ELSEVIER

The Omega-3 Index: a new risk factor for death from coronary heart disease?[☆,☆☆]

William S. Harris, Ph.D.[a,*] and Clemens von Schacky, M.D.[b]

[a] Lipid and Diabetes Research Center, Mid America Heart Institute of Saint Luke's Hospital, University of Missouri-KC School of Medicine, Kansas City, MO 64111, USA
[b] Medizinische Klinik and Poliklinik Innenstadt, University of Munich, Munich, Germany

Available online 2 April 2004

Abstract

Background. Low intakes or blood levels of eicosapentaenoic and docosahexaenoic acids (EPA + DHA) are independently associated with increased risk of death from coronary heart disease (CHD). In randomized secondary prevention trials, fish or fish oil have been demonstrated to reduce total and CHD mortality at intakes of about 1 g/day. Red blood cell (RBC) fatty acid (FA) composition reflects long-term intake of EPA + DHA. We propose that the RBC EPA + DHA (hereafter called the Omega-3 Index) be considered a new risk factor for death from CHD.

Methods. We conducted clinical and laboratory experiments to generate data necessary for the validation of the Omega-3 Index as a CHD risk predictor. The relationship between this putative marker and risk for CHD death, especially sudden cardiac death (SCD), was then evaluated in several published primary and secondary prevention studies.

Results. The Omega-3 Index was inversely associated with risk for CHD mortality. An Omega-3 Index of $\geq 8\%$ was associated with the greatest cardioprotection, whereas an index of $\leq 4\%$ was associated with the least.

Conclusion. The Omega-3 Index may represent a novel, physiologically relevant, easily modified, independent, and graded risk factor for death from CHD that could have significant clinical utility.
© 2004 The Institute for Cancer Prevention and Elsevier Inc. All rights reserved.

Keywords: Omega-3 fatty acids; Eicosapentaenoic acid; Docosahexaenoic acid; Coronary heart disease; Sudden cardiac death; Risk factors

Introduction

Coronary heart disease (CHD) claims roughly 500,000 deaths/year in the USA. Of these, about 50% are sudden cardiac deaths (SCDs) [1]. Recent evidence from secondary prevention trials suggests that supplementation with 850 mg/day of omega-3 fatty acids (FA), that is, eicosapentaenoic acid (EPA; C20:5 ω-3) and docosahexaenoic acid (DHA: C22:6 ω-3), can reduce the risk of CHD death by 25% and SCD by about 45% [2]. The American Heart Association (AHA) now recommends about 1.0 g/day of EPA + DHA to reduce risk for death from CHD in the secondary prevention setting [3]. In addition, for individuals without known disease (primary prevention), the AHA recommends the consumption of at least two, preferably oily, fish meals per week. This amount of fatty fish would provide about 500 mg of EPA + DHA per day. Because red blood cell (RBC) membranes reflect cardiac membrane omega-3 FA content, we propose that the content of EPA + DHA in RBC membranes (expressed as a percent of total FA) be considered a new risk factor for death from CHD and especially SCD. This biomarker is hereafter called the "Omega-3 Index". The goals of this study were (1) to explore the potential utility of the Omega-3 Index as a risk factor for CHD mortality and (2) to define ranges for the Omega-3 Index corresponding to high, medium, or low

risk. Identification of a cardioprotective Omega-3 Index could provide physicians with a new therapeutic target with the ultimate potential of reducing risk for death from CHD.

The following steps were taken to achieve these goals:

1. We conducted a randomized prospective, double-blind, dose-response study of moderate EPA + DHA supplementation to validate the Omega-3 Index as a marker of EPA + DHA intake.
2. We determined correlations between the Omega-3 Index and other biomarkers of omega-3 FA intake that had been used in prospective cohort studies examining the relationship between omega-3 FA and risk for CHD death.
3. We estimated the average Omega-3 Index associated with the lowest and highest risk for death from CHD in a variety of previous epidemiological studies and randomized controlled trials.

Methods

Dose-response study to assess the effects of moderate intake of EPA + DHA on the Omega-3 Index

To translate reported intakes of EPA + DHA into the Omega-3 Index, we conducted a randomized, prospective, double-blind trial to determine the effects of relatively small intakes of omega-3 FA on the Omega-3 Index. Healthy adults on a stable background diet (taking no drugs known to affect lipid metabolism, digestion, or absorption; with serum triglycerides between 100 and 300 mg/dL, LDL-cholesterol <130 mg/dL and HDL-cholesterol >40 mg/dL) were enrolled. They were excluded if the previous month's intake of oily fish (e.g., salmon, sardines, albacore tuna, and mackerel) exceeded one serving. Subjects provided written informed consent for this study after its approval by the Saint Luke's Hospital Institutional Review Board.

Subjects were randomized to 0 (placebo), 0.5, 1.0, and 2.0 g of EPA + DHA per day for 5 months following a 1-month placebo run-in period. They were given bottles containing 1-g capsules and instructed to take seven per day for the entire 6-month study. The placebo capsules contained corn oil, and the EPA + DHA capsules contained ROPUFA '30' omega-3 Food Oil (Roche Vitamins, Parsippany, NJ). ROPUFA was given full-strength (2-g group), pre-blended with corn oil 50:50 (1-g group), or 25:75 (0.5-g group). The ROPUFA oil contained 11% EPA and 18% DHA. Subjects were instructed to completely avoid consumption of oily fish for the duration of the study but otherwise to make no changes in their diets. Compliance was assessed by capsule count. The 5-month treatment period was sufficiently long to allow RBC FA composition to stabilize [4].

Correlations between other blood omega-3 FA biomarkers and the Omega-3 Index

To link the Omega-3 Index with risk in several published studies, we compared plasma phospholipid EPA + DHA and whole blood long-chain omega-3 FA (EPA + DHA plus docosapentaenoic acid, DPA, C22:5) to the Omega-3 Index in a random set of 65 (phospholipid study) and 38 (whole blood study) fasting blood samples using methods described below. The equations describing these relationships were then applied to the whole blood values reported by Albert et al. [5] and plasma phospholipid data from Lemaitre et al. [6].

Laboratory methods

Blood was drawn into EDTA tubes and centrifuged at $4°C$ to separate cells from plasma. The plasma and buffy coat were removed, and RBCs were washed three times with cold saline and frozen in distilled water (50:50 v/v) at $-70°C$ until analyzed for FA composition by gas chromatography [7]. Whole blood FA composition was determined as described for RBC. The analytical coefficient of variation for the Omega-3 Index was $10–12%$. Plasma phospholipid FAs were analyzed following separation by thin layer chromatography as described previously [8].

Statistical methods

The statistical package from Excel 97 (Microsoft Corporation) was used to calculate correlation coefficients and regression equations (for conversion of other biomarkers into the Omega-3 Index), and to perform paired t tests and one-way ANOVAs for the dose-response study. Differences with p values of <0.05 were considered statistically significant.

Results

Dose response study

The clinical characteristics of the 57 subjects in this study were as follows: mean age, $45 ± 15$; 70% female; baseline lipids (mg/dL): cholesterol, $206 ± 36$; triglycerides, $156 ± 72$; HDL-cholesterol, $47 ± 15$; LDL-cholesterol, $128 ± 30$. There were no differences among the four treatment groups at baseline. The effects of the 0–2 g of omega-3 FA on the Omega-3 Index are illustrated in Fig. 1. For the placebo group ($n = 22$), levels decreased slightly but significantly ($P < 0.001$), possibly because all subjects were instructed to avoid oily fish all together. The index rose from $4.7 ± 0.9%$ to $7.9 ± 1.7%$ in the 0.5 g/day group ($n = 22$; $P < 0.001$), to $9.9 ± 2.9%$ with 1 g/day ($n = 9$; $P < 0.001$), and $11.6 ± 2.4%$ with 2 g/day ($n = 4$; $P = 0.02$). The changes from baseline among groups were significantly different for all but the 1-g vs. 2-g groups.
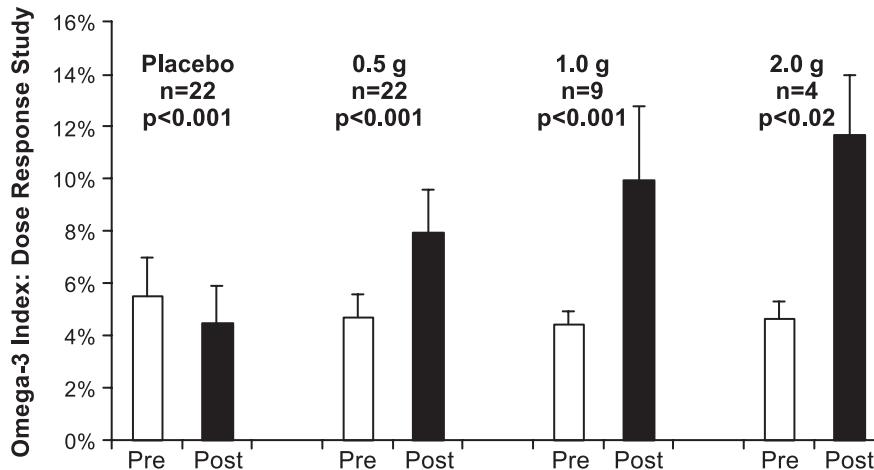
Fig. 1. The Omega-3 Index (mean $\pm$ SD) measured in 57 healthy volunteers given placebo, 0.5, 1, or 2 g of EPA + DHA per day for 20 weeks. Within each group, post-levels are significantly different from pre-levels as indicated; all changes from baseline are significantly different among groups ($P < 0.05$), except for the 1- vs. 2-g groups.

Comparison of the Omega-3 Index with other biomarkers

Correlation coefficients between the Omega-3 Index and whole blood omega-3 FA (Fig. 2) and plasma phospholipid EPA + DHA (Fig. 3) were both >0.9. These equations were applied to the data of Albert et al. [5] for whole blood and Lemaitre et al. [6] for plasma phospholipids to relate the Omega-3 Index to the risk for CHD death.

Relationship between the Omega-3 Index and risk for CHD death

In three US studies, the Omega-3 Index (or biomarkers convertible into it) was related to risk. Siscovick et al. [7–9] 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 Omega-3 Index was determined

in these samples and related to risk for primary cardiac arrest. The multivariate-adjusted odds ratios for primary cardiac arrest in the highest the Omega-3 Index quartile were about 10% of that in the lowest quartile (95% confidence interval, 0.1–0.4). The mean Omega-3 Index in the highest quartile was 6.5% with a range of 5.5–10.9%. Levels in the lowest quartile (highest risk) averaged 3.3% with a range of 2.0–4.0%.

Albert et al. [5] utilized data from the Physicians' Health Study (PHS). In this study, 14,916 healthy male physicians were screened for a wide variety of risk factors 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 FA (i.e., percent of total FA as EPA + DHA + DPA) in these cases was compared to that of 184 age and smoking status-matched controls. As in the Seattle study, risk for sudden cardiac death was reduced
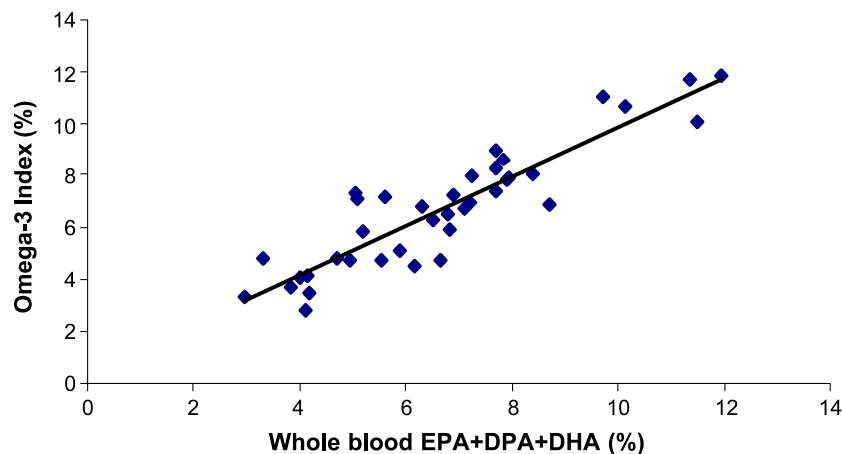

Fig. 2. The Omega-3 Index vs. whole blood long chain omega-3 FA. Fasting blood samples ($n = 38$) were analyzed for these two parameters. Omega-3 Index (%) = Whole blood EPA + DPA + DHA (%) $\times$ 0.95 + 0.35 ($r = 0.91$; $P < 0.0001$).
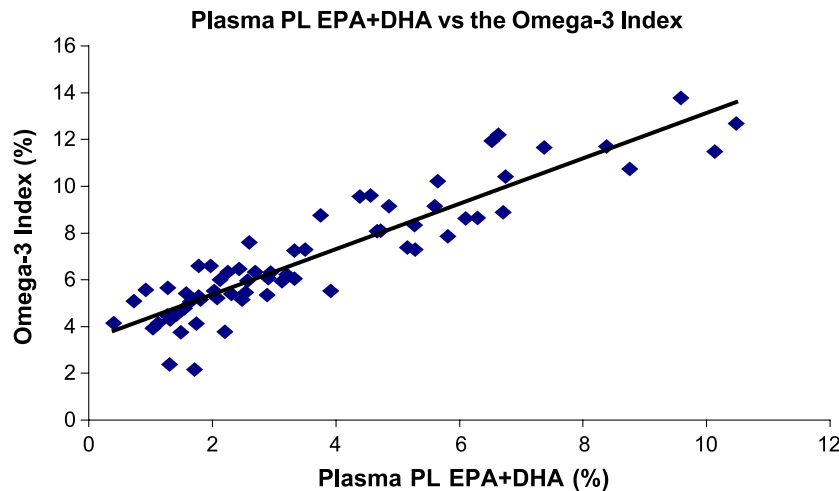
Plasma PL EPA+DHA vs the Omega-3 Index

Fig. 3. RBC vs. plasma phospholipid (PL) EPA + DHA. These two parameters were measured in 65 pairs of blood samples. Omega-3 Index (%) = Plasma PL (%) EPA + DHA $\times$ 0.97 + 3.43 ($r = 0.91$; $P < 0.001$).

by about 90% in those subjects with the highest blood EPA + DHA levels compared with those with the lowest levels. To help define the target Omega-3 Index, the whole blood values from the PHS were converted into this parameter using the equation illustrated in Fig. 2. Transformation of the data revealed that the average Omega-3 Index for the highest quartile was 6.9% with a range of 6.1–10.1%. Levels in the lowest quartile were about 3.8% with a range of 2.4–4.5%.

The third study to examine the relationship between a blood measure of omega-3 FA and risk for CHD death was reported by Lemaitre et al. [6] utilizing data from the Cardiovascular Health Study. These investigators found a strong, protective relationship between (in this case) serum phospholipid EPA + DHA and risk for fatal ischemic heart disease. The odds ratio associated with a 1 SD increase in this biomarker was 0.30 (95% CI, 0.12 to 0.76). Converting the reported phospholipid EPA + DHA values to the Omega-3 Index (Fig. 3) revealed that those subjects with an Omega-3 Index of about 8.9% were at 70% lower risk for fatal ischemic disease than those with an index of about 6.9%.

Four other studies [10–13] found strong inverse relationships between CHD events and other biomarkers of EPA + DHA intake (coronary artery, serum cholesterol ester, or total plasma FA). How these biomarkers relate mathematically to the Omega-3 Index has yet to be determined. One study reported no relationship between plasma omega-3 FAs and the 5-year risk of myocardial infarction [14].

The Omega-3 Index in randomized controlled trials

The study on the prevention of coronary atherosclerosis by intervention with marine omega-3 fatty acids (SCIMO) [15] was a randomized, placebo-controlled, prospective trial in which quantitative coronary angiography was utilized to follow the effects of EPA + DHA supplementation on

plaque progression and regression in 223 CHD patients over 2 years. SCIMO is the only omega-3 FA intervention trial that followed clinically meaningful CHD endpoints in which the Omega-3 Index was assessed. In this trial, omega-3 FA supplementation (3 g EPA + DHA/day for 3 months followed by 1.5 g/day for 18 months) resulted in a slower rate of disease progression ($P = 0.04$) and a trend towards fewer cardiovascular events (6.25% vs. 1.8%, $P = 0.1$). The Omega-3 Index increased from baseline values of 3.4% to 8.3% ($P < 0.05$) after 2 years on trial.

The Omega-3 Index estimated from EPA + DHA intakes in secondary prevention studies

In two major prospective intervention studies with EPA + DHA (derived either from capsules or oily fish) conducted in patients with known CHD [16,17], neither the Omega-3 Index nor any biomarker reducible to it was reported. Therefore, how reported intakes would likely have impacted the Omega-3 Index (based on the dose-response study reported here) will be used, again, to estimate the cardioprotective levels of the Omega-3 Index.

The first study was the Diet and Reinfarction Trial (DART) [16]. In this study, 2,033 men were randomized to either receive or not receive advice to increase their oily fish intake to about 300 g/week. After 2 years of follow-up, those receiving the fish advice experienced a 29% reduction in all-cause mortality and a 32% decrease in ischemic heart disease mortality compared to controls. The authors estimated an intake of 2.5 g of EPA per week (357 mg/day). Assuming that EPA contributes about 40% of the total EPA + DHA in oily fish, the intake in this study was about 900 mg/day.

The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico Prevenzione (GISSI-P) study [17] tested the effects of EPA + DHA supplementation on death from CHD. Patients ($n = 11,324$) receiving modern cardiac
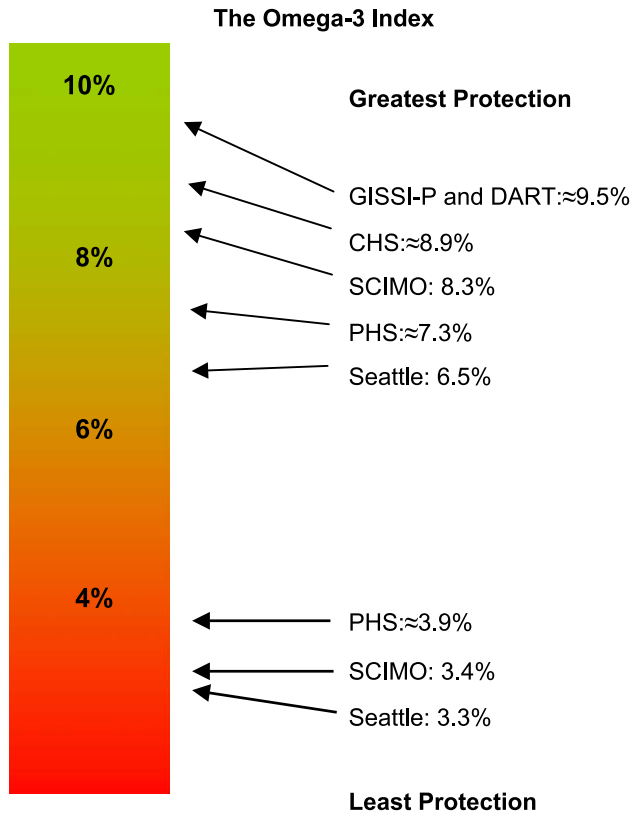
The Omega-3 Index

Fig. 4. Summary of evidence for the proposed cut points for the Omega-3 Index. Values in the vertical bar are the Omega-3 Index (%) from lowest to highest. The following data sets are represented: the Seattle study [9], the Physicians' Health Study (PHS) [5], the Cardiovascular Health Study (CHS) [6], the Diet and Reinfarction Trial (DART) [16], the study on the prevention of coronary atherosclerosis by intervention with marine omega-3 fatty acids (SCIMO) [15], and the GISSI-Prevenzione study [17]. Taken together, these data suggest a desirable target value for the Omega-3 Index of ≥8% and an undesirable level of ≤4%.

pharmacotherapy were randomized to 850 mg/day of EPA + DHA, 300 mg/day of vitamin E, both, or to the unsupplemented control group. After 3.5 years of follow up, the group given just the EPA + DHA (n = 2,836) experienced a 20% reduction in all-cause mortality, a 35% decrease in cardiac death, and a 45% reduction in sudden death (all $P < 0.01$) compared to the control group (n = 2,828). These effects became statistically significant within 3–4 months of randomization [2]. Thus, similar intakes of EPA + DHA in the DART and the GISSI-P study resulted in similar protection. The results of the dose-ranging study (Fig. 1) would suggest that intakes of about 900 mg/day of EPA + DHA would produce an Omega-3 Index of about 9.5%.

Based upon the data presented above, we were able to make an informed estimate of the Omega-3 Indexes associated with low and high risk for death from CHD. We found that the average Omega-3 Index associated with the lowest risk for death from CHD was about 8%, whereas the index associated with the highest risk was <4% (Fig. 4).

Discussion

The Omega-3 Index has the characteristics of a risk factor

There are several requirements that a putative risk factor or marker must meet to be clinically useful [18] (Table 1). The Omega-3 Index fulfills many of these. The epidemiological data, both between and within populations, as well as from prospective cohort studies, are quite consistent [3]. Since that time, other studies have been published that generally continue to support the rationale for measuring blood EPA + DHA to estimate risk for death from CHD [6,19–21]. A notable exception would be the unsuccessful attempt to reproduce the results of the DART in patients with stable angina [22]. Why a recommendation to consume more oily fish or to take fish oil capsules failed to reduce risk in this trial is not known.

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 [23]. In addition, EPA + DHA may enhance plaque stability [24], and may be anti-atherosclerotic via a variety of other mechanisms [25]. Finally, perhaps the most important question to be asked of a putative risk factor is whether changing the risk factor alters disease outcomes. The intervention trials described above suggest that EPA + DHA fulfill this critical criterion as well. One requirement that remains to be satisfactorily addressed before the Omega-3 Index can be widely implemented is the establishment of standardized laboratory methods of analysis (see Limitations and future directions).

The Omega-3 Index compared with other CHD risk factors

Using data from the Physicians' Health Study, Albert et al. [26] have published the relative risk for sudden cardiac death across quartiles of several risk factors (Fig. 5). Only two risk factors demonstrated statistically significant trends: C-reactive protein and the Omega-3 Index (estimated, as noted above, from the blood omega-3 FA composition). Of these, the latter had the steeper gradient

Table 1
The Omega-3 Index as a risk factor

Consistency of epidemiological data
Between populations—Yes [30,46,47]
Within populations—Yes [9,48]
Prospective cohorts—Yes [5,6,20,21,49–56]
Strong association between biomarker and disease—Yes [5]
Independence from other known risk factors—Yes [5,9]
Biological plausibility—Yes [23]
Modifiable (Safely, quickly, and cheaply)—Yes [15]
Modification Reduces Risk—Yes [24,16]
Standardized measure—No
Biological variability—Low [41]
Analytical reproducibility—Fair (CV = 10–12%)

in risk reaching 90% reduction at the highest quartile. Moreover, whereas the relative risk reductions for the Omega-3 Index were adjusted for several potential confounders, those for C-reactive protein were only adjusted for age and smoking status. Thus, the Omega-3 Index may be a more informative risk factor than C-reactive protein. Importantly, the associations between the Omega-3 Index and disease are not known to be influenced by other known CHD risk factors. In the studies by Albert et al. [5] and Siscovick et al. [9], the index remained a statistically significant risk predictor after multivariate adjustment. With the relative risk reduced by approximately 90% in the highest quartiles, the Omega-3 Index is both a strong and an independent predictor of risk for sudden cardiac death.

Modifying the Omega-3 Index: safety and cost

The clinical utility of a risk factor is greatly enhanced if it is modifiable. Modifiable risk factors (cholesterol, blood pressure, smoking, etc.) have a practical value that unmodifiable ones (age, gender, family history) lack. The Omega-3 Index can quickly and easily be increased simply by consuming more long-chain omega-3 FA. It is important to consider the associated safety and cost issues.

Safety

In 1997, the US Food and Drug Administration (FDA) granted generally recognized as safe (GRAS) status to refined menhaden fish oil [27]. In doing so, the agency indicated that the consumption of up to 3 g/day of EPA + DHA from all sources would be considered safe for American adults. This has been supported by population data (e.g., the Japanese [28,29] and Inuits [30,31]), and data from controlled clinical trials providing between 7 and 30 g/day [32,33]. Anecdotally, a bleeding tendency has been reported in Eskimos chronically consuming high quantities [34]. There is, however, no evidence that EPA and DHA increase risk for clinically significant bleeding [35], nor that they interact adversely with other drugs used to treat CHD, including anti-platelet agents [36,37]. Thus, consumption of up to 3 g/day of EPA + DHA is unlikely to be associated with any adverse effects, and as noted above, intakes this high may not even be needed to achieve a cardioprotective effect.

Cost

An EPA + DHA intake of about 1 g/day can be achieved with oily fish, fish oil capsules, cod liver oil, or fortified foods. The costs associated with these strategies can be as low as $0.06 per day using fish oil capsules purchased at wholesale buying clubs in the US [38]. Thus, an adequate intake can be achieved very economically.

Rationale for measuring the Omega-3 Index

The case has been made by the AHA that all adults should consume more oily fish to obtain 500–1,000 mg of EPA + DHA per day (depending on their CHD risk status), and this advice is well-taken. However, it is difficult to know exactly how much EPA + DHA one is actually consuming from fish. The EPA + DHA content of a serving
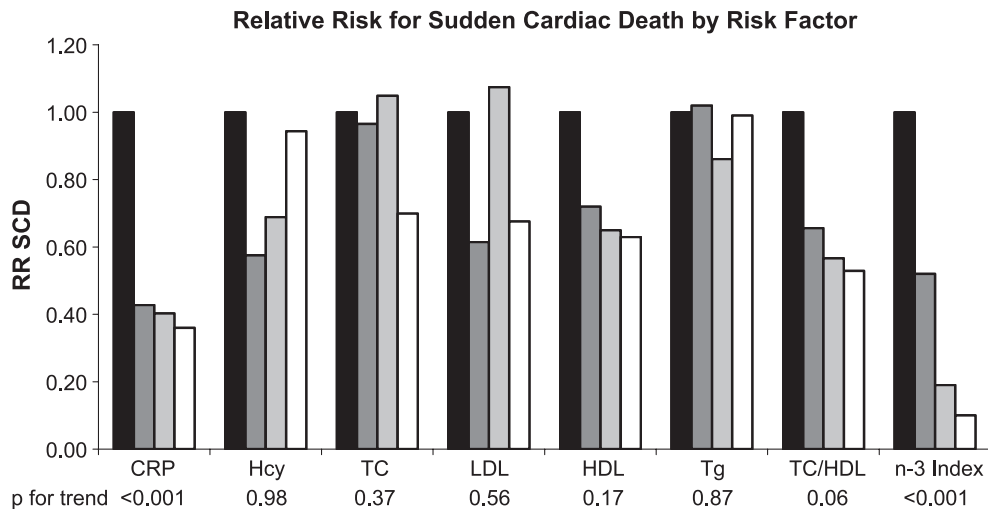
Fig. 5. The relative risk for sudden cardiac death (RR SCD) by quartile of the Omega-3 Index compared to other, more traditional blood-borne risk factors. The quartiles at highest risk (black bars) are set at a relative risk of 1.0 regardless of whether that represents the highest or the lowest blood levels of the marker. Each subsequent lighter bar represents the risk at each decreasing (or, for HDL and the Omega-3 Index, increasing) quartile. All analytes besides the Omega-3 Index were adjusted for age and smoking status. The Omega-3 Index was adjusted for age; smoking status; assignment to aspirin/beta-carotene/placebo; body mass index; history of diabetes, hypertension, or hypercholesterolemia; alcohol consumption; exercise frequency; parental history of MI before age 60 years; and trans- and monounsaturated FA intake. Adapted from the Physicians' Health Study, Albert et al. [5,26]. CRP = C-reactive protein; Hcy = homocysteine; TC = total cholesterol; LDL = low density lipoprotein cholesterol; HDL = high density lipoprotein cholesterol; Tg = triglycerides; n-3 Index = Omega-3 Index.

Table 2
Advantages of RBCs as biomarkers for omega-3 FA intakes

Lipid bilayer—reflects tissue FA composition
The Omega-3 Index half-life is 4–6 times longer than serum
  EPA + DHA [41], better reflecting long-term exposure
Not influenced by fasting or fed state (unpublished data)
Responsive to increasing intakes (Fig. 1)
Correlates well with other biomarkers of omega-3 FA intake
Less influenced by dyslipidemias than serum FA [57]
Less variable than serum EPA + DHA composition[a]
Laboratory assessment is simpler than lipoprotein or lipid fraction FA
Resilient to variations in pre-analytical storage conditions [58]
Usually discarded

[a] In 78 samples, the coefficient of variation of the Omega-3 Index was 23% and of whole plasma EPA + DHA was 66%.

of any given fish is unknown. Tables of EPA + DHA content by species [3] present average levels that can vary markedly depending on season, maturity, the fish's diet, post-catch processing, and cooking methods [39]. In addition, the truth of label claims for encapsulated oils is not regulated, and so the consumer cannot necessarily know how much EPA + DHA is being consumed from supplements. Nevertheless, a recent Consumers' Report analysis of 16 fish oil brands found that label claims were generally accurate [38]. Even if the omega-3 intake were known, each person is metabolically unique, with idiosyncrasies in digestion, absorption, tissue distribution, and cellular metabolism. Individual variations in the in vivo conversion of α-linolenic acid (the plant-derived omega-3 FA) into EPA and DHA as well as other dietary variables (e.g., kcalories, omega-6 FA) can also influence tissue EPA + DHA levels [39,40]. These factors conspire together to produce different levels in people all consuming the same amount of EPA + DHA. This is illustrated by the results of the dose-response study reported here (Fig. 1). At the end of the study, the Omega-3 Index varied from 3% to 7% in the placebo group, from 4% to 10% in the 500 mg/day group, and from 5% to 13% in the 1 g/day group. Consequently, knowledge of baseline levels will guide the physician's recommendations—not surprisingly, low baseline values may require a larger dose than a high baseline value. Therefore, the Omega-3 Index may be useful for assessing both baseline risk and a change in risk as a function of intake.

Limitations and future directions

Algorithms that include other risk factors should be developed to quantify the incremental predictive value obtained by measuring the Omega-3 Index. This could be achieved retrospectively by analysis of current data sets for which traditional risk factors and the Omega-3 Index have already been measured, or prospectively by analysis of stored RBCs from past or ongoing clinical trials. The proposed Omega-3 Index target of 8–10% will need to be defined more precisely in future CHD endpoint trials.

Although RBCs appear to be an ideal biomarker for EPA + DHA intake (Table 2), to date, the Omega-3 Index has not been measured using a standardized method, and the development of such an assay and quality control materials is a high priority. In addition, larger studies examining the dose-response relationship between EPA + DHA and the Omega-3 Index utilizing different groups of patients with differing background diets are needed. The effects of fish vs. capsules on this biomarker will need to be clarified, and precisely how rapidly steady state levels can be achieved is not known with certainty [41]. The impact upon the Omega-3 Index of different ratios of dietary EPA and DHA as well as α-linolenic acid and other dietary FA will need to be examined. Finally, how the Omega-3 Index correlates with risk in other disease states (e.g., Alzheimer's disease [42], depression [43], arthritis [44], prostate cancer [45], etc.) is currently unknown. Therefore, the use of a marker like the Omega-3 Index as a modifiable risk predictor may open several new avenues of research.

Conclusions

We have presented a case for the use of the Omega-3 Index as a risk stratification tool for CHD death. In addition, we have suggested that an Omega-3 Index level of ≥8% is a reasonable preliminary target value for reducing risk. The Omega-3 Index may represent a novel, physiologically relevant, modifiable, and independent marker of risk for death from CHD.

Acknowledgments

References

[1] Zheng ZJ, Croft JB, Giles WH, et al. Sudden cardiac death in the United States, 1989 to 1998. Circulation 2001;104:2158–63.

[2] Marchioli R, Barzi F, Bomba E, et al. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 2002;105:1897–903.

[3] Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 2002;106:2747–57.

[4] von Schacky C, Fischer S, Weber PC. Long-term effects of dietary marine w-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. J Clin Invest 1985;76:1626–31.

[5] Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. N Engl J Med 2002;346:1113–8.

[6] Lemaitre RN, King IB, Mozaffarian D, et al. N-3 polyunsaturated fatty acids, fatal ischemic heart disease and non-fatal myocardial infarction in older adults. The Cardiovascular Health study. Am J Clin Nutr 2002;76:319–25.

[7] Park Y, Harris WS. EPA, but not DHA, decreases mean platelet volume in normal subjects. Lipids 2002;37:941–6.

[8] Park Y, Harris WS. Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. J Lipid Res 2003;44:455–63.

[9] Siscovick DS, Raghunathan TE, King I, et al. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. J Am Med Assoc 1995;274:1363–7.

[10] Luostarinen R, Boberg M, Saldeen T. Fatty acid composition in total phospholipids of human coronary arteries in sudden cardiac death. Atherosclerosis 1993;99:187–93.

[11] Hallgren CG, Hallmans G, Jansson JH, et al. Markers of high fish intake are associated with decreased risk of a first myocardial infarction. Br J Nutr 2001;86:397–404.

[12] Erkkila AT, Lehto S, Pyorala K, et al. n-3 fatty acids and 5-y risks of death and cardiovascular disease events in patients with coronary artery disease. Am J Clin Nutr 2003;78:65–71.

[13] Rissanen T, Voutilainen S, Nyyssönen K, et al. Fish oil-derived fatty acids, docosahexaenoic acid and docosapentaenoic acid, and the risk of acute coronary events. The Kuopio Ischaemic Heart Disease Risk Factor study. Circulation 2000;102:2677–9.

[14] Guallar E, Hennekens CH, Sacks FM, et al. A prospective study of plasma fish oil levels and incidence of myocardial infarction in US male physicians. J Am Coll Cardiol 1995;25:387–94.

[15] von Schacky C, Angerer P, Kothny W, et al. The effect of dietary ω-3 fatty acids on coronary atherosclerosis. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 1999;130:554–62.

[16] Burr ML, Fehily AM, Gilbert JF, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet 1989;2:757–61.

[17] GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E in 11,324 patients with myocardial infarction: results of the GISSI-Prevenzione trial. Lancet 1999;354:447–55.

[18] Manolio T. Novel risk markers and clinical practice. N Engl J Med 2003;349:1587–9.

[19] Paganelli F, Maixent JM, Duran MJ, et al. Altered erythrocyte n-3 fatty acids in Mediterranean patients with coronary artery disease. Int J Cardiol 2001;78:27–32.

[20] Mozaffarian D, Lemaitre RN, Kuller LH, et al. Cardiac benefits of fish consumption may depend on the type of fish meal consumed: the Cardiovascular Health study. Circulation 2003;107:1372–7.

[21] Hu FB, Cho E, Rexrode KM, et al. Fish and long-chain omega-3 fatty acid intake and risk of coronary heart disease and total mortality in diabetic women. Circulation 2003;107:1852–7.

[22] Burr ML, Ashfield-Watt PA, Dunstan FD, et al. Lack of benefit of dietary advice to men with angina: results of a controlled trial. Eur J Clin Nutr 2003;57:193–200.

[23] Leaf A, Kang JX, Xiao YF, et al. Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 2003;107:2646–52.

[24] Thies F, Garry JM, Yaqoob P, et al. Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. Lancet 2003;361:477–85.

[25] von Schacky C. The role of omega-3 fatty acids in cardiovascular disease. Curr Atheroscler Rep 2003;5:139–45.

[26] Albert CM, Ma J, Rifai N, et al. Prospective study of C-reactive protein, homocysteine, and plasma lipid levels as predictors of sudden cardiac death. Circulation 2002;105:2595–9.

[27] Food and Drug Administration, H. Substances affirmed as generally recognized as safe: menhaden oil. Fed Regist 1997;30751–7.

[28] Kobayashi M, Sasaki S, Kawabata T, et al. Single measurement of serum phospholipid fatty acid as a biomarker of specific fatty acid intake in middle-aged Japanese men. Eur J Clin Nutr 2001;55:643–50.

[29] Kuriki K, Nagaya T, Imaeda N, et al. Discrepancies in dietary intakes and plasma concentrations of fatty acids according to age among Japanese female dietitians. Eur J Clin Nutr 2002;56:524–31.

[30] Dewailly E, Blanchet C, Lemieux S, et al. n-3 fatty acids and cardiovascular disease risk factors among the Inuit of Nunavik. Am J Clin Nutr 2001;74:464–73.

[31] Bang HO, Dyerberg J, Hjorne N. The composition of food consumed by Greenland Eskimos. Acta Med Scand 1976;200:69–73.

[32] Leaf A, Jorgensen MB, Jacobs AK, et al. Do fish oils prevent restenosis after coronary angioplasty? Circulation 1994;90:2248–57.

[33] Phillipson BE, Rothrock DW, Connor WE, et al. Reduction of plasma lipids, lipoproteins and apoproteins by dietary fish oils in patients with hypertriglyceridemia. N Engl J Med 1985;312:1210–6.

[34] Dyerberg J, Bang HO. Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. Lancet 1979;433–5.

[35] Knapp HR. Dietary fatty acids in human thrombosis and hemostasis. Am J Clin Nutr 1997;65 (Suppl):1687S–98S.

[36] Harris WS, Silveira S, Dujovne CA. The combined effects of omega-3 fatty acids and aspirin on hemostatic parameters in men. Thromb Res 1990;57:517–26.

[37] Svaneborg N, Kristensen SD, Hansen LM, et al. The acute and short-time effect of supplementation with the combination of n-3 fatty acids and acetylsalicylic acid on platelet function and plasma lipids. Thromb Res 2002;105:311–6.

[38] Omega-3 oil: fish or pills? Consum Rep 2003;30–2.

[39] Ackman RG. Nutritional composition of fats in seafoods. Prog Food Nutr Sci 1989;13:161–289.

[40] Gronn M, Gorbitz C, Christensen E., et al. Dietary n-6 fatty acids inhibit the incorporation of dietary n-3 fatty acids in thrombocyte and serum phospholipids in humans: a controlled dietetic study. Scand J Clin Lab Invest 1991;51:255–63.

[41] Katan MB, Deslypere JP, van Birgelen AP, et al. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. J Lipid Res 1997;38:2012–22.

[42] Kyle DJ, Schaefer E, Patton G, et al. Low serum docosahexaenoic acid is a significant risk factor for Alzheimer's dementia. Lipids 1999;34:S245.

[43] Tiemeier H, van Tuijl HR, Hofman A, et al. Plasma fatty acid composition and depression are associated in the elderly: the Rotterdam study. Am J Clin Nutr 2003;78:40–6.

[44] Volker D, Fitzgerald P, Major G, et al. Efficacy of fish oil concentrate in the treatment of rheumatoid arthritis. J Rheumatol 2000;27:2343–6.

[45] Terry PD, Rohan TE, Wolk A. Intakes of fish and marine fatty acids and the risks of cancers of the breast and prostate and of other hormone-related cancers: a review of the epidemiologic evidence. Am J Clin Nutr 2003;77:532–43.

[46] Davidson M, Bulkow LR, Gellin BG. Cardiac mortality in Alaska's indigenous and non-Native residents. Int J Epidemiol 1993;22:62–71.

[47] Kromann N, Green A. Epidemiological studies in the Upernavik District, Greenland. Acta Med Scand 1980;208:401–6.

[48] Burchfiel CM, Reed DM, Strong JP, et al. Predictors of myocardial lesions in men with minimal coronary atherosclerosis at autopsy. The Honolulu Heart Program. Ann Epidemiol 1996;6:137–46.

[49] Kromhout D, Bosschieter EB, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Engl J Med 1985;312:1205–9.

[50] Rodriguez BL, Sharp DS, Abbott RD, et al. Fish intake may limit the increase in risk of coronary heart disease morbidity and mortality among heavy smokers. The Honolulu Heart Program. Circulation 1996;94:952–6.

[51] Daviglus ML, Stamler J, Orencia AJ, et al. Fish consumption and the 30-year risk of fatal myocardial infarction. N Eng J Med 1997;336:1046–53.

[52] Yuan JM, Ross RK, Gao YT, et al. Fish and shellfish consumption in relation to death from myocardial infarction among men in Shanghai, China. Am J Epidemiol 2001;154:809–16.

[53] Oomen CM, Feskens EJ, Rasanen L, et al. Fish consumption and coronary heart disease mortality in Finland, Italy, and The Netherlands. Am J Epidemiol 2000;151:999–1006.

[54] Dolecek TA. Epidemiological evidence of relationships between dietary polyunsaturated fatty acids and mortality in the multiple risk factor intervention trial. Proc Soc Exp Biol Med 1992;200:177–82.

[55] Albert CM, Hennekens CH, O'Donnell CJ, et al. Fish consumption and risk of sudden cardiac death. JAMA 1998;279:23–8.

[56] Hu FB, Bronner L, Willett WC, et al. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. JAMA 2002;287:1815–21.

[57] Tynan MB, Nicholls DP, Maguire SM, et al. Erythrocyte membrane fatty acid composition as a marker of dietary compliance in hyperlipidaemic subjects. Atherosclerosis 1995;117:245–52.

[58] Giltay EJ, Geleijnse JM, Schouten EG, et al. High stability of markers of cardiovascular risk in blood samples. Clin Chem 2003;49:652–5.