n-3 Polyunsaturated Fatty Acids and Cardiovascular Disease: Principles, Practices, Pitfalls, and Promises – A Contemporary Review

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Significance of the Study

- There is immense interest in the cardiovascular use of n-3 polyunsaturated fatty acids (n-3 PUFA). Many potential roles in coronary disease, arrhythmias, heart failure (HF), and dyslipidemia have been identified, but clinical recommendations have been elusive. Hence, mechanistic and experimental evidence and reasons for prior mixed findings were reexamined. In 2 large, recent meta-analyses it was suggested that n-3 PUFA were associated with modest but statistically significant improvements in outcomes which might increase over time. In this review, a unique reinterpretation of evidence and the essentiality of using plasma or tissue n-3 PUFA levels are provided for future research.

Keywords

n-3 polyunsaturated fatty acids · Cardiovascular disease · Cardiovascular outcomes · Arrhythmias · Coronary heart disease · Myocardial infarction · Atherosclerosis · Endothelial function · Heart failure · Inflammation

Abstract

Amidst voluminous literature, inconsistencies and opposing results have confused rather than clarified cardiologists' ability to assess the potential benefits of n-3 polyunsaturated fatty acids (n-3 PUFA). In perspective, there are common themes that emerge from n-3 PUFA studies, even as imperfect as they may be. The approach taken was to identify and unite these themes into a manageable, cohesive, evidence-based, yet useful synthesis. In all reviews and meta-analyses,

the selection of component studies and assumptions influences outcomes. This overarching principle must be combined with the totality of the data, particularly when evidence is incompletely understood and gaps in knowledge must be bridged. Both the older literature and the most recent rigorous meta-analyses indicate that n-3 PUFA are highly pleiotropic agents with many documented positive physiological effects. Concordance among preclinical, observational, randomized clinical trials and meta-analyses is impressive. These agents have modest, statistically significant benefits which accrue over time. Given their favorable safety profile, a risk reduction of about 10% justifies their potential use in cardiovascular disease.

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Introduction

The debate about whether or not long-chain n-3 polyunsaturated fatty acids (n-3 PUFA) are evidence-based therapeutic agents in cardiovascular disease (CVD) has been argued for over half of a century. Morbidity and mortality from this disease, particularly coronary heart disease (CHD), is unacceptably high in every country, and even when state-of-the-art treatment is achieved, deadly events continue [1–3]. Additional data are also valuable for public health policymakers, since consumption of these agents is popular among 8% of the American population, and the global omega-3 market amounts to USD 31.4 billion (KWD 9,460,820,000.00), as questions swirl about the wisdom of this practice [4]. Indeed, “fish oil” has consistently been one of the most popular subjects in the lay press during the past decades. For these reasons, it is appropriate to update the evidence supporting potential benefits of these molecules for individuals with and without heart disease.

Background

Interest in n-3 PUFA and CVD grew after a published association of the high n-3 PUFA intake of the Greenland Eskimo population and relatively low incidence of CHD [5]. This observation was remarkable in view of their traditional diet, which was relatively high in saturated fat and cholesterol, but low in fruit, vegetables, and complex carbohydrates. The theme was further developed by several authors, as in the work of Bang and Dyerberg [6]. Kromhout et al. [7] reported the inverse dose-response relation between fish consumption and adjusted CHD mortality over 20 years of follow-up. Individuals who ate ≥ 30 g of fish per day exhibited less than half the mortality than those who did not, prompting the authors to conclude that consumption of just 1–2 fish servings per week could significantly affect CHD mortality [7]. In studying 20,551 participants in the US Physicians’ Health Study followed for up to 11 years, Albert et al. [8] reported that in healthy men consuming ≥ 1 serving of fish per week the relative risk (RR) for sudden cardiac death (SCD) was 0.48 (95% CI 0.24–0.96; $p = 0.04$) as compared with those who ate < 1 serving/month (p for trend = 0.03). Although intake was not associated with a lower risk of total myocardial infarction (MI), non-SCD, or total cardiovascular (CV) mortality, there was a significantly reduced association with total mortality. Albert et al. [9] extended this work by using n-3 PUFA blood levels in a prospective,

nested case-control analysis in healthy men followed for approximately 17 years in the Physicians’ Health Study for SCD as the first indication of CVD. Using 94 cases and 184 matched controls, baseline levels were inversely related to risk of SCD; the RR of SCD was significantly lower among those with levels in the 3rd quartile (adjusted RR 0.28, 95% CI 0.09–0.87) and in the 4th quartile (adjusted RR 0.19, 95% CI 0.05–0.71) as compared with the lower quartiles. Interestingly, adjustment for n-6 PUFA and saturated fats did not materially change this relationship, and adjustment for monounsaturated fat strengthened it. Harris et al. [10], combining 25 studies of n-3 PUFA levels in blood, plasma phospholipids, or tissue, reported that CHD events were inversely related with tissue levels of n-3 PUFA.

Since this earlier work, the literature examining the effects of n-3 PUFA upon CVD has exploded; the sophistication in experimental approach has improved, larger studies are available, and the scope of conditions has widened. At the same time, CV treatment has also progressed, and the standards against which n-3 PUFA effects are compared are different from those that existed in an earlier era. Space does not permit an exhaustive presentation of the voluminous data, but many reviews are available for the interested reader [11–22].

A relatively neglected area of omega-3 research was inattention to n-3 PUFA levels in both the preclinical and clinical experimental conditions explored. A recent review of 298 studies provided levels of fatty acids as plasma total lipids, plasma phospholipid, in erythrocytes and in whole blood [23]. For comparison, these data were converted to “relative weight percentages.” The authors documented widespread differences in blood levels in adults geographically, but importantly, levels were extremely low in the Middle East. In view of the many clinicopathological correlations of such shortfalls in Middle Eastern countries, a higher level of awareness with a view toward intervention is in order. This is especially true since repleting low levels of n-3 PUFA may be more beneficial than simply giving more to individuals with full stores of the agent [24].

Membrane and Electrophysiological Actions

The cardioprotective actions attributed to n-3 PUFA could be due to the interaction of a number of pleiotropic effects, which included anti-inflammatory, antiatherogenic, antiarrhythmic, anti-inflammatory, antithrombotic/antiplatelet, antioxidant, antimitogenic, vasodilatory

and hypotensive properties, associated improvements in endothelial function, and favorable lipid changes [11, 12, 14]. Hence, n-3 PUFA was studied for reductions in all-cause mortality, primordial, primary, and secondary prevention, acute atherothrombotic events, perisurgical protection, in arrhythmias, especially atrial fibrillation, type 2 diabetes (T2D), hypertension, HF, and other arterial and venous diseases. In several instances, these mechanisms have been linked and act synergistically.

A common theme in the study of beneficial effects was the approximate 50% reduction in SCD from relatively small amounts of fish: 1–2 servings/week or its equivalent in purified n-3 PUFA oil [8, 9]. Myocardial membranes may contain up to 2,000 lipids, and their fluidity is a function of the contents of PUFA, cholesterol, and phospholipids. There are highly assembled membrane areas in which saturated acyl tails of sphingolipids, cholesterol, and phospholipids coalesce to form structured microdomains called “lipid rafts” [25–27]. Substantial evidence now indicates that perturbations in such microdomains, typified by caveolae, react to physical and chemical changes, thereby altering cell signaling, intracellular transport, and ion channels [28]. Caveolae could be regarded as spatial organizers of signal transduction molecules. Minute changes in conformations or concentrations may therefore be accompanied by alterations in flexibility, compressibility, curvature, or thickness of the platforms integral to successful signaling or the completion of reactions. The partially enclosed flask shape of caveolae promotes a far higher concentration of ions inside it as compared with extracellular fluid, providing a fertile environment for the direct and indirect actions of lipids. Indeed, n-3 PUFA have potent effects upon ion channel proteins of nearly all types associated with lipid microdomains, including calcium regulatory proteins involved in additional processes, such as excitation-contraction coupling and inotropy [29–33].

Whether n-3 PUFA act directly or indirectly with the channel proteins remains unknown. When n-3 PUFA are given acutely and ion channel changes occur immediately, a direct action, or action in the membrane close to an ion channel, is more likely; longer-term administration allowing for incorporation into the sarcolemma may be associated with indirect effects [26, 27]. Marine (eicosapentaenoic acid [EPA; 20:5n-3], docosahexaenoic acid [DHA; 22:6n-3], and the metabolite docosapentaenoic acid [DPA; 22:5n-3]) and plant (α -linolenic acid [ALA; 18:3n-3]) n-3 PUFA influence many sodium, calcium, and potassium currents, but also modulate α - and β -adrenoreceptors. In addition, these agents are associ-

ated with beneficial effects on the autonomic system function, heart rate variability (HRV), and rate recovery after exercise. Intrinsic pacemaker rate alterations appear to be more important than rises in HRV, but HRV remains a predictor of mortality after MI and an index of cardiac health [34].

The n-3 PUFA given acutely block Kv1.5 and Kv11.1 ion channels at physiological concentrations [35], and depress the ultra-fast Nav1.5 [36] and L-type Ca channels [37]. These actions on the K-channels should lengthen the action potential duration (APD), but the actions on the Na- and Ca-channels should shorten the APD [35]. In addition, whether the net effect goes either way, the APD change may be antiarrhythmic or proarrhythmic, depending upon the cause of the arrhythmia. Prolonging the APD can be antiarrhythmic if the arrhythmia is due to reentry, but may also produce early afterdepolarizations (EAD) and Torsade de Pointes. On the other hand, shortening the APD may be antiarrhythmic if the arrhythmia is due to triggered EAD or delayed afterdepolarizations, but favors reentry by shortening the refractory period [37]. With respect to n-3 PUFA, these generalizations may not apply in the specific electrophysiological preparation, the method of application, the animal, the dose, and the heart disease (substrate) under study. Furthermore, unlike EPA and DHA, the shorter-chain plant ALA does not block Kv1.5 [35]. This said, the ion channel and antiadrenergic effects of n-3 PUFA noted above are similar to those of amiodarone, except that they do not widen QRS or prolong the QT interval, but they do prolong the APD, suppress Torsade de Pointes, and reverse triangulation, reverse use-dependence, instability, and dispersion (TRIaD) [36]. In fact, the reduction in SCD is greater with n-3 PUFA than with amiodarone in similar populations, while the adverse effects are not as limiting [36].

Even as compelling as some studies appear [35, 37–39], the absence of recent strong confirmation of the decrease in SCD associated with n-3 PUFA administration is responsible for uncertainty and lack of recommendations in guidelines. In part, this may reflect methodological inhomogeneity and gaps in knowledge. In view of the recent interest in prescription n-3 PUFA, several studies in progress may clarify open questions. Remarkably, one must note that EPA and DHA are among the most studied of all therapeutic substances, and use of n-3 PUFA is strikingly prevalent, yet arrhythmic deaths associated with acute administration are extremely rare events, even when used during monitored parenteral and enteral feeding in critical care units [40]. Searching through several

databases, finding documented examples of this phenomenon for study is difficult. Lands [41] expertly discussed the challenges in assigning weights to the: (i) confounding in existing studies concerning pharmacokinetics, pharmacodynamics, forms of n-3 PUFA, timescale factors, and topology, (ii) positive epidemiological and other evidence, (iii) particular use of n-PUFA in clinical settings, and (iv) safety.

The extent of both electrical and anatomic remodeling in heart muscle is a key determinant of the action of administered n-3 PUFA, which may partially account for minimal or inconsistent effects [34]. For example, in atrial fibrillation there are other variables, including cytokines and hypercoagulability involved in pathogenesis, in addition to the extent of pericardial fat and atrial fibrofatty infiltrates promoting electrical dissociation that become self-perpetuating. As was observed with drugs, remodeling beyond critical thresholds may drastically limit responsiveness. The GISSI-Prevenzione trial [42] with 11,323 participants provided the most recent confirmation of n-3 PUFA benefits post-MI in a large randomized trial, and again, effect sizes were greater than in comparable amiodarone studies [36, 43]. The arm treated with the modest amount of 850 mg of EPA+DHA daily enjoyed a 45% reduction in SCD, and 21 and 30% falls in total and CV mortality, respectively. As in other such studies, reductions in the rate of SCD account for much of the mortality benefit in primary and secondary prevention. Nonetheless, there were reports, largely theoretical, of potentially adverse n-3 PUFA actions in ischemic canine and porcine heart models [26, 29–31]. It is important to note that in no human study in which n-3 PUFA levels were measured has this been confirmed. In contrast, the total number of all participants in studies examining SCD is now impressive [8–14, 34–37]. Finally, relying upon dietary recall and study designs that preclude comparison may account for some discrepancies [44, 45].

Another important factor in n-3 PUFA research is the rapidity, dose, preparation, and duration of administration. Experimentally, it may take months for full incorporation into membranes, and effects of acute and longer administration (days vs. weeks vs. months) may differ. However, as previously suggested, the full benefits of a high n-3 PUFA intake may require years to accrue. In a meta-analysis of 11 studies, 39,044 patients, including post-MI subjects, consumed an average dose of EPA/DHA 1.8 ± 1.2 g/day for a mean duration of 2.2 ± 1.2 years [46]. There was a significantly reduced risk of CV deaths (odds ratio [OR] 0.87, 95% CI 0.79–0.95, $p = 0.002$), SCD (OR 0.87, 95% CI 0.76–0.99, $p = 0.04$), all-cause mortality

(OR 0.92, 95% CI 0.85–0.99, $p = 0.02$), and nonfatal CV events (OR 0.92, 95% CI 0.85–0.99, $p = 0.02$). Of note, the greatest mortality benefit was contributed by high-risk patients, and the improvement in nonfatal events occurred chiefly in the moderate-risk patients in the secondary prevention group. Although beyond the scope of this review, favorable metabolic actions of n-3 PUFA have persisted and/or resulted from sustained high intake over decades.

Based on some of the aforementioned, favorable data in primary prevention, about 7,125 CHD deaths could be prevented by sustained consumption of approximately 1 g/day of n-3 PUFA from farmed salmon per 100,000 individuals [9, 18, 47]. Since this number is offset by a small risk of deaths due to carcinogens consumed in the fish, this estimate for the equivalent in purified oils would be correspondingly greater.

Clinical Studies of n-3 PUFA

For CV outcomes in primary and secondary prevention and using either fish or n-3 PUFA oil, a surprisingly large number of participants have been included in a myriad of meta-analyses of randomized clinical trials (RCTs) and prospective cohort studies over the years [17, 46, 48–57], in addition to innumerable individual studies. Collectively, these studies showed significant reductions in mortality and SCD [14, 18, 58]. One meta-analysis [19] with a large study population and number of events had low statistical significance ($p = 0.0063$), included many heterogeneous component studies, and did not employ an individual-patient meta-analysis. Of the studies [7, 59–66] that did not show positive results, the designs used had limitations; in some instances, these were considerable. In addition, several were underpowered to identify a statistically significant effect on cardiac mortality [7, 62–65]. The Risk and Prevention RCT of patients at high CVD risk but without prior MI was limited to low event rates, which necessitated a change in endpoints during the trial [66]. In others, the rigor in controlling n-3 PUFA intake varied from none to several grams daily, and often remained unknown [7, 51–58]. As mentioned, inaccurate self-reported data and poor adherence can be misleading and introduce appreciable bias which may remain ignored, particularly in meta-analyses [67]. This problem was also accompanied by variation in the background consumption of EPA+DHA in the populations studied, and additionally by the inclusion of plant n-3 ALA, which has different properties. Different doses used in each of

the studies, ethnic variation in sensitivity to different n-3 PUFA properties, and even higher interindividual variability all impaired the credibility of the results. It is the preponderance and remarkable agreement from experimental work, data from preclinical trials of n-3 PUFA on risk factors, prospective observational studies, and fully powered RCTs that continue to drive interest.

A meta-analysis of 3 double-blind RCTs of CHD patients with implantable cardioverter-defibrillators examined the effects of n-3 PUFA versus placebo on spontaneous ventricular tachyarrhythmias; all of the 3 studies showed null results [55]. The rationale was that in CHD, triggered EADs and delayed afterdepolarizations and prolonged APDs are common proarrhythmogenic mechanisms, n-3 PUFA might suppress ventricular tachyarrhythmias, but in HF where the mechanism is predominantly reentry, the reverse would be expected. However, aside from the usual dose heterogeneity, underpowering left the meta-analysis unable to detect the risk reduction hypothesized, largely due to small sample sizes. However, these agents were previously found to be safe in HF patients [60]. GISSI-HF found that 1 g per day of n-3 PUFA lowered all-cause mortality in New York Heart Association (NYHA) class IV HF patients [63]. While the absolute reduction in mortality for an average of 4 years was 1.8%, the results were significant and achieved in patients who were being treated with multiple prescription drugs. The hypothesis followed that perhaps ventricular remodeling had been responsible. Prevention of ventricular hypertrophy in concert with improved cardiac dysfunction by n-3 PUFA has been documented in several animal preparations; postinfarction, a reorganization of junctional areas in association with changes in the protein connexin 43, involved in growth and differentiation, have been documented [68, 69].

Nodari et al. [70] randomized nonischemic dilated cardiomyopathy HF patients with minimal symptoms to 2 g of n-3 PUFA or placebo. After 1 year, the n-3 PUFA group had a significant rise in left ventricular ejection fraction (LVEF), peak oxygen consumption (VO_2), and exercise duration, accompanied by a fall in mean NYHA functional class and rates of rehospitalization. While this study also had limitations, an editorial speculated about a greater effect if higher n-3 PUFA doses were used [71]. Use of n-3 PUFA was rated as level of evidence B in the ACCF/AHA HF guidelines, which considered such therapy reasonable in patients with NYHA class II-IV symptoms and either HF with a reduced ejection fraction or HF with a preserved ejection fraction, to reduce both mortality and recurrent hospitalizations [72]. In a meta-analysis

of 7 trials with 825 chronic HF participants, treatment with n-3 PUFA significantly increased LVEF and reduced left ventricular end-systolic volume [73]. This effect was more pronounced in patients with nonischemic HF along with improvements in peak VO_2 and NYHA functional classification. The degree of changes in these indices would correspond to a 6% lower risk of overall mortality, but the additional import is that, once again, n-3 PUFA was associated with improved left ventricular function and restorative remodeling. Reversal of adverse remodeling involving fibrosis-, hypertrophy-, and inflammation-related genes were previously reported in a model of atrial cardiomyopathy [74].

Several other mechanisms contribute to the beneficial effects of n-3 PUFA in HF patients, which traditionally include a hypotensive action, reduction in collagen deposition, suppression of arrhythmias, and improvements in cardiac energetics. Omega-3 PUFAs could preserve cardiac mitochondrial function by stimulating the expression of proteins that improve cardiac lipid metabolism, lowering myocardial oxygen consumption, and raising mechanical efficiency. Endothelial function improves through the many pleiotropic properties of n-3 PUFA that are interrelated: increasing nitric oxide (NO) production, decreasing arterial stiffness, quelling inflammation, and molecular antiatherosclerotic mechanisms [75, 76]. For instance, not only is NO production important, but reducing plasma triglycerides (TG) may also modify arterial flexibility independently. When TG-rich lipoproteins (TRL) are hydrolyzed, remnant-like lipoprotein particles form, also contributing to endothelial dysfunction by impairing NO synthase; both mechanisms can be attenuated by n-3 PUFA. By raising levels of adiponectin, n-3 PUFA indirectly cause additional NO expression. n-3 PUFA also protect the endothelium against angiotensin II- and glucose-mediated injury, lower membrane arachidonic acid content, and displace ceramide, thereby opposing ceramide signaling that induces vasoconstriction mediated by thromboxane [77]. Insertion of EPA into membranes interrupts waves of peroxidation, protects low-density lipoprotein cholesterol (LDL-C), and lowers the level of small, dense LDL-C (sdLDL), which itself causes endothelial dysfunction. Inflammatory agonists can raise monocyte adhesion to the endothelium. This is reversed with EPA, but accentuated by arachidonic acid. EPA augments the bioavailability and function (migration and integration into tubules) of endothelial progenitor cells, i.e., stimulates neovasculation. Inflammation and high sympathetic drive play a significant role in HF, and levels of C-reactive protein are among many proin-

flammatory mediators that are suppressed by n-3 PUFA, along with a fall in nuclear factor-kappa B activity, TNF- α , IL-1, IL-6, and oxidative stress [78, 79]. Another meta-analysis recently confirmed a rise in LVEF in dilated cardiomyopathy patients, but also emphasized 2 additional mechanisms of interest: decreased brain natriuretic peptide levels and serum norepinephrine levels [80].

In summary, several processes could be responsible for improved function of the failing heart observed after n-3 PUFA administration. One of many that need further research is gene modulation in 2 pathways, one involved with lipid transport and metabolism, and the other with the downregulation of extracellular and cardiac matrix remodeling, inflammation, and cell growth. One dose schedule that has been advocated for the early treatment of HF is 5 g per day for 30 days followed by a maintenance dose of 2 g per day of n-3 PUFA in the absence of contraindications [71].

Role of Measuring n-3 PUFA Levels

Measuring n-3 PUFA levels constitutes a major advance in the methodology of conducting clinical n-3 PUFA trials, which may minimize some sources of bias and decrease heterogeneity among studies. Both blood and tissue levels could facilitate comparisons of findings, especially if differences are due to poor adherence to dietary, supplement, and prescription n-3 PUFA instructions, when there is a variation in the content of supplements, adulteration and mislabeling, digestive or absorption issues, or varying bioavailability [81]. Not infrequently, patients will substitute an over-the-counter (OTC) brand for a prescription version without informing physicians; contents of commercial brands vary appreciably, and often patients will not understand that the total marine oil in a bottle is not the same as the actual weights listed. This challenge has greater relevance than appreciated: when OTC brands were tested for the ability to inhibit copper-induced oxidation of human sdLDL *in vitro*, ≥ 30 other fatty acids were present [82]. In all, 10–14 different saturated species accounted for up to 36% of the total fatty acid content. A substantial percentage of these samples failed to inhibit sdLDL, likely due to saturated or peroxidized fats which remained undetected. Measurement of n-3 PUFA levels may not help identify this more complex type of error. More important than simply failing to inhibit sdLDL oxidation, the levels of saturated fat and peroxides were deemed sufficient to inflict harm themselves. Finally, OTC n-3 PUFA consumption is

prevalent, and may not be revealed in intake questionnaires or interviews. Hence, even “control” patients may have higher background n-3 PUFA levels than expected, potentially confounding calculations of effect sizes between groups.

In the Danish Diet, Cancer and Health study of 57,053 healthy enrollees unselected for cardiac risk factors, a buttock adipose biopsy was taken at baseline, along with demographic and medical information [83]. Incident cases of acute coronary syndrome (ACS) were matched, and levels of EPA, DHA, and DPA in the tissue were determined. After a mean follow-up period of 7.6 years, there were 1,012 cases of ACS. A significant inverse monotone relationship between the total n-3 PUFA in the adipose tissue, which accurately reflects dietary n-3 PUFA consumed over the prior 1–3 years, and incidence of ACS was found in men. The same negative relationship held for DHA, but not for EPA or DPA. Fewer women than men participated in the study, and the number of events in women was smaller, probably attributable to the later age at which women suffer ACS. The levels of n-3 PUFA in adipose biopsies were lower than expected, and interindividual differences were not as great – characteristics that may or may not be unique. Such low levels may have impaired the detection of an association between n-3 PUFA and ACS.

In contrast with many prior n-3 PUFA-ACS studies, the association was primarily driven by a lower incidence of nonfatal ACS. The use of tissue levels in this study enabled high-quality exposure assessment to describe the relationship with outcomes, bypassing food frequency questionnaires, and the potential for recall bias. These results agree with prior studies showing that the EPA+DHA content of red blood cell (RBC) membranes, as a percent of total FAs, is lower in ACS patients compared to matched controls (20% lower in this instance); EPA+DHA levels vary inversely with the OR for an ACS event [84]. Moreover, even in populations with high dietary consumption of n-3 PUFA, the ratios of EPA and DHA to arachidonic acid predict early ACS [85]. The “omega-3 index” (O3I), the sum of RBC (EPA+DHA) as a percent of total fatty acids, is a common measurement which is inversely related to risk for SCD, ACS, all-cause mortality, and many of the pathophysiological biomarkers investigated [86]. Interestingly, the explained variability in the adjusted O3I was 73%, with heritable (24%), dietary intake (25%), and supplements (15%) as major components [86].

The importance of measuring actual levels of n-3 PUFA cannot be overemphasized; the strength of any preclinical or clinical association with these agents de-

depends upon confirming the presence of a suitable intervention, and documented elevations in blood or tissue levels are reliable means of doing so. Simply assigning individuals to groups, supplying n-3 PUFA, or giving prescriptions to participants invites lack of rigor and impairs validity in trials.

Recent Major Meta-Analyses

The Study by Del Gobbo et al. [87]

In 19 studies (prospective [cohort, nested case-control] or retrospective) in which tissue or blood levels of n-3 PUFA were measured, 45,637 participants (mean age 59 years, 63% male) were followed for the development of incident CHD, nonfatal and fatal CHD, and the results were pooled [87]. Participants were excluded if they had a prior history of CHD, angina, or coronary revascularization. There were 7,973 MIs leading to 2,781 deaths and 7,157 nonfatal MIs.

The meta-analysis included EPA, DHA, DPA (22:5n-3), chiefly derived from endogenous metabolism, and plant ALA. For many CV actions, ALA is not as potent as EPA or DHA, but does have effects of its own. ALA can be converted to EPA, and EPA to DHA, but this process amounts to a few percent, less for DHA, and conversion is further compromised in cardiometabolic disease [88]. As is the case for EPA/DHA and their n-6 counterparts in membranes, the n-3 and n-6 series compete for 2 enzymes responsible for metabolism and transformation. Since the Western diet is high in n-6 PUFA, especially from vegetable oils, the ratio of n-6 to n-3 intake has fallen from approximately 1:1 in Paleolithic man to the current value of approximately 1:20, with deleterious effects on health [89].

Each component study conducted standardized, individual-level analyses using harmonized models, exposures, outcomes, and covariates. n-3 PUFA were measured in total plasma, phospholipids, cholesterol esters, and in adipose tissue, and were recorded as the weight percentage of total fatty acids. In addition, heterogeneity was analyzed by age, gender, ethnicity, the presence of T2D, use of statins or aspirin, n-6 levels, and FADS desaturase genes ($\Delta 5$ -desaturase gene [*FADS1*] catalyzes the conversion of 20:4n-3 [eicosatetraenoic acid] to 20:5n-3 [EPA], but also 20:3n-6 to 20:4n-6, arachidonic acid, in the n-6 series; $\Delta 6$ desaturase [*FADS2*] assists with the 18:3n-3 [ALA] to 18:4n-3 [stearidonic acid] and 24:5n-3 [tetracosapentaenoic acid] to 24:6n-3 [tetracosahexaenoic acid] conversions, which form DHA upon β -oxidation). Other covariates were age, body mass index, educational

level, smoking, physical activity, alcohol intake, T2D, and treated hypertension.

After adjustment, for every 1-standard deviation (SD) increase in the n-3 PUFA reported, there was a significant association with a decreased risk of fatal CHD (RR: ALA, 0.91 [95% CI 0.84–0.98]; DPA, 0.90 [95% CI 0.85–0.96], and DHA, 0.90 [95% CI 0.84–0.96]). Of the 3 main n-3 PUFA, only DPA was linked to a lower risk of total CHD (RR 0.94, 95% CI 0.90–0.99); there were no significant associations with nonfatal MI per 1 SD. The upper quintiles of EPA and DHA were associated with a reduced risk of nonfatal MI (RR 0.71 [95% CI 0.56–0.90] vs. 0.87 [95% CI 0.78–0.97]). The highest quintiles of DPA and DHA were associated with a significantly lower risk of fatal CHD (DPA [RR 0.76, 95% CI 0.65–0.90] and DHA [RR 0.77, 95% CI 0.64–0.89]).

This meta-analysis was unique in several respects. Results indicated that in vivo levels of ALA, DPA, and DHA were associated with a lower incidence of fatal CHD – a significant approximate 9% fall per 1 SD rise in these values. The population was diverse, not restricted, and effects were independent of age, sex, and n-6 PUFA (linoleic acid [18:2n-6] and arachidonic acid [20:4n-6]) levels. The variation was due only to dietary fish intake, avoiding limitations due to the use of self-reported amounts of fish or capsules consumed. The sample size was large, assuring a sufficient number of events for analysis. The number of component studies lowered the probability of publication bias, and inclusion criteria minimized reverse causation. Although some component studies used plasma levels, plasma phospholipids or cholesteryl esters, RBC levels, and others adipose tissue, comparing highest to lowest quintiles captured the essence of the effect, since there is good correlation between these biomarkers. Analysis was prespecified, and standardized definitions and modeling for all variables and outcomes were employed.

The data agree with many prior prospective cohort studies, RCTs, meta-analyses, and recent additional large-scale contributions that increased n-3 PUFA intake is associated with a lower risk of fatal MI or major events [8, 14, 16, 17, 58, 90, 91].

The Study by Alexander et al. [91]

From January 1947 through November 2015 a literature search was conducted to identify RCTs and prospective cohort studies relating EPA+DHA consumption and CHD outcomes [91]. Participants were nonhospitalized adults with and without CHD but free of serious noncardiac disease. The study was composed of 2 meta-analyses: in the first, 18 RCTs randomly assigned approximately

93,000 participants to consume a specific amount of n-3 PUFA. The second analyzed 16 trials and approximately 732,000 participants, followed for CHD events over a long period of time.

For inclusion, CHD outcomes, specifically MI (fatal or nonfatal), angina, SCD, coronary death, and CHD incidence (for prospective cohort studies) were required, as well as hazard ratios (HR) or rate ratios of outcomes and measures of variance (i.e., 95% CIs), or data to calculate these measures. For RCTs, a composite variable, “any CHD event,” was defined as a combination of fatal or nonfatal MI, coronary death, SCD, and angina. For prospective analyses, “any CHD event” was considered to be the same, with the addition of CHD incidence.

Random-effects meta-analysis models were employed to derive summary RR estimates and 95% CIs. Analysis was performed for larger samples of “any CHD event” and component, specific events. Heterogeneity was evaluated in subgroup and sensitivity analyses, and by meta-regression. Dose-response was evaluated in either stratified dose or intake analyses. The Cochran Q was used to assess between-study statistical variation. Finally, 2 methods of assessing publication bias were used.

The meta-analysis of RCT data revealed that n-3 PUFA supplements in ≥ 1 g per day doses (different forms) given for primary and secondary prevention were associated with a statistically insignificant 6% fall in CHD risk for any event (HR 0.94, 95% CI 0.85–1.05). However, in the subgroup with TG levels >150 mg/dL, the CHD risk fell by 16% (HR 0.84, 95% CI 0.72–0.98), and in those with LDL-C levels >130 mg/dL the risk fell by 14% (HR 0.86, 95% CI 0.76–0.98). In the meta-analysis of prospective cohort studies, additional n-3 PUFA intake (all intake categories, follow-up range from 4.3 to 40 years) significantly reduced CHD risk of any event by 18% (HR 0.82, 95% CI 0.74–0.92).

The final data in these meta-analyses not only add further to the momentum of positive evidence favoring the cardioprotective effects of n-3 PUFA, but do so in a different, important direction. The current pandemics of obesity, T2D, and metabolic syndrome reflect molecular phenotypes with a high cardiometabolic risk [2, 3]. Plasma TG <100 mg/dL is generally considered an index of metabolic health, but up to 33% of the adult US population have levels in excess of 150 mg/dL. Current guidelines do not recommend treatment for concentrations <500 mg/dL. Strong data using Mendelian randomization designed to minimize confounding and reverse causation now show that elevated TG levels are causes of low-grade inflammation, CHD, and all-cause mortality [92]. Further genetic

studies have also clearly established TRLs as causes of CHD, ischemic stroke, and all-cause mortality [93]. As already mentioned, remnant cholesterol, i.e., the cholesterol content of particular TRLs called remnants (including chylomicron remnants, and very low-density lipoproteins and intermediate-density lipoproteins in the nonfasting state and in the fasting state) also cause CHD and low-grade inflammation [94]. These relationships are independent from vascular damage attributable to LDL-C, are present in individuals without T2D or obesity, are substantial, and partially account for the residual risk remaining in patients being treated with evidence-based protocols [95–98]. In this respect it is appropriate to note that each additional 1 g per day of EPA-DHA consumed is associated with a fall in TG levels by -5.9 mg/dL (95% CI -2.5 to -9.3) [58]. Past meta-analyses did not stratify according to levels of TG, TRLs, or cholesterol remnants.

An editorial accompanying this study [91] acknowledged that it is the most comprehensive in the medical literature, and improvements in outcomes were impressive since they occur in statin-treated patients with no clinically important side effects. They too endorsed use of n-3 PUFA levels or the omega-3 index, a convenient and increasingly available tool [16, 23, 86]. Users should become familiar with reports in the various fatty acid profiles, with particular care in interpreting effects of the composition and properties of the n-3 PUFA administered, especially plant ALA, and the individual fatty acid levels reported. Due to the biochemical pathways involved, effects of ALA intake upon EPA levels will usually be minimal.

Updating a prior scientific statement [18], a recent American Heart Association Advisory survey [99] was limited in scope to large RCTs using supplements and major clinical CVD end-points, including diabetes. By definition, observational, mechanistic, physiological, or electrophysiological studies in humans, and those involving n-3 PUFA levels or fish, were excluded. Not surprisingly, in view of these restrictions, no recommendation could be made in several categories. However, on the basis of their deliberations, and comments that for many clinical situations “a lack of evidence of a benefit differs from evidence of a lack of effect,” treatment was considered reasonable in 2 subgroups: (a) secondary prevention of SCD and CHD among patients with prevalent CHD (class IIa, benefit much stronger than risk; level of evidence high [“A”], multiple populations involved/more than 1 RCT/meta-analysis of high-quality RCTs/ ≥ 1 RCTs corroborated by high-quality registry studies), and (b) secondary prevention of outcomes in HF patients (9% reduction in mortality and hospitalizations in HF pa-

tients with a reduced ejection fraction; class IIa; level of evidence moderate [“B-R,” ≥ 1 or more RCTs or meta-analyses of moderate-quality RCTs]).

A large meta-analysis of 14 RCTs involving 71,899 participants compared cardiac mortality in an n-3 PUFA arm (pharmaceuticals and supplements for ≥ 6 months) with a control arm [100]. There were 1,613 cardiac deaths (4.48%) in the intervention arm and 1,746 cardiac deaths (4.87%) in the control arm. Pooled RR estimates showed an 8.0% (95% CI 1.6–13.9%, $p = 0.015$) lower risk in the n-3 PUFA group versus the controls. In subset analyses there were numerically larger effects (12.9–29.1% lower risks, all $p < 0.05$) in subsets of RCTs with EPA+DHA dosages > 1 g per day and higher risk samples (in secondary prevention, baseline mean or median TG ≥ 1.69 mmol/L [150 mg/dL], LDL-C ≥ 1.69 mmol/L [130 mg/dL], statin use $< 40\%$ of subjects). These data lend further credence to the AHA Science Advisory that n-3 PUFA treatment is reasonable in secondary prevention to prevent SCD and in those with HF [99]. This particular meta-analysis, however, suggests that patients at higher risk, as reflected by TG and LDL-C levels, benefit more from n-3 PUFA than others [101]. In comparing the results of meta-analyses, the choice of study design, which RCTs to include, and outcomes, clearly has a strong influence on result consistency, with effect sizes varying from 0.45 to 1.45 [101]. The data from the recent trials considered herein strongly support a significant modest improvement of n-3 PUFA therapy in CHD patients, which vary according to subgroups [87, 91, 99, 100].

Three relatively unaddressed issues have been raised regarding the available data and interpretation of results, namely n-3 PUFA levels, additional CV risk from circulating nontraditional atherogenic moieties, and optimal doses in subsets of patients. As mentioned above, in the AHA Advisory, studies using n-3 PUFA levels were deliberately excluded [99]. Yet the variation of levels within populations is striking [23]. In many instances, administering a fixed physiological dose of n-3 PUFA to a cohort in which background levels randomly vary may result in overlap between baseline levels and postintervention levels. In an RCT, this would translate to permitting the uncontrolled contamination of groups with an active agent prior to administering the same agent, and then claiming to study the interventional effect of the latter. It is also possible that increases in differences in background intake of n-3 PUFA, for instance due to a steep rise in supplement use, may account for decreases in effect sizes in clinical trials over the past 2 decades. Obviously, any statements made concerning the existence of an interventional effect or its

size would be invalid. Use of n-3 PUFA levels would also identify nonresponders whose levels do not rise with exogenous dosing. Moreover, benefits of n-3 PUFA therapy are greater in patients with low baseline stores [24, 101]. The inclusion of studies that rely upon self-recall of n-3 product use, while rejecting those that rely upon n-3 PUFA levels, is particularly concerning. In the future, perhaps knowledge of n-3 PUFA levels will be considered as an advantage, not as a confounder to be ignored [14, 23, 81–84, 86–88]. Second, as discussed above, as the dual epidemics of obesity and T2D progress, glucose intolerance and atherogenic dyslipidemia follow, with increases in levels of TG, TRL, and cholesterol remnants. Greater characterization of these dyslipidemias prior to n-3 PUFA may assist in defining subpopulations with greater residual risk that could benefit the most from n-3 PUFA interventions. Third, more research needs to focus upon the optimum dose and forms of n-3 PUFA in various contexts since some investigators have reported greater response rates with higher doses of n-3 PUFA [91, 102–105].

Conclusion

In recent reports, imperfections in prior n-3 PUFA studies included nonlinear and decreasing reported death rates, variations in the background of n-3 PUFA intake, heterogeneity in study designs, dose, rapidity, and duration of administration, purity, peroxidation, and adulterating products, underpowering, short follow-up periods, disparate findings, variation in disease substrates, individual responsiveness and adherence, difficulties comparing component studies in meta-analyses, and confounding by cotherapies.

The available evidence tends to refute the impression that improving n-3 PUFA stores has little utility in the current era of cardiology care. Rather, for those interested in avoiding vascular damage and preventing the need for these therapies, n-3 PUFA may provide a relatively inexpensive, beneficial adjunctive approach. Optimal amounts for the general population are unknown, and given the heterogeneity in responsiveness, it may be impossible, even after performing blood level tests, to answer this question in a particular individual.

Even so, there are now many large-scale studies showing benefits, including the most recent high-quality meta-analyses discussed above. n-3 PUFA are highly pleiotropic agents with many documented physiological effects. Concordance between preclinical, observational RCTs and meta-analyses is impressive. These agents have mod-

est, statistically significant benefits, which may accrue over time. In all systematic reviews and meta-analyses, the selection of component studies influences outcomes. This overarching principle must be incorporated into a synthesis of the totality of data, particularly when evidence is inconsistent or conflicting. Reasons for inconsistencies between studies are still incompletely understood, and gaps in knowledge are evident. This report has included several sources of evidence that inform practice. In addition, combined with the impressive safety profile, the reward/risk ratio of n-3 PUFA is extremely high.

Some authorities prefer to favor n-3 PUFA consumption in the form of fish, but many bodies, including the American Heart Association, advise caution because of the mercury and other chemical pollutants present in the fish supply [106]. Indeed, although restriction of fish intake other than perhaps for pregnant/breastfeeding women is not universally advised, serious questions remain unanswered and are not inconsequential in CV patients [107–109].

Based on the totality of evidence, physicians and public health agencies should encourage the attainment of recommended amounts of n-3 PUFA in the population and, in particular, optimum levels in CV patients. Effect sizes in selected secondary prevention populations range up to an 18% reduction in RR, likely depending upon the lipid profile. There is a suggestion that effect sizes may vary with baseline n-3 levels. In this respect, it is agreed that even potential reductions in CHD deaths of 10% “would justify treatment” of n-3 PUFA administration due to the favorable safety profile [87, 91, 99, 100].

Studies in progress may provide further welcome information. In the interim, considering n-3 PUFA consumption as an intervention associated with moderate outcome improvements appears to be reasonable, particularly using the AHA Advisory recommendations [99]. Optimal use of n-3 PUFA requires individualization according to pathophysiology and biomarkers, and is yet another reminder that medical practice remains both an art and a science.

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