arrhythmic effects seen in experimental studies could extend to humans. Several smaller trials have attempted to address this hypothesis by studying patients at higher risk for arrhythmias, including patients with implantable cardioverter-defibrillators (ICDs) for recurrent tachyarrhythmias, patients with recurrent paroxysmal atrial fibrillation (AF), and patients undergoing cardiac surgery. As reviewed in the following text, findings have been mixed, with some trials demonstrating lower risk of arrhythmias and others finding no significant effects (141–147). Overall, although evidence from in vitro studies, animal-experiments, and at least some human studies remains compelling, confirmation of clinically relevant anti-arrhythmic effects of n-3 PUFA has remained elusive. It is also unclear whether such benefits, if present, are due to direct effects on myocyte electrophysiology or
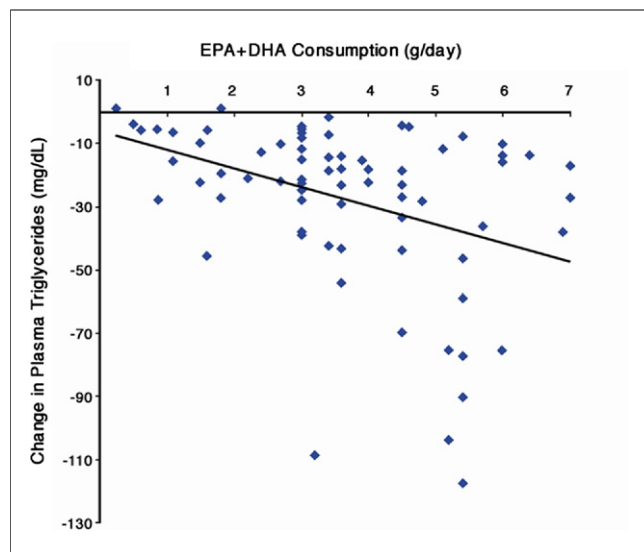
Figure 4   **Dose-Response Effects of n-3 PUFA Consumption on Fasting Plasma Triglycerides in RCTs**

Based on 55 placebo-controlled trials of n-3 PUFA consumption for 2 or more weeks as extracted from a prior systematic review (276) as well as 3 additional RCTs of fish or n-3 PUFA consumption (169,277,278) to provide additional dose-response information at doses of <1 g/day EPA+DHA. Each point represents the change in plasma triglycerides from baseline for each individual study arm, as compared with control. The **solid line** represents the line of best fit calculated from linear regression. Overall, each 1-g/day increase of EPA+DHA reduced triglycerides by −5.9 mg/dl (95% confidence interval [CI]: −2.5 to −9.3 mg/dl). This effect was significantly greater in trials of individuals with higher starting triglyceride levels (p interaction <0.001). Among trials of individuals with mean baseline triglycerides below the median (<83 mg/dl), each 1 g/day EPA+DHA decreased triglycerides by −1.7 mg/dl (95% CI: −3.1 to −0.2 mg/dl). Among trials of individuals with mean baseline triglycerides above the median (>83 mg/dl), each 1 g/day EPA+DHA decreased triglycerides by −8.4 mg/dl (95% CI: −13.7 to −3.2 mg/dl). Abbreviations as in Figure 1.

more indirect influences such as improvements in myocardial efficiency, autonomic tone, local inflammatory responses, and the like.

Molecular Mechanisms

Fatty acids play important and diverse roles in cellular and organelle membrane structure and function, tissue metabolism, and genetic regulation. With unique chemical structures and 3-dimensional configurations (Fig. 1), n-3 PUFA influence multiple relevant molecular pathways (Fig. 5), which individually or in sum might contribute to the observed effects on physiological risk factors and clinical events.

Cell and organelle membrane structure and function. Cellular and organelle functions are strongly influenced by membrane lipid environments. Lipid microdomains—for example, cholesterol and sphingolipid enriched "rafts" and caveolae in membranes—function as operational "platforms" to modulate numerous cellular functions, including signal transduction, protein and membrane trafficking, and ion channel kinetics (148–150). In cell culture and animal studies, the incorporation of n-3 PUFA into membrane phospholipids alters the physicochemical properties of

membrane rafts and caveolae, thereby influencing membrane-associated protein localization and function. Many such experimentally observed effects have been seen, including changes in caveolae-associated signaling protein H-Ras (151); suppression of protein kinase C-theta signaling and production of interleukin-2 (152,153); and disruption of dimerization and recruitment of toll-like receptor-4 with subsequent inhibition of lipopolysaccharide-induced inflammation (154,155). Membrane-bound n-3 PUFA might also enhance protein signaling efficiency as exemplified by the interaction between DHA and rhodopsin, a G-protein–coupled receptor critical in the visual system (156,157). Incorporation of n-3 PUFA into cellular membranes with subsequent alteration of protein function and signaling might contribute to potential anti-inflammatory and anti-arrhythmic effects (see the following text).

Ion channels and electrophysiology. In animal-experimental and in vitro studies, n-3 PUFA directly affect myocyte electrophysiology (e.g., altering the function of membrane sodium channel, L-type calcium channel, and sodium–calcium exchanger) (158–165). Such effects might contribute to reduced myocyte excitability and cytosolic calcium fluctuations, particularly in ischemic or damaged cells susceptible to partial depolarization and triggered arrhythmia (56). However, specific effects in experimental studies have not always been consistent and might depend on experimental models used (e.g., type of animal species) or method of n-3 PUFA administration (e.g., acute intravenous vs. long-term dietary incorporation into tissues) (166,167). Accumulating evidence suggests that lipid microenvironments modulate ion channel function (150). Thus, as described previously, incorporation of n-3 PUFA into and resultant changes in lipid membranes could contribute to effects on ion channels. Additionally, some evidence suggests that n-3 PUFA might also directly interact with membrane channels and proteins (156,162,168). For example, the inhibitory effects of EPA on the human cardiac sodium cation channels were reduced by a single amino acid point mutation in the protein alpha-subunit, suggesting a potential direct interaction between EPA and the ion channel (162). Whereas modulation of ion channels would be consistent with anti-arrhythmic effects seen in animal models (55) and suggested by at least some human studies (146,169–171), the potential relevance of these experimentally observed influences on ion channels to health effects in humans is not established.

Nuclear receptors and transcription factors. n-3 PUFA are natural ligands of several nuclear receptors and transcription factors that regulate gene expression in multiple tissues (122,172). Nonesterified n-3 PUFA or their acyl-CoA thioesters can bind and directly modulate activities of such receptors (173–176). Cytoplasmic lipid-binding proteins likely play important regulatory roles in this process by shuttling free fatty acids or fatty acyl-CoA into the nucleus to interact with the receptors (177,178). These receptors are central regulators of vital cellular functions related to CVD,
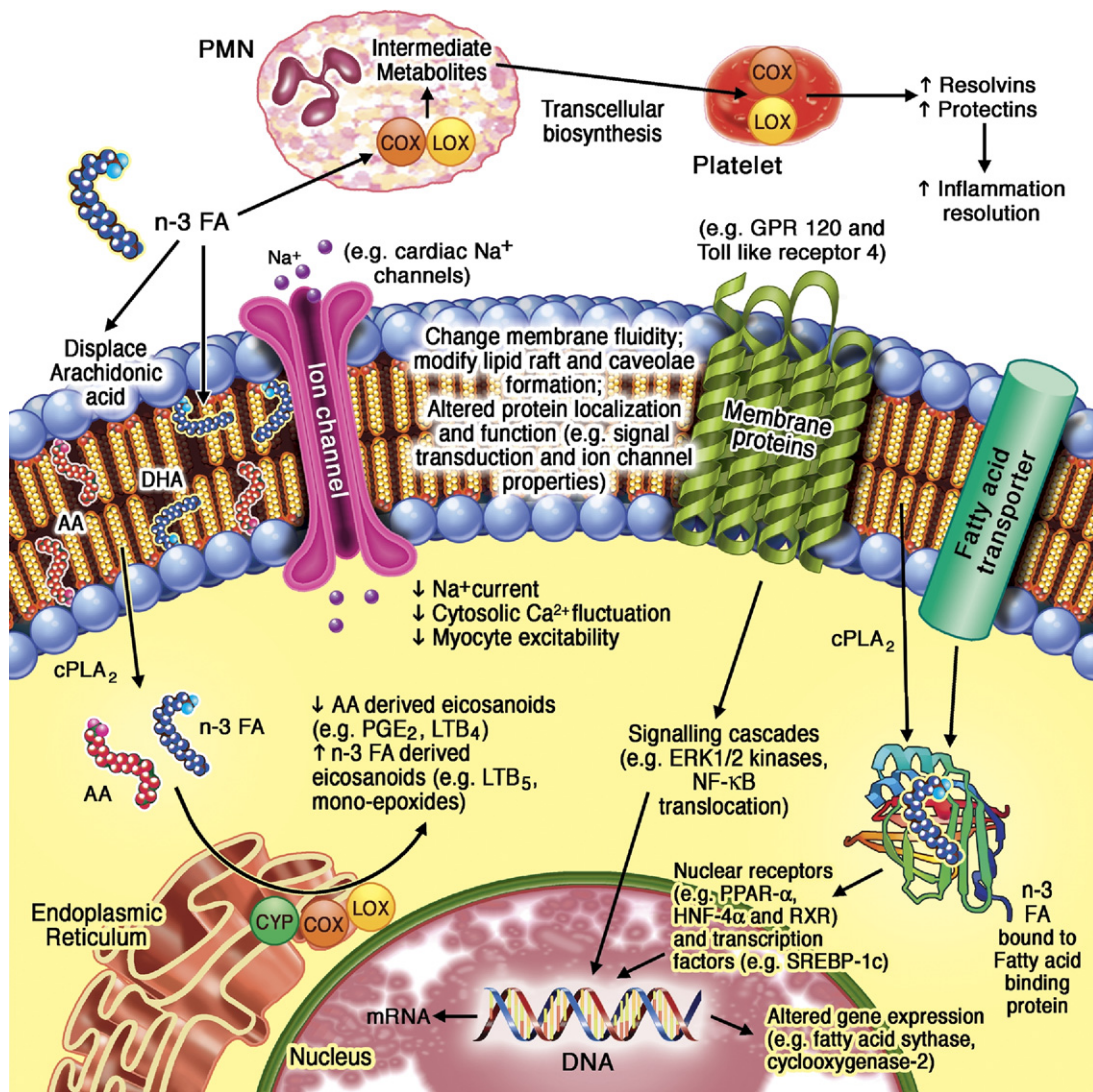
Figure 5   **Molecular Pathways Affected by n-3 PUFA**

n-3 polyunsaturated fatty acids (n-3 PUFA) modulate multiple molecular pathways that together contribute to their physiological effects. First, the physicochemical properties of cellular and organelle membranes are influenced by their lipid composition **(center)**. Incorporation of n-3 PUFA into these membranes alters membrane fluidity and biophysics of lipid rafts that modulate protein function and signaling events. For example, enrichment of cellular membranes with n-3 PUFA disrupts dimerization and recruitment of toll-like receptor-4, which might contribute to anti-inflammatory effects by down-regulation of nuclear factor-kappaB (NF-κB) activation. Ion channels such as sodium (Na^+), L-type calcium (Ca^{2+}), and Na^+–Ca^{2+} exchangers might be similarly modulated by n-3 PUFA incorporation into lipid membranes. Second, n-3 PUFA seem to directly interact with membrane channels and proteins **(center)**. For example, direct modulation of ion channels or G-protein-coupled receptor 120 (GPR 120) might contribute to anti-arrhythmic or anti-inflammatory effects, respectively. Third, n-3 PUFA directly regulate gene expression via nuclear receptors and transcription factors **(lower right)**. n-3 PUFA are natural ligands of many key nuclear receptors in multiple tissues, including peroxisome proliferator-activated receptors (PPAR; -alpha, -beta, -delta, and -gamma), hepatic nuclear factors (HNF-4; -alpha and -gamma), retinoid X receptors (RXR), and liver X receptors (alpha and beta). Interactions between n-3 PUFA and nuclear receptors are modulated by cytoplasmic lipid binding proteins (e.g. fatty acid [FA] binding proteins) that transport the FAs into the nucleus. n-3 PUFA also alter function of transcription factors such as sterol regulatory element binding protein-1c (SREBP-1c). Such genetic regulation contributes to observed effects of n-3 PUFA on lipid metabolism and inflammatory pathways. Fourth, after release from phospholipids by cytosolic phospholipase A_2 (cPLA$_2$), PUFA including n-3 PUFA are converted to eicosanoids by cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP450) enzymes **(lower left)**. n-3 PUFA displace arachidonic acid (AA) in membrane phospholipids, reducing the production of AA-derived eicosanoids (e.g., prostaglandin E2 [PGE2]) while increasing those generated from n-3 PUFA. This altered eicosanoid profile might influence inflammation, thrombosis, and vascular function. Fifth, emerging evidence suggests that n-3 PUFA play an important role in inflammation resolution via specialized pro-resolving mediators (SPMs), including resolvins or protectins that are n-3 PUFA metabolites derived from actions of COX and LOX **(top)**. Biosynthesis of SPMs seems to require involvement of 2 or more cell types ("transcellular biosynthesis"), with 1 cell type converting the n-3 fatty acid to metabolic intermediates, and the second cell type converting these intermediates into the SPMs. n-3 PUFA-derived SPMs seem to be key drivers of inflammation resolution programs that reduce chronic inflammation in a wide range of animal models. The roles of each of these molecular pathways in the cardiovascular protection of n-3 PUFA represent promising areas for future investigation. DNA = deoxyribonucleic acid; ERK = extracellular signal-regulated kinase; mRNA = messenger ribonucleic acid; PMN = polymorphonuclear leukocyte.

including lipid metabolism, glucose-insulin homeostasis, and inflammation. For example, effects of n-3 PUFA on these pathways likely contribute to triglyceride-lowering (3,179,180) and increased production of beneficial adipocytokines (181,182). n-3 PUFA can also affect activation of transcription factors (Fig. 5) (3,122,179). For example, by means of peroxisome proliferator-activated receptor-gamma activation or reduced protein kinase-C translocation to the plasma membrane, n-3 PUFA can reduce translocation of nuclear factor-kappaB to the nucleus and inflammatory cytokine generation (183,184).

AA-derived eicosanoids. Eicosanoids are bioactive lipid mediators derived from metabolism of PUFA by cyclooxygenases, lipoxygenases, cytochrome P450s, and nonenzymatic pathways. Although the term "eicosanoids" has traditionally referred to the n-6 PUFA AA and its 20-carbon metabolites, it has also been applied to similar n-3 PUFA–derived metabolites (185), a practice we will follow. n-3 PUFA consumption decreases production of AA-derived 2-series prostaglandins, thromboxanes, and 4-series leukotrienes in humans (123,126,186−192) (Fig. 5).

Because several AA-derived eicosanoids are considered to be pro-inflammatory or pre-thrombotic (e.g., leukotriene-B_4, thromboxane-A_2), their lowering by n-3 PUFA has been considered important for health benefits. Growing evidence argues that this hypothesis is overly simplistic. First, the anti-inflammatory effects of n-3 PUFA may be independent of AA (e.g., via direct interactions with G-protein-coupled receptors [168]). Furthermore, several AA-derived eicosanoids, such as epoxyeicosatrienoic acid (EET) and lipoxins, may protect against CHD. EETs and lipoxins exhibit anti-inflammatory activities; lipoxins also function as pro-resolution mediators of inflammation (136) (see the following text), and EETs are also potent vasodilators and modulate several ion channels (193). Higher EET levels protect against hypertension and cardiac injury in several animal models (194). In support of the benefits of AA-derived metabolites, higher AA levels were associated with lower systemic inflammation and lower CHD risk in some prospective observational studies (195,196). Thus, the significance and consequences of altered AA-derived metabolites following n-3 PUFA consumption appears complex. Future studies must investigate the interplay between n-3 PUFA and both traditional and novel AA-derived metabolites, as well as eicosanoids generated from n-3 PUFA themselves (see the following text).

n-3 PUFA-derived eicosanoids. Recently identified classes of n-3 PUFA-derived eicosanoids (e.g., specialized pro-resolving mediators [SPMs] [136] and CYP450-generated mono-epoxides from EPA and DHA [MEFAs] [197]) possess unique bioactivities that might influence CVD (Fig. 5). Traditionally, it was thought that the breakdown of local pro-inflammatory mediators (e.g., prostaglandins, thromboxanes) was sufficient to end the inflammatory response (198). However, specific cellular "resolution programs" have recently been identified, the efficient functioning of which appears to be essential to ensure timely inflammation resolution and return to tissue homeostasis (136). Both n-3 PUFA-derived SPMs, such as resolvins, protectins, and maresins, and AA-derived lipoxins are key drivers of these resolution programs. SPMs and lipoxins reduce chronic inflammation in a range of animal models; models of CHD are still limited (136,199). MEFAs are potent vasodilators (200−203), modulate several ion channels (200,202,204−206), and reduce inflammation (207) in vitro, with similar or stronger potency than analogous AA-derived EETs. In recent experiments, n-3 PUFA-derived MEFAs possessed nearly 1,000-fold greater potency than their parents EPA or DHA in reducing effects of calcium overload in rat ventricular myocytes; interestingly, AA-derived EETs antagonized this effect (208). Short-term n-3 PUFA consumption (4 g/day for 4 weeks) increased EPA- and DHA-derived MEFAs by ~5- and 2-fold, respectively (123). Robust effects of SPMs and MEFAs in multiple tissues and animal models suggest that they could play a key role in cardiovascular protection of n-3 PUFA—a highly promising area for future research.

Cardiovascular Outcomes

CHD mortality. More prospective observational studies and large RCTs have investigated potential effects of fish or n-3 PUFA consumption on CVD outcomes than any other food or nutrient. Numerous meta-analyses have been performed (Fig. 6) (1,18,20,141,209−215). Overall, the findings indicate that consumption of fish or fish oil significantly reduces CHD mortality, including fatal myocardial infarction and sudden cardiac death, in populations with and without established CVD (1,209,214,215). In meta-analyses of RCTs of n-3 PUFA (1,214,215), significant reductions or trends toward reductions have been seen for total mortality, with effect sizes consistent with expected benefits if n-3 PUFA consumption were to reduce CHD death but have little effect on other causes of mortality. These studies, together with ecologic evidence of n-3 PUFA consumption and CHD death rates across populations (216,217), provide strong concordant evidence that consumption of fish or n-3 PUFA reduces CHD mortality. More modest relationships have been seen with total CHD or nonfatal coronary syndromes, suggesting that, at usual dietary doses, n-3 PUFA might principally reduce ischemia-related cardiac death (218). The final common pathway for most cardiac deaths is arrhythmia. In in vitro and animal models, n-3 PUFA stabilize partially depolarized ischemic myocytes, reducing susceptibility to triggered ventricular arrhythmias (55,56). These findings are consistent with clinical reductions in cardiac death. Other modest physiologic benefits of n-3 PUFA, such as on blood pressure, triglycerides, or inflammation, could over many years or at higher doses alter chronic atherogenesis and/or acute plaque rupture, modestly lowering nonfatal coronary syndromes (1,209,218). However, clinical effects on nonfatal coronary events cannot yet be considered established.
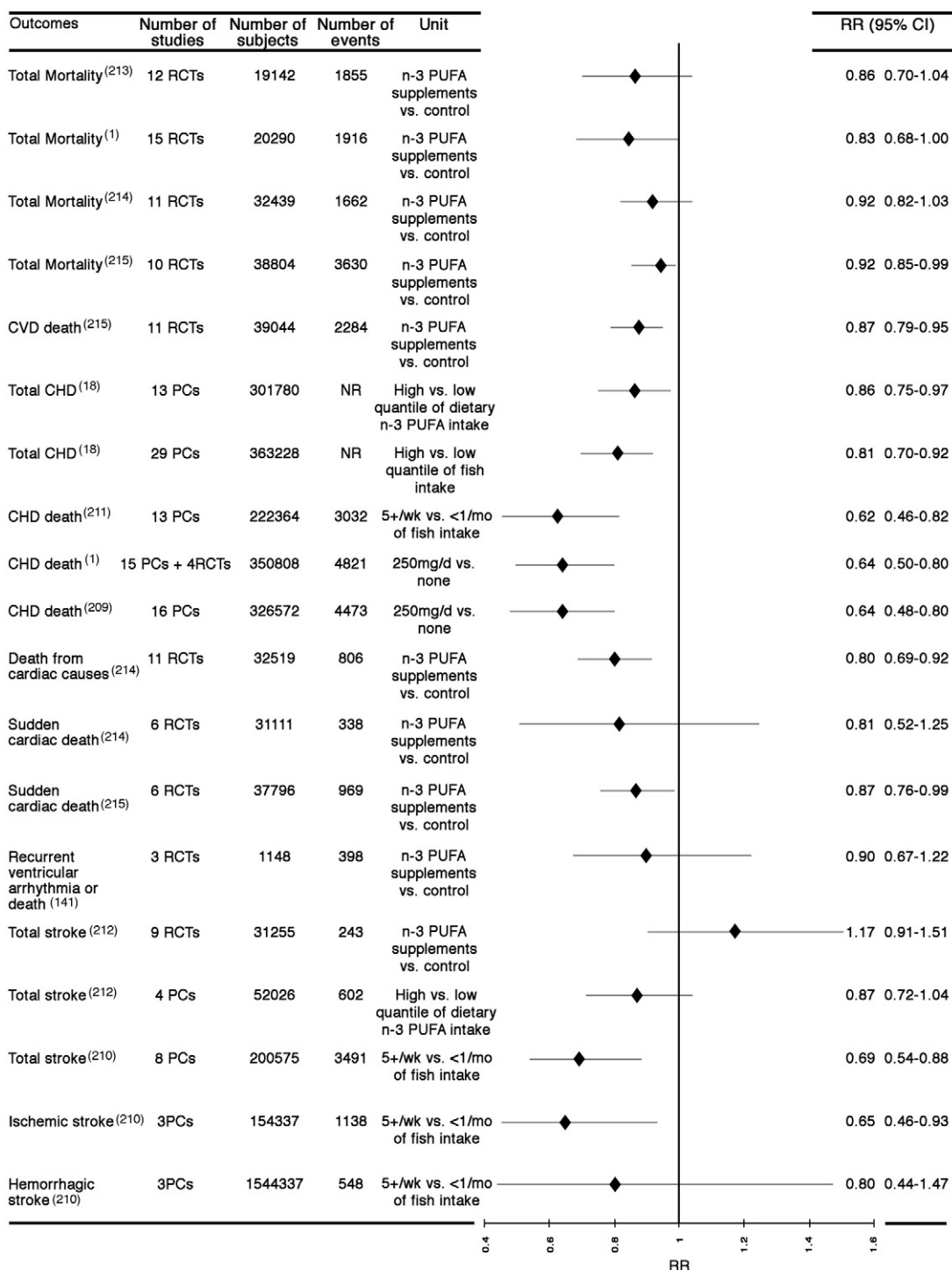
Outcomes	Number of studies	Number of subjects	Number of events	Unit		RR (95% CI)
Total Mortality [213]	12 RCTs	19142	1855	n-3 PUFA supplements vs. control		0.86 0.70-1.04
Total Mortality [1]	15 RCTs	20290	1916	n-3 PUFA supplements vs. control		0.83 0.68-1.00
Total Mortality [214]	11 RCTs	32439	1662	n-3 PUFA supplements vs. control		0.92 0.82-1.03
Total Mortality [215]	10 RCTs	38804	3630	n-3 PUFA supplements vs. control		0.92 0.85-0.99
CVD death [215]	11 RCTs	39044	2284	n-3 PUFA supplements vs. control		0.87 0.79-0.95
Total CHD [18]	13 PCs	301780	NR	High vs. low quantile of dietary n-3 PUFA intake		0.86 0.75-0.97
Total CHD [18]	29 PCs	363228	NR	High vs. low quantile of fish intake		0.81 0.70-0.92
CHD death [211]	13 PCs	222364	3032	5+/wk vs. <1/mo of fish intake		0.62 0.46-0.82
CHD death [1]	15 PCs + 4RCTs	350808	4821	250mg/d vs. none		0.64 0.50-0.80
CHD death [209]	16 PCs	326572	4473	250mg/d vs. none		0.64 0.48-0.80
Death from cardiac causes [214]	11 RCTs	32519	806	n-3 PUFA supplements vs. control		0.80 0.69-0.92
Sudden cardiac death [214]	6 RCTs	31111	338	n-3 PUFA supplements vs. control		0.81 0.52-1.25
Sudden cardiac death [215]	6 RCTs	37796	969	n-3 PUFA supplements vs. control		0.87 0.76-0.99
Recurrent ventricular arrhythmia or death [141]	3 RCTs	1148	398	n-3 PUFA supplements vs. control		0.90 0.67-1.22
Total stroke [212]	9 RCTs	31255	243	n-3 PUFA supplements vs. control		1.17 0.91-1.51
Total stroke [212]	4 PCs	52026	602	High vs. low quantile of dietary n-3 PUFA intake		0.87 0.72-1.04
Total stroke [210]	8 PCs	200575	3491	5+/wk vs. <1/mo of fish intake		0.69 0.54-0.88
Ischemic stroke [210]	3PCs	154337	1138	5+/wk vs. <1/mo of fish intake		0.65 0.46-0.93
Hemorrhagic stroke [210]	3PCs	1544337	548	5+/wk vs. <1/mo of fish intake		0.80 0.44-1.47

RR scale: 0.4 0.6 0.8 1 1.2 1.4 1.6

Figure 6   **Meta-Analyses of Studies of Fish or Long-Chain n-3 PUFA Consumption and Risk of CVD Outcomes**

Numerous PCs and RCTs from around the world have investigated the potential effects of fish or n-3 PUFA consumption on CVD outcomes. Meta-analyses of these studies indicate that fish and n-3 PUFA consumption reduce the risk of CHD events, primarily due to prevention of CHD death (1,18,20,140,206–212). Potential effects on total CVD events or total mortality are more modest, consistent with anticipated benefits that would occur from reduced CHD mortality alone. Results of PCs also demonstrate inverse associations between fish consumption and stroke, in particular ischemic stroke, but RCTs of n-3 PUFA supplementation have not confirmed these benefits, perhaps related to few numbers of strokes in these trials. Potential effects of fish or n-3 PUFA consumption on other outcomes, such as atrial fibrillation, recurrent ventricular arrhythmias, or congestive heart failure, require further investigation; few studies with relatively limited numbers of events have evaluated these endpoints. Abbreviations as in Figures 1 and 2.

| Table 2 | RCTs of n-3 PUFA and Clinical Cardiovascular Events |

Trials, Year (Ref. #)	Population	Intervention	Duration of Follow-Up, yrs	Events	RR (95% CI)	Achieved Power*
DART, 1989 (222)	2,033 men with recent (average ~1 month prior) MI	Advice to consume fatty fish 2 servings/week vs. usual care	2	IHD events, n = 276 IHD deaths, n = 194	0.84 (0.66–1.07) 0.68 (0.49–0.94)	0.69 0.57
GISSI-Prevenzione Trial, 1999 (169)	11,324 men with recent (≤3 months prior) MI	882 mg/day EPA+DHA vs. usual care	3.5	Cardiac deaths, n = 520 Sudden deaths, n = 286	0.78 (0.65–0.92) 0.74 (0.58–0.93)	0.91 0.69
DART 2, 2003 (221)	3,114 men with angina	Advice to consume fatty fish 2 servings/week vs. usual care	3–9	Cardiac deaths, n = 319 Sudden deaths, n = 120	1.26 (1.00–1.58) 1.54 (1.06–2.23)	0.65 0.26
JELIS, 2007 (220)	18,645 men and women with total cholesterol ≥6.5 mmol/l	1.8 g/day EPA vs. usual care	5	Major coronary events, n = 586 Coronary deaths, n = 60 Sudden deaths, n = 35	0.81 (0.69–0.95) 0.94 (0.57–1.56) 1.06 (0.55–2.07)	0.93 0.17 0.13
GISSI-Heart Failure 2008 (223)	6,975 patients with chronic congestive heart failure	882 mg/day EPA+DHA vs. placebo	3.9	Total mortality, n = 1,969 Cardiovascular death, n = 1,477 Sudden deaths, n = 632	0.91 (0.83–0.99) 0.90 (0.81–0.99) 0.93 (0.79–1.08)	>0.99 >0.99 0.94
Alpha-Omega, 2010 (17)	4,837 patients with a history of past (average ~4.3 yrs prior) MI	376 mg/day EPA+DHA vs. a combined control group receiving either placebo or ALA 1.9 g/day	3.3	Major cardiovascular events, n = 671 CHD deaths, n = 138	1.01 (0.87–1.17) 0.98 (0.68–1.32)	0.96 0.36
Omega, 2010 (219)	3,851 patients with recent (≤2 weeks prior) MI	840 mg/day EPA+DHA vs. placebo	1	Major cardiovascular events, n = 331 Sudden deaths, n = 57	1.21 (0.96–1.52) 0.95 (0.56–1.60)	0.72 0.17
SU.FOL.OM3, 2010 (224)	2,501 patients with a history of past (average ~100 days prior) acute coronary or cerebral ischemic event	600 mg/day EPA+DHA vs. a combined control group receiving either placebo or B vitamins (5-methyltetrahydrofolate, 560 μg; B-6, 3 mg; and B-12, 20 μg)	4.2	Major cardiovascular events, n = 157 CHD deaths, n = 40	1.08 (0.79–1.47) Not reported	0.4 0.14

*On the basis of the actual number of events, 2-sided alpha = 0.05, and a relative risk (RR) reduction of 25% for n-3 fatty acid treatment.

CHD = coronary heart disease; CI = confidence interval; DART = Diet and Reinfarction Trial; IHD = ischemic heart disease; JELIS = Japan EPA Lipid Intervention Study; MI = myocardial infarction; RCT = randomized controlled trial; SU.FOL.OM3 = Supplementation en Folates et Omega-3; other abbreviations as in Table 1.

There is currently little evidence that effects of n-3 PUFA on CHD differ by sex, age, or race/ethnicity. Prospective cohort studies among both men and women, in those of middle age and older adults, and in different races/ethnicities have demonstrated similar findings (1). Women convert ALA to EPA in modestly greater amounts (10), but the clinical significance of this is not established.

Conflicting evidence for CHD mortality. Not all RCTs have demonstrated reductions in CHD mortality with n-3 PUFA consumption (Table 2) (17,169,219–224). These include the Alpha-Omega (17), Omega (219), JELIS (Japan EPA Lipid Intervention Study) (220), DART (Diet and Reinfarction Trial) 2 (221), and SU.FOL.OM3 (Supplementation en Folates et Omega-3) (224) trials. Among these, only the DART 2 trial, an open-label, dietary advice trial among men with stable angina, was adequately powered to detect such effects (Table 2). Several limitations of this trial were reported, including lack of participant blinding, inadequate funding that interrupted recruitment over 7 years, little follow-up to reinforce dietary advice or evaluate long-term compliance, and no evaluation of changes in medications or other behaviors. Additionally, the control group was provided "sensible eating" advice, which resulted in similar or better outcomes in comparison with all intervention groups, which included dietary advice to consume fish in 1 arm and oats, fruits, and vegetables in another arm. These limitations make it difficult to interpret this trial's null findings.

The JELIS, Alpha-Omega, Omega, and SU.FOL.OM3 trials were each substantially underpowered to detect effects on CHD mortality (Table 2), although the JELIS trial did find modest benefits for nonfatal coronary events (see the following text). Also, due to lower than expected event rates, the Alpha-Omega trial compared the effects of low-dose EPA+DHA (376 mg/day) not with placebo, but with a combined control group that received either placebo or active treatment with the plant-derived n-3 ALA (1.9 g/day). In this European study, subjects also consumed relatively high background dietary EPA+DHA (median 120 to 130 mg/day), which could minimize effects of the additional low-dose n-3 PUFA supplement, on the basis of evidence for a threshold of benefit for CHD mortality at approximately 250 mg/day (1). Both the Omega and SU.FOL.OM3 trials were markedly underpowered, with 57 and 40 CHD deaths, respectively (219,224), providing only approximately 17% and approximately 14% power to detect a 25% risk reduction.

Overly optimistic estimates of benefits of n-3 PUFA could continue to foster implementation of small, underpowered RCTs, which would contribute to further confusion about cardiovascular effects of these fatty acids. Potent drugs such as statins produce modest (25% to 30%) reductions in CVD risk (225); expectations that n-3 PUFA should have much larger benefits are unrealistic, especially in patients receiving modern medical and interventional therapies. The apparent nonlinear dose-responses for some health effects of n-3 PUFA are also relevant to trial design.

Observational studies typically compare risk among individuals consuming higher levels of fish or n-3 PUFA versus individuals having little or no consumption. In contrast, RCTs generally enroll a broad cross-section of subjects, resulting in placebo groups that include comparatively high background levels of dietary fish and n-3 PUFA intakes. Because at least some benefits of n-3 PUFA seem to diminish as consumption increases (Fig. 3), including for CHD mortality (1), this difference in the comparison group of observational studies versus trials will necessitate greater numbers of subjects in trials to detect additional benefits of n-3 PUFA supplementation above and beyond background diet. These issues could be relevant to interpreting ongoing n-3 PUFA trials, including the ORIGIN (Outcome Reduction with an Initial Glargine Intervention) trial, Risk and Prevention Study, and VITAL (Vitamin D and Omega-3 Trial) (226–228).

In comparison with other isolated nutrients for which RCTs of clinical CVD events have been null (e.g., folate/B vitamins, vitamin D, vitamin E) or with other well-established behavioral and dietary targets for which no RCTs of clinical CVD events have even been performed (e.g., smoking cessation, physical activity, obesity reduction, salt or trans-fat reduction, or consumption of dietary fiber, fruits, vegetables, or whole grains), the presence of several confirmatory clinical trials of n-3 PUFA and CVD events lends considerable support to the total body of findings. Overall, there is concordance of evidence from experimental studies, controlled trials of physiological risk factors, prospective observational studies of clinical endpoints, and adequately powered RCTs of clinical endpoints that modest n-3 PUFA consumption—compared with little or no consumption—reduces CHD mortality.

Ischemic stroke. In comparison with CHD mortality, effects of n-3 PUFA on other CVD outcomes are less well established. For instance, meta-analyses of observational studies suggest that fish consumption reduces risk of ischemic stroke (210), but stroke incidence has not been significantly affected in fish oil trials (212,229). Reasons for these differing findings remain unclear, with possibilities including residual confounding (bias) in the observational studies, inadequate statistical power in the trials (which were not designed to evaluate stroke as an endpoint), insufficient duration of treatment in the trials, or benefits for stroke from other nutrients present in fish but not fish oil. Established effects on several risk factors (Fig. 2) provide biological plausibility that n-3 PUFA could reduce stroke. Appropriately powered RCTs of fish or fish oil consumption and ischemic stroke are needed.

AF. In vitro and animal experiments suggest that n-3 PUFA could reduce onset of AF (230–235). Mechanistically, such effects could derive from putative direct antiarrhythmic effects or favorable changes in left ventricular performance, autonomic tone, or inflammation (see the preceding text). A growing number of human studies are evaluating n-3 PUFA and AF. Estimated fish or dietary n-3 PUFA consumption was associated with lower AF incidence in some (236) but not other (237–239) large observational cohorts. In a prospective evaluation of plasma n-3 PUFA biomarkers, DHA levels were inversely associated with incident AF (240). Five small RCTs have evaluated perioperative n-3 PUFA supplementation and postoperative AF after cardiac surgery (142–145,147). Two trials found benefit and the other 3 showed no effect, with the varying designs and small sizes (n = 108 to 200) limiting strong conclusions. Another trial demonstrated no effects of n-3 PUFA supplements on AF recurrence in 663 patients with pre-existing paroxysmal AF (241). A second similar trial is ongoing (242), which we believe similarly may not demonstrate benefits due to the low likelihood that n-3 PUFA (or most any treatment) can appreciably counteract the pro-arrhythmogenic cardiac structural changes already present in patients with pre-existing AF. To date, the mixed evidence limits inference about whether n-3 PUFA can prevent AF. The ongoing OPERA (Omega-3 Fatty Acids for the Prevention of Post-operative Atrial Fibrillation) trial is a large, double-blind, placebo-controlled, multicenter RCT that will help answer this important question (76,243).

Recurrent ventricular tachyarrhythmias. Based partly on antiarrhythmic effects in animal experiments, 3 placebo-controlled RCTs have evaluated whether n-3 PUFA reduce recurrent ventricular tachycardia or fibrillation (VT/VF) in patients with ICDs and pre-existing VT/VF. One of these trials showed 31% reduction in definite or probable recurrent VT/VF (p = 0.03) (244), whereas 2 others showed no statistically significant effects (245,246); meta-analysis of all 3 trials found significant pooled effects (141,247). Heterogeneity of results could relate to varying patient populations or n-3 PUFA dosing (from 0.8 to 2.6 g/day EPA+DHA). Overall, the relatively small sizes (n = 200 to 546) and brief treatment durations (1 to 2 years) of these trials limit definitive conclusions for effects of n-3 PUFA on recurrent VT/VF in patients with ICDs. Benefits seen in at least 1 of these trials, when considered with in vitro and animal-experimental evidence, lend further support to antiarrhythmic effects of n-3 PUFA. Confirmation of such effects would be important for this patient subset and also mechanistically relevant. However, the presence or absence of effects on recurrent VT/VF (or recurrent AF) might have relatively little generalizability to effects of fish or n-3 PUFA consumption on primary arrhythmias in less-selected populations, for example, ischemia-induced ventricular fibrillation that causes the majority of sudden cardiac deaths and fatal myocardial infarctions in CHD patients and the general population.

Congestive heart failure. Physiological effects in humans (248) and animal models (107,249,250) suggest that n-3 PUFA could prevent heart failure. Fish or dietary n-3 PUFA consumption has been associated with lower incidence of heart failure in 4 of 5 prospective observational studies (251–255). A recent investigation using objective

circulating biomarkers found this relationship to be strongest for EPA, with 50% lower incidence of CHF in the highest versus lowest quartile (RR: 0.52, 95% CI: 0.38 to 0.72, p trend = 0.001) (256). In a double-blind, placebo-controlled clinical RCT among nearly 7,000 patients with established heart failure, n-3 PUFA supplementation (1 g/day) reduced total mortality by 8% (p = 0.009) (223), accompanied by significant improvements in left ventricular ejection fraction (102), when given in addition to maximal modern drug therapies. On the basis of this trial, recommendations for fish consumption or fish oil use should be considered in patients with established heart failure. Potential effects of n-3 PUFA on preventing heart failure incidence require further study.

Fish Versus Fish Oil

Most studies of CHD death in generally healthy populations have evaluated fish consumption, not fish oil supplementation. As described in the preceding text, fish contain several potentially beneficial nutrients not contained in fish oil. Thus, we agree with clinical and policy recommendations that focus on dietary n-3 PUFA from fish consumption rather than from supplements. For individuals who cannot consume sufficient amounts of fish or who consume fish but wish to further supplement their dietary n-3 PUFA consumption, use of fish oil is reasonable.

Many over-the-counter supplements and 1 prescription formulation are available. Depending on the brand, the combined content of EPA plus DHA per 1 g capsule varies from approximately 300 to 850 mg (257–259). Among commonly sold brands, quality assessments have confirmed that listed and actual contents of n-3 PUFA are similar (257,258). Fish oil supplements contain no mercury (which is tightly protein-bound) and contain low absolute quantities of dioxins/PCBs (because fish oil is consumed only in g quantities). Thus, the choice among different brands can be determined by other factors such as price, availability, and (for flavored brands) taste. If significant triglyceride-lowering is a goal, then concentrated over-the-counter or prescription formulations are preferable to facilitate sufficiently high doses (>3 g/day EPA+DHA) with reasonable daily numbers of capsules.

A common symptom from fish oil is "fishy" taste or eructation. From our experience, taking the capsule frozen, switching to a different formulation, or taking the capsule with meals or at a different time of day can minimize this symptom in most people.

Dietary Guidelines

Several national and international organizations have recommended minimum levels of fish or n-3 PUFA consumption for the general population (Table 3) (15,87,260–272). Recommendations for ALA or total n-3 consumption are generally based on prevention of essential fatty acid defi-

ciency; available evidence does not allow more specific recommendations for CVD or other chronic diseases (15). Recommendations for EPA+DHA are typically based on the prevention of CHD mortality. There is remarkable convergence of these guidelines to recommending at least 250 mg/day EPA+DHA or at least 2 servings/week of fish, preferably oily fish. For women who are or might become pregnant and nursing mothers and young children, additional specific recommendations are available for avoidance of selected higher-mercury fish species (15) as well as for minimum DHA consumption (272) to optimize brain development in their children.

Dietary guidelines for n-3 PUFA consumption are otherwise not different by sex or race/ethnicity. The American Heart Association 2020 Strategic Impact Goals defined consumption of at least 2 3.5-oz servings/week of fish, preferably oily fish, as 1 of 5 primary dietary metrics that characterized ideal cardiovascular health (271). The 2010 U.S. Dietary Guidelines for Americans recommended that individuals at both higher and average CVD risk consume 2 4-oz seafood servings/week, with types of fish selected to provide an average of at least 250 mg/day EPA+DHA (1,750 mg/week) (15) On the basis of the available evidence for prevention of CHD death, we agree with these recommendations. As additional prospective studies and clinical trial data are obtained, dietary guidelines might require updating to reflect dose-responses for other specific CVD endpoints. Data are insufficient on relative importance of EPA versus DHA or any specific "ratio" of their intakes for CVD benefits, and thus guidelines are based on their combined consumption.

Current consumption levels in most countries do not meet recommended intakes. Among U.S. adults in 2005 to 2006, mean EPA+DHA intakes were approximately 125 mg/day in non-Hispanic whites, approximately 160 mg/day in blacks, and approximately 130 mg/day in Hispanics (273). Because many people do not consume seafood at all, median EPA+DHA intakes are far lower, with approximately one-half of U.S. adults consuming <60 mg/day.

Conclusions

n-3 PUFA consumption improves vascular and cardiac hemodynamics, triglycerides, and possibly endothelial function, autonomic control, inflammation, thrombosis, and arrhythmia. Experimental studies confirm multiple relevant molecular effects, including on membrane structure and associated functions, ion channel properties, genetic regulation, eicosanoid synthesis, and production of novel inflammation-resolving mediators. Further experimental studies will be valuable to improve our understanding of which molecular mechanisms relate to specific effects of n-3 PUFA on risk factors and clinical endpoints. Not all trials of n-3 PUFA have demonstrated reductions in CVD, but several adequately powered clinical trials have documented

Table 3   National and International Recommendations for Consumption of n-3 PUFA in the General Population

Source (Ref. #)	Year	Recommendations		
		EPA+DHA	ALA	Total n-3 PUFA
International Society for the Study of Fatty Acids and Lipids Workshop (269)	1999	Target: 650 mg/day Adequate: ≥220 mg/day EPA Adequate: ≥220 mg/day DHA	Target: 2.2 g/day	Target: 1.3% energy
European Commission Eurodiet Core Report (260)	2000	Target: 200 mg/day	Target: 2 g/day	
Health Council of Netherlands (262)	2001	Adequate: 200 mg/day	Adequate: 1% energy	
U.S. National Academy of Sciences (263)	2002	AMDR: 0.06%–0.12% energy	AMDR: 0.6%–1.2% energy	
French Agency for Food Environmental and Occupational Health Safety Omega-3 Report (261)	2003	Target: 400–500 mg/day, including AA; 100–120 mg/day DHA	Target: 1.6–2 g/day	Target: 1% energy
European Society of Cardiology (270)	2003	Recommendation: ~1 g/day*		
Joint United Nations Food and Agricultural Organization/ World Health Organization Expert Consultation. Diet, Nutrition and the Prevention of Chronic Diseases (264)	2003	Target: 400–1,000 mg/day (1–2 fish servings/week)		Target: 1%–2% energy
International Society for the Study of Fatty Acids and Lipids Policy Statement 3 (265)	2004	Minimum: 500 mg/day	Target: 0.7% energy	
United Kingdom Scientific Advisory Committee on Nutrition (266)	2004	Minimum: 450 mg/day (≥2 fish servings/week)		
American Heart Association (87,268,271)	2002, 2006, 2010	Minimum: 2 3.5-oz fish servings/week, especially oily fish ~1 g/day*		
National Health and Medical Research Council (Australia and New Zealand) (267)	2006	Adequate: 90–160 mg/day Target: 430–610 mg/day	Adequate: 0.8–1.3 g/day Target: 2.7 g/day	
United Nations Food and Agricultural Organization Report on Fats and Fatty Acids in Human Nutrition (272)	2008	AMDR: 250–2,000 mg/day†	Minimum: 0.5% energy	AMDR: 0.5%–2% energy
U.S. Department of Agriculture, 2010 Dietary Guidelines for Americans (15)	2010	Minimum: 2 4-oz fish servings/week, providing an average of ≥250 mg/day	0.6%–1.2% energy	

Adapted, with updates, from Harris (275). *For secondary prevention of CHD. †Consumption of at least 300 mg/day EPA+DHA, with at least 200 mg being DHA, is recommended for pregnant women or nursing mothers.

AA = arachidonic acid; AMDR = Acceptable Macronutrient Distribution Range; other abbreviations as in Tables 1 and 2.

significant benefits. When combined with the robust global evidence from observational studies, the documented effects on risk factors in short-term trials, and the experimental and mechanistic evidence, it is clear that n-3 PUFA are bioactive nutrients that play an important role in cardiovascular health, in particular for reducing risk of cardiac mortality. Additional appropriately designed and powered clinical trials are needed to assess effects of n-3 PUFA on other cardiovascular endpoints, including nonfatal coronary events, ischemic stroke, recurrent ventricular arrhythmias, AF, and heart failure. If the apparent nonlinear dose-response for CHD mortality extends to these other CVD outcomes, such trials might be most effective in individuals with little or no background fish intake. Additional studies should also address the potential of ALA and DPA to improve CVD risk factors and outcomes. As part of achieving a healthier overall dietary pattern that includes fruits, vegetables, whole grains, nuts, vegetable oils, and dairy (271), physicians should recommend fish consumption for their patients, and government and public health agencies should implement strategies to improve attainment of the recommended levels of fish and n-3 PUFA consumption to reduce population burdens of CHD mortality and sudden cardiac death.

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