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November 5, 2014

U.S. Food and Drug Administration
Office of Nutritional Products
Labeling and Dietary Supplements (HFS-800)
5100 Paint Branch Pkwy
College Park, MD 20740

RE: Response to FDA Request for Comments related to the Petition filed by the Global Organization for EPA and DHA Omega-3s' (GOED)

The North American Branch of the International Life Sciences Institute appreciates the opportunity to share science-based conclusions from an ILSI North America supported 2008 expert workshop, entitled "Towards Establishing Dietary Reference Intakes for Eicosapentaenoic and Docosahexaenoic Acids." ILSI North America is a public, non-profit organization that works to identify and resolve scientific issues important to the health of the public. The organization carries out its mission by sponsoring relevant research, professional education programs and workshops, seminars and publications, as well as, providing a neutral forum for government, academic, and industry scientists to discuss and resolve scientific issues of common concern for the well-being of the general public.

Given that food label statements and claims to achieve and maintain good health have in some cases been contingent on established Dietary Reference Intakes (DRI's), it is relevant that a panel of 10 experts evaluated scientific evidence and concluded that "…consistent evidence from multiple research paradigms demonstrates a clear, inverse relationship between EPA+DHA intake and risk of fatal (and possibly nonfatal) CHD [coronary heart disease], providing evidence that supports a nutritionally achievable DRI for EPA+DHA between 250 and 500 mg/d…". These daily intake levels were based on evidence available through 2008 and for health broadly, and therefore are not specific to the GOED petition which is based on more current evidence specific to blood pressure, but are relevant to the relationship between EPA+DHA and overall health.

The expert panel addressed many of the obstacles noted previously in 2002 by the Institute of Medicine, as detailed in the published workshop summary [Harris WS, Mozaffarian D, Lefevre M, Toner CD, Colombo J, Cunnane SC, Holden JM, Klurfeld DM, Morris MC, Whelan J. Towards Establishing Dietary Reference Intakes for Eicosapentaenoic (EPA) and Docosahexaenoic Acids (DHA). *Journal of Nutrition* 2009;139:804S–819S]. We trust that this more recent evaluation of evidence, including the

recommendation to establish a DRI for EPA+DHA based on low conversion rates of dietary alpha-linolenic acid to EPA+DHA, a history of safe use at typical intakes, and emerging evidence of health benefits, is helpful in the more specific deliberations regarding EPA+DHA and blood pressure.

The ILSI North America, and its Dietary Lipids Committee which sponsored the Expert Workshop described above, appreciate this opportunity to provide information that may be relevant.

Sincerely,

Eric Hentges, Ph.D.
Executive Director
ILSI North America

The Journal of Nutrition
Supplement: Towards Dietary Reference Intakes for EPA and DHA

Towards Establishing Dietary Reference Intakes for Eicosapentaenoic and Docosahexaenoic Acids[1,2]

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Abstract

There is considerable interest in the impact of (n-3) long-chain PUFA in mitigating the morbidity and mortality caused by chronic diseases. In 2002, the Institute of Medicine concluded that insufficient data were available to define Dietary Reference Intakes (DRI) for eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), noting only that EPA and DHA could contribute up to 10% toward meeting the Adequate Intake for α-linolenic acid. Since then, substantial new evidence has emerged supporting the need to reassess this recommendation. Therefore, the Technical Committee on Dietary Lipids of the International Life Sciences Institute North America sponsored a workshop on 4–5 June 2008 to consider whether the body of evidence specific to the major chronic diseases in the United States—coronary heart disease (CHD), cancer, and cognitive decline—had evolved sufficiently to justify reconsideration of DRI for EPA+DHA. The workshop participants arrived at these conclusions: *1*) consistent evidence from multiple research paradigms demonstrates a clear, inverse relation between EPA+DHA intake and risk of fatal (and possibly nonfatal) CHD, providing evidence that supports a nutritionally achievable DRI for EPA+DHA between 250 and 500 mg/d; *2*) because of the demonstrated low conversion from dietary ALA, protective tissue levels of EPA+DHA can be achieved only through direct consumption of these fatty acids; *3*) evidence of beneficial effects of EPA+DHA on cognitive decline are emerging but are not yet sufficient to support an intake level different from that needed to achieve CHD risk reduction; *4*) EPA+DHA do not appear to reduce risk for cancer; and *5*) there is no evidence that intakes of EPA+DHA in these recommended ranges are harmful. J. Nutr. 139: 804S–819S, 2009.

Introduction and background

The (n-3) PUFA and foods from which they are derived have long been recognized as beneficial to human health. There is considerable interest in the potential ability of both the short-chain [α-linolenic acid (ALA)[13] 18:3(n-3)] and the long-chain [eicosapentaenoic acid (EPA) 20:5(n-3); docosahexaenoic acid (DHA) 22:6(n-3)] (n-3) PUFA to mitigate risk for chronic diseases, particularly those of the cardiovascular (CV) system

[1] Published in a supplement to *The Journal of Nutrition*. Presented at the workshop ''Towards Dietary Reference Intakes for Omega-3 Fatty Acids,'' held in Washington, DC June 4–5 2008. The workshop was cosponsored by the Technical Committee on Dietary Lipids of the International Life Sciences Institute North America (ILSI North America). The Supplement Coordinators for this publication were William S. Harris and Michael Lefevre. The views expressed in this article are not necessarily those of ILSI North America, the Supplement Coordinators, the Technical Committee on Dietary Lipids, nor the publisher, Editor, or Editorial Board of *The Journal of Nutrition*. Supplement Coordinator disclosures: William S. Harris: Grant support from Monsanto Co. and Reliant Pharma (now GSK), Speakers' Bureau, GSK, Scientific Advisory Committee, Monsanto and GSK; Michael Lefevre: receives compensation from ILSI North America as scientific consultant to the Technical Committee on Dietary Lipids and for organizing and attending workshops.

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and brain. In 2002, the Institute of Medicine (IOM) concluded that insufficient data were available to set a recommended dietary allowance or an adequate intake (AI) for EPA and/or DHA (1). The IOM report reviewed the available data on the physiology of absorption, metabolism, and excretion of (n-3) PUFA as well as evidence for deficiency and impact on 2 clinical outcomes: growth and neural development. An estimated average requirement was not established because of the lack of data in support of an (n-3) PUFA requirement for healthy individuals and for adequate growth and neural development. The IOM concluded that an AI for ALA could be set based on current population ALA intake in the apparent absence of dietary deficiency and that up to 10% of the AI for ALA may be provided by EPA+DHA. Again, the conclusion reflected current intakes of long-chain (LC) (n-3) PUFA. The IOM report formed the foundation for the 2005 Dietary Guidelines for Americans (2).

Since the publication of the IOM report, substantial new evidence has emerged to warrant a reassessment of the data for effects of EPA+DHA on health, particularly with respect to reducing risk for chronic diseases. To address this issue, the Technical Committee on Dietary Lipids of the International Life Sciences Institute (ILSI) North America sponsored and held a workshop on June 4–5, 2008 in Washington, DC. This workshop examined the rationale and process that governed the development of dietary reference intakes (DRI) for (n-3) LCPUFA in 2002 and considered whether the body of evidence specific to cardiovascular disease (CVD), cancer, and cognitive decline produced since that time justifies reconsideration of a DRI for EPA+DHA. An overview, rather than a thorough review, of the potential role of these fatty acids in human neurodevelopment was also included. The workshop also addressed the state of the science specific to the methods used to assess both intake and exposure, and identified research needs critical for DRI establishment.

EPA+DHA and health outcomes

Cardiovascular disease. Since the 2002 IOM report on the DRI for macronutrients (1), substantial new evidence has accumulated on the health effects of EPA+DHA with respect to coronary heart disease (CHD) and CVD. CHD is not a distinct single event but a complex and multifaceted set of processes and clinical outcomes (**Fig. 1**). The first manifestation is the slow development and chronic progression of atherosclerotic plaques in response to chronic arterial injury. In contrast to this slow progression of plaque burden, characteristics related to plaque stability can change over weeks to months, increasing vulnerability to acute rupture, which can be followed by acute thrombosis. In up to half of plaque rupture cases, the resulting myocardial ischemia or injury can rapidly induce ventricular arrhythmia, which is nearly always fatal. Such fatal ventricular arrhythmias can be defined, depending upon clinical characteristics, as fatal myocardial infarction (MI), CHD death, or sudden cardiac death (SCD).

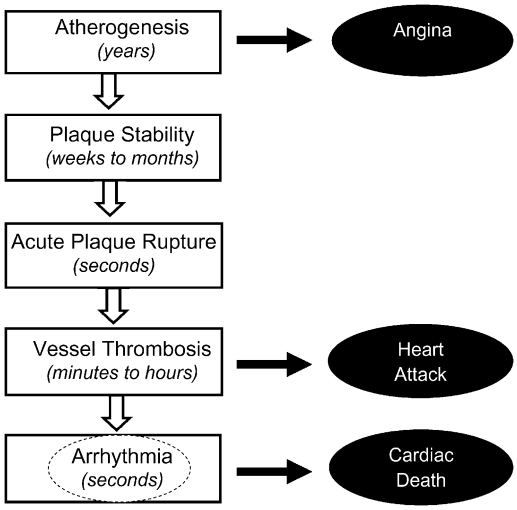

FIGURE 1 Development of coronary heart disease and potential sites of action of (n-3) fatty acids. At typical dietary doses (up to 1 g/d), the likely major site of action is the final pathway of ischemia-related cardiac arrhythmia (*dotted circle*), reducing the risk of cardiac death from fatal heart attack or sudden death. More modest effects on other pathways, such as atherosclerosis and plaque stability, may also reduce the risk of nonfatal myocardial infarctions (heart attacks) and acute coronary syndrome to a smaller extent.

Thus, CHD involves a spectrum of biologic processes and related risk factors and mechanistic pathways, each of which can be affected in different ways by particular risk factors, treatments, and interventions, including (n-3) LCPUFA. The most consistent effect of (n-3) LCPUFA consumption is to reduce cardiac death, based on strongly concordant evidence from numerous prospective cohort studies in generally healthy populations (3–20), a retrospective case-control study of SCD (21), 4 large randomized controlled trials with fish or fish oil in patients with and without known heart disease (22–25), and

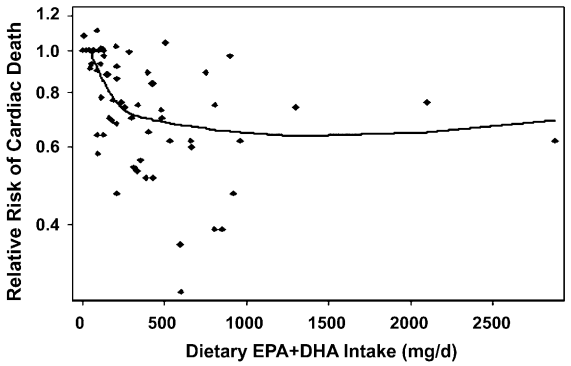

FIGURE 2 Meta-analysis of estimated dietary EPA+DHA consumption from seafood and risk of cardiac death in generally healthy populations of individuals without known heart disease. The pooled analysis includes 4473 cardiac deaths in 326,572 generally healthy individuals in 16 prospective cohort studies (3–18) from multiple countries in Europe, the United States, China, and Japan, using methods described by Mozaffarian and Rimm (28).

[13] Abbreviations used: AA, arachidonic acid; ACS, acute coronary syndrome; AD, Alzheimer's disease; AI, adequate intake; ALA, α-linolenic acid); CAD, coronary artery disease; CHAP, Chicago Health and Aging Project; CHD, coronary heart disease; CHS, Cardiovascular Health Study; CIMT, carotid intima-media thickness; CNS, central nervous system; CRP, C-reactive protein; CSFII, Continuing Survey of Food Intakes by Individuals; CV, cardiovascular; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DRI, Dietary Reference Intakes; EPA, eicosapentaenoic acid; FA, fatty acids: GISSI, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico; ICD, implantable cardioverter-defibrillator; ILSI, International Life Sciences Institute; IOM, Institute of Medicine; JELIS, Japan Eicosapentaenoic Acid Lipid Intervention Study; LA, linoleic acid; LC, long-chain; MI, myocardial infarction; MMSE, Mini-Mental Status Examination; RR, relative risk; SCD, sudden cardiac death; SDA, stearidonic acid; SR, National Nutrient Database for Standard Reference; TC, total cholesterol.

numerous in vivo and animal experimental studies (26,27). Based on a previously reported pooled analysis of observational studies in adults (primary prevention) and of randomized controlled trials in patients with and without established CHD (primary and secondary prevention), this effect is seen at relatively low doses and is nonlinear (28). Effects of EPA+DHA in generally healthy individuals would be most relevant for setting population DRI. Thus, a new pooled analysis was performed and is presented in **Figure 2**. This analysis of prospective cohort studies of CHD death including only generally healthy individuals without established CHD (3–18) supported an intake of at least 250 mg/d of EPA+DHA to materially reduce risk for CHD. A recent meta-analysis (29) limited only to U.S. prospective studies (9,12,13,21,30,31) identified intakes of ~500 mg/d as affording the greatest protection. One notable exception to these positive trials was 1 in men with angina that reported no benefit of recommending increased fish consumption (32).

In comparison, magnitudes of benefit and overall evidence for effects of (n-3) LCPUFA on other CV events, such as atherosclerosis progression and nonfatal acute coronary syndrome (ACS)/MI, are more modest. The much stronger effects on cardiac death, together with consistent antiarrhythmic effects of EPA+DHA from in vivo studies in rats, dogs, and primates and in vitro experiments (26,27,33), indicate that, at least for dietary doses (<1 g/d), the main cardiovascular effect of EPA+DHA is to reduce risk of fatal CHD, likely by reducing cardiac arrhythmias. Thus, based on the pathophysiology of CHD (Fig. 1), the likely major mode of action of (n-3) LCPUFA is on development of ischemia-related cardiac arrhythmia, with lesser effects on earlier processes (i.e., atherosclerosis progression, plaque instability, or thrombosis).

Largely on the basis of studies utilizing estimated dietary intake from questionnaires (Fig. 2), cardiac mortality is reduced ~35% by modest EPA+DHA consumption (~250–500 mg/d), an effect at least as great, for example, as that of statin therapy (34,35). Given random errors (and thus bias toward the null) inherent in dietary questionnaire estimates of EPA+DHA consumption (36), tissue levels of EPA+DHA (e.g., in erythrocytes, plasma, phospholipids, whole blood, or adipose) are more objective biomarkers of exposure that provide more precise estimates of effects. In both retrospective and prospective biomarker studies among generally healthy individuals without known CHD, compared with individuals in the highest quartile of EPA+DHA tissue levels, individuals in the lowest quartile had 10-fold higher risk of SCD independent of other known risk factors. This suggests that findings based on dietary questionnaires alone substantially underestimate the true benefits of (n-3) LCPUFA on SCD. Furthermore, the magnitudes of risk reduction seen with biomarkers of EPA+DHA are rarely seen in epidemiology—comparable to associations of smoking or asbestos with incidence of lung cancer—and are nearly impossible to explain solely by residual confounding by other factors.

As mentioned, numerous studies have evaluated potential effects of (n-3) LCPUFA on nonfatal CV outcomes, with more mixed results than those for cardiac death. These include studies showing equivocal effects of fish oil on atherosclerosis progression (37–40), and observational studies (12,13,18,41,42), and a large randomized controlled open-label trial [Japan Eicosapentaenoic Acid Lipid Intervention Study (JELIS)] (24) that suggest a modest reduction in risk of nonfatal ACS/MI. Evidence for effects on stroke is also mixed. Meta-analyses indicate a 30% lower risk of ischemic stroke with fish ≥1/wk compared with <1/mo (43) but no effect of fish oil consumption on stroke risk (44). Conversely, a post-hoc analysis from the JELIS trial found that EPA supplementation reduced recurrent stroke in individuals with a history of stroke (45). Overall, a growing body of evidence suggests that EPA+DHA consumption may benefits CV outcomes other than cardiac death, but the magnitudes of effect and breadth and concordance of evidence are not yet definitive compared with the clear reduction in cardiac death.

In randomized controlled trials, short-term EPA+DHA consumption at pharmacologic levels (>1–2 g/d) favorably affects many physiological measures of CV risk including blood pressure, resting heart rate, triglyceride levels, and possibly heart rate variability (28,46,47). For most of these risk factors, particularly those related to cardiac function, concordant findings have been seen in observational studies with habitual dietary EPA+DHA consumption at lower levels (<1 g/d) (48–52), suggesting that such lower levels of consumption may provide benefits in regard to these risk factors long-term. Because modest effects of dietary EPA+DHA on several of these risk factors could produce the moderate reductions in risk of nonfatal MI/ACS and ischemic stroke seen in some observational studies and controlled trials, these studies provide plausible physiological mechanisms to support the observed benefits of EPA+DHA on nonfatal cardiovascular events.

Unfortunately, effects of EPA+DHA on arrhythmic risk are difficult to quantify in a physiological study, as no widely accepted single modifiable physiological measure of arrhythmic risk exists. Three randomized trials in patients with implanted cardioversion defibrillator (ICD) devices have produced mixed results, with 2 trials (53,54) reporting no effects, and 1 reporting a benefit (55). Post-hoc subgroup analyses in 1 trial (53) suggested potential adverse effects in patients with a diagnosis of ventricular tachycardia who received the ICD, but this finding was not confirmed in the 2 larger studies (33,54). Established CHD risk factors such as plasma cholesterol levels, blood pressure, and C-reactive protein only modestly predict risk of arrhythmic cardiac death (55), and these risk factors are not strongly affected by dietary EPA+DHA. Resting heart rate, the QT interval, and heart rate variability each predict risk of fatal cardiac arrhythmia, and each has been favorably affected by fish oil consumption (47,49,50). The underlying variability in these markers makes them relatively insensitive for routine clinical evaluation of physiological effects on arrhythmic risk. Perhaps the best biochemical measure of arrhythmic risk is the tissue level of EPA+DHA, which is associated with 10-fold differences in risk of SCD across quartiles of tissue levels, as discussed previously (19,21). The omega-3 index, a measure of tissue (erythrocyte) EPA+DHA level, is discussed further below.

Both EPA and DHA are present in seafood and all fish oil supplements, although the ratio between the 2 typically differs, with fish usually having more DHA and supplements more EPA (56). Nearly all observational studies and randomized clinical trials of disease outcomes have evaluated the summed effects of combined EPA+DHA. Although some studies indicate that DHA may be preferentially antiarrhythmic (26), many experimentally characterized effects of EPA and DHA are similar (27,33,57), and the JELIS trial, which used pure EPA, reduced major coronary events by 19% ($P = 0.01$) (24). Thus, current evidence does not allow strong conclusions about whether EPA or DHA, or a specific ratio of the 2, is most beneficial. Based on this uncertainty, both should be consumed (in ratios between ~1:2 to 2:1) to maximize cardiac health.

The evidence for a benefit of ALA in CV risk is much less well established than that for EPA+DHA (16,58,59). Although

several cohort studies support such a connection (60), especially in the context of a low-linoleic acid (LA) diet (16), the lack of convincing data from randomized, controlled trials (61) leaves the role of ALA in cardioprotection unresolved. Hence, at present, ALA should not be considered as a replacement for EPA+DHA in reducing risk of cardiac death or other CVD.

In conclusion, evidence from randomized controlled trials of disease outcomes, prospective observational studies, retrospective case-control studies of disease outcomes, and randomized controlled trials of physiological measures, along with mechanistic insights from in vitro and in vivo animal-experimental studies, indicates that modest EPA+DHA consumption markedly reduces the risk of cardiac death. The quality, strength, and concordance of this evidence are remarkable, meeting and indeed generally exceeding those for any other dietary factor for which a DRI has been set based on reducing risk for chronic disease, including saturated fat, dietary cholesterol, salt, and dietary fiber. For primary prevention of cardiac death, the leading cause of death in both men and women in the United States and nearly all other developed nations, current evidence supports a DRI for EPA+DHA between 250 and 500 mg/d.

Cognitive development. The brain and retina contain relatively large quantities of PUFA, specifically DHA and arachidonic acid (AA) (62,63). The accumulation of these fatty acids in central nervous system (CNS) tissue occurs during fetal development, primarily through placental transfer from maternal sources (64,65) and, in infancy and toddlerhood, largely through dietary sources (66,67). A consistent literature indicating that mammalian diets deficient in (n-3) PUFA adversely affect the development of visual acuity (68) and learning (69–75) and that replacing DHA in these diets prevents or corrects these behavioral and functional deficits implies a direct and specific role for DHA in normal neurological development.

Initial studies, both observational (76) and experimental (77–78), examining the impact of (n-3) LCPUFA supplementation, showed that (n-3) LCPUFA levels were related to the development of visual acuity in human infants. Reviews of research on the effects of (n-3) LCPUFA on visual function in human infants (79–82) indicate that supplementing infant formula with (n-3) LCPUFA improves visual development of preterm infants, although perhaps not cognitive development (83). The evidence for benefit in full-term infants is more equivocal.

Further evidence for the effects of (n-3) LCPUFA on cognitive development comes from studies of early attention and recognition memory in infancy. Research using measures of attention in infancy (expressed in terms of duration of looking) is fairly consistent, showing shorter looking times (consistent with more mature cognitive development) during the first year with (n-3) LCPUFA-supplemented infants (84–87) or infants from mothers with higher erythrocyte (n-3) LCPUFA levels (88). In addition, longer looking and greater attention during free play has been reported in a separate analysis of the same sample (88). Although a few studies have reported better visual recognition memory in infants supplemented with (n-3) LCPUFA (89), most studies have not (85,87,90–92).

Measures of receptive language (ability to perceive, process, and understand various aspects of language), communicative development, and vocabulary have also been used as outcomes. The (n-3) LCPUFA status in breast-fed infants was found to be greatly correlated with retention of the ability to discriminate a nonnative speech contrast (valid speech sounds not used in the individual's native language) at 9 mo (93) and with receptive and expressive vocabulary (words actually spoken) at 14 mo (89). Recently, it was reported that blood levels of DHA were highly positively correlated with performance on the Peabody Picture Vocabulary Test (94). The domain of language has yielded the only negative findings to date with respect to (n-3) LCPUFA status (95), although the negative outcome (slightly lower vocabulary in supplemented infants) did not persist in follow-up evaluations (95,96).

Results of studies using standardized indices of cognitive status as outcome variables in observational and experimental studies of (n-3) LCPUFA status are equivocal (89,90,97–102). These studies were recently reviewed and noted to vary across many parameters that could account for differences (103).

Dosage has not generally been evaluated in a comparable manner across studies (90,96,98,104,105). Although findings from these studies may not allow for extrapolation to daily intake, they do suggest that the current maximum for DHA (0.35%) in infant formula may actually represent a minimum. A consensus document in 2007 recommended at least 200 mg/d of DHA for pregnant women (80).

Cognitive decline. There is wide variability in age-related cognitive decline in the population, a large portion of which represents various stages of dementia (106,107). Dementia is defined as the loss of cognition of sufficient severity to interfere with everyday function. The primary form of dementia is Alzheimer's disease (AD), which accounts for ~60–80% of the cases and affects 10–13% of the population aged 65 y and older (108). To date there is no cure for AD, no widely accepted prevention strategy, and no effective treatment. The economic impact is enormous, with annual costs exceeding US$100 billion.

AD is characterized by a gradual decline in memory and at least 1 other cognitive domain (109). A primary theory of AD is that oxidative and inflammatory processes damage neuronal cells, causing severe disruption in their functioning, which leads to DNA damage, the accumulation of amyloid plaques, and loss of neurons and synapses (110). The established risk factors of AD include older age, apolipoprotein E-ε4 genotype, and low education (111), but risk factors common to CHD, including a high intake of saturated fat, low consumption of fish and (n-3) LCPUFA, hyperlipidemia, and hypertension, may also be implicated. Cerebral infarcts more than double the risk of dementia and AD (112).

Interest in fish consumption and the (n-3) LCPUFA as risk factors for dementia and AD is fairly recent, stemming mostly from the literature on DHA and neurocognitive development (113). DHA is concentrated in the most metabolically active areas of the brain, such as the cerebral cortex, synaptosomes, and mitochondria (114). Studies with aging animals have found that DHA composition of the brain decreases with age (115), and that loss of DHA is associated with decreases in antioxidant enzymes, scavengers of amyloid protein, hippocampal nerve growth (116), fluidity of synaptic membranes (117), and with increased oxidation of lipid membranes (118), ischemic damage (119), synaptic loss (120), and amyloid burden (121).

The epidemiological literature on (n-3) LCPUFA and dementia is fairly limited. Among 4 prospective studies (122–125) that examined fish consumption and the risk of developing dementia, 3 observed statistically significant decreased risks of 30–70% with consumption of 1 fish meal/wk after adjustment for age, sex, educational level, and various factors. The Chicago Health and Aging Project (CHAP) is 1 of the largest studies to date on the effects of fish and (n-3) LCPUFA intake on risk of incident AD and cognitive decline. In a sample of 815 CHAP participants, there was a 60% reduction in the risk of developing

incident AD over 3.9 y among persons who consumed 1 fish meal/wk compared with persons who never or rarely consumed fish after adjustment for age, sex, race, apolipoprotein E-ε4, education, and total energy intake (125). The CHAP study also investigated the risk of incident AD by type of (n-3) LCPUFA consumed. Intake of EPA was not associated with AD risk. However, persons in quintiles 3 through 5 (median DHA intakes, 0.06 and 0.10 g/d, respectively) had a 60–80% lower risk compared with persons in quintile 1. Only 1 other prospective study (126) examined (n-3) LCPUFA intake and risk of dementia and found no evidence of an association. Studies reporting (n-3) LCPUFA levels in plasma or brain in subjects with various degrees of dementia have not shown a consistent relation between low DHA levels and clinical evidence of cognitive decline or dementia (127–129).

Four cohort studies have reported a protective effect against cognitive decline with higher (n-3) LCPUFA intakes (130–133). In a 4-y follow-up of 246 participants of the EVA study, a 1 standard deviation increase in erythrocyte total (n-3) LCPUFA and DHA were associated with a statistically significant 40% reductions in cognitive decline [as measured by a 2-point drop of on the Mini-Mental Status Examination (MMSE)] (130). In the CHAP study of 3718 persons, the annual rate of cognitive decline over 6 y was greatly reduced by 10% and 13%, respectively, for persons who consumed 1 and 2 fish meals/wk when compared with the rate among persons who consumed fish less often (131). Compared with the fish consumers among 210 participants of the Zutphen Elderly Study, nonconsumers had 4 times the risk of a 2-point decline in MMSE score over 5 y (132). Plasma phospholipid levels of (n-3) LCPUFA were closely associated with slower decline in verbal fluency among 2251 participants of the Atherosclerosis Risk in Communities study, but there was no association with levels and measures of verbal fluency, perceptual speed, or a global measure of cognitive function (133).

To date, there is only 1 published large randomized clinical trial of (n-3) LCPUFA and cognitive decline in which 204 patients with AD were randomized to daily intake of 1.7 g DHA and 0.6 g EPA or placebo for 6 mo. Whereas there was no overall effect on MMSE score or the Alzheimer Disease Assessment Scale, in post-hoc analyses, positive effects were observed in a small group of patients with very mild AD (134).

In summary, the available evidence for protective effects of (n-3) LCPUFA from fish on risk of dementia is promising but limited. Epidemiological evidence is positive for benefits of just 1 fish meal/wk on risk of AD, dementia, and cognitive decline. Further, the animal evidence and biologic plausibility support a protective relation of (n-3) LCPUFA on neurodegeneration of the brain with aging. However, tissue DHA levels do not seem to be consistently lower in AD or any form of cognitive decline associated with aging (127). Several primary and secondary prevention trials are in progress in the United States and Europe that will greatly inform the field. Further research is necessary to examine the effects of different dose levels, benefits of DHA, EPA, and ALA, and the importance of relative intakes of (n-3) vs. (n-6) fatty acids.

Cancer. The preponderance of evidence for anticarcinogenic effects of EPA+DHA in humans is weak despite persuasive data from animal models and cultured tumor cell lines. An Agency for Healthcare and Research Quality review (135) and a meta-analysis reviewing 10 randomized controlled trials [relative risk (RR) 1.07, 95% CI 0.88–1.30] and 7 cohort studies (44) each concluded that there was little evidence for an effect of dietary (n-3) LCPUFA intake on cancer incidence (44,135). In a more recent meta-analysis examining (n-3) LCPUFA and colorectal cancer risk, the pooled RR from 14 studies comparing highest and lowest intakes was 0.88 (95% CI 0.78–1.00) (136). No significant associations were found between total fish intakes and breast cancer risk in the multicenter European Prospective Investigation into Cancer and Nutrition study (RR 1.01, 95% CI 0.99–1.02, $P = 0.28$) (137) or colon cancer risk in the Norwegian Women and Cancer study ($n = 63{,}914$) (138).

A review of hormone-related cancers (prostate and breast) also revealed no evidence for an effect (beneficial or harmful) of fish and marine fatty acids (139–141). Although some studies suggested that intakes or blood levels of marine-derived (n-3) PUFA may be inversely associated with risk for prostate cancers, particularly at advanced, late, and/or metastatic stages than at earlier stages (142,143), others have reported the opposite (144). Unfortunately, the stage of cancer is not usually taken into account by systematic reviews (44,139,145).

Recently, there has been some concern that dietary ALA might increase the risk of prostate cancer, particularly advanced and late stage (140,145,146). Although this was supported by a meta-analysis (60), more recent studies with other cohorts found no association (141,147). Nevertheless, because ALA is the predominant dietary (n-3) PUFA, clarifying the potential for interactions between dietary ALA and (n-3) LCPUFA and advanced prostate cancer risk merits further investigation.

The discordant results between animal and human studies for cancer effects have yet to be reconciled. This is an area of concern, particularly because the data generated from animal models and cell culture experiments have traditionally had preclinical utility as supporting evidence for the generation of DRI (1). Issues, separately or combined, that could explain the differential effects observed between clinical trials and experimental models include the inability to accurately assess (n-3) LCPUFA intakes in epidemiological studies, the supraphysiological doses often used in animal studies, the lack of established guidelines for background diets in animal studies (148), and the greater potential tissue need for DHA in rodents compared with humans (149).

In summary, there are no clear relations between dietary intakes of EPA and DHA and risk for cancer. The epidemiological data, and particularly prospective studies, are relatively consistent on this issue. Inconsistencies in dose and duration could be problematic as evidenced by the improvement in symptoms in animals that already have the disease and that are fed very high levels of (n-3) LCPUFA. Generating a standard dietary protocol that results in human-equivalent tissue levels of (n-3) LCPUFA will be essential to maximize the preclinical value of experimental animal models (148).

Total mortality. Higher intakes of (n-3) LCPUFA have been associated with a reduced risk for death from any cause, largely related to their benefit on CHD mortality. In 3 prospective cohort studies that provided data on EPA+DHA consumption (11,30,150), significant reductions in all-cause mortality were associated with higher intakes; this, however, was not observed in a study in women initially free of heart disease (15). In a meta-analysis of the effects of treatments for dyslipidemia (diets and drugs), Studer et al. (151) reported that total mortality was reduced by only 2 interventions: (n-3) PUFA and statins. In 2 large randomized trials in cardiac patients (23,25), treatment with EPA+DHA reduced risk for death from any cause. Finally, in a meta-analysis of randomized controlled trials, fish oil treatment greatly reduced total mortality (28).

The conversion of ALA to EPA+DHA

As addressed by the IOM Panel on Macronutrients (1), the efficiency of conversion of ALA to EPA and on to DHA is a complex yet critical question. Uncertainty surrounds the extent of conversion in humans of the parent (n-3) PUFA, ALA, to its long-chain products, the potential health effects of ALA per se, and how ALA compares to stearidonic acid [SDA, 18:4(n-3)] as a source of EPA, docosapentaenoic acid (DPA, 22:5(n-3)], or DHA.

With the rare exception of genetic anomalies such as Zellweger syndrome, humans have a functional desaturation-chain elongation pathway in the liver (as well as the gut and brain) that can convert some ALA or LA to the respective LCPUFA. This has been established by several groups using in vitro assays in cultured human cells or assays of semipurified liver microsomal preparations of the $\Delta 6$ and $\Delta 5$ desaturases (152–155). Various model systems have been used to elucidate the generally acknowledged features of this desaturation-chain elongation pathway (156), which unequivocally exists in humans.

The relative health effects of the various (n-3) LCPUFA hinge not on the *existence* of desaturation-chain elongation activity but rather on its *capacity*. Two research approaches have been used in humans to study the conversion of the 18-carbon PUFA substrates to their 20- and 22-carbon products: tracer studies and dietary supplementation studies in which changes in levels of the LCPUFA in serum lipids are assessed. There are ~18 papers reporting 1 of these approaches, and with few exceptions, they broadly agree that ALA conversion to DHA is no greater than ~0.5% (156).

Given these data, the in vitro performance of the purified desaturases cannot be directly linked to their functional capacity in humans. In fact, because the capacity of the desaturation-chain elongation pathway in healthy, nonvegetarian humans is <0.5%, [perhaps somewhat greater in women than men (157)], even large amounts of dietary ALA have a negligible effect on plasma DHA, an effect paralleled by the (n-6) PUFA pathway, where dietary LA has only a negligible effect on plasma AA levels (158). Data on SDA or EPA conversion to DHA are extremely limited, but they confirm the inefficiency of DHA synthesis in healthy, adult, nonvegetarian humans (159).

It has also been reported that higher background intakes of LA can reduce plasma content of EPA (160). Whether this is a result of reduced EPA synthesis from ALA or of increased competition for acylation sites on plasma lipids is not clear. In addition, desaturation-chain elongation depends on several enzymes the activity of which can be inhibited by inadequate intakes of several cofactor nutrients including iron, zinc, and pyridoxine (161). The ability of ALA to slightly raise EPA and DPA (but not DHA) levels may partly explain the inverse associations between ALA and CHD noted earlier. Clearly, raising EPA alone can reduce risk for CHD (24). In addition, the presence in tissues of more DPA than EPA suggests that the former, as well as their precursor ALA, may have an as yet undefined physiological role (162).

Assessing (n-3) PUFA intake and exposure

What are we eating?. Dietary sources of the plant-derived (n-3) PUFA, ALA, include canola and soybean oils, walnuts, and flaxseed oil, the latter being 1 of the most concentrated sources of ALA known (56). Although flaxseed oil is much richer in ALA than soybean oil (55 vs. 8% of fatty acids), the latter is the largest contributor of ALA to the U.S. diet, accounting as it does for more than two-thirds of the edible oil consumed (163).

Oils found naturally in fish are the richest dietary sources of EPA and DHA. There is typically more DHA than EPA in fish oils, but their ratios can vary widely across species (and in fish oil supplements). Even though U.S. fish consumption has increased slightly in recent years, intake is still quite low compared with other countries. In addition, many of the more popular varieties (e.g., shrimp, pollock, catfish, cod, clams, crabs, scallops, and tilapia) are not particularly good sources of (n-3) LCPUFA. The U.S. PUFA intake has been estimated using NHANES data, for which respondents provide dietary intake information over a 24-h period. According to the 2005–2006 survey, total PUFA [(n-6) and (n-3)] intake in adults was ~19 g/d (164), of which ~9% came from (n-3) PUFA, 8.2% (~1.5 g/d) from ALA, and 0.7% (~135 mg/d) from EPA+DHA. These values have changed little over the last 6 y (164,165). Fortified foods and fish oil supplements are large market growth areas by commercial sales figures; however, there are no reliable data indicating the amounts of EPA and DHA these products contribute to the American diet.

Per capita food disappearance data indicate that fish intake in the United States constitutes ~6% of total meat consumption (166,167). In the context of a very low national fish intake, terrestrial meats (e.g., beef, pork, mutton, poultry, game) may actually contribute significant amounts of DHA and DPA. In the Australian diet, it has been estimated that terrestrial meats contribute almost 50% of the (n-3) LCPUFA, primarily in the form of DPA (168). Assessment of the impact of terrestrial meat consumption on (n-3) LCPUFA intake in the United States has been limited by the lack of complete data on these fatty acids in some terrestrial meats in food composition databases before 2008. The contribution of (n-3) LCPUFA from meat in the Australian diet may be higher than that in the U.S. diet because Australian cattle are more often fed grass (which contains some ALA), whereas U.S. cattle are more commonly finished on corn (very low in ALA).

Continual improvement of food composition databases is critical to accurate assessment of (n-3) LCPUFA intake. These databases form the ultimate foundation for U.S. food policy. Food composition databases, especially the USDA National Nutrient Database for Standard Reference (SR) (56), catalogue the (n-3) LCPUFA content of foods. The SR is used to estimate intakes in studies based on both 24-h recall and FFQ methods. Under the National Food and Nutrient Analysis Program, the USDA, NIH, and FDA are currently collaborating to expand the (n-3) LCPUFA composition data for foods (seafood as well as other foods, such as terrestrial meats and eggs) and dietary supplements in the SR (169). The new analytical data will be released in ~2010. Although these efforts will greatly improve the accuracy of (n-3) LCPUFA intake estimates in the future, studies currently in the published literature (all of which used earlier versions of the database) probably underestimated intakes.

Even with a perfect database, all methods of estimating food consumption are limited by participant recall error, difficulties in describing amounts and composition of foods eaten, and assessment-induced changes in dietary patterns. Although the 24-h recall is less likely to accurately capture occasional foods (e.g., fish), FFQ are limited by crude categorization of fish species and portion size. Together, these factors can lead to inaccurate intake estimates, which when used in cohort studies of disease endpoints, can result in substantial underestimation of the true magnitude of benefit or risk (36). The need for validated biomarkers of (n-3) LCPUFA exposure is critical and is discussed further below.

What should we be eating?. Recommendations for (n-3) LCPUFA intake have been put forth by several organizations globally (**Table 1**) (170–178). Most focus on primary prevention

TABLE 1 Recommendations for fish and/or EPA+DHA intakes for healthy adults from government and health organizations worldwide

Organization	Year	Recommendation
Eurodiet Conference (170)	2000	200 mg/d
Agence Française de Sécurité Sanitaire des Aliments, Centre national de coordination des études et recherches sur la nutrition et l'alimentation, and Centre national de la recherche scientifique (France) (171)	2001	500 mg/d
UK Scientific Advisory Committee on Nutrition (172)	2004	Fish twice/wk, 1 serving of which should be oily, minimum intake 450 mg/d
International Society for the Study of Fatty Acids and Lipids (173)	2004	500 mg/d
Australia Department of Health and Ageing (Australia and New Zealand) (174)	2005	442 mg/d for men, 318 mg/d for women*
American Heart Association (175)	2006	2 servings/wk of (preferably oily) fish
Health Council of the Netherlands (176)	2006	Fish twice/wk, 1 serving of which should be oily, to achieve the DRI of 450 mg/d of (n-3) LCPUFA
Superior Health Council of Belgium (177)	2006	Minimum of 0.3% energy for adults (~667 mg/d)
American Dietetic Association/ Dietitians of Canada (178)	2007	Fish twice/wk, both servings oily or 500 mg/d

* The Australia and New Zealand recommendation is for EPA+DHA+DPA. The portion provided by EPA+DHA alone is given here per Howe et al. (168).

of CHD, whereas others aim only to prevent nutritional deficiencies. (The extent to which premature CHD can be considered a manifestation of EPA+DHA deficiency remains a point of discussion.) Some (e.g., American Heart Association), recommend foods (i.e., oily fish), whereas others (e.g., IOM) deal with nutrients. Whether the recommendation is for fish or for EPA+DHA, the resulting EPA+DHA values typically fall between 200 and 600 mg/d.

An AI of ALA was defined as ~0.6% of energy by the IOM Panel on Macronutrients (1). This translates into ~0.5 g/d for infants, 1.1 g/d for women, and 1.6 g/d for men. The AI is based on the median intake of ALA in the United States, according to the Continuing Survey of Food Intakes by Individuals (CSFII) 1994–1996 and 1998 (now replaced by NHANES), and concomitant lack of apparent deficiency in the population. It should be appreciated that the IOM Panel did not make a recommendation for EPA and DHA, per se. Rather, the Panel concluded that, because roughly 10% of the total (n-3) PUFA intake currently comes from EPA and DHA, 10% of the AI for ALA could be met by these 2 (n-3) LCPUFA.

Assessing (n-3) status. Given the uncertainties surrounding accurate assessment of (n-3) LCPUFA intakes, a biomarker-based approach to defining target intakes may be more rational. In other words, if a certain blood level of (n-3) LCPUFA can be clearly linked to an important health benefit (e.g., reduced risk for CHD), then that blood level could be considered a surrogate endpoint, and the intakes needed to achieve that blood level may be recommended. The omega-3 index (55) is an example of a marker of (n-3) LCPUFA biostatus. The omega-3 index is the sum of EPA+DHA in erythrocyte membranes and is expressed as a percentage of total erythrocyte fatty acids (179). This marker has been validated against dietary intake (180) and has been shown to correlate strongly with reduced risk for mortality from CHD (as described above). The choice of membrane (n-3) LCPUFA levels, as opposed to plasma (n-3) LCPUFA composition or concentration, as a biomarker of intake is based on the fact that the effects of these fatty acids on basic cellular function appear to arise primarily from their effects on and in membranes. Still, whole plasma (and plasma phospholipid and cholesteryl ester) levels do correlate closely with erythrocyte levels (55). The omega-3 index is strongly associated with human cardiac membrane EPA+DHA levels ($r = 0.81$, $P < 0.0001$), and in cardiac transplant patients supplemented with (n-3) LCPUFA, both cardiac and RBC EPA+DHA increase to the same extent (162). Depending on the dose, it takes 3 to 6 mo for a new steady-state omega-3 index to be established (181,182). In addition, the omega-3 index has been shown to reflect the intake of both oily fish and fish oil supplements and to be less variable than plasma EPA+DHA levels (183).

The blood content of (n-3) LCPUFA [which is highly correlated with the omega-3 index (179)] may actually be a better predictor of risk for SCD than traditional CHD risk factors. This may be seen by comparing 2 reports from the

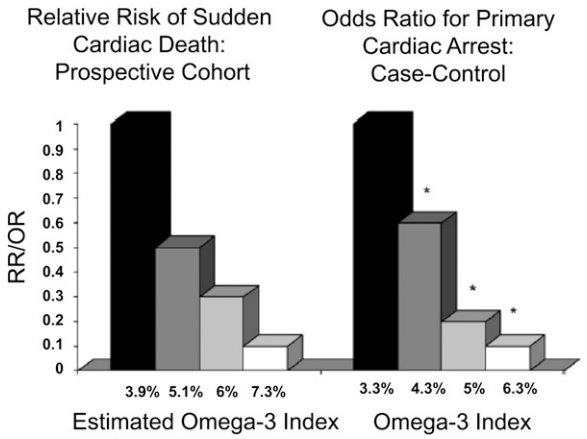

FIGURE 3 Relation between the omega-3 index and risk for primary cardiac arrest (*right*) and the estimated omega-3 index and SCD (*left*). The former were derived from a population-based case-control study (21), and the latter from a case-control study nested in the prospective Physicians' Health Study (P-trend = 0.001) (19). [The omega-3 index was estimated from the whole-blood (n-3) LCPUFA content using equations described by Harris and von Schacky; $*P < 0.05$ vs. quartile 1.] Reproduced with permission from William S. Harris (162).

Physicians' Health Study (**Fig. 3**) (184). Albert et al. (185) published the relative risk for SCD across quartiles of C-reactive protein (CRP), total cholesterol (TC), LDL- and HDL-cholesterol, triglycerides, homocysteine and the TC/HDL ratio. In the second article (19), the relations between blood (n-3) LCPUFA content and the same endpoints were reported. Only 2 risk factors demonstrated statistically significant relations with risk for SCD after controlling for age and smoking status: CRP and (n-3) LCPUFA. Blood (n-3) LCPUFA content was related to risk in a dose-dependent manner, unlike CRP, which was more of a dichotomous variable. In addition, the risk reduction at the highest levels of the omega-3 index (90%) was greater than that associated with the lowest levels of CRP (65%). Therefore, in the case of SCD [which is responsible for about half of all CHD deaths (186)], the omega-3 index may be more informative than other currently accepted risk factors.

In proposing that the omega-3 index may serve as a new risk marker for CHD mortality, Harris and von Schacky (179) estimated what a "healthy" omega-3 index target might be by extrapolating from studies of other (n-3) LCPUFA biomarkers (e.g., whole blood, plasma phospholipids) and of (n-3) LCPUFA intakes. They arrived at a proposed cardioprotective target level of 8% of erythrocyte fatty acids as EPA+DHA. An omega-3 index of 4% or less was associated with the greatest risk for CHD death. These values have recently been shown to distinguish between ACS patients and controls (41). One of the major obstacles to defining target cardioprotective levels of (n-3) LCPUFA is the lack of standardized methods for their assessment. In the 1980s, the National Heart Lung and Blood Institute and CDC undertook to establish the Lipid Standardization Program, which has led to worldwide uniformity in lipid and lipoprotein testing. A similar effort is needed for blood fatty acid testing.

Obstacles to setting a DRI

Overarching issues. One of the major goals of the workshop was to understand the obstacles preventing the establishment of a DRI for (n-3) LCPUFA. In 2002, the IOM Committee concluded that there was insufficient evidence available to set a DRI for (n-3) LCPUFA (1). Some of the concerns were as follows:

1. Because ALA can be converted to (n-3) LCPUFA, the latter could not strictly be considered as essential nutrients.
2. EPA and DHA are distinct chemical species, not 1 nutrient (like ascorbic acid), and absent data showing a specific need for 1 or the other (or a defined ratio of the 2), it was unclear how a combined recommendation could be made.
3. The evidence linking higher intakes of (n-3) LCPUFA to reduced CVD risk was not based on studies with these fatty acids per se but on population estimates of fish intake, which provides multiple other nutrients and displaces other foods associated with higher CVD risk.
4. The trial evidence for cardioprotection was derived from patients with CHD, not from the general population, so the application of these intakes to primary prevention was unclear.
5. The extent to which increased intakes of (n-3) LCPUFA were safe (particularly with respect to risks for bleeding) was poorly defined.

Six years later, many of these concerns have been addressed (if not resolved). To summarize:

1. The rate of conversion of ALA to EPA is low (~5%), and to DHA even lower (~0.5%). This degree of conversion is insufficient to achieve tissue levels of (n-3) LCPUFA that will protect against premature CHD.
2. Although specific or optimal ratios among the (n-3) LCPUFA cannot yet be defined, both their natural ratios in fish (typically DHA > EPA) and in the prescription (n-3) product used in the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI) studies (23,25) (EPA > DHA) appear to provide substantial protection against fatal CHD. Although EPA and DHA have different physiological roles (187) that will need to be more precisely defined, EPA:DHA ratios ranging from ~1:2 to 2:1 are expected to be effective. In addition, there is significant precedent for treating macronutrients as complex mixtures of multimolecular species, the individual components of which often have differing physiological effects (e.g., dietary fiber, total fat, saturated fat, carbohydrate, protein). Despite the inability to define the "optimal" composition of each one, a DRI has been set for each of these macronutrient complexes. Consequently, concerns that the "right" ratio of EPA and DHA is not yet precisely known or that all of their physiological effects have not been discovered should not be an obstacle to defining a DRI.
3. The effects on CHD first observed with fish intake [e.g., in the Diet and Reinfarction Trial (22)] have now been replicated in 3 major clinical trials using only (n-3) LCPUFA (23–25). Hence, although fish provides many nutrients, the (n-3) LCPUFA content is clearly a major mediator of the observed CHD benefits of fish seen in healthy populations (primary prevention).
4. The wealth of data from multiple observational cohort studies in numerous countries strongly support a primary prevention benefit for (n-3) LCPUFA on fatal CHD. Furthermore, JELIS (24), the first large prevention trial to include a majority of patients without established CHD, demonstrated a 19% reduction in major coronary events with EPA. Although the trial was not powered to separately detect benefits in both the primary and secondary prevention arms, subgroup analyses indicated very similar risk reduction estimates in these 2 groups. It is questionable, in the face of similar results from both observational studies of hundreds of thousands of generally healthy individuals and randomized trials of patients with and without established CHD, to conclude that secondary prevention data does not inform primary prevention. Indeed, contention that secondary prevention data cannot support efficacy in primary prevention may be challenged by the fact that there has never been a treatment regimen in CVD that, after being documented to be effective in the secondary setting, failed to be effective when ultimately tested in the primary setting. The basic pathophysiology of CVD is the same, whether for the first heart attack or the second. Concerns regarding the extrapolation of secondary to primary prevention efficacy are quite appropriate when evaluating drugs whose benefits must outweigh potential side effects. These are of considerably less concern with agents such as (n-3) LCPUFA, which have a strong safety profile. Finally, the requirement that a nutrient be shown to reduce clinical events in primary prevention, randomized trials before the assigning of a DRI has not been applied to other nutrients or food components. Neither increases in dietary fiber (discussed in more detail below) nor reductions in total fat, saturated fat, or cholesterol have ever been shown, in single-variable, placebo-controlled, prospective

trials, to reduce risk for CHD events in either primary or secondary populations, and yet DRI exist for them all. Because the requirement for definitive evidence of primary prevention has not been applied to other nutrients, it is not clear why it should be required for establishing a DRI for (n-3) LCPUFA, nutrients for which the highest levels of evidence for benefit have been assembled.

5. With regard to safety, JELIS (24), GISSI-Prevenzione (23), and GISSI-Heart Failure (25) reported no clinically relevant adverse effects in over 35,000 individuals. In addition, a review of the effects of pharmacologic doses (3 g/d or greater, even in combination with anticoagulants) on clinically significant bleeding found no reason for concern (188). Over 10 y ago, the FDA determined that intakes of EPA+DHA of up to 3 g/d are safe for the general population (189). Hence, there should be no concerns about the safety of targeting an EPA+DHA intake of up to 500 mg/d.

The dietary fiber precedent. The 2002 IOM report on macronutrients included a DRI for dietary fiber (1), which provides a useful precedent for EPA+DHA. An AI for adults was established based on CHD risk reduction, as observed from 3 prospective observational studies of substantial scientific quality. The basis for selecting the AI was, "[T]he AI of 14 g of fiber per 1000 kcal was calculated using the average intake of fiber in the 'protected group' in each of the studies (i.e., the highest quintile of fiber intake)" (1). The same principles may be applied to EPA+DHA, for which a reduction in CHD was documented in several prospective observational studies in North America (9,12,13,19,21,30). The average intake in the highest quintile in these studies was 566 mg/d (29). In addition, there are 4 randomized controlled trials (22–25) with hard CHD endpoints for (n-3) LCPUFA and none for fiber. Hence, the evidence for a CHD benefit with EPA+DHA is considerably stronger than that for fiber.

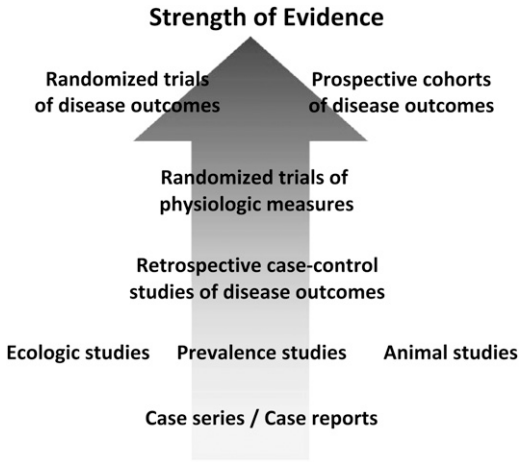

FIGURE 4 Hierarchy of quality and strength of evidence of different study designs for evaluating causation of chronic disease. Studies of physiological endpoints may not fully account for all effects of an intervention, and thus, evidence is strongest when derived from well-conducted studies of disease endpoints. Well-controlled prospective observational studies and well-performed randomized clinical trials have different, and highly complementary, strengths and limitations. The major strength of randomized trials is to minimize residual confounding; limitations include potential lower generalizability to external populations, the inability to evaluate the effects of many risk factors of interest (e.g., smoking, biomarker levels) due to ethical or practical limitations, inadequacy of duration and/or dose tested, challenges in testing multiple doses, noncompliance, unblinding, differential loss to follow-up, and treatment crossover. The major limitation of prospective observational studies is potential residual confounding, whereas major strengths generally include the converse of each of the major limitations of randomized trials. Thus, evidence is most robust when studies of both designs provide concordant results, as is the case for EPA+DHA consumption and (especially fatal) CHD.

Evidence supporting a CHD benefit for increased intakes of EPA+DHA is available from studies across the hierarchy of trial designs and qualities (**Fig. 4**). Since the 2002 IOM report was issued, over 50 studies addressing the relations between CHD and EPA+DHA have been published (**Table 2**). It is largely on the strength of this dataset that the health organizations listed in Table 1 have made recommendations for EPA+DHA to reduce risk for CHD. In the view of the authors, a literature of this depth should be sufficient to support a reevaluation of the need to establish a U.S. DRI for EPA+DHA.

Research needs

Additional areas of research that would help to further define optimal intakes for EPA+DHA are below:

1. Expand investigation into the impact of (n-3) LCPUFA on other CVD outcomes, such as atherosclerosis progression and plaque stability.
2. Further define the individual effects of ALA, SDA, EPA, DPA, and DHA.
3. Improve tools for dietary assessment, including food composition databases used to quantify the (n-3) LCPUFA content of foods, fortified foods, and dietary supplements (including lesser known sources, such as terrestrial animals, microorganisms, and technology-enhanced oils) and FFQ instruments with greater specificity for seafood varieties and preparations.
4. Validate biomarkers for exposure and health outcomes (e.g., the omega-3 index), establish a dose-response relation between dietary intakes and biomarker levels, and standardize methods for laboratory analysis.
5. Further document the safety of (n-3) LCPUFA and identify populations susceptible to potential adverse effects.

Potential impact of a DRI for EPA+DHA on food policy and industry

A defined nutrient recommendation can have an important impact on food policy. Potential avenues of policy implementation include Dietary Guidelines for Americans; MyPyramid; food labeling; fortification; and feeding programs (i.e., Women, Infants and Children). If a DRI for EPA+DHA were to be set, it would be important to consider the impact of such a recommendation on the ability of suppliers of (n-3) LCPUFA to meet the increased demand in a sustainable manner. Serious concerns about overfishing and aquaculture practices have been raised (190) and must be considered. Aquaculture has the potential to increase the global availability of seafood and, likewise, the intake of (n-3) LCPUFA. Although there are tonnage and dollar values for species raised through farming available through the Food and Agriculture Organization (191), data on fatty acid composition and actual consumption in the United States and other countries are quite limited. The extent to which aquaculture may influence (n-3) LCPUFA intake depends on many factors, not least of which is whether an increased availability of fish would generate an increased demand. If the demand for a

TABLE 2 Examples of studies related to (n-3) LCPUFA and CHD published since the 2002 IOM Report on Macronutrients

First author (reference)	Year	Study topic
Marchioli (196)	2002	GISSI Prevenzione update
Albert (19)	2002	Blood (n-3) FA and risk for SCD
Hu (12)	2002	Fish intake and CHD death: Nurse's Health Study
He (197)	2002	Fish intake and incident stroke
Bucher (198)	2002	(n-3) FA trials and death: meta-analysis
Nestel (199)	2002	(n-3) FA and systemic arterial compliance
Thies (200)	2003	(n-3) FA and plaque stability
Burr (32)	2003	Diet and Reinfarction Trial-II
Mozaffarian (13)	2003	(n-3) FA and CHD: Cardiovascular Health Study (CHS)
Erkkilä (201)	2003	Serum (n-3) FA and CHD death
Dallongeville (202)	2003	Fish intake and heart rate
Lemaitre (20)	2003	Serum (n-3) FA and CHD death: CHS
Pischon (203)	2003	(n-3) FA intake and inflammatory markers
Hino (204)	2004	(n-3) FA intake and carotid intima-media thickness (CIMT)
Mozaffarian (205)	2004	(n-3) FA and incident atrial fibrillation: CHS
He (5)	2004	Fish and CHD mortality: meta-analysis
Schrepf (206)	2004	Infusion of (n-3) FA and arrhythmic inducibility
Harris (162)	2004	Cardiac and RBC (n-3) FA levels in transplant patients
Harris (55)	2004	Omega-3 index as a CHD risk marker
He (43)	2004	Fish and incident stroke: meta-analysis
Erkkilä (37)	2004	Serum (n-3) FA and coronary artery disease (CAD) progression in women
Hooper (207)	2004	Cochrane (n-3) FA and CHD: meta-analysis
Mozaffarian (47)	2005	(n-3) FA and heart rate: meta-analysis
Mozaffarian (208)	2005	(n-3) FA and incident stroke: CHS
Raitt (53)	2005	(n-3) FA and ICD discharge: Portland
Leaf (33)	2005	(n-3) FA and ICD discharge: Boston
Studer (151)	2005	Antilipidemic agents and death: meta-analysis
Calò (209)	2005	(n-3) FA and atrial fibrillation post-coronary artery bypass graft
Iso (18)	2006	Japan Public Health Center: fish/(n-3) FA and CHD
Wang (210)	2006	(n-3) FA and CHD: systematic review
Mozaffarian (28)	2006	(n-3) FA risk/benefit review
Mozaffarian (50)	2006	(n-3) FA and electrocardiogram: CHS
Mozaffarian (51)	2006	(n-3) FA and cardiac structure and function: CHS
Brouwer (54)	2006	(n-3) FA and ICD discharge: Holland
Radaelli (211)	2006	(n-3) FA and baroreceptor reflex
Hjerkinn (212)	2006	Fish and (n-3) FA and pulse wave velocity and CIMT
Harris (213)	2006	(n-3) FA and heart rate in cardiac transplant patients
Yokoyama (24)	2007	JELIS: EPA and CHD events
Harris (179)	2007	(n-3) FA and bleeding risk
Harris (214)	2007	(n-3) FA biomarkers and CHD risk
Wennberg (215)	2007	(n-3) intake and stroke
Mita (216)	2007	EPA and CIMT: arterial compliance in type 2 diabetes mellitus
Harris (183)	2007	Fish vs. fish oil capsules on RBC (n-3) levels
Block (41)	2008	Blood cell (n-3) PUFA and ACS: case control
GISSI-HF (25)	2008	GISSI-Heart Failure
Harris (29)	2008	(n-3) FA intake for primary prevention
Sekikawa (217)	2008	Serum (n-3) FA and CAD: Japan vs. U.S.
Cohen (218)	2008	RBC (n-3) FA and depression in ACS
Aarsetoy (219)	2008	RBC (n-3) FA and post-MI ventricular fibrillation
Mozaffarian (49)	2008	Fish intake and heart rate variability: CHS
Sun (42)	2008	Serum (n-3) FA and nonfatal MI: Nurse's Health Study
Tanaka (45)	2008	JELIS-EPA and stroke
Walser (220)	2008	(n-3) FA effects on stroke volume
Wilhelm (221)	2008	Blood (n-3) FA and risk for ventricular arrhythmia
Hall (222)	2008	Acute effects of EPA on digital pulse volume
Yamagishi (223)	2008	(n-3) FA intakes and CV death in Japan

food grows, systems and technologies needed to produce it will need to be developed that are both economically viable and sustainable. In addition to potential expansion of aquaculture and improved methods for sustainable wild catch fishing, other novel approaches have potential to increase availability of (n-3) LCPUFA to consumers. These include *1*) the cultivation of single-celled organisms, with or without genetic modification, to produce EPA and/or DHA (192); *2*) the creation of new plant cultivars that contain either more readily convertible precursors of LC (n-3) PUFA (159) or EPA and DHA (193,194); and *3*) the use of deodorized and stabilized oils derived from inedible (n-3) PUFA-rich marine organisms (e.g., menhaden, krill) to produce a wide variety of fortified foods (195). In each case, the (n-3) LCPUFAs thus produced could be used either in human foods or to expand aquaculture. Nevertheless, challenges in implementing a nutrient recommendation should not deter federal agencies from setting a DRI if the scientific support and the potential health benefits are substantial. Additional sources of and new approaches to providing (n-3) LCPUFA will undoubtedly be found once a need is codified.

Conclusion

From this workshop the following conclusions were reached:

- Based on the strength of the current literature (which includes over 50 additional observational and interventional studies published since the 2002 IOM review), there is a clear, inverse relation between EPA+DHA intake and risk of fatal (and possibly nonfatal) CHD. Accordingly, there is a compelling rationale for reconsidering the establishment of a nutritionally achievable DRI for EPA+DHA. Based on these studies, international recommendations have been made for intakes of ~500 mg/d to reduce risk for CHD.
- Because of the demonstrably low conversion of dietary ALA to EPA and especially to DHA, protective tissue levels of EPA+DHA can currently be achieved only through direct intake of these fatty acids.
- Beneficial effects of EPA+DHA on cognitive decline are emerging, but the data are not yet sufficiently compelling

to support an intake level different from that needed to achieve CHD risk reduction.

Higher intakes of EPA+DHA do not appear to reduce risk for cancer.

There is no evidence that intakes of EPA+DHA in the range recommended are harmful.

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