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Omega-3 Fatty Acids and Cardiovascular Disease: A Case for Omega-3 Index as a New Risk Factor

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Abstract

The omega-3 fatty acids (FAs) found in fish and fish oils (eicosapentaenoic and docosahexaenoic acids, EPA and DHA) have been reported to have a variety of beneficial effects in cardiovascular diseases. Ecological and prospective cohort studies as well as randomized, controlled trials have supported the view that the effects of these FAs are clinically-relevant. They operate via several mechanisms, all beginning with the incorporation of EPA and DHA into cell membranes. From here, these omega-3 FA alter membrane physical characteristics and the activity of membrane-bound proteins, and once released by intracellular phospholipases, 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. In as much as blood levels are a strong reflection of dietary intake, it is proposed that an omega-3 FA biomarker, the omega-3 index (erythrocyte EPA+DHA) be considered at least a marker, if not a risk factor, for coronary heart disease, especially sudden cardiac death. The omega-3 index fulfils many of the requirements for a risk factor including consistent epidemiological evidence, a plausible mechanism of action, a reproducible assay, independence from classical risk factors, modifiability, and most importantly, the demonstration that raising tissue levels will reduce risk for cardiac events. For these and a number of other reasons, the omega-3 index compares very favourably with other risk factors for sudden cardiac death.

Keywords

Eicosapentaenoic acid; docosahexaenoic acid; biomarkers; risk factors; cardiovascular disease; coronary heart disease; sudden cardiac death; fish oils

Introduction

The American Heart Association [1], the European Society for Cardiology [2], the Scientific Advisory Committee on Nutrition (UK) [3], the Australian Health and Medical Research Council [4] and a host of other health agencies and professional organizations have issued recommendations for increased intakes of omega-3 fatty acids (FAs). These recommendations are based on strong evidence derived from a variety of scientific approaches linking dietary deficiency of long chain omega-3 FAs with risk for cardiovascular events, notably sudden

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death. These have been recounted in detail in several recent publications [1,5-11]. The primary purpose of this paper is to make a case for the use of a biomarker for omega-3 FA intake, “the omega-3 index,” in coronary heart disease (CHD) risk stratification. In so doing, the results of omega-3 epidemiological and interventional studies, and their apparent mechanisms of action will be briefly reviewed.

Omega-3 epidemiology

In order to summarize the fish/omega-3 ecological studies, He *et al.* [12] performed a meta-analysis of 13 cohorts including over 222,000 individuals followed for CHD death for an average of about 12 years. They found that the consumption of only one fish meal per week (vs. <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 enjoyed a 40% reduction in risk. Similar findings were reported for stroke [13].

An inverse relation between fish intake and risk for CHD has also been recently reported in Greek [14] and in Japanese cohorts [15]. The latter study examined the association between fish (and omega-3 FA) intake and various CHD endpoints in 41,578 Japanese men and women age 50-70 over a 10 year follow-up period [15]. The lowest quintile of intake was about 300 mg/d, which is about twice the median intake in the US [16]. At the high end, intakes averaged 2.4 g/d. The median intake in this cohort was about 900 mg/d, about 6-fold higher than US intakes. Across this intake gradient there was a significant reduction in risk for non-fatal coronary events and total myocardial infarctions (Figure 1). Hence, the intake of omega-3 FA at which benefits plateau is not yet defined.

Omega-3 interventions

There have been several intervention studies of varying quality (see references [7,11]). The largest and most well-controlled was the GISSI Prevenzione study which tested the hypothesis that relatively small intakes of omega-3 FA (<1 g) could reduce risk for death from CHD in high risk patients. Over 11,000 post-myocardial infarction patients were randomized to either one capsule of omega-3 FA ethyl esters (Omacor, Pronova Biocare, Norway; 850 mg of EPA +DHA) or usual care and then followed them for 3.5 years. In this study, the risk for death from any cause was reduced by 20% and risk for sudden death by 45% in the supplement group [17].

To bring a perspective to the beneficial effects of omega-3 FA, Studer *et al.* [18] computed the relative reduction in risk for death from any cause in trials of anti-lipidemic drugs and lipid-lowering diets. These regimens are obviously prescribed not just to reduce serum lipid levels, but ultimately to reduce risk for death, typically from CHD. Over 137,000 patients receiving treatment for lipid disorders were compared to controls in a total of 97 studies. There were 35 trials with statins, 7 studies with fibrates, 8 with bile acid binding resins, 14 with omega-3 FA and 18 examining the effects of global dietary changes. Only two interventions were associated with significant reductions in total mortality: statins (risk ratio 0.87, 95% CI 0.81-0.94) and omega-3 FA (risk ratio 0.77, 95% CI 0.63-0.94). Although a fascinating analysis that underscores the fact that risk for serious adverse health outcomes can be reduced even without lowering cholesterol, one could argue that two studies included with the omega-3 group were not strictly omega-3 studies but overall dietary interventions. In these two studies [19,20], the active agent(s) cannot be identified with confidence because so many dietary variables differed between groups. Whether removing these studies from the omega-3 group would leave the latter still associated with reduced risk for death is not clear. Nevertheless, the preponderance of the data suggests that for most individuals, increasing the intake of long-chain omega-3 FA

is a safe and inexpensive way to significantly reduce risk for CHD, especially sudden cardiac death.

Omega-3 mechanisms

The fundamental mechanism by which omega-3 FA appear to mitigate risk for CHD begins with the enrichment of membrane phospholipids with EPA and DHA. Once these long chain n-3 FA are resident in cell membranes, they may have at least four separate effects. The relative importance of each, their coordinated interaction and their sufficiency to explain the clinical observations have yet to be determined.

First, because of their highly unsaturated nature, they may alter membrane properties [21]. This can have the secondary effect of changing the microenvironment of transmembrane proteins (e.g., receptors) altering the manner in which they interact with their ligands [22]. Altering membrane FA composition can also affect the ability of membrane-associated proteins to actually associate with the membrane and consequently to interact with other multi-protein complexes involved with cell signaling systems [23]. In addition, a variety of cell stressors (e.g., inflammatory mediators) interact with transmembrane receptors and subsequently initiate intracellular G-protein linked responses, one of which is the activation of phospholipase A₂ (PLA₂). This enzyme hydrolyzes long-chain omega-6 and omega-3 FA esterified to inner leaflet phospholipids, liberating them and making them available for conversion to a wide variety of eicosanoids via cyclo-oxygenase, lipoxygenase, and cytochrome P-450 mono-oxygenases [24]. These molecules powerfully influence cellular metabolism. PLA₂-liberated omega-3 FA may directly modify the activity of ion channel themselves, resulting in altered resting membrane potentials [25]. Finally, intracellular omega-3 FA are also able to serve as ligands for a variety of nuclear receptors [e.g., peroxisome proliferation activated receptors (PPARs), sterol receptor element binding protein (SREBP)-1c, retinoid X receptor, farnesol X receptor, and hepatocyte nuclear factor- α [26,27]] which impact inflammatory responses and lipid metabolism.

Presumably as a consequence of these membrane effects, omega-3 FA can diminish the activity of inflammatory cells and reduce levels of certain inflammatory mediators [24], which may ultimately result in reduced arterial plaque fragility [28]. Mild effects on blood pressure [29] are likely to be the result of an improvement in endothelial function [30]. The decrease in serum triglycerides that is produced by intakes of 3-4 g/d of EPA+DHA [31] appears to be secondary to increased hepatic beta-oxidation and decreased lipogenesis [32], which themselves are the result of modulation of the nuclear receptor actions noted above. Although the precise mechanisms by which omega-3 FA reduce risk for cardiac events are not known in detail, their presence in membranes and the coordinated downstream effects outlined here undoubtedly play a role.

Omega-3 biomarkers

Another approach to linking omega-3 FA (and not just fish) with CHD risk has been to examine the relationships between omega-3 biomarkers and risk. Two studies are particularly relevant in this regard. Siscovick *et al.* [33] obtained blood samples from 80 adults experiencing primary cardiac arrest in the Seattle area and from 108 healthy matched controls. The cases did not have known CHD at the time of their events. The percent of total FA in red blood cell (RBC) membranes present as EPA+DHA was determined in these samples and related to the odds of being in the primary cardiac arrest group. Albert *et al.* [34] conducted a prospective case control study within the Physicians' Health Study. Healthy male physicians (n=14,916) were recruited and provided baseline blood samples between 1982 and 1984. Over the next 17 years, 94 men experienced sudden cardiac death. Whole blood long-chain omega-3 levels in the cases were compared to those of 184 matched controls. In both this and the Siscovick study, multivariable-

adjusted odds for sudden cardiac death were reduced by 90% in those subjects with the highest blood omega-3 levels compared with those with the lowest levels (Figure 2). These studies both concluded that increased omega-3 FA intakes (as reflected by higher biomarker levels) were associated with a significant reduction in risk for sudden cardiac death. However, neither study directly recommended that blood omega-3 FA could be used as a risk factor.

Moving beyond sudden cardiac death, we recently tested the hypothesis that lower blood n-3 FA are associated with increased risk for acute coronary syndrome (ACSs). We analyzed the FA composition of whole blood from 94 ACS patients and 94 age- gender- and race-matched controls [35]. Omega-3 FA associations with ACS were assessed using multivariable models adjusting for smoking status, alcohol use, diabetes, body mass index, serum lipids, and history of myocardial infarction or revascularization. Whole EPA+DHA content was 29% lower in cases than controls (1.7 ± 0.9 vs. $2.4\pm 1.4\%$, $p<0.001$). Thus, low blood EPA+DHA content was an independent predictor of increased risk for ACS.

The omega-3 index

Clemens von Schacky and I recently proposed that the “omega-3 index” (EPA+DHA as a percent of total RBC FA) be considered a new risk factor for death from CHD [36]. We also suggested preliminary targets or cut-points to segregate those at low, intermediate and high risk based on a combination of a survey of the literature and correlations between intakes and omega-3 index levels determined experimentally. We estimated that a cardioprotective target level for the omega-3 index appeared to be about 8%, and the level associated with the increased risk for CHD death was <4% (Figure 3). These proposed cutpoints have yet to be prospectively tested, however. We recommended measuring the EPA+DHA content of RBC membranes (as opposed to whole plasma or plasma phospholipids) for the reasons outlined in Table 1.

From biomarker to risk factor

There are several requirements that a putative risk factor/marker must meet in order to be clinically useful [37] (Table 1). The omega-3 index, even at this early stage of development, appears to fulfill many. The epidemiological data, both between and within populations, from prospective cohort and case-control studies are strong. A relationship between membrane EPA +DHA levels and risk for sudden cardiac death is biologically plausible. Currently, the most likely mechanism by which they appear to operate is via a reduction in myocardial susceptibility to lethal arrhythmias [25,38]. In addition, EPA+DHA may enhance plaque stability [28], and may be anti-atherosclerotic [39,40] via a variety of other mechanisms [1]. The omega-3 index is modifiable by increasing the dietary levels of omega-3 FA, and it has been shown to correlate with human myocardial omega-3 FA content [41]. The omega-3 index also appears to be independent of other known risk factors. In the ACS case-control study described above, the omega-3 index was associated with ACS case status even after taking serum lipids, age, sex, and a history of CHD into account. The same applies to the studies by Siscovick *et al.* [33] and Albert *et al.* [34]. Future studies are still needed to explore the question of independence more thoroughly. Finally, perhaps the most important question to be asked of a putative risk factor is whether changing it alters disease outcomes. The intervention trials described above indicate that EPA+DHA may fulfill this critical criterion as well.

Omega-3 index compared with other CHD risk factors

Using data from the Physicians' Health Study, Albert *et al.* [34,42] published the relative risk for sudden cardiac death across quartiles of several risk factors (Figure 4). Only two risk factors demonstrated statistically significant trends after controlling for age and smoking status: C-reactive protein and blood omega-3 FA content (which is highly correlated with the omega-3 index). 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 adds predictive value beyond the other cannot be deduced from this analysis. Nevertheless, the omega-3 index was clearly related to risk in a dose dependent manner which was not the case for CRP. 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%). Thus, for the case of sudden cardiac death (which is responsible for about half of all CHD deaths [43] and is the first- and last- symptom of CHD in about 1/3rd of cases [44]), the omega-3 index may be more informative than any other known risk factor.

Conclusion

The potential for the omega-3 index (or any validated marker of tissue omega-3 FA status) to add clinically-important prognostic information regarding risk for death from CHD is significant. The omega-3 index has been validated as a surrogate for myocardial omega-3 FA composition in the human and as such reflects the omega-3 status of the most critical organ. It can be used to both assess baseline omega-3 status and to check for compliance with recommendations to increase omega-3 intake. Altering the omega-3 index is simple, safe and inexpensive and has been shown in randomized trials to reduce risk for CHD death. The widespread clinical implementation of the omega-3 index will allow clinicians to detect omega-3 “insufficiency”, to better stratify patients with respect to risk for SCD, and could ultimately contribute to a reduced burden of CHD.

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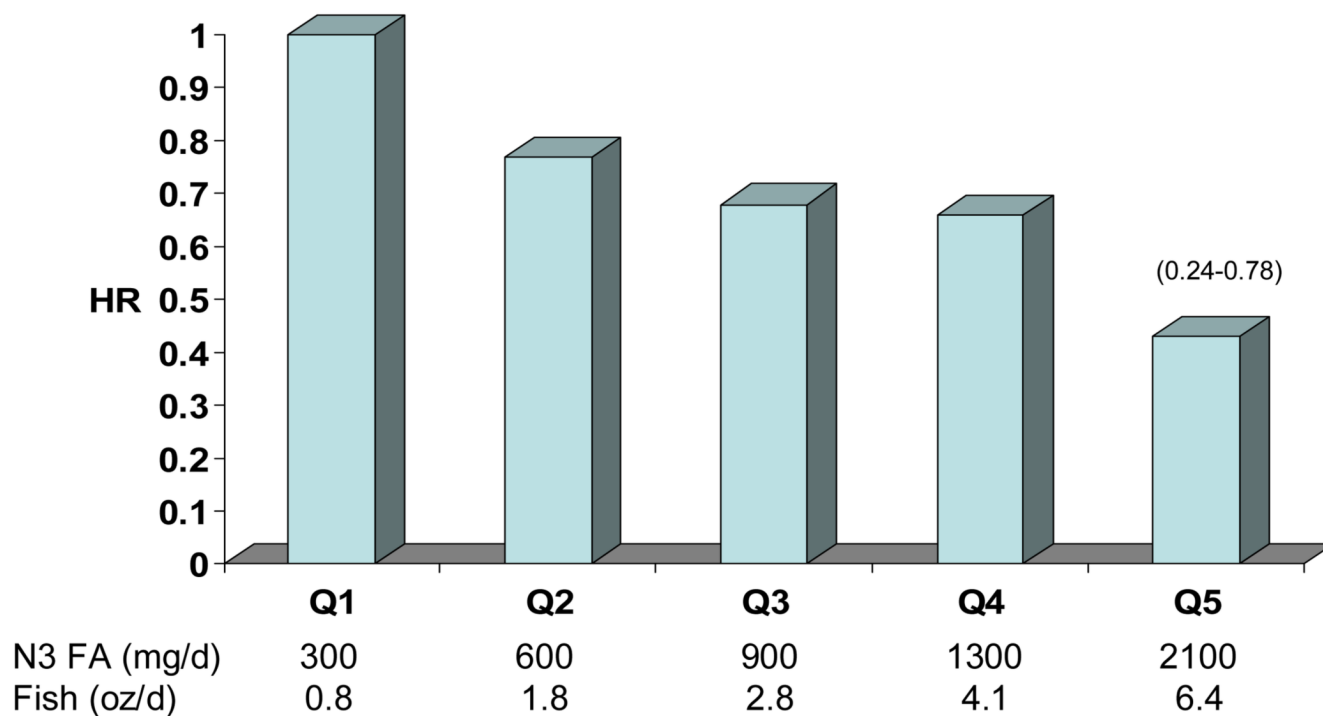


Fig 1.

Risk for MI by median omega-3 FA intake estimated from reported fish intake in the Japan Public Health Center-Based Study Cohort I. 41,578 subjects; 40-59 yrs; 10 yr f/u. Age, sex, smoking, alcohol, BMI, Hx HTN and diabetes, drug use for elevated cholesterol, educational level, sports in leisure time, and quintiles of dietary fruits, vegetables, sat fat, mono fat, n-6 fat, cholesterol and kcal. P for trend =0.02. Adapted from Iso *et al.* [15].

Relative Risk of Sudden Cardiac Death: Prospective Cohort

Odds Ratio for Primary Cardiac Arrest: Case-Control

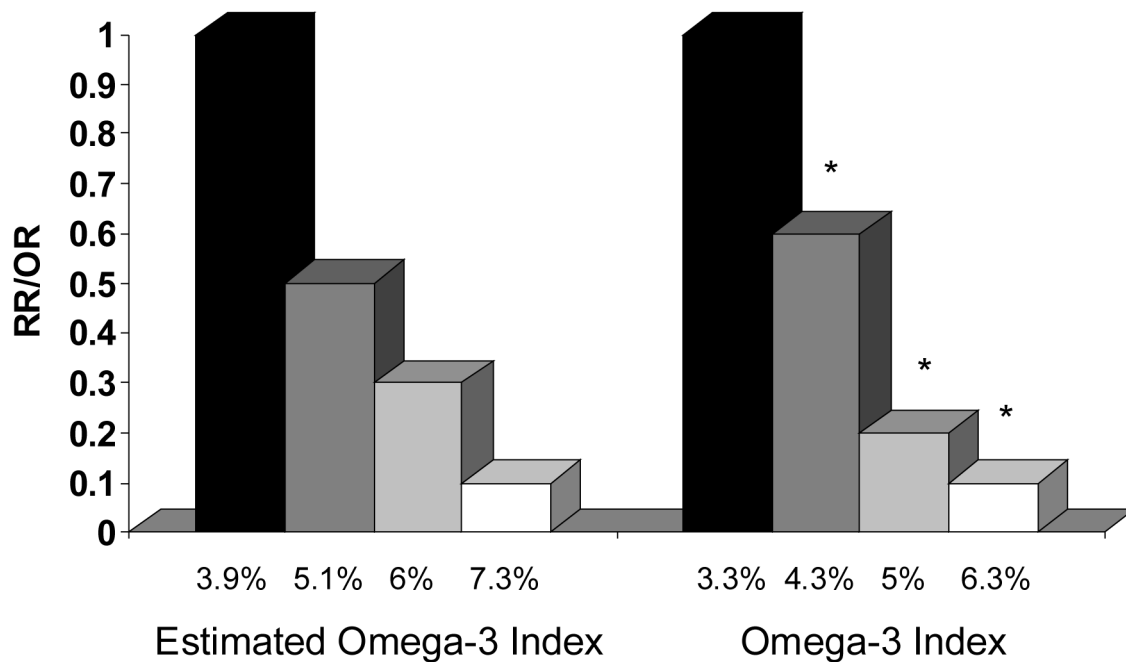


Fig 2.

The relationship between RBC EPA+DHA content and risk for primary cardiac arrest (right) or sudden cardiac death (left). The former data were derived from Siscovick *et al.* [33] from a population-based case control study, and the latter from Albert *et al.* [34] from a case-control study nested in the prospective Physicians' Health Study. The omega-3 index was estimated from the latter data set (which measured whole blood long-chain omega-3 FA content) using equations described by Harris and von Schacky [36].

Proposed Risk Zones for the Omega-3 Index

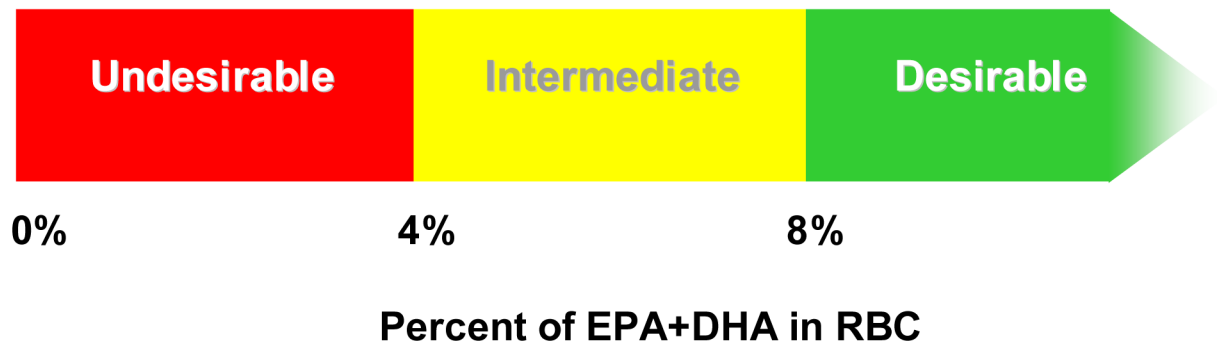


Fig 3. Proposed risk zones for the omega-3 index (RBC EPA+DHA). Proposed cutpoints were estimated from a review of the literature [36].

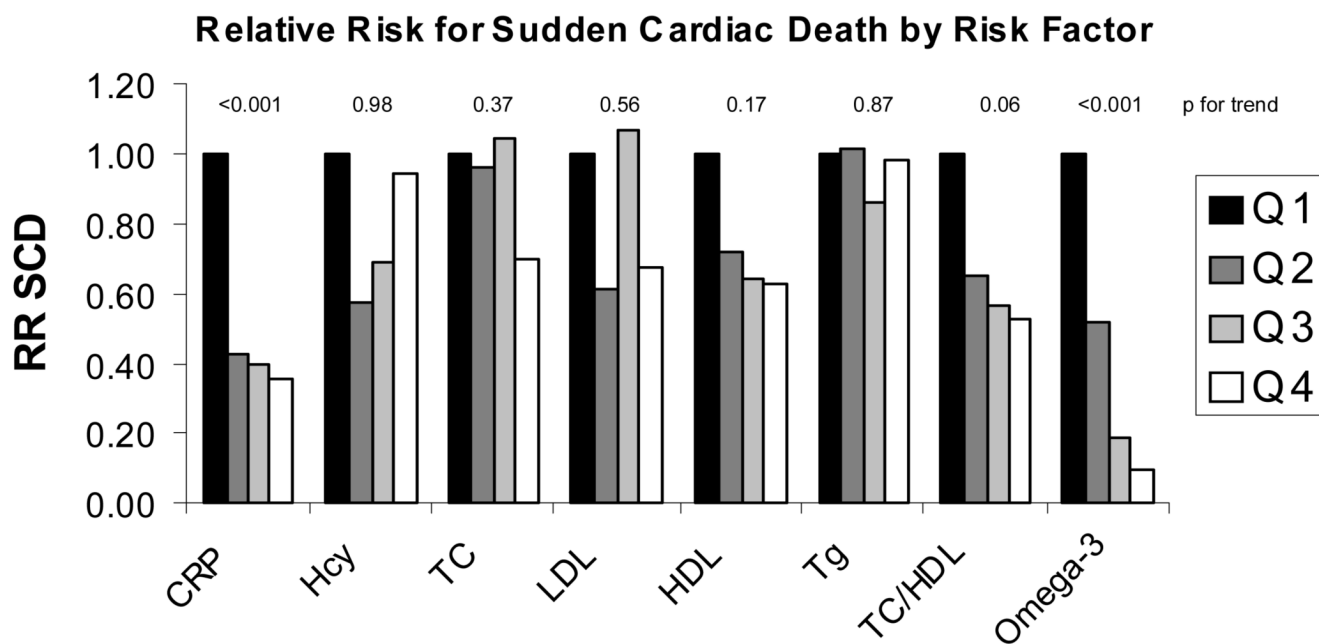


Fig 4.

The multivariable-adjusted relative risk for sudden cardiac death (RR SCD) by quartile of blood omega-3 FA compared to other, more traditional blood-borne risk factors. The quartiles at highest risk (black bars) are set at a relative risk of 1.0 for the presumed highest-risk levels of each marker. Each subsequent lighter bar represents the risk at each decreasing (or, for HDL and omega-3 FA, increasing) quartile. Adapted from the Physicians' Health Study, Albert *et al.* [34,42]. CRP=C reactive protein; Hcy=homocysteine; TC=total cholesterol; LDL=low density lipoprotein cholesterol; HDL=high density lipoprotein cholesterol; Tg=triglycerides.

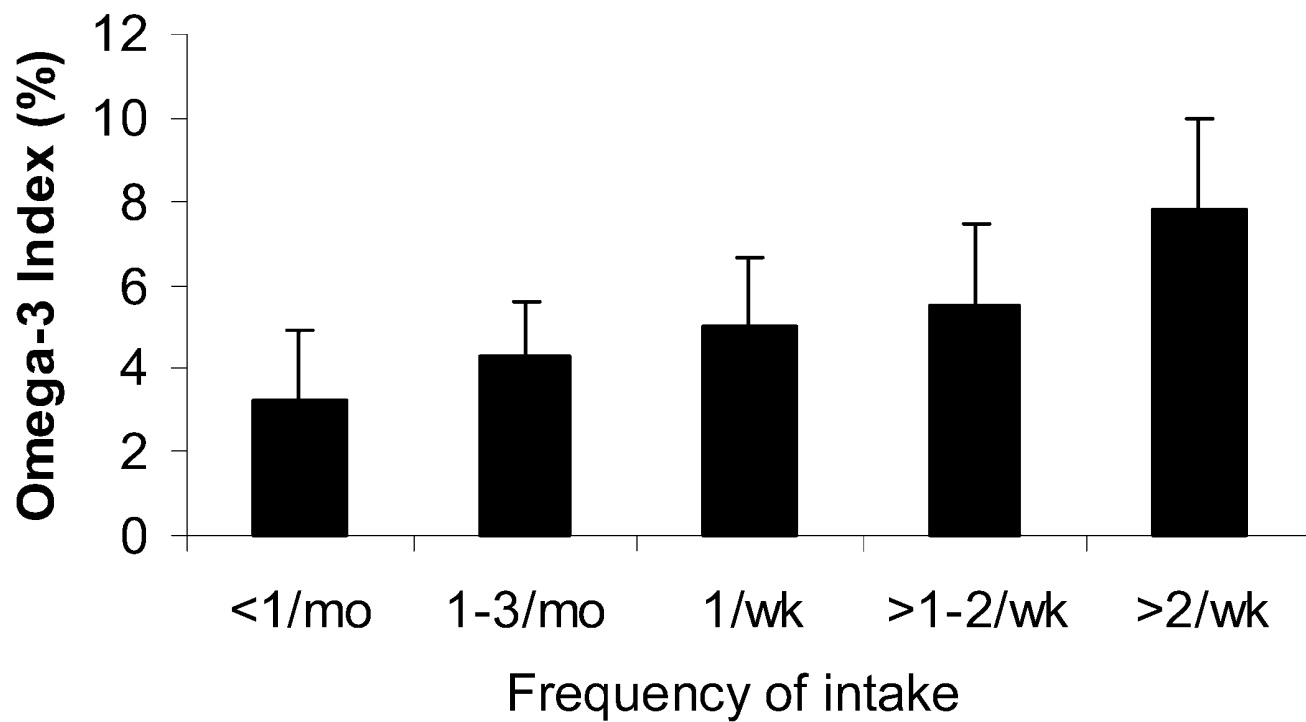


Fig 5. RBC EPA+DHA by category of reported consumption of tuna or non-fried fish per month. Groups differed by ANOVA ($p < 0.0001$), with the highest RBC EPA+DHA group differing significantly from all others ($p < 0.05$), and the second highest differing from the first ($p < 0.05$) by Tukey's post-hoc test. Reprinted from Sands *et al.* [45] with permission.

Table 1

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

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- Consistency of epidemiological data for fish intake/omega-3 biomarkers and CHD risk
 - between populations – Yes [46-49]
 - within populations – Yes [33,50];
 - prospective cohorts – Yes [34,51-61]; No [62]
 - case-control studies – Yes [63,64]; No [62,65]
 - Raising biomarker levels with fish or omega-3 FAs reduces risk – Yes [66,67]; No [68]
 - Strong association between biomarker and disease – Yes [34]
 - Biomarker independent of other known risk factors – Yes [33,34,59]
 - Adds predictive value to currently-available risk markers - Unknown
 - Biological plausibility for elevated biomarker levels – Yes [25]
 - Biomarker is modifiable by diet – Yes [39] (Figure 5)
 - Omega-3 index is highly correlated with intake of oily fish [69] and fish oil supplements [36]
 - Advantages of RBC
 - Standardized methodology –Yes
 - Low biological variability – Yes [70]
 - High analytical reproducibility – Yes (CV=4%-7%)
 - Pre-analytical stability – Yes (7 days at room temperature; unpublished observation)
 - Measurable in fasting or fed samples (unpublished observation)
 - RBC FA composition stable for at least 4 years frozen at -80°C [71,72]
 - RBC FA composition is less influenced by day-to-day variations and by dyslipidemias than are plasma FAs [73] [(coefficient of variation for plasma phospholipid EPA+DHA = 32% vs 22% for RBC EPA+DHA (N=207; unpublished observation)]
 - 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 [70]
 - RBC EPA+DHA is highly correlated with other omega-3 biomarkers such as whole serum, serum phospholipids, and whole blood (which can be measured in a dried blood spot [74])
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