

THEME: CARDIOLOGY

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Use of red blood cell fatty-acid profiles as biomarkers in cardiac disease



The Omega-3 Index is defined as the percentage of eicosapentaenoic acid plus docosahexaenoic acid in red blood cell fatty acids, assessed by a standardized methodology. Better than fatty-acid compositions in other compartments, the Omega-3 Index represents a person's status in eicosapentaenoic acid plus docosahexaenoic acid. An Omega-3 Index less than 4% is associated with a tenfold risk for sudden cardiac death in comparison to an Omega-3 Index greater than 8%. Mechanisms of action are plausible and large-scale intervention studies in humans support causality. A low Omega-3 Index may also be a risk factor for coronary artery disease and for complications of congestive heart failure. Ongoing research will define the value of the Omega-3 Index as a risk factor and treatment parameter more precisely.

KEYWORDS: congestive heart failure, coronary artery disease, docosahexaenoic acid, eicosapentaenoic acid, Omega-3 Index, sudden cardiac death

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Sudden cardiac death (SCD) is defined as: "Death from unexpected circulatory arrest, usually due to a cardiac arrhythmia, occurring within an hour of the onset of symptoms" [1]. According to this definition, 13% of all natural deaths are due to SCD in Western countries [1]. Established risk factors for SCD identify a very small group of high-risk patients, and necessitate the implantation of a cardioverter-defibrillator [1]. However, the vast majority of SCDs occur in persons not yet identified as being at risk for the condition [1].

Conventional risk factors (e.g., age, blood pressure, low-density lipoproteins [LDLs] and so on) predict coronary artery disease, but not SCD, fairly well [2]. Conventional risk factors are used in risk stratifications such as the Framingham Score or the Score of the European Society for Cardiology [3]. These risk stratifications could be improved substantially by the incorporation of new cardiovascular risk determinants [4,5].

Chronic congestive heart failure is a heterogeneous syndrome, which mainly affects the elderly [6]. Heart failure can be caused by ischemic heart disease, hypertension, dilative cardiomyopathy and a host of other etiologies [6]. Despite conventional therapy, mortality from heart failure remains high, and is mainly due to worsening of heart failure or SCD, indicating an unresolved medical problem [6]. While brain natriuretic peptide can be used to predict death and postdischarge outcomes, many other biomarkers are currently being evaluated [7].

Taken together, there is a need for novel risk factors for SCD, coronary artery disease and for complications of congestive heart failure. An ideal risk factor should be easy to assess (e.g., a routine laboratory parameter), have a high predictive value, have a large incremental informative value, be amenable to treatment with a proven, cheap and low risk intervention, and a change in risk should be assessable by repeat measurement of the risk factor. In this article, the use of the Omega-3 Index as a biomarker in cardiac disease will be discussed.

The Omega-3 Index

The Omega-3 Index was defined in 2004 as the sum of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) acids in red blood cells as a percent of total fatty acids [8]. Clearly, this definition calls for both standardized analytical procedure and evaluation of analytical results, currently available in a very limited number of laboratories. Previously, differences in procedures and evaluation of fatty-acid analyses have resulted in vastly different results reported, obviating a comparison of reports [9]. A joint effort towards standardization of analytical procedures has already borne fruit [10], demonstrating that application of quality assurance measures comparing clinical routine laboratory analyses with fatty-acid analyses is feasible, and ensures results obtained in different laboratories are identical. Applying a standardized methodology, it was found that analytical reproducibility is high (coefficient

of variation: 4–7%), and that biologic variability is low, and in fact much lower than plasma measurements [11].

The Omega-3 Index was proposed because previous work had indicated that red blood cell fatty-acid composition reflects the long-term intake of Omega-3 fatty acids [12]. Although there is some exchange with the surrounding milieu, fatty acids are largely incorporated into blood cell membranes during cell maturation [12]. Other blood cells, such as mononuclear cells, polymorphonuclear leukocytes or platelets, have a shorter lifespan, and therefore reflect a shorter timeframe [12–15]. Plasma-free fatty acids can change in quantity and composition within minutes, whereas some fractions, such as plasma phospholipids, take hours to days to reflect the incorporation of dietary Omega-3 fatty acids [15]. There is a correlation between the Omega-3 Index and cardiac cell EPA plus DHA levels ($r = 0.81$; $p < 0.0001$), which was found to be true for an unaltered diet as well as for a change in Omega-3 fatty acid intake [16,17]. Intake of EPA and DHA is only one determinant of the Omega-3 Index [18]: the Omega-3 Index increases by 0.24 units with each additional monthly serving of tuna or nonfried fish [18]. However, it also increases by 0.5 units for each additional decade

in age [18]. The Omega-3 Index was found to be 1.13% units lower in subjects with diabetes, and decreased by 0.3% units with each 3-unit increase in BMI. In Japan, females had a higher Omega-3 Index than males, whereas in the USA, a gender difference was not seen [18,19]. Apparently owing to genetic determinants, a third of humans convert α -linolenic acid to EPA (maximum 5%), whereas another third of humans cannot perform this metabolic step, also indicating a genetic influence on the Omega-3 Index [20]. The possibility of finding more determinants that influence the Omega-3 Index is likely [18,21].

Epidemiology

In Kansas City (KS, USA), an average Omega-3 Index of 4.9% was found in the general population [18], which is similar to values in Munich (Germany) [VON SCHACKY C ET AL., UNPUBLISHED DATA]. As mentioned previously, the incidence of SCD in the general population in Western countries is approximately 150/100,000 person-years [1]. However, in Japan, the corresponding number is 7.8/100,000 person-years, which is paralleled by an Omega-3 Index among 10% in this population [19,22]. Moreover, a case–control study conducted in Seattle (WA, USA) and published in 1995 indicated that there is an inverse relation between red blood cell Omega-3 fatty-acid concentration and risk for SCD: 3.3% was associated with a relative risk (RR) of 1.0, whereas 6.5% was associated with a RR of 0.1 [23]. This is corroborated by findings from the Physicians Health study [24]. Combining the numbers mentioned results in FIGURE 1; this figure is partly hypothetical because the Omega-3 Index was not measured using standardized methodology in the studies compiled. Studies currently in progress will add precision to this figure.

In patients with coronary artery disease receiving current standard care in Italy, the incidence of SCD was 828/100,000 patient-years [25]. By contrast, in Japan it was 40/100,000 patient-years in hyperlipidemic patients, 20% of whom had established coronary artery disease along with other risk factors [26]. Thus, there is a 20-fold difference in the incidence of SCD between Italy and Japan in persons with roughly comparable cardiovascular risk. As mentioned previously, a similar difference exists between general populations in Western countries and Japan (FIGURE 1).

The incidence of nonfatal myocardial infarction in patients at risk for this condition was 1151/100,000 patient-years in Italy, but 156/100,000 patient-years in Japan [25,26]. This is

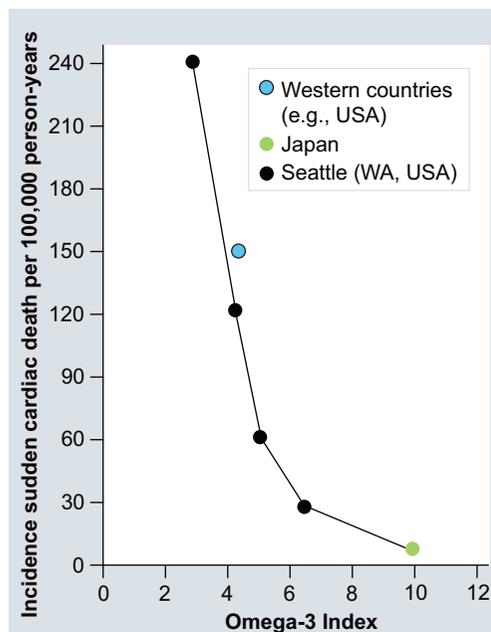


Figure 1. Relation between Omega-3 Index and incidence of sudden cardiac death in the general population. Seattle data represent a case–control study within a population. Owing to the heterogeneous analytical methods used for the Omega-3 Index, this figure remains to be validated. Taken from [1,20–22].

substantiated by the observation within a population in Kansas City that a low Omega-3 Index is a risk factor for acute coronary syndrome [27]. Supportive data have been published independently, although in this case, plasma EPA plus DHA appeared to be a better predictor for non-fatal myocardial infarctions than erythrocyte EPA plus DHA [28].

Recent evidence indicates that intake of Omega-3 fatty acids is inversely related to cardiovascular mortality, which was found to be mainly due to heart failure mortality [29].

Taken together, a high Omega-3 Index is associated with a low risk for SCD or nonfatal myocardial infarctions. This phenomenon cannot be replicated in all populations, since a preponderance of SCDs obscures differences in nonfatal events. Whether a low Omega-3 Index predicts risk of developing heart failure and/or its complications remains to be investigated.

Mechanisms

In vitro, Omega-3 fatty acids prolong the refractory period of the action potential and thus increase electrical stability by specific actions on various ion channels [30]. In animal models of myocardial infarction, infusing Omega-3 fatty acids prevented ventricular tachyarrhythmias [31].

In vivo, Omega-3 fatty acids are incorporated into cell membranes, which is likely to have profound effects on cell signaling and function [31]. Since these effects tend to be species specific, measurements need to be performed in humans, and therefore will be indirect. What has been demonstrated, however, was reduced inducibility of ventricular arrhythmias after infusion or ingestion of Omega-3 fatty acids [32,33]. Other mechanisms that may prevent arrhythmias, specifically supraventricular arrhythmias, are a reduced heart rate, accelerated normalization of heart rate after exercise and increased heart rate variability, all demonstrated in randomized intervention studies in humans [34,35]. Based on these mechanistic findings and promising first results [36], several studies currently investigate the effect of dietary Omega-3 fatty acids on atrial fibrillation and its prevention.

A host of mechanisms, as studied *in vivo* or *ex vivo* in randomized intervention studies in humans with Omega-3 fatty acids, have been suggested to mediate an anti-atherosclerotic effect. Among them are reductions in pro-inflammatory or pro-atherogenic cytokines, a triglyceride-lowering effect, reductions in parameters indicating interaction between blood cells and endothelium and others [37–40]. These

mechanisms may underlie the plaque-stabilizing effect that has been demonstrated in a randomized intervention study *ex vivo* [41]. Moreover, an increase in the Omega-3 Index from 3.4 to 8.3% mitigated coronary lesion progression in our 2-year, randomized, double-blind angiographic intervention study [42]. Therefore, mechanistic studies and studies with intermediate end points indicate that Omega-3 fatty acids, or an increase in the Omega-3 Index, may also reduce non-fatal coronary events. Of note, however, findings in coronary arteries have not been replicated in carotid arteries, maybe owing to a site-specificity of action of Omega-3 fatty acids [42].

Some of the mechanisms mentioned previously have been invoked to make a positive effect of EPA plus DHA in patients with congestive heart failure likely [43].

Intervention studies with clinical end points

Randomized, controlled intervention studies with clinical end points such as mortality and morbidity were conducted in populations at risk for cardiovascular events. These studies used either a dietary advice approach (i.e., the advice to eat fatty fish twice weekly in the Diet and Reinfarction Trial [DART] or DART-2) or purified preparations of Omega-3 fatty acids (1 g EPA + DHA/day in the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico [GISSI] and 1.8 g/day in the Japan EPA Lipid Intervention Study [JELIS]). Whereas some studies were open (DART [44], GISSI-Prevenzione [25], DART-2 [45] and JELIS [26]), GISSI-heart failure (GISSI-HF) was placebo controlled [46].

In a meta-analysis comprising DART, DART-2, GISSI and the literature published up to April 2006, a reduction in total mortality of 17% was found, largely driven by a reduction in fatal reinfarctions and SCD [47]. Two large studies were published later, and will be discussed specifically.

JELIS was a 5-year study on 18,624 hyperlipidemic persons (average LDL 182 + 30 mg/dl), of whom 20% had established coronary artery disease, 35% were hypertensive, 16% had diabetes and 18% were smokers [26]. A total of 35 SCDs and 25 fatal myocardial infarctions occurred during the study, which was not different between intervention and control groups [26]. The reduction in the primary end point of the study by 19% (fatal and nonfatal events) was entirely driven by a difference in nonfatal events. The incidence of SCD seen in the cardiovascular risk population of

JELIS (40/100,000 person-years) was lower than the incidence of SCD in a Western population (~150/100,000). The Omega-3 Index was not measured in JELIS, but plasma fatty-acid measurements indicate that the population studied in JELIS was typically Japanese in terms of fatty acids (i.e., Omega-3 Index among 10%), with a doubling of EPA in the intervention group, while DHA remained constant in both groups [26].

GISSI-HF was presented at the 2008 congress of the European Society for Cardiology, and recently published [46]. A total of 6975 patients with heart failure due to ischemic heart disease (~50%), dilative cardiomyopathy (~29%), hypertensive cardiopathy (~15%) and other diseases (~7%) participated for 3.9 years. Participants were NYHA II (~64%) or III–IV (~36%). Mean left ventricular ejection fraction was $33 \pm 8.5\%$. Patients received current guideline-oriented background therapy. Baseline inequalities called for a prespecified adjustment of results [43]. In the control group, all-cause mortality was 29.1%, whereas it was 27.3% in the intervention group, a 9 relative % reduction (adjusted hazard ratio [HR]: 0.91; 95.5% confidence interval [CI]: 0.833–0.998; $p = 0.041$). The prespecified combined end point (death and/or cardiovascular hospitalization) was reached by 59.0% of the control group, but only 56.7% of the intervention group (adjusted HR: 0.92; 99% CI: 0.849–0.999; $p = 0.009$). Predefined secondary outcomes were cardiovascular death, which occurred in 22.0% of the control group versus 20.4% of the intervention group (adjusted HR: 0.90; 95% CI: 0.81–0.99; $p = 0.045$), and SCD, which occurred in 9.3% of the control group versus 8.8% of the intervention group (not significant). Nonfatal end points (e.g., hospitalization for cardiovascular reasons or for ventricular arrhythmias) also occurred significantly less frequently in the intervention group. The incidence of SCD was less frequent than expected in GISSI-HF, which was ascribed to the very high percentages of guideline-conforming treatments. This lower than expected incidence was only nonsignificantly reduced, nevertheless the greatest proportion of reduction in total mortality was attributed to presumed arrhythmic death [46].

From the Omega-3 Index perspective, the data from the intervention studies support causality, since the dose of Omega-3 fatty acids provided increased the Omega-3 Index on average, although it was not measured in the studies mentioned [8]. Patients were recruited into the studies irrespective of their Omega-3 Index.

Studies performed in Western populations with a high incidence of SCD demonstrate a reduction in SCD. In studies where there was already a relatively low incidence of SCD, such as JELIS or in GISSI-HF, reductions in other end points such as nonfatal cardiac events were visible.

Omega-3 fatty acids & the Omega-3 Index in current cardiologic guidelines

In current 'Guidelines for Management of Patients with Ventricular Arrhythmias and the Prevention of SCD', Omega-3 fatty acids are recommended at a level B IIB, indicating that 'the recommendations usefulness/efficacy is less well established' and 'greater conflicting evidence from single randomized trial or nonrandomized study' [1]. This statement was based on three mid-sized studies in carriers of an implantable cardiovascular defibrillator (ICD) that yielded inconclusive results with respect to their primary end point: action of the device, for instance, anti-tachycardia pacing and shocks [48–50]. However, 25 participants died in the fish oil groups versus 36 participants in the placebo groups [48–50], a nonsignificant 30% risk reduction, according to a recent meta-analysis (RR: 0.70; 95% CI: 0.42–1.15; $p = 0.15$) [51].

Of note, while ICDs acted more frequently on ventricular tachycardias in persons with a high Omega-3 Index [52], ICDs acted less frequently on ventricular fibrillations caused by ischemia in persons with a high Omega-3 Index [53]. Similarly, in coronary artery patients prone to developing ischemic arrhythmias, the ICDs tended to act less frequently in the three ICD studies mentioned (HR: 0.79; 95% CI: 0.6–1.06; $p =$ not submitted) [54]. Thus, taken together, simply counting the actions of ICDs may be inadequate to evaluate the effects of Omega-3 fatty acids.

The guideline committee based its recommendation only on the three studies that examined the actions of the ICD (antitachycardia pacing and shocks, and intermediate end points), but did not take into account the results of the clinical end point studies that demonstrated reductions in mortality (especially SCD) and morbidity following ingestion of Omega-3 fatty acids [1]. This is highly unconventional and, in the authors' personal opinion, wrong. It is hoped that the next edition of the guidelines will use a more conventional approach towards assessing strategies to prevent SCD.

In guidelines concerning treatment after a myocardial infarction or cardiovascular prevention, EPA plus DHA are recommended at 1 g/day in the form of fish or as dietary supplements [3,55].

Guidelines on the treatment of congestive heart failure have yet to incorporate EPA plus DHA [6]. Guidelines on the treatment of supraventricular arrhythmias do not mention Omega-3 fatty acids [56].

To the author's knowledge, no current cardiologic guideline recommends assessing the Omega-3 Index.

Omega-3 Index versus conventional risk markers

As demonstrated in the Physicians' Health Study, whole blood levels of Omega-3 fatty acids may be more informative in predicting SCD than levels of C-reactive protein, homocysteine, total cholesterol, LDL, high-density lipoprotein and triglycerides [8,24,57]. This is one of the lines of evidence underlying an ongoing project aimed at validating the Omega-3 Index (<4%) as a risk factor for SCD [VON SCHACKY C ET AL., UNPUBLISHED DATA]. In this project, the relative contributions of the parameters mentioned (plus the criteria for implantation of an ICD) to SCD risk can be calculated and compared with the predictive value of the Omega-3 Index. Moreover, the incremental informative value of the Omega-3 Index can be estimated.

A number of novel risk factors for coronary artery disease have been suggested; most of them, however, add little to the area under the curve of the c statistic or in statistical models reflecting clinical decision-making [58]. The Omega-3 Index was assessed as a risk factor for acute coronary syndrome in a case-control study of 768 patients with acute coronary syndrome and 768 matched controls. In a multivariate logistic regression analysis (matched for age, sex and race), which adjusted for differences in lipid levels, BMI, diabetes, hypertension, family history of coronary artery disease, personal history of myocardial infarction, alcohol consumption, smoking habits, use of statins, aspirin or antiplatelet drugs, and education level, the Omega-3 Index was inversely associated with odds for acute coronary syndrome [27]. For a one-unit increase in the Omega-3 Index, the odds ratio (OR) decreased by 23% (OR: 0.77; 95% CI: 0.72–0.84; $p < 0.0001$). Therefore, the Omega-3 Index appeared to provide an independent assessment of risk for acute coronary syndrome [27]. Preliminary data from this study indicate that the Omega-3 Index added incremental informative value to the Framingham Risk Score assessed in these acute coronary syndrome patients [59]. Ongoing investigations in collaboration with the Framingham group seek to expand on these results.

Biomarkers in heart failure were recently reviewed, and only the natriuretic peptides appeared useful [7]. The results of GISSI-HF make it likely that the Omega-3 Index may be a risk factor for the development of complications of congestive heart failure. Only after validation as a risk marker in congestive heart failure can the Omega-3 Index be compared with natriuretic peptides.

Strengths, limitations & future perspective

As mentioned previously, assessing the Omega-3 Index calls for strictly standardized methodology and quality assurance algorithms that are currently used in only three laboratories worldwide. This limits the availability of the test. However, pre-analytical stability of the required EDTA samples has been repeatedly demonstrated to be up to 7 days in the tubes in which the blood was taken, even while samples are being transported [11]. Moreover, a transportation method was developed and validated using a specially prepared filter paper, thus facilitating international transportation of samples [10]. When frozen at -80°C , samples have been demonstrated to be stable for at least 4 years [11]; analyses after 10 years were also plausible [VON SCHACKY C ET AL., UNPUBLISHED DATA].

Work is currently in progress to assess the Omega-3 Index in various populations, such as in Korea, Alaska and other parts of the world, and relate the results of these measurements to the incidence of SCD. Prospective evaluations are currently carried out to assess a low Omega-3 Index as a predictor for the development of coronary artery disease (in collaboration with the Framingham Group). Whether a low Omega-3 Index is a risk factor for clinical events in heart failure patients is also currently under investigation. Investigations will be expanded to other cardiologic disease entities. Moreover, the Omega-3 Index is increasingly being used as an end point in intervention studies with Omega-3 fatty acids [60]. Taken together, in the next few years, a large body of evidence will appear based on standardized measurements of the Omega-3 Index, adding precision to the value of the Omega-3 Index.

As yet, no intervention studies using the Omega-3 Index as an entry criterion for study participants have been published to the authors' knowledge. Since every individual thus far studied had a measurable Omega-3 Index, study participants with a high Omega-3 Index are less likely to show an effect of Omega-3 fatty-acid

supplementation. The opposite is probably true for persons with a low Omega-3 Index. Therefore, in order to more clearly delineate the mechanistic and therapeutic effects of Omega-3 fatty acids, study participant selection according to their Omega-3 Index is a logical consequence. The author is unaware of published studies to date using the Omega-3 Index as an entry and target criterion. It would be logical to recruit study participants based on a disease plus a defined Omega-3 Index, calibrate the intervention to a target Omega-3 Index and record the effect on the disease. In areas already known to respond to treatment with Omega-3 fatty acids, this approach would have to be compared with a fixed-dose approach.

Conclusion

The Omega-3 Index is a biomarker that best represents a person's status in terms of Omega-3 fatty acids in a number of circumstances and for a number of purposes. A low Omega-3 Index has been suggested as a risk factor for SCD, which is supported by mechanistic and intervention studies using Omega-3 fatty acids. Accumulating evidence from several lines indicates that a low Omega-3 Index may also be a risk factor for

nonfatal cardiovascular events, such as acute coronary syndrome, and for complications of congestive heart failure. Among the drawbacks of the Omega-3 Index is the necessity of a highly standardized methodology, which is currently implemented in only three laboratories worldwide. Ongoing research will add precision to the predictive value of the Omega-3 Index, a prerequisite for comparing it to other biomarkers.

Financial & competing interests disclosure

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Executive summary

- Sudden cardiac death (SCD) is a major cause of death in Western countries, whereas coronary artery disease and congestive heart failure are costly chronic diseases with a high mortality rate.

The Omega-3 Index

- The Omega-3 Index is defined as the sum of eicosapentaenoic plus docosahexaenoic acid in red blood cell fatty acids, determined using a standardized method. The Omega-3 Index represents a person's status in Omega-3 fatty acids.

Epidemiology

- The incidence of SCD, and less so of acute coronary syndrome, is inversely related to the Omega-3 Index. Less is known in congestive heart failure.

Mechanisms

- Omega-3 fatty acids have been demonstrated to be anti-arrhythmic, anti-inflammatory and anti-atherogenic.

Intervention studies with clinical end points

- Large-scale randomized, controlled intervention studies have demonstrated that Omega-3 fatty acids:
 - Reduce total mortality and SCD in persons with coronary artery disease.
 - Reduce total mortality and morbidity in persons with congestive heart failure.

Omega-3 fatty acids & the Omega-3 Index in current cardiologic guidelines

- Cardiac societies recommend increased intake of Omega-3 fatty acids for cardiovascular prevention and after a myocardial infarction. Data from intervention studies also support the increased intake of Omega-3 fatty acids for the prevention of SCD as well as complications of congestive heart failure.

Omega-3 Index versus conventional risk markers

- Data published so far indicate that the Omega-3 Index may be a powerful risk factor for SCD, less so for acute coronary syndrome. Whether the Omega-3 Index is a risk factor for complications of congestive heart failure is not known.

Strengths, limitations & future perspective

- Ongoing research will add precision to the value of the Omega-3 Index in terms of validity, incremental informative value and others. Present research topics are SCD, coronary artery disease and congestive heart failure.

Bibliography

Papers of special note have been highlighted as:
 ■ of interest

- 1 Zipes DP, Camm AJ, Borggrefe M *et al.*: ACC/AHA/ESC guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death – executive summary: a report of the American College of Cardiology/American Heart Association Task Force and the European Society for Cardiology Committee for Practice guidelines (writing committee to develop guidelines for management of patients with ventricular Arrhythmias and the prevention of sudden cardiac death). *Eur. Heart J.* 27, 2099–2140 (2006).
- 2 Anand SS, Islam S, Rosengren A *et al.*: INTERHEART investigators: risk factors for myocardial infarction in women and men: insights from the INTERHEART study. *Eur. Heart J.* 29, 932–940 (2008).
- 3 Fourth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts); Graham I, Atar D, Borch-Johnsen K *et al.*: European guidelines on cardiovascular disease prevention in clinical practice: executive summary. *Eur. Heart J.* 28, 2375–2414 (2007).
- 4 Smulders YM, Thijs A, Twisk JW: New cardiovascular risk determinants do exist and are clinically useful. *Eur. Heart J.* 29, 436–440 (2008).
- 5 Wang TJ: New cardiovascular risk factors exist, but are they clinically useful? *Eur. Heart J.* 29, 441–444 (2008).
- 6 Dickstein K, Cohen-Solal A, Filippatos G *et al.*: ESC guidelines for the diagnosis and treatment of acute and chronic heart failure (2008): the task force for the diagnosis and treatment of acute and chronic heart failure (2008) of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). *Eur. Heart J.* 29, 2388–2442 (2008).
- 7 Braunwald E: Biomarkers in heart failure. *N. Engl. J. Med.* 358, 2148–2159 (2008).
 - **Review on the development of biomarkers in heart failure.**
- 8 Harris WS, von Schacky C: The Omega-3 Index: a new risk factor for death from coronary heart disease? *Prev. Med.* 39, 212–220 (2004).
 - **Seminal paper for a new field, opened the door to considering the Omega-3 Index as a risk factor for sudden cardiac death.**
- 9 Harris WS, Poston WC, Haddock CK: Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. *Atherosclerosis* 193, 1–10 (2007).
- 10 Harris WS, von Schacky C, Park Y: Establishing uniform analytical methods for RBC Omega-3 fatty acids: an intercontinental pilot study. *ISSFAL* 78 (2008) (Abstract).
- 11 Harris WS: The Omega-3 Index as a risk factor for coronary heart disease. *Am. J. Clin. Nutr.* 87, S1997–S2002 (2008).
 - **Updates the field to mid-2007, discussing developments in the literature and methodological issues.**
- 12 von Schacky C, Fischer S, Weber PC: Long-term effects of dietary marine ω -3 fatty acids upon plasma and cellular lipids, platelet function and eicosanoid formation in humans. *J. Clin. Invest.* 76, 1626–1631 (1985).
- 13 von Schacky C, Fahrner C, Fischer S: Catabolism of leukotriene B5 in humans. *J. Lipid Res.* 31, 1831–1838 (1990).
- 14 Kaminski WE, Jendraschak E, Kiefl R, von Schacky C: Dietary Omega-3 fatty acids lower levels of PDGF mRNA in human mononuclear cells. *Blood* 81, 1871–1879 (1993).
- 15 von Schacky C, Weber PC: Metabolism and effects on platelet function of the purified eicosapentaenoic and docosahexaenoic acids in humans. *J. Clin. Invest.* 76, 2446–2450 (1985).
- 16 Harris WS, Sands SA, Windsor SL, Ali HA *et al.*: Omega-3 fatty acids in cardiac biopsies from heart transplantation patients: correlation with erythrocytes and response to supplementation. *Circulation* 110, 1645–1649 (2004).
- 17 Metcalf RG, James MJ, Gibson RA *et al.*: Effects of fish-oil supplementation on myocardial fatty acids in humans. *Am. J. Clin. Nutr.* 85, 1222–1228 (2007).
- 18 Sands SA, Reid KJ, Windsor SL, Harris WS: The impact of age, body mass index, and fish intake on the EPA and DHA content of human erythrocytes. *Lipids* 40, 343–347 (2005).
- 19 Itomura M, Fujioka S, Hamazaki K *et al.*: Factors influencing EPA+DHA levels in red blood cells in Japan. *In vivo* 22, 131–135 (2008).
- 20 Plourde M, Cunnane SC: Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements. *Appl. Physiol. Nutr. Metab.* 32, 619–634 (2007).
- 21 Friedman AN, Moe SM, Perkins SM, Li Y, Watkins BA: Fish consumption and Omega-3 fatty acid status and determinants in long-term hemodialysis. *Am. J. Kidney Dis.* 47, 1064–1071 (2006).
- 22 Iso H, Kobayashi M, Ishihara J *et al.*: JPHC Study Group. Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: the Japan Public Health Center-Based (JPHC) Study Cohort I. *Circulation* 113, 195–202 (2006).
- 23 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.* 275, 836–837 (1995).
- 24 Albert CM, Campos H, Stampfer MJ: Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N. Engl. J. Med.* 346, 1113–1118 (2002).
- 25 Marchioli R, Barzi F, Bomba E *et al.*: On behalf of the GISSI Prevenzione Investigators. 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 Miocardio (GISSI)-Prevenzione. *Circulation* 105, 1897–1903 (2002).
- 26 Yokoyama M, Origasa H, Matsuzaki M *et al.*: Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet* 369, 1090–1098 (2007).
- 27 Block RC, Harris WS, Reid KJ, Sands SA, Spertus JA: EPA and DHA in blood cell membranes from acute coronary syndrome patients and controls. *Atherosclerosis* 197, 821–828 (2008).
 - **First paper describing the use of the Omega-3 Index for coronary risk prediction.**
- 28 Sun Q, Ma J, Campos H *et al.*: Blood concentrations of individual long-chain n-3 fatty acids and risk of nonfatal myocardial infarction. *Am. J. Clin. Nutr.* 88, 216–223 (2008).
- 29 Yamagishi K, Iso H, Date C *et al.*: Fish, Omega-3 polyunsaturated fatty acids, and mortality from cardiovascular diseases in a nation-wide, community-based cohort of Japanese men and women. *J. Am. Coll. Card.* 52, 988–996 (2008).
- 30 Leaf A, Xiao YF, Kang JX, Billman GE: Prevention of sudden cardiac death by n-3 polyunsaturated fatty acids. *Pharmacol. Ther.* 98, 355–377 (2003).
- 31 London B, Albert C, Anderson ME *et al.*: Omega-3 Fatty acids and cardiac arrhythmias: prior studies and recommendations for future research: a report from the National Heart, Lung, and Blood Institute and Office of Dietary Supplements Omega-3 fatty acids and their role in cardiac arrhythmogenesis workshop. *Circulation* 116, E320–E335 (2007).

- 32 Schrepf R, Limmert T, Claus Weber P, Theisen K, Sellmayer A: Immediate effects of n-3 fatty acid infusion on the induction of sustained ventricular tachycardia. *Lancet* 363, 1441–1442 (2004).
- 33 Metcalf RG, Sanders P, James MJ, Cleland LG, Young GD: Effect of dietary n-3 polyunsaturated fatty acids on the inducibility of ventricular tachycardia in patients with ischemic cardiomyopathy. *Am. J. Cardiol.* 101, 758–761 (2008).
- 34 O'Keefe JH Jr, Abuissa H, Sastre A, Steinhaus DM, Harris WS: Effects of Omega-3 fatty acids on resting heart rate, heart rate recovery after exercise, and heart rate variability in men with healed myocardial infarctions and depressed ejection fractions. *Am. J. Cardiol.* 97, 1127–1130 (2006).
- 35 Christensen JH, Schmidt EB: Autonomic nervous system, heart rate variability and n-3 fatty acids. *J. Cardiovasc. Med. (Hagerstown)* 8(Suppl. 1), S19–S22 (2007).
- 36 Calò L, Bianconi L, Colivicchi F *et al.*: n-3 fatty acids for the prevention of atrial fibrillation after coronary artery bypass surgery: a randomized, controlled trial. *J. Am. Coll. Cardiol.* 45, 1723–1728 (2005).
- 37 von Schacky C: A review of Omega-3 ethyl esters for cardiovascular prevention and treatment of increased blood triglyceride levels. *Vasc. Health Risk Manag.* 2, 251–262 (2006).
- 38 Yaqoob P, Calder PC: Fatty acids and immune function: new insights into mechanisms. *Br. J. Nutr.* 98(Suppl. 1), S41–S45 (2007).
- 39 De Caterina R, Zampoll A: Omega-3 fatty acids, atherogenesis, and endothelial activation. *J. Cardiovasc. Med. (Hagerstown)* 8(Suppl. 1), S11–S14 (2007).
- 40 von Schacky C: n-3 PUFA in CVD: influence of cytokine polymorphism. *Proc. Nutr. Soc.* 66, 166–170 (2007).
- 41 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* 361, 477–485 (2003).
- 42 von Schacky C, Harris WS: Cardiovascular benefits of Omega-3 fatty acids. *Cardiovasc. Res.* 72, 310–315 (2007).
- 43 Tavazzi L, Tognoni G, Franzosi MG *et al.*: Rationale and design of the GISSI heart failure trial: a large trial to assess the effects of n-3 polyunsaturated fatty acids and rosuvastatin in symptomatic congestive heart failure. *Eur. J. Heart Fail.* 6, 635–641 (2004).
- 44 Burr ML, Fehily AM, Gilbert JF *et al.*: Effects of changes in fat, fish, and fibre intakes on death and myocardial infarction diet and reinfarction trial (DART). *Lancet* 2(8666), 757–761 (1989).
- 45 Burr ML, Ashfield-Watt PAL, Dunstan FDJ *et al.*: Lack of benefit of dietary advice to men with angina, results of a controlled trial. *Eur. J. Clin. Nutr.* 57, 193–200 (2003).
- 46 GISSI-HF investigators: Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomized, double-blind, placebo-controlled trial. *Lancet* 372, 1223–1230 (2008).
- 47 Mozaffarian D, Rimm EB: Fish intake, contaminants, and human health: evaluating the risks and the benefits. *J. Am. Med. Assoc.* 296, 1885–1899 (2006).
- 48 Leaf A, Albert CM, Josephson M *et al.*: For the fatty acid antiarrhythmia trial investigators. Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. *Circulation* 112, 2762–2768 (2005).
- 49 Raitt MH, Connor WE, Morris C *et al.*: Fish oil supplementation and risk of ventricular tachycardia and ventricular fibrillation in patients with implantable defibrillators. A randomized controlled trial. *J. Am. Med. Assoc.* 293, 2884–2891 (2005).
- 50 Brouwer IA, Zock PL, Camm AJ *et al.*: SOFA Study Group. Effect of fish oil on ventricular tachyarrhythmia and death in patients with implantable cardioverter defibrillators: the Study on Omega-3 Fatty Acids and Ventricular Arrhythmia (SOFA) randomized trial. *JAMA* 295, 2613–2619 (2006).
- 51 Jenkins DJ, Josse AR, Beyene J *et al.*: Fish-oil supplementation in patients with implantable cardioverter defibrillators: a meta-analysis. *CMAJ* 178, 157–164 (2008).
- 52 Wilhelm M, Tobias R, Asskali F *et al.*: Red blood cell Omega-3 fatty acids and the risk of ventricular arrhythmias in patients with heart failure. *Am. Heart J.* 155, 971–977 (2008).
- 53 Aarsetøy H, Pönitz V, Nilsen OB, Grundt H, Harris WS, Nilsen DW: Low levels of cellular Omega-3 increase the risk of ventricular fibrillation during the acute ischaemic phase of a myocardial infarction. *Resuscitation* 78, 258–264 (2008).
- 54 Brouwer IA, Raitt MH, Dullemeyer C *et al.*: Effect of fish oil on life-threatening arrhythmias in patients with implantable cardioverter defibrillators: a combined analysis of 3 trials. *ISSFAL* 58 (2008) (Abstract).
- 55 Van der Werf F, Ardissino D, Betriu A *et al.*: Task force on the management of acute myocardial infarction of the European Society of Cardiology. Management of acute myocardial infarction in patients presenting with ST-segment elevation. *Eur. Heart J.* 24, 28–66 (2003).
- 56 Fuster V, Rydén LE, Cannon DS: ACC/AHA/ESC 2006 guidelines for the management of patients with atrial fibrillation – executive summary: a report of the American College of Cardiology/American Heart Association task force on practice guidelines and the European Society of Cardiology Committee for practice guidelines (writing committee to revise the 2001 guidelines for the management of patients with atrial fibrillation). *Eur. Heart J.* 27, 1979–2030 (2006).
- 57 Albert CM, Ma J, Rifai N, Stampfer MJ, Ridker PM: Prospective study of C-reactive protein, homocysteine, and plasma lipid levels as predictors of sudden cardiac death. *Circulation* 105, 2595–2599 (2002).
- 58 Cook NR: Statistical evaluation of prognostic versus diagnostic models: beyond the ROC curve. *Clin. Chem.* 54, 17–23 (2008).
- 59 Shearer GC, Pottala JV, Harris WS: Blood cell fatty acid profiles discriminate acute coronary syndrome case better than standard cardiovascular risk factors. *ISSFAL* 179 (2008) (Abstract).
- 60 Harris WS, Lemke SL, Hansen SN *et al.*: Stearidonic acid-enriched soybean oil increased the Omega-3 Index, an emerging cardiovascular risk marker. *Lipids* 43, 805–811 (2008).