

The omega-3 index as a risk factor for coronary heart disease¹⁻³

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ABSTRACT

Because blood concentrations of n-3 (or omega-3) fatty acids (FAs) (eicosapentaenoic and docosahexaenoic acids) are a strong reflection of dietary intake, it is proposed that a n-3 FA biomarker, the omega-3 index (erythrocyte eicosapentaenoic acid plus docosahexaenoic acid), be considered as a potential risk factor for coronary heart disease mortality, especially sudden cardiac death. The omega-3 index fulfills many of the requirements for a risk factor including consistent epidemiologic evidence, a plausible mechanism of action, a reproducible assay, independence from classic risk factors, modifiability, and, most important, the demonstration that raising levels will reduce risk for cardiac events. Measuring membrane concentrations of n-3 FAs is a rational approach to biostatus assessment as these FAs appear to exert their beneficial metabolic effects because of their actions in membranes. They alter membrane physical characteristics and the activity of membrane-bound proteins, and, once released by intracellular phospholipases from membrane stores, they can interact with ion channels, be converted into a wide variety of bioactive eicosanoids, and serve as ligands for several nuclear transcription factors, thereby altering gene expression. The omega-3 index compares very favorably with other risk factors for sudden cardiac death. Proposed omega-3 index risk zones are (in percentages of erythrocyte FAs): high risk, <4%; intermediate risk, 4-8%; and low risk, >8%. Before assessment of n-3 FA biostatus can be used in routine clinical evaluation of patients, standardized laboratory methods and quality control materials must become available. *Am J Clin Nutr* 2008;87(suppl):1997S-2002S.

THE OMEGA-3 INDEX

The omega-3 index is the sum of 2 prominent long-chain n-3 fatty acids [ie, eicosapentaenoic acid (EPA) and docosahexaenoic acids (DHA)] in erythrocyte membranes and is expressed as a percentage of total erythrocyte fatty acids (FAs). The choice of membrane n-3 FA concentrations (rather than plasma n-3 FA composition or concentration) as a biomarker of intake is based on the fact that the effects of these FAs on basic cellular function appear to arise primarily from their effects on and in membranes. They act in at least 4 separate ways, but the relative importance of each, their coordinated interaction, and their sufficiency to explain the clinical observations have yet to be precisely determined.

First, because of their highly unsaturated nature, n-3 FAs may alter membrane biophysical properties (1). This alteration can have the secondary effect of changing the microenvironment of transmembrane proteins (eg, receptors), altering the manner in which they interact with their ligands (2) (**Figure 1**). Changing membrane FA composition can also affect the affinity of

membrane-associated proteins for the membrane and consequently their interaction with other multiprotein complexes involved with cell signaling systems (3). In addition, a variety of cell stressors (eg, inflammatory mediators) interact with transmembrane receptors and subsequently initiate intracellular G-protein-linked responses, one of which is the activation of phospholipase A₂. This enzyme hydrolyzes long-chain n-6 and n-3 FAs esterified to inner leaflet phospholipids, liberating them and making them available for conversion to a wide variety of eicosanoids via cyclooxygenase, lipoxygenase, and cytochrome P-450 monooxygenases (4). These molecules powerfully influence cellular metabolism. Phospholipase A₂-liberated n-3 FAs may directly modify the activity of the ion channel themselves, resulting in altered resting membrane potentials (5). Finally, intracellular n-3 FAs liberated from membranes are also able to serve as ligands for a variety of nuclear receptors [eg, peroxisome proliferation-activated receptors, sterol receptor element-binding protein-1c, retinoid X receptor, farnesol X receptor, and hepatocyte nuclear factor- α (6, 7)], which have an impact on inflammatory responses and lipid metabolism.

Presumably via an orchestrated set of membrane effects, n-3 FAs can diminish the activity of inflammatory cells and reduce concentrations of certain inflammatory mediators (4), which may ultimately result in reduced arterial plaque fragility (8). Alterations in the susceptibility of excitable membranes to inappropriate electrical patterns appear to be due to the effects of n-3 FAs released from membranes (5). The mild effects of these FAs on blood pressure (9) are likely to be the result of an improvement in arterial compliance (10) and endothelial function (11), possibly arising from enhanced nitric oxide availability (12). The decrease in serum triacylglycerol concentrations that is produced by intakes of 3-4 g/d EPA + DHA (13) appears to be due to increased hepatic β -oxidation and decreased lipogenesis (14), which themselves are the result of modulation of the nuclear receptor actions noted above. Although the precise mechanisms by which n-3 FAs reduce the risk for cardiac events are not known in detail, their presence in membranes and the coordinated downstream effects outlined here undoubtedly play a role.

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² Presented at the symposium "Beyond Cholesterol: Prevention and Treatment of Coronary Heart Disease with n-3 Fatty Acids," held in New York, NY, June 9, 2007.

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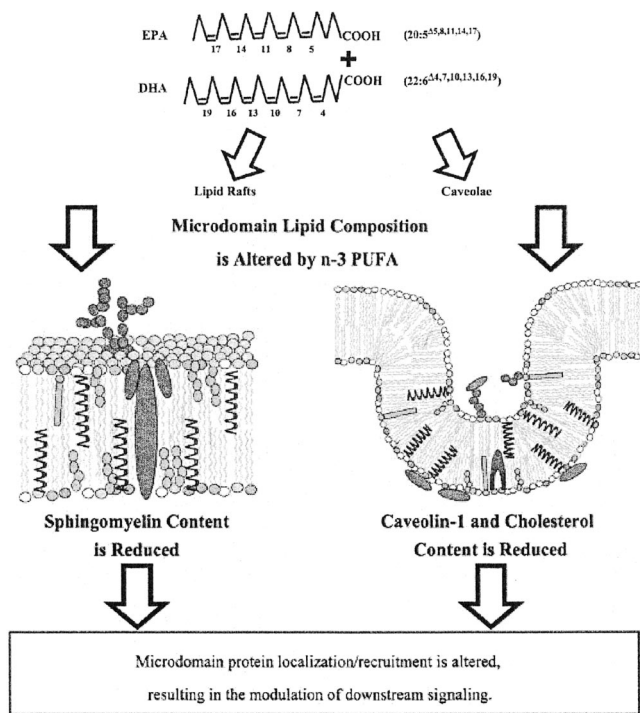


FIGURE 1. Modifications of membrane composition with increased eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) intake. When EPA and DHA become incorporated into membranes, they affect the properties of both lipid rafts (left) and caveolae (right), reducing the sphingomyelin content of the former and the cholesterol and caveolin content of the latter. These changes can influence the function and binding of membrane-associated proteins, ultimately altering cell function. PUFA, polyunsaturated fatty acids. From Ma et al (2).

n-3 EPIDEMIOLOGY

Primary compared with secondary prevention of coronary heart disease (CHD) with n-3 fatty acids

Although there are secondary prevention trials demonstrating CHD and total mortality benefits of n-3 FAs (15, 16), there are no primary prevention, prospective randomized controlled trials with n-3 FAs including clinical CHD endpoints, with the possible exception of the primary prevention subset of the Japan EPA Lipid Intervention Study (JELIS) (17). This was a prospective, open-label, randomized trial with masked endpoint evaluation. The study cohort consisted 18 645 hypercholesterolemic men and women who either did (20%) or did not (80%) have a history of CHD. All patients were given statins (10 mg pravastatin or 5 mg simvastatin) and then randomly assigned to a control or EPA group, with the latter receiving 1.8 g/d EPA ethyl esters (Epadel, Mochida, Japan). Patients were followed for a mean of 4.6 y for clinical endpoints. In the entire cohort, the major adverse coronary event rate was 19% lower in patients who received EPA (2.8% compared with 3.5%; $P = 0.011$). In the primary prevention subset, the event rate reduction was 18%, but it was not statistically significant ($P = 0.13$). The event rate reduction in the secondary prevention subset was 19% ($P = 0.048$). Because the effect sizes were virtually the same in both subsets, these findings suggest that EPA is as effective in reducing relative risk for CHD events in both primary and secondary prevention settings. The study was complicated, however, by

the very high background intake of both EPA and DHA, which is estimated to be between 800 and 1000 mg/d in the adult Japanese population (18). Thus, the application of these findings to Western populations consuming lower amounts of n-3 FAs is not clear. In addition, the authors did not measure the omega-3 index in this study (only serum concentrations of EPA), so the relations between the index and risk cannot be directly ascertained.

In the absence of prospective randomized intervention trials in primary prevention populations, we may turn to prospective observational cohort studies in which either dietary estimates of n-3 FA intake or, apropos to the omega-3 index, blood or blood cell membrane n-3 FA concentrations were measured. The former studies have been summarized by He et al (19), who performed a meta-analysis of 13 cohorts including >222 000 individuals followed for CHD death for an average of ≈ 12 y. They found that the consumption of only 1 fish meal/wk compared with <1/mo was associated with a statistically significant 15% reduction in risk. When subjects were classified into categories of increasing fish consumption (<1/mo, 1-3/mo, 1/wk, 2-4/wk, and ≥ 5 /wk), those in the highest intake group showed a 40% reduction in risk. An inverse relation between fish intake and risk for CHD has also been recently reported in Greek (20) and in Japanese cohorts (18). Relations between n-3 FA biomarkers and risk for CHD events was recently analyzed by Harris et al (21). We showed in a meta-analysis that across a wide variety of FA biomarkers (plasma, erythrocyte, adipose tissue, and others) DHA and DHA + EPA were inversely related to risk. A recent case-control study in which the omega-3 index was measured in patients admitted to the hospital with acute coronary syndromes showed that this biomarker was a significant predictor of case status, independent of multiple clinical covariates including plasma lipids and lipoproteins (22). Furthermore, across the 3 proposed omega-3 index risk categories, there was an approximate 70% reduction in odds for acute coronary syndrome status (Figure 2). Siscovick et al (23) were the first to show in a case-control setting that erythrocyte EPA + DHA was a powerful discriminator of cases experiencing primary cardiac arrest compared with controls (Figure 3). Long-chain n-3 FAs measured in whole blood samples collected at baseline in the Physicians' Health Study were compared between participants who ultimately experienced sudden cardiac death and matched control subjects who did not (24) (Figure 3). Here again, this biomarker of n-3 FA status [which correlates highly with the omega-3 index (25)] was shown to be an independent predictor of risk. Both of these studies were conducted in subjects who were free of known CHD at the time of blood sampling, and thus they constitute evidence that a higher omega-3 index is predictive of CHD risk in the primary prevention setting.

OMEGA-3 INDEX COMPARED WITH OTHER CHD RISK FACTORS

The relative predictive value for sudden death of blood n-3 FA concentrations compared with other more established CHD risk factors can be estimated by comparing 2 reports from the Physicians' Health Study. Albert et al (26) published the relative risk for sudden cardiac death across quartiles of C-reactive protein (CRP), total cholesterol, LDL cholesterol,

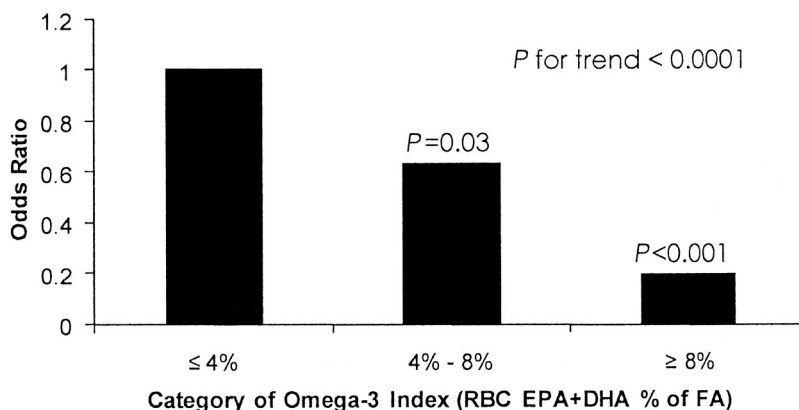


FIGURE 2. Odds ratios for acute coronary syndrome case status is inversely associated with proposed omega-3 index categories. Low (<math><4\%</math>), intermediate, and high (>math>8\%</math>) omega-3 index values are expressed as a percentage of total blood cell membrane fatty acids (FAs). Odds ratios were calculated in a multivariable logistic regression model including the following additional factors: age, race, sex, diabetes, hypertension, family history of coronary artery disease, history of myocardial infarction, and serum lipids (LDL, HDL, and log-triacylglycerols). Odds *P* values above columns reference the <math><4\%</math> category. Odds ratios were higher in the intermediate versus the high omega-3 index categories ($P = 0.012$). EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; RBC, erythrocyte. From Block et al (22).

HDL cholesterol, triacylglycerols, homocysteine, and the total cholesterol-to-HDL cholesterol ratio. In a second paper (24) they reported the relations between blood n-3 FA content and the same endpoint in the same study (Figure 4). Only 2 risk factors demonstrated statistically significant relations with risk for sudden cardiac death after controlling for age and smoking status: CRP and n-3 FAs. Unfortunately, neither of these was adjusted for the other nor was either adjusted for homocysteine or other lipid/lipoproteins classes. Thus, whether either of these add predictive value beyond the other is unclear. Nevertheless, blood n-3 FA content was related to risk in a dose-dependent manner, which was not the case for

CRP, which was more of a dichotomous predictor. In addition, the risk reduction at the highest levels of the omega-3 index (90%) was greater than that associated with the lowest concentrations of CRP (65%). Therefore, in the case of sudden cardiac death [which is responsible for about half of all CHD deaths (27)], the omega-3 index may be more informative than other currently accepted risk factors.

PROPOSED CUTOFFS FOR THE OMEGA-3 INDEX

In proposing that the omega-3 index may serve as a new risk marker for CHD mortality, von Schacky and I (25) estimated what a reasonable target “healthy” omega-3 index might be. We surveyed the literature to determine what estimated omega-3 index values were associated with the lowest risk for CHD mortality by either extrapolating from other n-3 FA biomarkers (eg, whole blood or plasma phospholipids) or, in studies that reported n-3 FA intakes only, by estimating how such intakes would affect the omega-3 index. Using this approach, we arrived at a proposed cardioprotective target concentration of 8% of erythrocyte FAs as EPA + DHA. It also appeared that an omega-3 index of $\leq 4\%$ was associated with the greatest risk for CHD death.

WHY ERYTHROCYTES?

Recommendations to use erythrocyte FA composition as a marker of dietary FA intake date back to 1963 (28). The phospholipids comprising the cell membranes become enriched with n-3 FAs not only during reticulocyte maturation in bone marrow but also via direct exchange in plasma (29). This enrichment appears to occur via transfer of DHA (and presumably also EPA)—containing lysophosphatidylcholine, which circulates associated with serum albumin (30), and these molecules are derived from the hydrolysis of n-3 FA-containing phospholipids in lipoproteins. Plasma exchange explains why erythrocytes can become enriched with n-3 FAs faster than would be predicted if they only appeared with new erythrocytes (31).

We recommend measuring the EPA + DHA content of erythrocyte membranes for several reasons (Table 1), not the least of

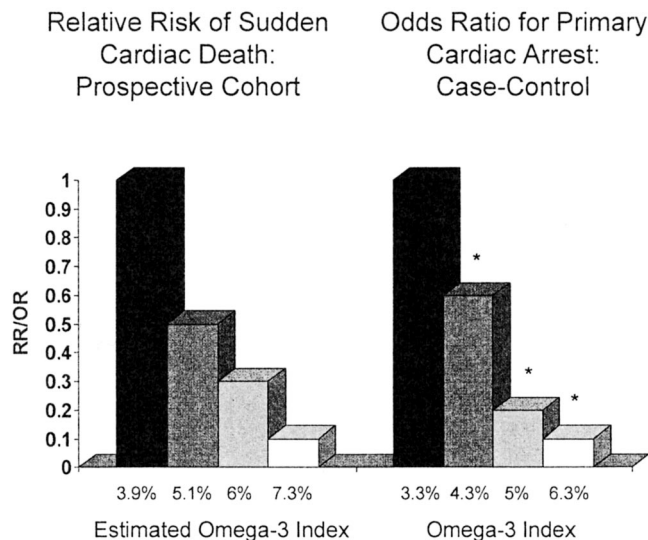


FIGURE 3. Relations between the omega-3 index and risk for primary cardiac arrest (left) and the estimated (below) omega-3 index and sudden cardiac death (right). The former data were derived from Siscovick et al (23) from a population-based case-control study, and the latter from Albert, et al (24) from a case-control study nested in the prospective Physicians’ Health Study. The omega-3 index was estimated from the latter data set from the whole-blood long-chain n-3 fatty acid content with use of equations described by Harris and von Schacky (25). * $P < 0.05$ compared with quartile 1. OR, odds ratio; RR, relative risk.

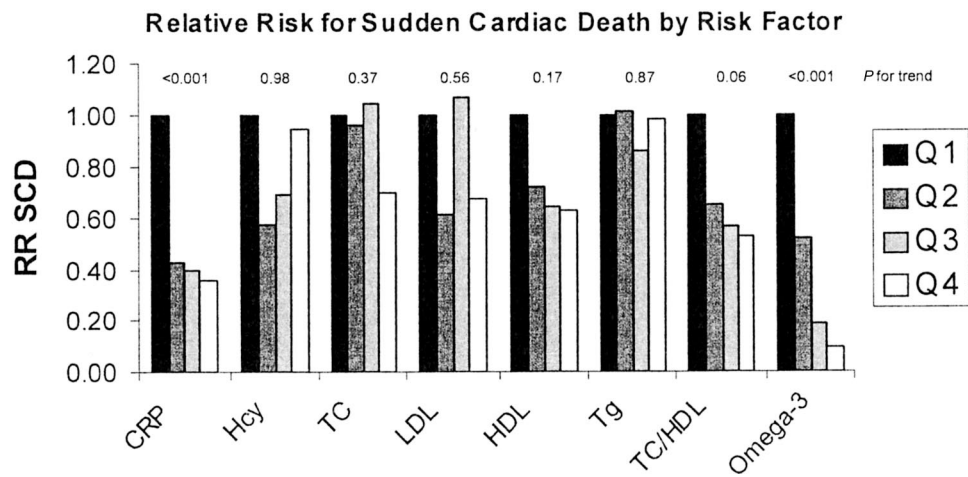


FIGURE 4. The multivariable-adjusted relative risk for sudden cardiac death (RR SCD) by quartile (Q) of blood n-3 fatty acids (FAs) compared with other, more traditional circulating risk factors. The quartiles at presumed highest risk (black bars) are set at a relative risk of 1.0. Each subsequent lighter bar represents the risk at each decreasing (or, for HDL and n-3 FAs, increasing) quartile. CRP, C-reactive protein; Hcy, homocysteine; TC, total cholesterol; Tg, triacylglycerols. Adapted from Albert et al (24, 26).

which is the demonstration that the omega-3 index is correlated with human cardiac membrane EPA + DHA levels ($r = 0.81$, $P < 0.0001$), and in cardiac transplant patients supplemented with n-3 FAs, both cardiac and erythrocyte EPA + DHA concentrations increase to the same extent (59). Depending on the dose, it takes 3–6 mo for a new steady-state omega-3 index to be established (60, 61). In addition, the EPA + DHA content of eryth-

rocyte membranes is highly correlated with other plasma-based measures of EPA + DHA content (**Figure 5**).

FUTURE RESEARCH NEEDS

There is a pressing need for standardization in FA analysis. At present, although gas chromatography is used for virtually all FA

TABLE 1

Omega-3 index (and other n-3 biomarkers) as potential risk markers

	Reference numbers
Consistency of epidemiological data for fish intake/omega-3 biomarkers and CHD risk	
Between populations	36–39
Within populations	23; 40
Prospective cohorts	24; 41–51
Case-control studies	22; 53; 54
Raising biomarker levels with fish or n-3 FAs reduces risk	15; 16
Strong association between biomarker and disease	24
Biomarker independent of other known risk factors	23; 24; 49
Adds discriminative value to currently-available risk markers	22
Biologically plausible relation between biomarker and risk	5
Biomarker is modifiable (safely, quickly, and cheaply)	33; 57
Omega-3 index is highly correlated with intake of oily fish and fish oil supplements	58; 25
Other advantages of RBC for n-3 FA analysis	
Standardized methodology - (In development in our laboratory)	
Low biological variability	31
High analytical reproducibility, CV = 4%–7%	
Pre-analytical stability; 7-d at room temperature, unpublished observation; can be mailed to lab unrefrigerated	59
Unaffected by fasting/fed state	Unpublished observation
RBC FA composition stable for at least 4 years frozen at -80°C	59; 60
RBC FA composition is less influenced by day-to-day variations and by dyslipidemias than are plasma FAs [(coefficient of variation for plasma phospholipid EPA+DHA = 32% vs 22% for RBC EPA+DHA (N=207; unpublished observation)]	61
Better reflection of long-term dietary n-3 FA intake than plasma	33; 62
The RBC is a readily-available byproduct of usual phlebotomy that, if shown to be useful, can be obtained without subjecting the patient to additional procedures	
The half-life of RBC EPA+DHA is 4–6 times longer than that of serum EPA+DHA	31
RBC EPA+DHA is highly correlated with other n-3 biomarkers such as whole serum, serum phospholipids, and whole blood (which can be measured in a dried blood spot; Figure 5)	35

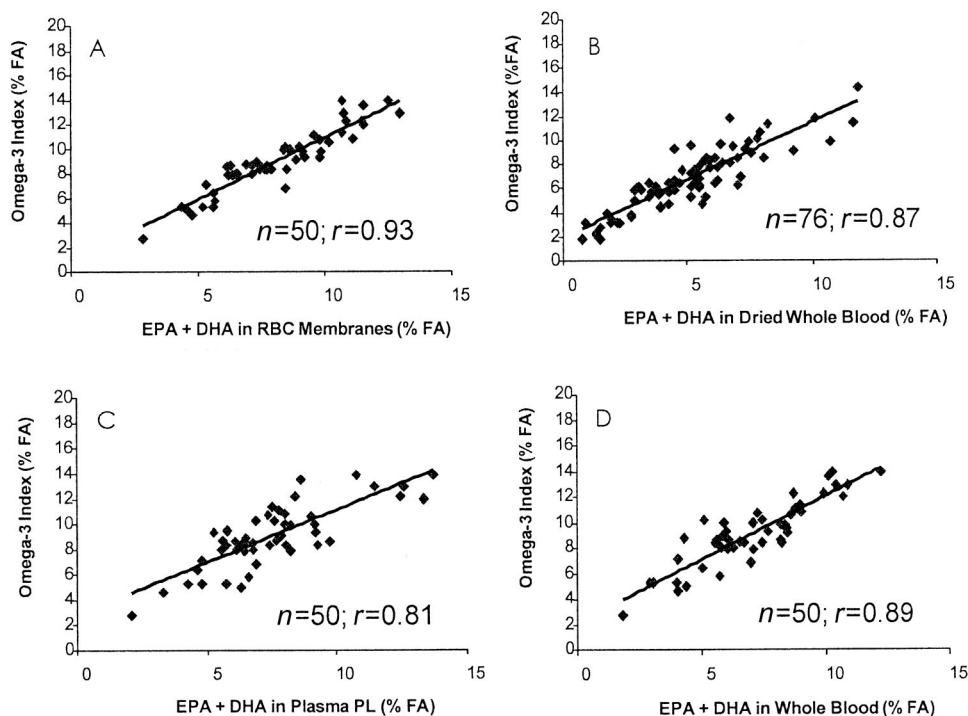


FIGURE 5. Correlations between the omega-3 index and (A) blood cell membranes isolated by ultracentrifugation from frozen whole blood [as used in (22)], (B) dried whole blood on filter paper (35), (C) plasma phospholipids, and (D) liquid whole blood. Sample size and correlation coefficients are given in each graph (WS Harris, unpublished observations). EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; RBC, erythrocyte; PL, phospholipids; FA, fatty acid.

analyses, there are a myriad of pre- and postanalytic variables that can all affect the outcome. Some of these factors include the choice of methylation reagent and time, extraction solvents, direct compared with postlipid extraction methylation, gas chromatography columns and program conditions, carrier gases, and post-run control for response factors. There is also a need for standardized quality-control materials that multiple laboratories can use to harmonize results. The use of dried blood spots on specially prepared filter papers (62) may facilitate the development of such interlaboratory assessment systems. Obviously, if the results of studies from various laboratories are to be compared (eg, to identify cardioprotective target n-3 FA biomarker concentrations), it will be essential to have standardized methods. Furthermore, if the analysis of blood n-3 FAs is ever to enter clinical practice, uniformity and standardization must be established. The day will probably come when a test such as the omega-3 index will be routinely included in lipid panels so that the clinician can address not only cholesterol, triacylglycerol, and lipoprotein abnormalities but also deficiencies in n-3 FAs. Opportunities to make a significant impact on not only CHD risk but also risk for cognitive decline, neuropsychiatric disorders, macular degeneration, inflammatory diseases, and other disorders await the development of harmonized methods of assessing n-3 FA status.

The author is a consultant to GlaxoSmithKline and Monsanto.

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