

## Exploring newer cardioprotective strategies: $\omega$ -3 fatty acids in perspective

Matteo Nicola Dario Di Minno<sup>1</sup>; Elena Tremoli<sup>2</sup>; Antonella Tufano<sup>1</sup>; Anna Russolillo<sup>1</sup>; Roberta Lupoli<sup>1</sup>; Giovanni Di Minno<sup>1</sup>

<sup>1</sup>Department of Experimental and Clinical Medicine, "Federico II" University, Naples, Italy; <sup>2</sup>Department of Pharmacological Sciences, Milan University, Milan, Italy

### Summary

In the 1980s, observational retrospective studies showed an inverse relation between coronary heart disease (CHD) and consumption of fish containing fatty acids that belong to the omega ( $\omega$ )-3 family. Large case-control studies and prospective intervention trials consistently showed that  $\omega$ -3 fatty acids supplementation lowers fatal myocardial infarction (MI) and sudden cardiac death. By analysing the strengths of the results of individual studies and how the meta-analyses agree with them, putting together relevant backgrounds, and identifying open questions, the following findings/directions emerge. (i) Dietary and non-dietary intake of  $\omega$ -3 fatty acids reduces overall mortality, mortality due to MI, and sudden death in patients with CHD; (ii) Fish oil consumption directly or indirectly affects cardiac electrophysiology. Fish oil reduces heart rate, a major risk factor for sudden death; (iii) Among patients with implantable cardioverter defibrillators,  $\omega$ -3 fatty acids do

not reduce the risk of ventricular tachycardia/ventricular fibrillation and may actually be pro-arrhythmic; (iv) The consumption of  $\omega$ -3 fatty acids leads to a 10–33% net decrease of triglyceride levels. The effect is dose-dependent, larger in studies with higher mean baseline triglyceride levels, and consistent in different populations (healthy people, people with dyslipidaemia, diabetes, or known cardiovascular risk factors); (v) Outcomes for which a small beneficial effect  $\omega$ -3 fatty acids is found include blood pressure (about 2 mmHg reduction), re-stenosis rates after coronary angioplasty (14% reduction), and exercise tolerance testing. Major experimental data provide strength (biological plausibility) for these findings, and define directions for newer clinical trials with  $\omega$ -3 fatty acids.

### Keywords

Prevention, acute myocardial infarction, nutrition

### Correspondence to:

Matteo Nicola Dario Di Minno  
via S. Pansini 5, 80131 Naples, Italy  
Tel./Fax: +39 0 81 7462060  
E-mail: dario.diminno@hotmail.it

Received: January 4, 2010

Accepted after major revision: May 29, 2010

Prepublished online: August 30, 2010

doi:10.1160/TH10-01-0008

Thromb Haemost 2010; 104: 664–680

## Introduction

Modern nutrition emphasises health benefits of eating sufficient levels of the very long chain polyunsaturated fatty acids that belong to the omega ( $\omega$ )-3 family: eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA], and  $\alpha$ -linolenic acid [ALA]. Such bioactive compounds, whose predominant sources are fish and vegetable oils, are not convertible and have very different biochemical roles.

Clinical data emphasise the efficacy of  $\omega$ -3 fatty acids supplementation over and above the usual GISSI strategy (aspirin, beta-blockers, nitrate, ACE-inhibitors, statins) for the prevention of coronary heart disease (CHD) and ischaemic events in survivors of a myocardial infarction (MI) (1). However, in the therapeutic strategy of such patients, the additional benefits  $\omega$ -3 fatty acids are presently poorly understood. By analysing the strengths of the findings and how the meta-analyses agree with the individuals reports on the prevention of CHD/sudden death by  $\omega$ -3 fatty acids; putting together relevant backgrounds; examining the evidence and identifying inconsistencies and open questions of single studies, we report here that the utility of  $\omega$ -3 fatty acids presently appears to be largely due to their triglyceride lowering capability and to their anti-arrhythmic benefits. We also report that, while the

mechanism by which triglycerides promote atherogenicity may be difficult to separate from its synergistic effect with lipoprotein and other cardiovascular variables, the additional benefits of  $\omega$ -3 fatty acids do not include the lowering of total or LDL cholesterol. Potential directions to be pursued in secondary care and post-MI patients are also discussed.

## Methods

We have approached the issue of  $\omega$ -3 fatty acids and their relevance in clinical practice, with emphasis on three comprehensively reviewed relevant issues. In all cases, a series of key terms has been identified for an appropriate search strategy:

- The actual strength of the evidence of the association of  $\omega$ -3 fatty acids and prevention/treatment of coronary heart disease (CHD);
- The actual strength of the evidence of the association of  $\omega$ -3 fatty acids and the prevention/treatment of (ventricular) arrhythmias;

- The biological plausibility and potential mechanisms of the benefits of  $\omega$ -3 fatty acids use in cardioprotection (including prevention/treatment of CHD and of arrhythmias).

As to the latter issue, it has been thoroughly reviewed in a series of papers concerning antiarrhythmic/antifibrillatory effects of  $\omega$ -3 fatty acids. As to the former issues, the analysis has been based on epidemiological and intervention studies published up to 2010.

## Search strategy and evidence acquisition

Using the key terms of:  $\omega$ -3 fatty acids, cardioprotection;  $\omega$ -3 fatty acids, mechanisms; plasma/cellular  $\omega$ -3 fatty acids, risk of CHD;  $\omega$ -3 fatty acids, randomised clinical studies (RCTs);  $\omega$ -3 fatty acids, prevention of CHD, observational studies;  $\omega$ -3 fatty acids, prevention of CHD, retrospective studies;  $\omega$ -3 fatty acids, primary prevention of CHD, RCTs;  $\omega$ -3 fatty acids, secondary prevention of CHD, RCTs;  $\omega$ -3 fatty acids, prevention of arrhythmias, patients with implantable cardioverter defibrillators (ICDs), RCTs;  $\omega$ -3 fatty acids, prevention of cardiovascular death, meta-analyses of RCTs; we searched in the Medline and Cochrane databases as well as the trial register of the Cochrane group to identify studies published in the area up to March 2010. For an in-depth scrutiny of the information provided by the individual papers, their references were also critically reviewed. In each case and for each report, in addition to clinical relevance, emphasis has been put and the inherent potential limitations of the individual analyses. We have found the following evidence synthesis.

## $\omega$ -3 fatty acids and prevention of CHD/sudden death

### Observational, retrospective studies

In the early 1960s and 1970s, dietary advice to prevent recurrence of CHD was inconclusive. This was likely to be due to the small ( $\approx$ 500) number of subjects examined. Moreover, in these studies, attempts to lower plasma cholesterol levels in survivors of MI were based on diets lower in fats or with high polyunsaturated/saturated ratios. Excessive consumption of foods containing  $\omega$ -3 fatty acids increases total fat intake. In view of this, the intake of saturated fats was decreased and that of unsaturated was increased. Thus, the study design and the sample size may have been inadequate to address the issue (1). In the 1980s, three retrospective studies (2–4) showed an inverse relation between fish consumption and CHD mortality. In parallel, fish fats containing EPA were shown to impair platelet aggregation and thromboxane formation, two major events in arterial thrombosis (5). The combined data fostered newer studies in the field.

### Prospective (nested case-control) studies

A series of nested case-control studies showed an inverse association between low plasma/cellular EPA/DHA and risk of MI, sudden cardiac death, or total cardiovascular mortality (► **Table 1**).

A strong inverse correlation between fish consumption and total mortality as well as sudden death was first found in the *Physicians' Health Study (PHS)* (6, 7). A total of 20,551 US male physicians 40 to 84 years of age and free of MI, cerebrovascular disease, and cancer at baseline were evaluated, after 11 years follow-up, for the incidence of sudden cardiac death (6). Dietary fish intake was associated with a reduced risk of total mortality as well as of sudden death, with an apparent threshold effect at a consumption level of one fish meal per week ( $p$  for trend=0.03). For men who consumed fish at least once per week, the relative risk (RR) of sudden death was 0.48 ( $p=0.04$ ) compared with men who consumed fish less than monthly. Neither dietary fish consumption nor  $\omega$ -3 fatty acid intake was associated with a reduced risk of total MI, non-sudden cardiac death, or total cardiovascular mortality. The extended follow-up (17 years) of the study (7) showed that  $\omega$ -3 fatty acids found in fish are strongly associated with a reduced risk of sudden death among men without evidence of prior cardiovascular disease (CVD). Baseline blood levels of long-chain  $\omega$ -3 fatty acids were inversely related to the risk of sudden death both before ( $p$  for trend = 0.004) and after ( $p$  for trend = 0.007) adjustment for potential confounders.

In the *Nurses Health Study (NHS)* (8), a cohort study in 84,688 female nurses aged 34 to 59 years and free from CVD and cancer at baseline (1980), dietary consumption and follow-up data were collected with validated questionnaires (24). Compared with women who seldom ate fish (<1 serving/month), those with a higher intake of fish had a lower risk of CHD. Similarly, women with a higher intake of  $\omega$ -3 fatty acids had a lower risk of CHD ( $p<0.001$  for trend) across quintiles of intake. For fish intake and  $\omega$ -3 fatty acids, the inverse association appeared to be stronger for CHD deaths than for non-fatal MI. A higher consumption of fish and  $\omega$ -3 fatty acids was associated with a 30% reduction in the risk of major coronary events. This reduction increased when only fatal events (coronary deaths) were considered.

Employing dietary interviews and local food tables, Oomen et al. (9) have evaluated the association between total, lean, and fatty fish consumption and the risk of CHD mortality in 1,088 Finnish, 1,097 Italian, and 553 Dutch men participants in the Seven Countries Study, who were aged 50–69 years and free of CHD. After 20 years of follow-up, 242 (22.2%) men in Finland, 116 (10.6%) men in Italy, and 105 (19.0%) men in the Netherlands had died of CHD. After adjustment for confounders, while lean fish consumption was not associated with CHD mortality in any of the three Countries, fatty fish was associated with lower CHD mortality, pooled RR for fatty fish consumers being 0.66.

The association of plasma phospholipid concentrations of DHA, EPA, and ALA as biomarkers of intake with the risk of incident fatal CHD and incident non-fatal MI in older adults was investigated in the Cardiovascular Health Study, a cohort study of adults aged  $\geq$ 65 years (10). Plasma phospholipid concentrations of

**Table 1:**  $\omega$ -3 fatty acids and cardioprotection: nested case-control studies in prevention of CHD/sudden death.

Author (ref)	Study description/results/conclusion/comments
Albert CM (6)	<p><b>Study description:</b> The Physicians' Health Study (PHS) evaluated the incidence of sudden cardiac death (death within 1 hour of symptom onset) in a total of 20,551 US male physicians 40 to 84 years of age, free of MI, cerebrovascular disease, and cancer at baseline, who completed a semiquantitative food frequency questionnaire on fish consumption.</p> <p><b>Results:</b> There were 133 sudden deaths over the course of the study. After controlling for age, randomised aspirin and <math>\beta</math>-carotene assignment and coronary risk factors, dietary fish intake was associated with a reduced risk of sudden death, with an apparent threshold effect at a consumption level of one fish meal per week (<math>p</math> for trend=0.03). For men who consumed fish at least once per week, the multivariate relative risk (RR) of sudden death was 0.48 (95% CI, 0.24–0.96; <math>p</math>=0.04) compared with men who consumed fish less than monthly. Estimated dietary <math>\omega</math>-3 fatty acid intake from seafood was also associated with a reduced risk of sudden death, without a significant trend across increasing categories of intake. Neither dietary fish consumption nor <math>\omega</math>-3 fatty acid intake was associated with a reduced risk of total MI, non-sudden cardiac death, or total cardiovascular mortality and total mortality.</p> <p><b>Conclusion:</b> Consumption of fish at least once per week may reduce the risk of sudden cardiac death in men.</p>
Albert CM (7)	<p><b>Study description:</b> A nested case-control analysis among apparently healthy men who were followed for up to 17 years in the PHS (see above). The fatty-acid composition of previously collected blood (from 94 men in whom sudden death occurred as the first manifestation of cardiovascular disease and from 184 controls matched for age and smoking status) was analysed by gas-liquid chromatography.</p> <p><b>Results:</b> Baseline blood levels of <math>\omega</math>-3 fatty acids were inversely related to the risk of sudden death both before (<math>p</math> for trend = 0.004) and after adjustment for potential confounders (<math>p</math> for trend = 0.007). As compared with men whose blood levels of long-chain <math>\omega</math>-3 fatty acids were in the lowest quartile, the RR of sudden death was significantly lower among men with levels in the third quartile (adjusted RR, 0.28; 95% CI, 0.09 to 0.87) and the fourth quartile (adjusted RR, 0.19; 95% CI, 0.05 to 0.71).</p> <p><b>Conclusion:</b> <math>\omega</math>-3 fatty acids found in fish are strongly associated with a reduced risk of sudden death among men without evidence of prior cardiovascular disease.</p>
Hu FB (8)	<p><b>Study description:</b> Incident non-fatal myocardial infarction (MI) and coronary heart disease (CHD) deaths were evaluated in the Nurses Health Study (NHS), a cohort study in 84,688 female nurses aged 34 to 59 years, free from cardiovascular disease and cancer at baseline (1980), in whom dietary consumption and follow-up data were compared from validated questionnaires.</p> <p><b>Results:</b> During 16 years of follow-up, there were 1513 incident cases of CHD (484 CHD deaths and 1,029 non-fatal MIs). Compared with women who seldom ate fish (&lt;1 per month), those with a higher intake of fish had a lower risk of CHD. After adjustment for age, smoking, and other cardiovascular risk factors, the RRs of CHD were 0.79 (95% CI, 0.64–0.97) for fish consumption 1–3 times per month, 0.71 (95% CI, 0.58–0.87) for once per week, 0.69 (95% CI, 0.55–0.88) for 2–4 times per week, and 0.66 (95% CI, 0.50–0.89) for five or more times per week (<math>p</math> for trend = 0.001). Women with a higher intake of <math>\omega</math>-3 fatty acids had a lower risk of CHD, with RRs of 1.0, 0.93, 0.78, 0.68, and 0.67 (<math>p</math>&lt;0.001 for trend) across quintiles of intake. For fish intake and <math>\omega</math>-3 fatty acids, the inverse association was stronger for CHD deaths (multivariate RR for fish consumption five times per week, 0.55 [95% CI, 0.33–0.90] for CHD deaths vs 0.73 [0.51–1.04]) than for non-fatal MI.</p> <p><b>Conclusion:</b> A higher consumption of fish and <math>\omega</math>-3 fatty acids is associated with a 30% reduction in the risk of major coronary events especially in people at high risk of CHD.</p>
Oomen et al. (9)	<p><b>Study description:</b> Analysis of the association between total, lean, and fatty fish consumption (dietary interviews) and the risk of CHD mortality in 1,088 Finnish, 1,097 Italian, and 553 Dutch men participants in the Seven Countries Study who were aged 50–69 years and free of CHD around 1970.</p> <p><b>Results:</b> After 20 years of follow-up, 242 (22.2%) men in Finland, 116 (10.6%) men in Italy, and 105 (19.0%) men in the Netherlands had died of CHD. Cox proportional hazards analysis showed no association between <u>total fish consumption</u> and CHD mortality. After adjustments for age, body mass index, smoking, energy intake, and relevant dietary variables, the pooled RR for the highest quartile of total fish compared with no fish consumption in the three countries was 1.08 (95% CI: 0.76, 1.53). <u>Lean fish consumption</u> was not associated with CHD mortality in any country. In contrast, <u>fatty fish</u> compared with non-fatty-fish consumption was associated with lower CHD mortality; the adjusted, pooled RR for fatty fish consumers was 0.66 (95% CI: 0.49, 0.90).</p> <p><b>Conclusion:</b> Fatty fish, rich in <math>\omega</math>-3 polyunsaturated fatty acids, protects from CHD mortality.</p>
Lemaitre RN (10)	<p><b>Study description:</b> The risk of incident fatal ischaemic heart disease and incident nonfatal MI in older adults was investigated among participants to the Cardiovascular Health Study, a cohort study of adults aged <math>\geq</math>65 years. Such risk was related with plasma phospholipid concentrations of DHA, EPA, and <math>\alpha</math>-linolenic acid. Plasma phospholipid concentrations of <math>\omega</math>-3 polyunsaturated fatty acids were measured in blood samples drawn approximately two years before the event.</p> <p><b>Results:</b> Cases experienced incident fatal MI, ischaemic heart disease death (<math>n</math>=54) and incident non-fatal MI (<math>n</math>=125). Matched controls were randomly selected (<math>n</math> = 179). A higher concentration of combined DHA and EPA was associated with a lower risk of fatal ischaemic heart disease, and a higher concentration of <math>\alpha</math>-linolenic acid with a tendency to lower risk, after adjustment for risk factors [OR: 0.32 (95% CI: 0.13, 0.78; <math>p</math> = 0.01) and 0.52 (0.24, 1.15; <math>p</math> = 0.1), respectively].</p> <p><b>Conclusion:</b> Higher combined dietary intake of DHA and EPA, and possibly <math>\omega</math>-linolenic acid, may lower the risk of fatal ischaemic heart disease in older adults.</p>
Simon JA (11)	<p><b>Study description:</b> In the Usual Care group of the Multiple Risk Factor Intervention Trial (MRFIT), DHA in plasma phospholipids was measured in stored serum samples from 94 men who subsequently developed CHD and in 94 matched controls who did not.</p> <p><b>Results:</b> In a multivariate model controlled for the ratio of HDL to LDL cholesterol, the concentration of DHA was inversely associated with CHD risk (OR: 0.57; 95% CI: 0.36, 0.90).</p>

$\omega$ -3 fatty acids were measured in blood samples drawn approximately two years before the event. After adjustment for risk factors, a higher concentration of combined DHA and EPA was associated with a lower risk of fatal ischaemic heart disease ( $p=0.01$ ). Since no association was found with non-fatal MI, the conclusion was that the data are consistent with possible antiarrhythmic effects of these fatty acids.

Finally, in the Usual Care group of the Multiple Risk Factor Intervention Trial, DHA in plasma phospholipids was measured in stored serum samples from 94 men who subsequently developed CHD (11). The concentration of DHA was inversely associated with CHD risk (odds ratio [OR]: 0.57; 95% confidence interval [CI]: 0.36, 0.90).

### RCTs in secondary prevention of CHD/sudden death

Prospective studies consistently showed that  $\omega$ -3 fatty acids supplementation lowers the risk of total MI, sudden cardiac death, or total cardiovascular mortality (► **Table 2**).

The *Diet and Reinfarction Trial* (DART) (12) randomised 2,033 men, who had recovered from a MI, to advice to eat at least two portions (200–400 g) of oily fish per week, to reduce their total fat and saturated fat intake, or to increase their intake of cereal fibre. The equivalent weekly consumption of EPA was about 2.5 g (300 g of oily fish). At two years, about 22% of patients who could not tolerate the recommended amount of fish, took fish oils as a partial or total substitute. At this time, when compared with other groups, about 30% fewer people in the fish advice group died, a reduction that was due to less CHD deaths (7.7% vs. 11.4%). There was no significant reduction in CHD events overall (CHD death or non-fatal MI) probably because the reduction in CHD death was counterbalanced by an increase in non-fatal MI.

The *Lyon Heart study* was a prospective, randomised single-blinded multicentre trial whose primary endpoints were death and non-fatal MI (13). Of the 600 survivors enlisted, half adopted a prudent diet and as many adopted a Mediterranean-type of diet, with more bread, vegetables, fish, fresh fruit and olive oil (or margarine enriched in ALA for subjects who did not like olive oil). At the two-year follow-up, there were 16 cardiovascular deaths in the control group and three in the experimental one. Similar to the DART study, the beneficial effect of this diet occurred early after randomisation and was not associated with changes in plasma cholesterol or HDL-cholesterol levels. After correction for other factors, a surprisingly high (76%) reduction in the risk of cardiac death was found during the observation period. The protective effect of the experimental diet was associated with enhanced plasma levels of EPA and ALA. In keeping with this, arachidonic acid was significantly reduced in subjects who had received the experimental diet. However, variables other than EPA were affected by the Mediterranean type of diet. The antioxidant vitamin E, was significantly increased while granulocyte count was lowered.

The *Gissi Prevenzione trial* (14) was a prospective, multicentre, open labelled trial, in which 11,324 recent (<3 months) survivors

of a first MI, were randomly assigned to receive, in addition to the usual GISSI strategy (aspirin, beta-blockers, nitrate, ACE-inhibitors, statins), a supplementation of  $\omega$ -3 fatty acids (1 g/day corresponding to 850 mg of a mixture of EPA+ DHA in a 1:1.4 ratio), vitamin E (300 mg/day), or the combination of  $\omega$ -3 fatty acids + vitamin E. While the treatment with vitamin E was not effective as to event-free survival ( $p=0.07$ ), the treatment with  $\omega$ -3 fatty acids significantly reduced the risk of new ischaemic events ( $p=0.009$ ). When the data were stratified as to individual endpoints, cardiovascular death (-30%), CHD death (-35%), total death (-20%) and sudden death (-45%) were all significantly reduced by the supplementation. Similar to the DART and the Lyon Studies, the beneficial effect of the supplementation occurred early after randomisation (3 months for total mortality and 4 months for sudden death) (15). As to the prevention of sudden death, it could be also documented in high-risk individuals such as those with systolic dysfunction (16). In 9,630 such patients, left ventricular systolic function was monitored by echocardiography (16).  $\omega$ -3 fatty acids treatment reduced significantly the total mortality (24%,  $p=0.02$ ) in those with ventricular systolic dysfunction (ejection fraction <40%), and non significantly (19%) in those without ventricular systolic dysfunction (ejection fraction >50). In particular, patients with ejection fractions <40%, had a four-fold greater benefit from  $\omega$ -3 fatty acids treatment in the reduction of sudden death compared to those with ejection fractions >50%.

The *DART-2 trial* (17, 18), included 3,114 men with stable angina and showed an excess of sudden and total cardiac deaths in these subjects. The excess was maximal in participants taking fish oil capsules rather than eating oily fish. This trial was described as 'well designed but sub-optimally conducted or reported' (19). Due to lack of funding, it was stopped midway during the trial, and it was unclear if the participants continued to adhere to the advice to increase their fish consumption or to take their fish oil supplements.

The objective of the *Nilsen study* (20) was to evaluate the effect of a high-dose ethylester concentrate of  $\omega$ -3 fatty acids administered early after an acute MI. No significant difference in prognosis was observed between groups for single or combined cardiac events. The authors concluded that no clinical benefit of a high-dose concentrate of  $\omega$ -3 fatty acids compared with corn oil was found despite a favorable effect on serum lipids. The lack of benefit in this setting has been thought to be due to the high background intake of fish oils in the Norwegian study participants, which could have masked the treatment effects.

Data from the *JELIS Study* (21) do not support this formulation. In the latter (21), in 18,645 patients with a total cholesterol of 6.5 mM or greater, recruited from local physicians throughout Japan, the hypothesis was tested that long-term use of EPA is effective for prevention of major coronary events. Out of them 14,981 were subject to primary prevention and 3,664 to secondary prevention. This study population was likely to have high levels of serum EPA because of the high fish consumption in the Japanese population (22). The patients were randomly assigned to receive either 1,800 mg of EPA daily plus statin (EPA group;  $n=9,326$ ) or statin only (controls;  $n=9,319$ ). At mean follow-up of 4.6 years, the authors documented the primary endpoint in 262 (2.8%) patients

Table 2:  $\omega$ -3 fatty acids and cardioprotection: RCTs in secondary prevention of CHD/sudden death.

Author (ref)	Study description/results/conclusion/comments
Burr ML et al. (12)	<p><b>Study description:</b> The Diet and Reinfarction (DART) Study randomised 2,033 survivors of a MI, advised to eat at least two portions (200–400 g) of oily fish per week; to reduce their total fat and saturated fat intake, or to increase their intake of cereal fibre.</p> <p><b>Results:</b> Two years later, about 22% of patients who could not tolerate the recommended amount of fish, took fish oils as a partial or total substitute. At this time, about 30% fewer people in the fish advice group died than those in other groups (9.3% vs. 12.8%; RR 0.71, 95% CI 0.54–0.93), which was due to a reduction in CHD death (7.7% vs. 11.4%). There was no significant overall reduction in CHD events probably because the reduction in CHD death was counterbalanced by an increase in nonfatal MI. The equivalent weekly consumption of EPA was about 2.5 g (300 g of oily fish).</p> <p><b>Conclusion:</b> This study first supports the possibility of a protective role of <math>\omega</math>-3 fatty acids in the secondary prevention of MI.</p>
de Lorgeril M et al. (13)	<p><b>Study description:</b> The Lyon Heart study was a prospective, randomised single-blinded trial whose primary endpoints were death and non-fatal MI. Of the 600 survivors enlisted, half adopted a prudent diet and as many adopted a Mediterranean-type of diet, with more bread, vegetables, fish, fresh fruit and olive oil (or margarine enriched in ALA for subjects who did not like olive oil). The subjects were analysed for five years, starting six months after the ischaemic event.</p> <p><b>Results:</b> At the two-year follow-up, there were 16 cardiovascular deaths in the control group and three in the experimental one. The overall mortality was 20% in the control group and 8% in the experimental group. After correction for other factors, a 76% reduction in the risk of cardiac death was found during the observation period.</p> <p><b>Conclusion:</b> Similar to the DART study, the beneficial effect of this diet occurred early after randomisation and was not associated with changes in plasma cholesterol or HDL-cholesterol.</p> <p><b>Comments:</b> The protective effect of the experimental diet was associated with enhanced plasma levels of EPA and ALA. AA was significantly reduced in subjects who had received the experimental diet, in whom vitamin E was significantly increased and granulocyte count was lowered.</p>
GISSI Prevenzione (14)	<p><b>Study description:</b> Prospective, multicentric, open labelled trial with a factorial design, in which 11,324 recent (&lt;3 months) survivors of a first MI aged 50–80 years, were randomly assigned to receive, in addition to the usual GISSI strategy (aspirin, beta-blockers, nitrate, ACE-I, statins), a supplementation of <math>\omega</math>-3 fatty acids (1 g/day corresponding to 850 mg of a mixture of EPA+ DHA in a 1:1.4 ratio), vitamin E (300 mg/day), or the combination of <math>\omega</math>-3 fatty acids + vitamin E. The clinical assessment and food questionnaires were obtained at 6, 12, 18, 30 and 42 months. Primary end-points were all cause death, CV death, non-fatal MI and stroke.</p> <p><b>Results:</b> The treatment with <math>\omega</math>-3 fatty acids significantly reduced the risk of CV disease (293/2828 vs. 236/2836, 10.4% two-way analysis, 15% four-way analysis, <math>p=0.009</math>). The effect of the combination of <math>\omega</math>-3 fatty acids + vitamin E (293/2828 vs. 236/2830, <math>p=0.01</math>) was comparable to the <math>\omega</math>-3 fatty acids supplementation alone. When the data were stratified as to individual endpoints, cardiovascular death (-30%), CHD death (-35%), total death (-20%) and sudden death (-45%) were all significantly reduced by the <math>\omega</math>-3 fatty acids supplementation.</p> <p><b>Conclusion:</b> In this population at intermediate risk, the protective effect of <math>\omega</math>-3 fatty acids diet was comparable on cardiovascular death, CHD death, total death and sudden death. No previous study showed the ability of drugs to reduce sudden death.</p> <p><b>Comment:</b> The 45% reduction in sudden death was not associated with changes in the use of drugs (e.g. antiplatelet agents, <math>\beta</math>-blockers, ACE-inhibitors or cholesterol-lowering agents) that may play a role in lowering the number of complex arrhythmias.</p>
Burr ML et al. (20)	<p><b>Study description:</b> Randomised controlled factorial trial of general practitioners in South Wales. A total of 3,114 men under 70 years of age with chronic angina were evaluated to assess whether their mortality can be reduced by dietary advice. The subjects were randomly allocated to four groups: 1) advised to eat two portions of oily fish each week, or to take three fish oil capsules daily; 2) advised to eat more fruit, vegetables and oats; 3) given both the above types of advice; and 4) given no specific dietary advice. Mortality was ascertained after 3–9 years.</p> <p><b>Results:</b> Compliance was better with the fish advice than with the fruit advice. All-cause mortality was not reduced by either form of advice, and no other effects were attributable to fruit advice. Risk of cardiac death was higher among subjects advised to take oily fish than among those not so advised; the adjusted hazard ratio was 1.26 (95% CI 1.00, 1.58; <math>p=0.047</math>), and even greater for sudden cardiac death (1.54; 95% CI 1.06, 2.23; <math>p=0.025</math>). The excess risk was largely located among the subgroup given fish oil capsules. There was no evidence that it was due to interactions with medication.</p> <p><b>Conclusions:</b> Advice to eat more fruit was poorly complied with and had no detectable effect on mortality. Men advised to eat oily fish, and particularly those supplied with fish oil capsules, had a higher risk of cardiac death.</p> <p><b>Comment:</b> Although it had the longest follow-up of all RCTs (randomised controlled trials); it was the only RCT that specifically enrolled men treated for angina; it was the first trial in which <math>\omega</math>-3 fatty acids from oily fish had a different effect to fish oil supplements (but this was found not to explain the differences), due to lack of funding, it was stopped midway during the trial. It is unclear if the participants continued to adhere to the advice to increase their fish consumption or to take their fish oil supplements. In addition, participants had a high baseline level of fish consumption.</p>

in the EPA group and in 324 (3.5%). Post-treatment LDL cholesterol concentrations decreased by 25%, in both groups. Unstable angina and non-fatal coronary events (including non-fatal MI, unstable angina, angioplasty, stenting, or coronary artery bypass)

were also significantly reduced in the EPA group. Sudden cardiac death and coronary death did not differ between groups.

Heart failure (HF), a major cause of morbidity and mortality in the Western Countries, is a major public health concern. Being

Table 2: Continued

Author (ref)	Study description/results/conclusion/comments
Nilsen et al. (22)	<p><b>Study description:</b> To evaluate the effect of a high-dose ethylester concentrate of <math>\omega</math>-3 fatty acids administered early after an acute MI on subsequent cardiac events and serum lipids. Three hundred patients with acute MI were randomly assigned to a daily dose of either 4 g highly concentrated <math>\omega</math>-3 fatty acids or corn oil, administered in a double-blind manner over 12–24 months. Median follow-up time was 1.5 years. Clinical follow-up, including the drawing of blood samples, was performed after six weeks of treatment and later at 0.5-year intervals.</p> <p><b>Results:</b> Forty-two (28%) patients in the <math>\omega</math>-3 group and 36 (24%) in the corn oil group experienced at least one cardiac event (cardiac death, resuscitation, recurrent MI, or unstable angina). No significant difference in prognosis was observed between groups for single or combined cardiac events. Total cholesterol concentrations decreased in both groups, with no significant intergroup differences. On average, the monthly increase in HDL cholesterol was 1.11% in the <math>\omega</math>-3 group and 0.55% in the corn oil group (<math>p=0.0016</math>). Triacylglycerol concentrations decreased by 1.30%/month in the <math>\omega</math>-3 group, whereas they increased by 0.35%/month in the corn oil group (<math>p&lt;0.0001</math>).</p> <p><b>Conclusions:</b> No clinical benefit of a high-dose concentrate of <math>\omega</math>-3 fatty acids compared with corn oil was found despite a favourable effect on serum lipids.</p> <p><b>Comment:</b> Since in the data obtained from the GISSI Prevenzione trial the evidence does support early protection against sudden death, it may be wise to make a distinction between patients with chronic disease, and those with acute MI. On the other hand, the lack of benefit in the latter setting might be due to the high background intake of fish oils in the Norwegian study participants, which could have masked the treatment effects.</p>
JELIS Study (23)	<p><b>Study description:</b> 18,645 patients with a total cholesterol of 6.5 mM or greater were recruited from local physicians throughout Japan between 1996 and 1999. Out of these patients, 14,981 were subject to primary prevention and 3,664 to secondary prevention. The patients were randomly assigned to receive either 1,800 mg of EPA daily with statin (EPA group; <math>n=9,326</math>) or statin only (controls; <math>n=9,319</math>) with a five-year follow-up. The primary endpoint was any major coronary event, including sudden cardiac death, fatal and non-fatal MI, and other non-fatal events including unstable angina pectoris, angioplasty, stenting, or coronary artery bypass grafting. Analysis was by intention-to-treat.</p> <p><b>Results:</b> At mean follow-up of 4.6 years, the primary endpoint was documented in 262 (2.8%) patients in the EPA group and in 324 (3.5%) controls, a 19% relative reduction in major coronary events (<math>p=0.011</math>) being found between cases and controls. Post-treatment LDL cholesterol concentrations decreased by 25%, from 4.7 mM in both groups. Serum LDL cholesterol was not a significant factor in a reduction of risk for major coronary events. Unstable angina and non-fatal coronary events were also significantly reduced in the EPA group. Sudden cardiac death and coronary death did not differ between groups. In patients with a history of coronary artery disease who were given EPA treatment, major coronary events were reduced by 19% as well (secondary prevention subgroup: 158 [8.7%] in the EPA group vs. 197 [10.7%] in the control group; <math>p=0.048</math>). In patients with no history of coronary artery disease, EPA treatment reduced major coronary events by 18%, but this finding was not significant (104 [1.4%] in the EPA group vs. 127 [1.7%] in the control group; <math>p=0.132</math>).</p> <p><b>Conclusions:</b> EPA is a promising treatment for prevention of major coronary events, and especially non-fatal coronary events, in Japanese hypercholesterolaemic patients.</p>
Tavazzi et al. GISSI HF trial (17, 18)	<p><b>Study description:</b> Long-term administration (3.9 years of follow-up) of 1 g/day <math>\omega</math>-3 fatty acids to a large population (<math>n=3,494</math>) of patients with heart failure (HF), all receiving treatments of proven efficacy for chronic HF (ACE-inhibitors, beta-blockers, diuretics, digitalis, spironolactone)</p> <p><b>Results:</b> <math>\omega</math>-3 fatty acids were effective in reducing all-cause mortality [adjusted HR (95.5% CI): 0.91 (0.833 – 0.998); <math>p</math>-value: 0.041], and hospitalisations for cardiovascular reasons [adjusted HR (99% CI): 0.92 (0.849 – 0.999); <math>p</math>-value 0.009]. The benefit was smaller than expected (RRR 7%–9% vs. assumed 15%) but it was supported by per-protocol analysis (RRR 12%–14%), and consistent across all the predefined subgroups. Major causes of death were: presumed arrhythmia, 7.8% vs. 8.7% in the placebo group; worsening HF, 9.1 vs. 9.5% in the placebo group (<math>n=3,481</math>). A similar benefit was not seen in the arm of the GISSI-HF Trial in which statin therapy did not reach the same clinical outcomes.</p> <p><b>Conclusion:</b> <math>\omega</math>-3 fatty acids are effective in reducing all-cause mortality in patients with chronic HF.</p>

marked by a rapid progression (impairment of quality of life, frequent hospital admissions etc), its prognosis is worse than that of some cancers.  $\beta$ -blockers, ACE inhibitors, diuretics, digitalis, and spironolactone, can delay or even prevent the progression of HF (1). Whether, on top of such treatments,  $\omega$ -3 fatty acids would improve mortality and morbidity among patients with chronic HF (NYHA class II–IV, of any cause and with any level of left ventricular systolic function), has been addressed in GISSI HF trial (23, 24). Long-term administration (3.9 years of follow-up) of 1 g/day  $\omega$ -3 fatty acids to a large population of patients with HF (3,494 vs. 3,481 randomised to placebo), all receiving ACE-inhibitors,  $\beta$ -blockers, diuretics, digitalis, and spironolactone, was effective in reducing all-cause mortality ( $p$ -value 0.041), and hospitalisations for car-

diovascular reasons ( $p$ -value 0.009). Compared to the placebo arm, the  $\omega$ -3 fatty acids group resulted in a 1.8% absolute reduction in all-cause mortality and in 2.3% reduction in mortality or admission for cardiovascular reasons. A similar benefit was not seen in the arm randomised to statin therapy (24).

### $\omega$ -3 fatty acids supplementations and prevention of arrhythmias

The results of the *GISSI Prevenzione* and those of the *PHS*, supported the idea that  $\omega$ -3 fatty acids prevent sudden death by reduc-

ing susceptibility to ventricular arrhythmia. Other data argue for this concept. In a population-based case-control study, whether the dietary intake of long-chain  $\omega$ -3 polyunsaturated fatty acids from seafood is associated with a reduced risk of primary cardiac arrest has been assessed (25). A total of 334 cases with primary cardiac arrest, aged 25 to 74 years, and 493 controls, matched for age and sex, were randomly identified from the community. Spouses of case patients and control subjects were interviewed to quantify dietary  $\omega$ -3 polyunsaturated fatty acid intake from seafood during the prior month. Blood specimens from 82 cases (collected in the field) and 108 controls were analysed to determine red blood cell membrane fatty acid composition, a biomarker of dietary  $\omega$ -3 polyunsaturated fatty acid intake. Compared with no dietary intake, an intake of 5.5 g of  $\omega$ -3 fatty acids per month was associated with a 50% reduction in the risk of primary cardiac arrest (OR; 0.5; 95% CI: 0.4 to 0.8). A red blood cell  $\omega$ -3 polyunsaturated fatty acid level of 5.0% of total fatty acids (the mean of the third quar-

tile) was associated with a 70% reduction in the risk of primary cardiac arrest (OR, 0.3; 95% CI, 0.2 to 0.6). Finally, in a study by Calo et al. (26), perioperative  $\omega$ -3 fatty acid administration was associated with a more favourable outcome than usual care alone in 160 patients undergoing elective bypass surgery. Patients randomised (open-label) to treatment with  $\omega$ -3 fatty acids received an average dose of 0.850 to 0.882 g/day starting five days before surgery and ending with discharge from the hospital after an average of seven postoperative days. Patients were followed for one month after discharge for assessment of the postoperative incidence of atrial fibrillation. There was a RR reduction of 54.4% and an absolute risk reduction of 18.1%, the effect being similar to that of sotalol and amiodarone. In multivariate analysis,  $\omega$ -3 fatty acids supplementation prior to and following bypass surgery was an independent predictor of postoperative atrial fibrillation at one month (OR: 0.32; 95% CI: 0.10–0.98;  $p = 0.013$ ).

**Table 3:**  $\omega$ -3 fatty acids and cardioprotection: RCTs in patients with implantable cardioverter defibrillators (ICDs).

Author (ref)	Study description/results/conclusion/comments
Leaf A (27) FAAT	<p><b>Study description:</b> To test the possibility that long-chain <math>\omega</math>-3 fatty acids may prevent potentially fatal ventricular arrhythmias in high-risk patients, 402 patients with ICDs were randomly assigned to double-blind treatment with either a fish oil or an olive oil daily supplement for 12 months. The primary end point, time to first ICD event for ventricular tachycardia or fibrillation (VT or VF) confirmed by stored electrograms or death from any cause, was analysed by intention to treat. Secondary analyses were performed for "probable" ventricular arrhythmias, "on-treatment" analyses for all subjects who had taken any of their oil supplements, and "on-treatment" analyses only of those who were on treatment for at least 11 months.</p> <p><b>Results:</b> Compliance with double-blind treatment was similar in the two groups; however, the non-compliance rate was high (35% of all enrollees). In the primary analysis, assignment to treatment with the fish oil supplement showed a trend toward a prolonged time to the first ICD event (VT or VF) or of death from any cause (risk reduction of 28%; <math>p=0.057</math>). When treatments for probable episodes of VT or VF were included, the risk reduction became significant at 31%; <math>p=0.033</math>. For those who stayed on protocol for at least 11 months, the anti-arrhythmic benefit of fish oil was improved for those with confirmed events (risk reduction of 38%; <math>p=0.034</math>).</p> <p><b>Conclusion:</b> For individuals at high risk of fatal ventricular arrhythmias, a regular daily ingestion of fish oil fatty acids may reduce potentially fatal ventricular arrhythmias.</p>
Raitt MH (28) SEATTLE	<p><b>Study description:</b> Randomised, double-blind, placebo-controlled trial performed at six US medical centers, with enrollment from February 1999 until January 2003, to determine whether <math>\omega</math>-3 fatty acids have beneficial antiarrhythmic effects in patients with a history of sustained VT or VF. Two hundred patients with an ICD and a recent episode of sustained VT or VF were randomly assigned to receive fish oil, 1.8 g/day, 72% <math>\omega</math>-3 fatty acids, or placebo and were followed up for a median of 718 days (range, 20–828 days). Time to first episode of ICD treatment for VT/VF, changes in red blood cell concentrations of <math>\omega</math>-3 fatty acids, frequency of recurrent VT/VF events, and predetermined subgroup analyses were determined.</p> <p><b>Results:</b> Patients randomised to receive fish oil had an increase in the mean % of <math>\omega</math>-3 fatty acids in red blood cell membranes (from 4.7% to 8.3%, <math>p&lt;0.001</math>), with no change observed in patients receiving placebo. At six, 12, and 24 months, 46% (SE, 5%), 51% (5%), and 65% (5%) of patients randomised to receive fish oil had ICD therapy for VT/VF compared with 36% (5%), 41% (5%), and 59% (5%) for patients randomised to receive placebo (<math>p=0.19</math>). In the subset of 133 patients whose qualifying arrhythmia was VT, 61% (SE, 6%), 66% (6%), and 79% (6%) of patients in the fish oil group had VT/VF at six, 12, and 24 months compared with 37% (6%), 43% (6%), and 65% (6%) of patients in the control group (<math>p=0.007</math>). Recurrent VT/VF events were more common in patients randomised to receive fish oil (<math>p&lt;0.001</math>).</p> <p><b>Conclusion:</b> Among patients with a recent episode of sustained ventricular arrhythmia and an ICD, fish oil supplementation does not reduce the risk of VT/VF.</p>
Brouwer IA (29) SOFA	<p><b>Study description:</b> Randomised, parallel, placebo-controlled, double-blind trial conducted at 26 cardiology clinics across Europe to study the effect of supplemental fish oil vs. placebo on ventricular tachyarrhythmia or death. A total of 546 patients with ICDs and prior documented malignant VT or VF were enrolled between October 2001 and August 2004. Patients were randomly assigned to receive 2 g/day of fish oil (<math>n = 273</math>) or placebo (<math>n = 273</math>) for a median period of 356 days (range, 14–379 days).</p> <p><b>Results:</b> The primary end point occurred in 81 (30%) patients taking fish oil vs. 90 (33%) patients taking placebo (hazard ratio [HR], 0.86; 95% CI, 0.64–1.16; <math>p=0.33</math>). In prespecified subgroup analyses, the HR was 0.91 (95% CI, 0.66–1.26) for fish oil vs. placebo in the 411 patients who had experienced VT in the year before the study, and 0.76 (95% CI, 0.52–1.11) for 332 patients with prior MIs.</p> <p><b>Conclusion:</b> No strong protective effect of intake of <math>\omega</math>-3 fatty acids from fish oil against ventricular arrhythmia in patients with ICDs is present in this report.</p>

In view of this, three double-blind, randomised intervention studies in patients with implantable cardioverter defibrillators (ICDs) investigated the direct effects of fish oil on ventricular tachyarrhythmia (27–29) (► **Table 3**). These studies have been reviewed in detail (30).

A total of 402 patients with ICDs were randomly assigned to a double-blind treatment with either a fish oil or an olive oil daily supplement for 12 months in the *FAAT trial* (27). At the end of the study, in both treatment groups, the number of patients that discontinued their prescribed supplements was high (35% of all en-

**Table 4:**  $\omega$ -3 fatty acids and cardioprotection: systematic reviews and meta-analyses.

Author (ref)	Study description/results/conclusion/comments
Bucher et al. (31)	<p><b>Study description:</b> By evaluating dietary and non-dietary (supplemental) intake of <math>\omega</math>-3 polyunsaturated fats of acids and CHD, 11 trials, published between 1966 and 1999, that included 7,951 patients in the intervention and 7,855 patients in the control groups were identified.</p> <p><b>Results:</b> The risk ratio of nonfatal MI in patients who were on <math>\omega</math>-3 fatty acid-enriched diets, compared with control diets or placebo, was 0.8 (95% CI: 0.5 to 1.2, <math>p=0.16</math>; Breslow-Day test for heterogeneity, <math>p=0.01</math>), and the risk ratio of fatal MI was 0.7 (95% CI: 0.6 to 0.8, <math>p&lt;0.001</math>; heterogeneity <math>p&gt;0.20</math>). In 5 trials, sudden death was associated with a risk ratio of 0.7 (95% CI: 0.6 to 0.9, <math>p&lt;0.01</math>; heterogeneity <math>p&gt;0.20</math>), whereas the risk ratio of overall mortality was 0.8 (95% CI: 0.7 to 0.9, <math>p&lt;0.001</math>; heterogeneity <math>p&gt;0.20</math>). There was no difference in estimates between dietary and non-dietary interventions of <math>\omega</math>-3 fatty acids for all investigated endpoints.</p> <p><b>Conclusion:</b> Dietary and non-dietary intake of <math>\omega</math>-3 polyunsaturated fatty acids reduces overall mortality, mortality due to MI, and sudden death in patients with CHD.</p>
Balk et al. (32)	<p><b>Study description:</b> Systematic review of the literature to assess the effect of the consumption of EPA, DHA, and ALA on various CVD risk factors and intermediate markers of CVD in healthy people, people with dyslipidaemia, diabetes, or known CVD. A total of 807 full text articles were screened and 123 studies that met inclusion criteria to address the key questions (i.e. studies in which the amount of fish or <math>\omega</math>-3 fatty acid intake was quantified, less than 6 g of <math>\omega</math>-3 fatty acid per day was consumed, and of at least 4 weeks' duration) were analysed.</p> <p><b>Results:</b> <math>\omega</math>-3 fatty acids showed a net decrease in triglycerides: 10–33%. The effect was dose-dependent, consistent in different populations, and was larger in studies with higher mean baseline triglyceride levels. The effect of <math>\omega</math>-3 fatty acids on other serum lipids was weaker (up to a 6% increase in HDL). Outcomes for which a small beneficial effect was found with fish oil supplementation included blood pressure (about 2 mm Hg reduction), restenosis rates after coronary angioplasty (14% reduction), exercise tolerance testing, and heart rate variability. A direct relationships between dose of consumed <math>\omega</math>-3 fatty acids and changes in measured levels of EPA+DHA, either as plasma or serum phospholipids, platelet phospholipids, or erythrocyte membrane phospholipids was found.</p> <p><b>Conclusion:</b> A large, consistent beneficial effect of <math>\omega</math>-3 fatty acids was found only for triglyceride levels. Little or no effect of <math>\omega</math>-3 fatty acids was found for a variety of other cardiovascular risk factors and markers of cardiovascular disease. The benefits of <math>\omega</math>-3 fatty acids on reducing cardiovascular disease are not well explained by the fatty acids' effects on the cardiovascular risk factors examined. A strong, linear association was found across studies between <math>\omega</math>-3 fatty acid intake and tissue levels. Heterogeneity of treatment effect was common among studies across the outcomes evaluated.</p>
Hopper et al. (33)	<p><b>Study description:</b> Meta-analysis to assess whether dietary or supplemental <math>\omega</math>-3 fatty acids affect total mortality, cardiovascular events or cancers. RCTs and cohort studies were used. RCTs where <math>\omega</math>-3 intake or advice was randomly allocated and unconfounded, and study duration was at least six months, were included. Cohorts were included where a cohort was followed up for at least six months and <math>\omega</math>-3 intake estimated.</p> <p><b>Results:</b> Forty-eight randomised controlled trials (36,913 participants) and 41 cohort analyses were included. Pooled trial results did not show a reduction in the risk of total mortality or combined cardiovascular events in subjects taking additional <math>\omega</math>-3 fats (with significant statistical heterogeneity). Sensitivity analysis, retaining only studies at low risk of bias, reduced heterogeneity and confirmed no significant effect of <math>\omega</math>-3 fats. Restricting analysis to trials increasing fish-based <math>\omega</math>-3 fats, or those increasing short chain <math>\omega</math>-3s, did not suggest significant effects on mortality or cardiovascular events in either group. Subgroup analysis by dietary advice or supplementation, baseline risk of CVD or <math>\omega</math>-3 dose suggested no clear effects of these factors on primary outcomes.</p> <p><b>Conclusion:</b> It is not clear whether dietary or supplemental <math>\omega</math>-3 fats affect total mortality, combined cardiovascular events or cancers in people with, or at high risk of, cardiovascular disease or in the general population. There is no evidence to advise people to stop taking <math>\omega</math>-3 fats. There is no clear evidence that <math>\omega</math>-3 fats differ in effectiveness according to sources or dose.</p>
He et al. (34)	<p><b>Study description:</b> Meta-analysis of cohort studies to examine the association between fish intake and CHD mortality. Studies were included if they provided a RR and corresponding 95% CI for CHD mortality in relation to fish consumption and to the frequency of fish intake. A database was developed on the basis of 11 eligible studies and 13 cohorts, including 222,364 individuals with an average 11.8 years of follow-up.</p> <p><b>Results:</b> Compared with those who never consumed fish or ate fish less than once per month, individuals with a higher intake of fish had lower CHD mortality. The multivariate RRs for CHD mortality were 0.89 (95% CI, 0.79 to 1.01) for fish intake 1-3 times per month, 0.85 (95% CI, 0.76 to 0.96) for once per week, 0.77 (95% CI, 0.66 to 0.89) for 2-4 times per week, and 0.62 (95% CI, 0.46 to 0.82) for five or more times per week. Each 20-g/day increase in fish intake was related to a 7% lower risk of CHD mortality (<math>p</math> for trend=0.03).</p> <p><b>Conclusion:</b> Fish consumption is inversely associated with fatal CHD. Mortality from CHD may be reduced by eating fish once per week or more.</p>

Table 4: Continued

Author (ref)	Study description/results/conclusion/comments
Studer M (35)	<p><b>Study description:</b> Systematic search of randomised controlled trials published up to June 2003, comparing any lipid-lowering intervention with placebo or usual diet, with respect to mortality. Outcome measures were mortality from all, cardiac, and non-cardiovascular causes. A total of 97 studies met eligibility criteria, with 137,140 individuals in intervention and 138,976 individuals in control groups.</p> <p><b>Results:</b> Compared with control groups, RR for overall mortality were 0.87 for statins (95% CI [CI], 0.81–0.94), 1.00 for fibrates (95% CI, 0.91–1.11), 0.84 for resins (95% CI, 0.66–1.08), 0.96 for niacin (95% CI, 0.86–1.08), 0.77 for <math>\omega</math>-3 fatty acids (95% CI, 0.63–0.94), and 0.97 for diet (95% CI, 0.91–1.04). Compared with control groups, RR for cardiac mortality indicated benefit from statins (0.78; 95% CI, 0.72–0.84), resins (0.70; 95% CI, 0.50–0.99) and <math>\omega</math>-3 fatty acids (0.68; 95% CI, 0.52–0.90). RR for non-cardiovascular mortality of any intervention indicated no association when compared with control groups, with the exception of fibrates (risk ratio, 1.13; 95% CI, 1.01–1.27).</p> <p><b>Conclusion:</b> Statins and <math>\omega</math>-3 fatty acids are the most favorable lipid-lowering interventions with reduced risks of overall and cardiac mortality.</p>
Mozaffarian et al. (36)	<p><b>Study description:</b> Meta-analysis of randomised, double-blind, placebo-controlled trials that evaluated the effect of fish oil on heart rate (HR), a major risk factor for sudden death. Predefined stratified meta-analyses and meta-regression were used to explore potential heterogeneity.</p> <p><b>Results:</b> Of 197 identified articles, 30 met inclusion criteria. In the overall pooled estimate, compared with placebo, fish oil decreased HR by 1.6 beats per min (bpm; 95% CI, 0.6 to 2.5; <math>p=0.002</math>). Fish oil reduced HR by 2.5 bpm (<math>p&lt;0.001</math>) in trials with baseline HR <math>\geq 69</math> bpm (median) but had little effect (0.04-bpm reduction; <math>p=0.56</math>) in trials with baseline HR <math>&lt;69</math> bpm (<math>p</math> for interaction=<math>0.03</math>). Fish oil reduced HR by 2.5 bpm (<math>p&lt;0.001</math>) in trials with duration <math>\geq 12</math> weeks but had less effect (0.7-bpm reduction; <math>p=0.27</math>) in trials with duration <math>&lt;12</math> weeks (<math>p</math> for interaction=<math>0.07</math>). HR reduction with fish oil intake did not significantly vary by fish oil dose (range, 0.81 to 15 g/day), type of HR measure, population age, population health, parallel vs. crossover design, type of control oil, or study quality.</p> <p><b>Conclusion:</b> In humans, fish oil reduces HR, particularly in those with higher baseline HR or on longer treatment duration.</p>
Hopper et al. (37)	<p><b>Study description:</b> Meta-analysis of the evidence for an effect of long chain and shorter chain <math>\omega</math>-3 fatty acids on total mortality, cardiovascular events, and cancer. Analysis of RCTs of <math>\omega</math>-3 intake for six months in adults (with or without risk factors for cardiovascular disease) with data on a relevant outcome. Cohort studies that estimated <math>\omega</math>-3 intake and related this to clinical outcome during at least six months were also included.</p> <p><b>Results:</b> 48 RCTs (36,913 participants) and 41 cohort studies were analysed. The pooled estimate showed no strong evidence of reduced risk of total mortality (RR 0.87, 95% CI 0.73 to 1.03) or combined cardiovascular events (0.95, 0.82 to 1.12) in participants taking additional <math>\omega</math>-3 fats. The few studies at low risk of bias were more consistent, but they showed no effect of <math>\omega</math>-3 on total mortality (0.98, 0.70 to 1.36) or cardiovascular events (1.09, 0.87 to 1.37). When data from the subgroup of studies of long chain <math>\omega</math>-3 fats were analysed separately, total mortality (0.86, 0.70 to 1.04; 138 events) and cardiovascular events (0.93, 0.79 to 1.11) were not clearly reduced. Neither RCTs nor cohort studies suggested increased risk of cancer with a higher intake of <math>\omega</math>-3 (trials: 1.07, 0.88 to 1.30; cohort studies: 1.02, 0.87 to 1.19).</p> <p><b>Conclusion:</b> Long chain and shorter chain <math>\omega</math>-3 fats do not have a clear effect on total mortality, combined cardiovascular events, or cancer.</p> <p><b>Comments:</b> A <math>t</math> variance with all previous meta-analyses, this included the very large negative clinical trial by Burr et al. (32), that appeared to strongly influence the overall effect and statistical significance of the pooled clinical trials.</p>
MacLean et al. (38)	<p><b>Study description:</b> Systemic review to determine estimates of the effect of <math>\omega</math>-3 fatty acids on cancer risk in prospective cohort studies. A total of 38 articles with a description of effects of consumption of <math>\omega</math>-3 fatty acids on tumour incidence, prospective cohort study design, human study population; and description of effect of <math>\omega</math>-3 among groups with different levels of exposure in the cohort were included.</p> <p><b>Results:</b> Across 20 cohorts from seven countries for 11 different types of cancer and using up to six different ways to categorise <math>\omega</math>-3 fatty acid consumption, 65 estimates of the association between <math>\omega</math>-3 fatty acid consumption were reported. Among these, only eight were statistically significant. The high degree of heterogeneity across these studies precluded pooling of data. For breast cancer one significant estimate was for increased risk (RR, 1.47; 95% CI, 1.10–1.98) and three were for decreased risk (RR, 0.68–0.72); seven other estimates did not show a significant association. For colorectal cancer, there was one estimate of decreased risk (RR, 0.49; 95% CI, 0.27–0.89) and 17 estimates without association. For lung cancer one of the significant associations was for increased cancer risk (IRR, 3.0; 95% CI, 1.2–7.3), the other was for decreased risk (RR, 0.32; 95% CI, 0.13–0.76), and four other estimates were not significant. For prostate cancer, there was one estimate of decreased risk (RR, 0.43; 95% CI, 0.22–0.83) and one of increased risk (RR, 1.98; 95% CI, 1.34–2.93) for advanced prostate cancer; 15 other estimates did not show a significant association. The study that assessed skin cancer found an increased risk (RR, 1.13; 95% CI, 1.01–1.27).</p> <p><b>Conclusion:</b> A large body of does not provide evidence of a significant association between <math>\omega</math>-3 fatty acids and cancer incidence. Dietary supplementation with <math>\omega</math>-3 fatty acids is unlikely to prevent cancer.</p>

rolles,  $n = 142$ ). In the primary analysis, assignment to treatment with the fish oil supplement showed a trend toward a prolonged time to the first ICD event or of death from any cause (risk reduction of 28%;  $p=0.057$ ). After adjusting for compliers who continued their supplements for at least 11 months, the antiarrhythmic

benefit of fish oil was improved, the risk reduction approaching 38% ( $p = 0.034$ ). When comparing these data with the ones published during the same year by Raitt et al. (28), the latter authors speculate that the positive effects they observed in the *FAAT*, in contrast to the negative results reported in their trial, may have

Table 4: Continued

Author (ref)	Study description/results/conclusion/comments
Mozaffarian et al. (39)	<p><b>Study description:</b> Reports published through April 2006 evaluating (1) intake of fish or fish oil and cardiovascular risk, (2) effects of methylmercury and fish oil on early neuro-development, (3) risks of methylmercury for cardiovascular and neurologic outcomes in adults, and (4) health risks of dioxins and polychlorinated biphenyls in fish. Emphasis was put on studies evaluating risk in humans.</p> <p><b>Results:</b> Modest consumption of fish (e.g. 1–2 servings/week), especially species higher in the <math>\omega</math>-3 fatty acids EPA and DHA, reduces risk of coronary death by 36% (95% CI, 20%-50%; <math>p &lt; 0.001</math>) and total mortality by 17% (95% CI, 0%-32%; <math>p = 0.046</math>) and may favourably affect other clinical outcomes. Intake of 250 mg/day of EPA and DHA appears sufficient for primary prevention. DHA appears beneficial for, and low-level methylmercury may adversely affect, early neuro-development. Women of childbearing age and nursing mothers should consume two seafood servings/week, limiting intake of selected species. Methylmercury may modestly decrease the cardiovascular benefits of fish intake, individuals with very high consumption (<math>\geq 5</math> servings/week) should limit intake of species highest in mercury levels. Levels of dioxins and polychlorinated biphenyls in fish are low, and potential carcinogenic and other effects are outweighed by potential benefits of fish intake and. Women of childbearing age should consult regional advisories for locally caught freshwater fish.</p> <p><b>Conclusion:</b> The benefits of fish intake exceed the potential risks in adults. For women of childbearing age, benefits of modest fish intake, excepting a few selected species, outweigh risks.</p>
Brouwer IA (40)	<p><b>Study description:</b> Meta-analysis on all three available trials on fish oil and ventricular arrhythmia in subjects with ICDs. Individual data of the two JAMA reports ( JAMA 2005; 293: 2884–2891 and JAMA 2006; 295: 2613–2619) were pooled together. The main outcome was time to first confirmed VF or VT combined with death, and time to first spontaneous confirmed VF or VT for the pooled analysis.</p> <p><b>Results:</b> The meta-analysis (<math>n = 1148</math>) showed no convincing protective effect of fish oil (RR 0.90; 95% CI 0.67–1.22). The hazard ratio for the subgroup of patients with coronary artery disease at baseline (0.79; 0.60–1.06) tended towards a protective effect. The pooled analysis (<math>n = 722</math>) showed that time to appropriate ICD intervention was similar for fish oil and placebo treatment (log-rank <math>p = 0.79</math>).</p> <p><b>Conclusion:</b> These findings do not support a protective effect of <math>\omega</math>-3 fatty acids from fish oil on cardiac arrhythmia in all patients with an ICD.</p>

been due to the lower levels of baseline  $\omega$ -3 fatty acids in the erythrocytes (3.3% vs. 4.75%) of the patients they enrolled, as well as the lower fatty fish consumption. An alternative explanation may be attributed to the inclusion criteria of the two studies. Raitt et al. (28) enrolled patients who did not have a recent MI, whereas patients enrolled in FAAT were not excluded if they had a recent MI. Thus, the greater benefits of  $\omega$ -3 fatty acid supplementation observed in FAAT may have been due to the presence of more ischaemia-driven arrhythmia in the study population (30).

The *Seattle trial* was a randomised, double-blind, placebo-controlled trial performed at six US medical centers to determine whether  $\omega$ -3 fatty acids have beneficial antiarrhythmic effects in patients with a history of sustained ventricular tachycardia (VT) or ventricular fibrillation (VF) (28). Two hundred patients with an ICD implanted because of a history of cardiac arrest, a history of sustained ventricular tachycardia or fibrillation during electrophysiologic studies and a recent episode of sustained VT or VF were randomly assigned to receive fish oil, 1.8 g/day, 72%  $\omega$ -3 fatty acids, or placebo and were followed up for a median of 718 days (range, 20–828 days). According to Kaplan-Meier estimates, 28% of patients in the fish oil group reached the primary endpoint of VT, VF, or death compared to 39% of patients in the olive oil control group. At the end of 12 months, the  $\omega$ -3 fatty acid group showed a trend toward a longer time to first ICD event (VT or VF) or death, with a risk reduction of 28% ( $p = 0.057$ ) that disappeared at 6, 12, and 24 months observations. Recurrent VT/VF events were more common in patients randomised to receive fish oil ( $p < 0.001$ ). Thus, fish oil supplementation did not reduce the risk

of VT/VF and was somehow proarrhythmic (28) among patients with a recent episode of sustained ventricular arrhythmia and an ICD.

The *Study on  $\omega$ -3 fatty acids and ventricular Arrhythmia (SOFA)* (29) was a randomised, placebo-controlled, double-blind trial conducted at 26 cardiology clinics across Europe, to study the effect of supplemental fish oil vs. placebo on ventricular tachyarrhythmia or death. A total of 546 patients with ICDs and prior documented malignant VT or VF (at least one episode during the previous year) were enrolled. Half of the patients were randomly assigned to receive 2 g/day of fish oil ( $n = 273$ ) containing 900 mg of  $\omega$ -3 fatty acids for 12 months, as many to a placebo of high-oleic acid sunflower oil ( $n = 273$ ) for a median period of 356 days (range, 14–379 days). The primary outcome was: incidence of recurrent spontaneous VT or VF and all-cause mortality. At one year, no significant difference in event-free survival was observed between treatment groups (70% vs. 67%, respectively). The primary end point occurred in 81 (30%) patients taking fish oil vs. 90 (33%) patients taking placebo ( $p = 0.33$ ). The hazard ratio [HR], R was 0.91 (95% CI: 0.66–1.26) for fish oil vs. placebo in the 411 patients who had experienced VT in the year before the study, and 0.76 (95% CI: 0.52–1.11) for 332 patients with prior MIs. At variance with the findings by Raitt et al. (28), benefits of fish oil supplementation were most pronounced for patients with VT at entry.

One of the possible explanations for the discrepancies in results between these three studies may relate to differences in types of patients enrolled in each study (30). The mix of patients in SOFA (29) may have favored a positive result from fish oil therapy: 63% had a

recent MI in the latter, compared with 55% who had a prior, but not recent, MI in the study conducted by Raitt et al. (28) In addition, fewer patients enrolled in SOFA (29) had baseline VT (55%) compared with patients enrolled by Raitt et al. (28) (66%).

Raitt et al. (28) suggested that although sodium channel blockade by  $\omega$ -3 fatty acids may be protective in acute ischaemia, it may be proarrhythmic in patients with premature ventricular contractions following an MI. Patients who were most adversely affected by  $\omega$ -3 therapy in their study had a qualifying rhythm of VT, which would not have been an ischaemia-related arrhythmic event in the absence of an MI. They also stressed that, in the studies on ICD, patients with ventricular arrhythmias without the ischaemic background or recent MI had been included (28). Whether  $\omega$ -3 fatty acids of cell membranes may exert opposite effects depending on the underlying mechanism of arrhythmia, and whether  $\omega$ -3 fatty acids are mostly effective in arrhythmias with ischaemic backgrounds is open to discussion. The trials published to date in arrhythmias without the ischaemic background have either been inconclusive or underpowered (1).

## $\omega$ -3 fatty acids and cardioprotection: Meta-analyses

In the past years, there have been several major meta-analyses and systematic reviews of the literature summarising the effects of  $\omega$ -3 fatty acids in cardiovascular prevention (31–40) (► Table 4).

By evaluating dietary and non-dietary (supplemental) intake, Bucher et al. (31) have investigated the effects of  $\omega$ -3 polyunsaturated fats of acids and CHD. The risk ratio of non-fatal MI in patients who were on  $\omega$ -3 polyunsaturated fatty acid-enriched diets compared with control diets or placebo was 0.8 and the risk ratio of fatal MI was 0.7 (95% CI: 0.6 to 0.8;  $p < 0.001$ ; heterogeneity  $p > 0.20$ ). In five trials, sudden death was associated with a risk ratio of 0.7 whereas the risk ratio of overall mortality was 0.8 There was no difference in summary estimates between dietary and non-dietary interventions of  $\omega$ -3 polyunsaturated fatty acids for all endpoints.

Balk et al. (32) performed a systematic review of the literature to assess the effect of consumption of EPA, DHA, and ALA on various CVD risk factors and intermediate markers of CVD in healthy people, people with dyslipidaemia, diabetes, or known CVD. Among the outcomes analysed,  $\omega$ -3 fatty acids showed a consistently large, significant net decrease in triglycerides (10–33%). The effect was dose-dependent, consistent in different populations, and larger in studies with higher mean baseline triglyceride levels. The effect of  $\omega$ -3 fatty acids on other serum lipids was weaker (up to a 6% increase in HDL). Outcomes for which a small beneficial effect was found with fish oil supplementation included blood pressure (about 2 mm Hg reduction), re-stenosis rates after coronary angioplasty (14% reduction), exercise tolerance testing, and heart rate variability. For other evaluated outcomes, including measures of glucose tolerance, the effects of  $\omega$ -3 fatty acids were either small or inconsistent across studies. Across studies, a direct

relationship between dose of consumed  $\omega$ -3 fatty acids and changes in measured levels of EPA+DHA was found. The correlation between dose and change in level appears to be fairly uniform, 1 g supplementation of EPA and/or DHA corresponding to approximately a 1% increase in EPA+DHA level. They also reported that the benefits of  $\omega$ -3 fatty acids on reducing cardiovascular disease are not well explained by the fatty acids' effects on the cardiovascular risk factors examined.

Hopper et al. (33) included RCTs where  $\omega$ -3 intake or advice was randomly allocated and unconfounded, and study duration was at least six months. Cohorts were included when followed-up for at least six months and  $\omega$ -3 intake estimated. Forty-eight randomised controlled trials (36,913 participants) and 41 cohort analyses were included. Pooled trial results did not show a reduction in the risk of total mortality or combined cardiovascular events in subjects taking additional  $\omega$ -3 fats. While reducing heterogeneity, sensitivity analysis confirmed the lack of any significant effect of  $\omega$ -3 fats. Neither RCTs nor cohorts suggested increased RR of cancers with higher  $\omega$ -3 intake but estimates were unreliable so a clinically important effect could not be excluded. Reviewers' conclusions were that: i) it is not clear that dietary or supplemental  $\omega$ -3 fats alter total mortality, combined cardiovascular events or cancers in people with, or at high risk of, cardiovascular disease or in the general population; ii) there is no evidence to advise people to stop taking rich sources of  $\omega$ -3 fats, but further high quality trials are needed to confirm suggestions of a protective effect of  $\omega$ -3 fats on cardiovascular health; iii) there is no clear evidence that  $\omega$ -3 fats differ in effectiveness according to fish or plant sources, dietary or supplemental sources, dose or presence of placebo.

A meta-analysis of cohort studies was conducted by He et al. (34) to examine the association between fish intake and CHD mortality. Compared with those who never consumed fish or ate fish less than once per month, individuals with a higher intake of fish had lower CHD mortality. The pooled multivariate RRs for CHD mortality were 0.85 (95% CI: 0.76 to 0.96) for fish intake once per week, 0.77 (95% CI: 0.66 to 0.89) for 2–4 times per week, and 0.62 (95% CI, 0.46 to 0.82) for five or more times per week. Each 20-g/day increase in fish intake was related to a 7% lower risk of CHD mortality ( $p$  for trend=0.03).

Studer et al. (35) carried out a systematic search of randomised controlled trials, in which any lipid-lowering intervention has been compared with placebo or to usual diet with respect to mortality. Outcome measures were mortality from all, cardiac, and non-cardiovascular causes (1). A total of 97 studies met eligibility criteria, with 137,140 individuals in intervention and 138,976 individuals in control groups. The selection of the trials was oriented towards comparison of individual drugs to placebo or usual care. Based on pharmacological characteristics, the trials were divided into statin, fibrate, resin, niacin, omega-3 PUFA, and dietary intervention groups. The  $\omega$ -3 fat group included 14 trials with nine trials on secondary cardiovascular disease prevention. The average relative reduction in total cholesterol was highest in the statin group and lowest in the  $\omega$ -3 group. Compared with control groups, risk ratios for overall mortality were 0.87 for statins (95% CI: 0.81–0.94), 1.00 for fibrates (95% CI: 0.91–1.11), 0.84 for resins

(95% CI: 0.66–1.08), 0.96 for niacin (95% CI: 0.86–1.08), 0.77 for  $\omega$ -3 fatty acids (95% CI: 0.63–0.94), and 0.97 for diet (95% CI: 0.91–1.04). Thus, the risk ratios for overall mortality were not correlated with reductions in cholesterol level. Compared with control groups, risk ratios for cardiac mortality indicated benefit from statins (0.78; 95% CI: 0.72–0.84), resins (0.70; 95% CI: 0.50–0.99) and  $\omega$ -3 fatty acids (0.68; 95% CI: 0.52–0.90). Risk ratios for non-cardiovascular mortality of any intervention, indicated no association when compared with control groups, with the exception of fibrates (1.13; 95% CI: 1.01–1.27). Their conclusion was that statins and  $\omega$ -3 fatty acids are the most favorable lipid-lowering interventions with reduced risks of overall and cardiac mortality. The estimated number to treat for one year in this group was 140 patients for secondary prevention to prevent one death, whereas for the statin group, the number to treat was 248 patients for secondary prevention. In primary prevention (with a mortality rate of below 1% per year) the number to treat was 855 patients to prevent one death. Similar to *JELIS*, this review also concluded that the benefits of  $\omega$ -3 fats were not related to reduction in the cholesterol level.

The effect of fish oil on heart rate (HR), a major risk factor for sudden death, was calculated by Mozaffarian (36) in a meta-analysis of randomised, double-blind, placebo-controlled trials of fish oil in humans. Predefined stratified meta-analyses and meta-regression were used to explore potential heterogeneity. They concluded that in randomised controlled trials in humans, fish oil reduces HR, particularly in those with higher baseline HR or longer treatment duration. These findings provide firm evidence that fish oil consumption directly or indirectly affects cardiac electrophysiology in humans.

To review systematically the evidence for an effect of long chain and shorter chain  $\omega$ -3 fatty acids on total mortality, cardiovascular events, and cancer, Hopper et al. (37) analysed RCTs of  $\omega$ -3 intake in adults with or without risk factors for cardiovascular disease. The authors concluded that long chain and shorter chain  $\omega$ -3 fats do not have a clear effect on total mortality, combined cardiovascular events, or cancer.

At variance with all previous meta-analyses, this controversial meta-analysis included the very large negative clinical trial by Burr et al. (20) that appeared to strongly influence the overall effect and statistical significance of the pooled clinical trials.

In addition to oils, fish contains also contaminants (e.g. dioxins, methyl-mercury; polychlorinated biphenyls) that might hamper its advantages (risk for cardiovascular and neurologic outcomes in adults, risk of cancer etc). Two systemic reviews/meta-analyses (38, 39) have been carried out to address the issue. To determine estimates of the effect of  $\omega$ -3 fatty acids on cancer risk in prospective cohort studies, MacLean et al. (38) screened a total of 38 articles with a description of effects of consumption of  $\omega$ -3 fatty acids on tumour incidence, prospective cohort study design, human study population; and description of effect of  $\omega$ -3 among groups with different levels of exposure in the cohort were included. The high degree of heterogeneity across these studies precluded pooling of data. For breast cancer, one significant estimate was for increased risk (incidence risk ratio [IRR]: 1.47; 95% CI: 1.10–1.98) and three

were for decreased risk (RR: 0.68–0.72); seven other estimates did not show a significant association. For colo-rectal cancer, there was one estimate of decreased risk (RR: 0.49; 95% CI: 0.27–0.89) and 17 estimates without association. For lung cancer, one of the significant associations was for increased risk (IRR: 3.0; 95% CI: 1.2–7.3), the other was for decreased risk (RR: 0.32; 95% CI: 0.13–0.76), and four other estimates were not significant. For prostate cancer, there was one estimate of decreased risk (RR: 0.43; 95% CI: 0.22–0.83) and one of increased risk (RR: 1.98; 95% CI: 1.34–2.93) for advanced prostate cancer; 15 other estimates did not show a significant association. The study that assessed skin cancer found an increased risk (RR: 1.13; 95% CI: 1.01–1.27). No significant associations between  $\omega$ -3 fatty acid consumption and cancer incidence were found for aerodigestive cancer, bladder cancer, lymphoma, ovarian cancer, pancreatic cancer, or stomach cancer. Thus, a large body of literature spanning numerous cohorts from many countries and with different demographic characteristics does not provide evidence to suggest a significant association between  $\omega$ -3 fatty acids and cancer incidence. Dietary supplementation with  $\omega$ -3 fatty acids is unlikely to prevent cancer.

Mozaffarian et al. (39) identified reports published through April 2006 evaluating intake of fish or fish oil and cardiovascular risk; effects of methylmercury and fish oil on early neurodevelopment; risks of methylmercury for cardiovascular and neurologic outcomes in adults, and health risks of dioxins and polychlorinated biphenyls in fish.

A modest consumption of fish (e.g. 1–2 servings/week), especially of species higher in EPA and DHA, reduces risk of coronary death by 36% ( $p < 0.001$ ) and total mortality by 17% ( $p = 0.046$ ) and may favourably affect other clinical outcomes. Intake of 250 mg/day of EPA and DHA appears sufficient for primary prevention. DHA appeared beneficial for, and low-level methylmercury may adversely affect, early neurodevelopment. Women of child-bearing age and nursing mothers should consume two seafood servings/week, limiting intake of selected species. Health effects of low-level methylmercury in adults are not clearly established; methylmercury may modestly decrease the cardiovascular benefits of fish intake. A variety of seafood should be consumed; individuals with very high consumption ( $\geq 5$  servings/week) should limit the intake of species highest in mercury levels. In conclusion, the benefits of fish intake exceed the potential risks. For women of childbearing age, benefits of modest fish intake, excepting a few selected species, also outweigh risks.

Since none of the three trials on  $\omega$ -3 fatty acids supplementations and prevention of arrhythmias convincingly showed whether or not supplementation with  $\omega$ -3 fatty acids has preventive effects in ICD patients, a meta-analysis was carried in which the results of these three trials were combined to assess the effect of fish oil on VT in the total group of ICD patients and in subgroups with different disease history (40). The authors concluded that the data available do not support a protective effect of  $\omega$ -3 fatty acids from fish oil on cardiac arrhythmia in all patients with an ICD.

## The biological plausibility (potential mechanisms) of the benefits of $\omega$ -3 fatty acids use in cardioprotection

It has long been known that the biological effects of  $\omega$ -3 fatty acids, some of which are related to DHA and EPA enrichment of membrane phospholipids (41), include lowering of monocyte adhesion to endothelial cells and of plasma triglycerides; LTB<sub>4</sub>, PDGF, and VLDL-cholesterol; enhancement of HDL-cholesterol and of membrane fluidity (42). By analysing the strengths of the findings and how the meta-analyses agree with the individuals trials, the following lines of evidence emerge.

- Dietary and non-dietary intake of  $\omega$ -3 fatty acids is inversely associated with fatal CHD: it reduces overall mortality, mortality due to MI, and sudden death in patients with CHD;
- Among patients with implantable cardioverter defibrillators, there is evidence that  $\omega$ -3 fatty acids do not reduce the risk of ventricular tachycardia/ventricular fibrillation and may actually be pro-arrhythmic;
- It is possible that  $\omega$ -3 fatty acids can have anti-arrhythmic or pro-arrhythmic effects depending on the origins of arrhythmias;
- Fish oil consumption directly or indirectly affects cardiac electrophysiology. There is good evidence that fish oil reduces HR, a major risk factor for sudden death. This is particularly relevant in individuals with higher baseline HR or longer treatment duration;
- The consumption of  $\omega$ -3 fatty acids leads to a large (10–33%), consistent decrease of triglyceride levels. The effect is dose-dependent, larger in studies with higher mean baseline triglyceride levels, and consistent in different populations (healthy people, people with dyslipidaemia, diabetes, or known cardiovascular risk factors). The effect of  $\omega$ -3 fatty acids on serum lipids other than triglycerides is weak (up to a 6% increase in HDL). The benefits of  $\omega$ -3 fatty acids are not related to reduction in the cholesterol level nor are they well explained by the effects on major cardiovascular risk factors;
- $\omega$ -3 fatty acids and statins are the most favorable lipid-lowering interventions, with reduced risks of overall and cardiac mortality;
- Outcomes for which a small benefit of  $\omega$ -3 fatty acids is found, include blood pressure (about 2 mm Hg reduction), re-stenosis rates after coronary angioplasty (14% reduction), and exercise tolerance testing.

Four major lines of evidence support the plausibility of these concepts.

- Antiarrhythmic/antifibrillatory effects. In almost 50% of cases, sudden death is preceded by life-threatening cardiac arrhythmias (43). *In vitro* and *in vivo* observations imply that the antiarrhythmic effect  $\omega$ -3 fatty acids is related to the modulation of the Na<sup>+</sup> and Ca<sup>2+</sup> currents in the myocyte sarcolemma, electrical stabilisation of cardiomyocytes, enhanced myocardial efficiency, and increased resistance to reperfusion arrhythmias

(44). In the *GISSI Prevenzione trial* (14), the supplementation of low/intermediate dose  $\omega$ -3 fatty acids (810 mg/day) given to stable coronary artery disease (CAD) patients decreased resting heart rate, increased post-exercise heart rate recovery, and increased beat-to-beat heart rate variability. These changes have been interpreted to be due to improved autonomic sympatho-vagal balance (45, 46). In keeping with this,  $\omega$ -3 fatty acids inhibit voltage-gated sodium channels in cardiomyocytes, resulting in a longer relative refractory period and an increased threshold voltage required for depolarization (47).  $\omega$ -3 fatty acids also maintain the integrity of L-type calcium channels, preventing cytosolic calcium overload during periods of ischaemic stress (48–50). Studies in patients undergoing heart transplants suggest that  $\omega$ -3 fatty acids can reduce heart rate independently of vagal activation (51).

- Effects on endothelial function, autonomic tone and blood pressure. In 14 healthy volunteers, a one-month supplementation of a preparation of EPA and DHA superimposable to that employed in the *GISSI Prevenzione study*, in parallel with changes in the plasma and platelet content of EPA and DHA, caused an impaired platelet aggregation in response to collagen or ADP that was independent of thromboxane biosynthesis. Such impaired aggregation correlated ( $p=0.036$  and  $0.068$ , respectively) with changes in the intracellular pH (pHi) of the Na<sup>+</sup>/H<sup>+</sup> reverse transport (52). In addition to platelet function, the latter mechanism is important as to lymphocyte function and blood pressure control (53, 54). As recently stressed in a comprehensive review on the issue (55),  $\omega$ -3 fatty acids are capable of reducing blood pressure (45, 46), to improve arterial and endothelial function (57), and to favourably affect the autonomic tone of the vessels (45, 57). In a meta-regression analysis of 22 double-blind randomised trials on blood pressure response to fish oil supplementation (58), consumption of ~4.0 g/day of  $\omega$ -3 fatty acids was associated with a significant 1.7- and 1.5-mm Hg reduction in systolic and diastolic blood pressure. Such reductions were maximal in older patients and in those with higher blood pressures. A 2 mm Hg reduction in blood pressure yields to a 4% reduction in mortality due to CAD (59). Changes in cell membrane composition that follow the supplementation of  $\omega$ -3 fatty acids are thought to be essential to achieve these effects, and DHA is reported to be more important than EPA in this respect. Although higher levels of DHA than of EPA are present in membrane phospholipids (60), data from JELIS challenge this formulation. Moreover, membranes typically contain 10 times as much AA as EPA (52), and EPA is a weaker substrate than AA for the production of cyclooxygenase- and lipoxygenase-derived eicosanoids (60). Changes in the lipid composition of the cellular membranes that occur following a dietary supplementation with EPA+DHA are likely to alter the activity of membrane-bound proteins (receptors, ion channels, etc), thus causing vascular effects (55).
- Antithrombotic and antiinflammatory effects. In RCTs in secondary prevention of CHD studies, a large proportion of individuals, in addition to  $\omega$ -3 fatty acids, was simultaneously receiving aspirin (> 80% in the *GISSI Prevenzione*). Since aspirin impairs

thromboxane formation, a major mechanism of platelet activation, and chronic  $\omega$ -3 fatty acids supplementation impair ADP-induced aggregation (52), another important mechanism of platelet activation, sudden death prevention may well be the result of a more intensive anti-platelet treatment. Studies in settings of coronary ischaemia (acute coronary syndrome, coronary stenting etc) where the synergistic effect of combinations of antiplatelet agents (e.g. aspirin + clopidogrel) with different antiplatelet mechanisms greatly reduced acute coronary deaths, support this formulation. When administered to obese people, high-dose  $\omega$ -3 fatty acids (1.8 g/day of EPA) increased the level of adiponectin that, besides improving insulin sensitivity, reduces inflammation indices (61). On the other hand, very high-dose  $\omega$ -3 fatty acids (8.0 g/day) have been shown to have anti-inflammatory effects in patients with HF (62). These data imply that, in addition to the well known anti-platelet effects (52, 63), the search for a biological plausibility has to take into consideration the anti-inflammatory properties of  $\omega$ -3 fatty acids.  $\omega$ -3 fatty acids have been shown to suppress the production of proinflammatory cytokines such as interleukin-6, interleukin-1 $\beta$ , and tumour necrosis factor- $\alpha$  (64–67). In a cross-sectional study of 5,677 men and women from the Multi-Ethnic Study of Atherosclerosis (MESA) cohort, including African Americans, Caucasians, Chinese, and Hispanics aged 45 to 84 years and free of clinical cardiovascular disease, He et al. (68) have documented the ability of long-chain  $\omega$ -3 fatty acid intake (as assessed by a self-administered food frequency questionnaire) to affect variables reflecting low levels of inflammation and endothelial activation. The intake was inversely associated with plasma concentrations of interleukin-6 ( $p = 0.01$ ) and of matrix metalloproteinase-3 ( $p = 0.03$ ). Such association was independent of age, body mass index, physical activity, smoking, alcohol consumption, and dietary variables. Non-fried fish consumption was inversely related to C-reactive protein ( $p = 0.045$ ) and interleukin-6 ( $p < 0.01$ ), and after adjustment for potential confounders, fried fish consumption was inversely related to soluble intercellular adhesion molecule-1 ( $p < 0.01$ ). However, the ability of  $\omega$ -3 fatty acids to consistently lower C-reactive protein levels has been challenged (69). In addition, other studies with  $\omega$ -3 fatty acids have not found significant changes in pro-thrombotic markers (70, 71).

- **Lipid lowering effects.** When triglycerides are raised, the cardiovascular risk from high serum cholesterol or low HDL cholesterol is enhanced. Some of the cardioprotective effects of high-dose  $\omega$ -3 fatty acids is likely to be due to their favorable effects on the lipid profile. In addition to the direct ability to lower plasma triglyceride levels, relatively high (3.4 g/day) amounts of  $\omega$ -3 fatty acids may enhance the effects of simvastatin therapy, by further lowering (~30%) triglyceride levels and VLDL cholesterol levels (~9.0%), with a significant increase in HDL levels (72). As to LDL cholesterol, the effect of high dose  $\omega$ -3 fatty acids is largely dependent on the baseline lipid profile: in hypercholesterolaemic subjects (e.g. those randomised in the *JELIS study*), LDL is reduced by ~10%; in moderately elevated and highly elevated baseline triglyceride levels, it is increased by

~10–30% (73, 74). Lp-PLA2 is an emerging independent risk factor for cardiovascular disease. Following oxidation of the LDL particle, Lp-PLA2 cleaves the oxidised phosphatidylcholine to lysophosphatidylcholine and oxidised free fatty acids, both proinflammatory compounds. Pedersen et al. (75) have evaluated the effect of marine  $\omega$ -3 fatty acids on plasma Lp-PLA2 levels in 60 healthy subjects randomised to a moderate dose (2 g) of  $\omega$ -3 fatty acids, a high dose (6.6 g) of  $\omega$ -3 fatty acids or olive oil (control) daily for 12 weeks. Plasma Lp-PLA(2) was measured at baseline and after the interventions. In the report, the authors called attention to the ability of  $\omega$ -3 fatty acids to exert antithrombotic effects by counteracting pro-atherosclerotic leukocyte activation and pro-inflammatory effects elicited by Lp-PLA2, which, in turn may hamper plaque instability suggested to be promoted by Lp-PLA2 (76, 77). Hypertriglyceridaemia is the most common hyperlipidaemia found survivors of MI. The concepts that i) raised triglycerides are an independent risk factor for CAD (78), and ii) that they act synergistically with other lipids and lipoproteins (mostly, lipoprotein transporting triglycerides) to create high-risk individuals (79), have been confirmed and extended. In a meta-analysis of 17 population-based studies, abnormally high triglycerides are associated with a 30% increase in cardiovascular disease in men and with a 75% increase in women (80). Despite the unsolved issue whether or not triglyceride concentrations should be measured in a fasting state (81), these data support the concept that triglyceride levels >1.7 mM are associated with raised cardiovascular risk (82). On the other hand, according to the results of secondary-prevention trials of CAD events (83–86), the efficacy of  $\omega$ -3 fatty acids for all-cause mortality may not only involve triglyceride lowering.

## Perspectives

In the era of poly-pills for CHD prevention, drugs with multifaceted mechanisms of action should be taken into serious consideration. The additional benefits of  $\omega$ -3 fatty acids over and above the usual *GISSI* strategy do not include the lowering of total or LDL cholesterol. Moreover, during the last decade, the interest for the antithrombotic hypothesis of  $\omega$ -3 fatty acids has progressively waned. In contrast, although the mechanism by which triglycerides promote atherogenicity is difficult to be split from its synergistic effects on lipids, lipoprotein and other cardiovascular variables, the triglyceride lowering and anti-arrhythmic effects of  $\omega$ -3 fatty acids appear to be major directions to be pursued. The implications of this possibility are discussed below.

- The metabolic syndrome is a clinical situation in which raised plasma triglycerides cluster together with other atherogenic dyslipidaemias, visceral fat accumulation, different degrees of hypertension, abnormal glucose metabolism, and pro-thrombotic and pro-inflammatory conditions (reviewed in [89, 90]). Such patients are at a higher than normal risk of myocardial ischaemia. In patients with hypertriglyceridaemia and abnormal

glucose tolerance,  $\omega$ -3 fatty acid supplementation does not lead to increased risk of diabetes (91). In overweight and obese adults,  $\omega$ -3 fatty acid supplementation improves insulin sensitivity (92). The antiarrhythmic action of  $\omega$ -3 fatty acids may well involve a hyperinsulinaemic effect in patients with MI (1).

- Non-alcoholic fatty liver disease (NAFLD) may be regarded as the hepatic expression of the metabolic syndrome: the more facets of the metabolic syndrome are present, the greater the chance of developing NAFLD (93, 94). NAFLD encompasses isolated hepatic steatosis, non-alcoholic steato-hepatitis and cirrhosis: it is characterised by the pathological accumulation of fat in the liver when no other explanatory disease is present (93, 94). NAFLD is the most common cause of liver disease in the US, and accounts for 11% of referrals to hepatology services (93, 94). NAFLD predicts the development of other features of the metabolic syndrome; it is independently associated with a raised risk of vascular events and, in the NHANES-III population-based study, with increased mortality (95). Inasmuch NAFLD affects 10–35% of the adult population worldwide, there is no consensus on its treatment (96).  $\omega$ -3 fatty acids are important regulators of hepatic gene transcription (93). Heterozygosity for the haemochromatosis gene is common in NAFLD; polymorphisms in the genes coding for the nuclear envelope protein lamin A as well as for PPAR- $\gamma$  have been identified in partial lipodystrophies (reviewed in [93, 96]). Animal studies show that  $\omega$ -3 fatty acids reduce hepatic steatosis, improve insulin sensitivity and reduce markers of inflammation, all major events in NAFLD development (93, 94, 96). In view of this,  $\omega$ -3 fatty acids have recently been suggested as a direction to be pursued to define appropriate strategies of treatment for NAFLD. A critical appraisal of the literature (96) shows that, in spite of inherent significant design limitations (sample size; follow-up etc), clinical trials in human subjects confirm experimental findings, and argue for  $\omega$ -3 fatty acids as being a promising treatment for NAFLD. Such direction needs to be tested in *ad hoc* randomised placebo-controlled trials.
- $\omega$ -3 fatty acids reduce HR, a major risk factor for sudden death. Low HR variability is associated with an abnormally high morbidity and mortality in post-MI patients (1).  $\omega$ -3 fatty acids exert a protective effect against fatal ventricular arrhythmias, particularly in post-MI patients, and against atrial fibrillation (AF) (14, 36). In addition to post-MI, AF is the most common complication after coronary artery bypass surgery (98). Presently, it is unclear whether  $\omega$ -3 fatty acids have a direct effect on the heart. Little is known about the influence of  $\omega$ -3 fatty acid supplements on energy homeostasis in post-MI patients, where the balance between glucose and non-esterified fatty acids may be crucial for the vitality of the myocardium (1, 99). The prevention of the adverse effects of fatty acid toxicity in the ischaemic myocardium (free radical formation) may be a direction to be pursued. In a dog model (100), infusion of  $\omega$ -3 fatty acids prevents ischaemia-induced sudden cardiac death by preventing ventricular fibrillation. The possibility of anti-arrhythmic and pro-arrhythmic effects  $\omega$ -3 fatty acids depending on the origins of arrhythmias, needs to be evaluated in *ad hoc* trials.

- Seventy five years ago, long chain  $\omega$ -3 fatty acids were added to the list of essential nutrients. Presently, their use in childhood, lactation and pregnancy is established (22). In individuals eating low amounts of fish, the optimal target EPA + DHA consumption has been suggested (101) to be at least 500 mg/day for individuals without underlying overt CV disease and at least 800 to 1,000 mg/day for individuals with CHD and HF. In spite of this, presently, there is no evidence as to whether concentrated preparations of  $\omega$ -3 fatty acids are interchangeable with individual preparations of DHA or EPA. Likewise, optimal dosing, duration and the relative ratio of DHA and EPA  $\omega$ -3 PUFA that provides maximal protection in primary and secondary vascular disease are little known.

## References

1. Patel JV, Tracey I, Hughes EA, et al. Omega-3 polyunsaturated fatty acids: a necessity for a comprehensive secondary prevention strategy. *Vasc. Health Risk Manag* 2009; 5: 801–810.
2. 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: 1205–1209.
3. Shekelle RB, Missell LV, Oglesby P, et al. Fish consumption and mortality from coronary heart disease. *N Engl J Med* 1985; 313(13): 820–4.
4. Norell SE, Ahloom A, Feychting M, et al. Fish consumption and mortality from coronary heart disease. *Br Med J* 1986; 293: 426.
5. Dyerberg J, Bang HO, Stoffersen E, et al. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet* 1978; 117–119.
6. Albert CM, Hennekens CH, O'Donnell CJ, et al. Fish consumption and risk of sudden cardiac death. *J Am Med Assoc* 1998; 279: 23–28.
7. Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain  $\omega$ -3 fatty acids and the risk of sudden death. *n-3 fatty acids and the risk of sudden death. N Engl J Med* 2002; 346: 1113–1118.
8. Hu FB, Bronner L, Willett WC, et al. Fish and  $\omega$ -3 fatty acids intake and risk of coronary heart disease in women. *J Am Med Assoc* 2002; 287: 1815–1821.
9. Oomen CM, Feskens EJ, Räsänen L, et al. Fish consumption and coronary heart disease mortality in Finland, Italy and the Netherlands. *Am J Epidemiol* 2000; 151: 999–1006.
10. Lemaitre RN, King IB, Mozaffarian D, et al. n-3 polyunsaturated fatty acids, fatal ischemic heart disease, and nonfatal myocardial infarction in older adults: the Cardiovascular Health Study. *Am J Clin Nutr* 2003; 77: 319–325.
11. Simon JA, Hodgkins ML, Browner WS, et al. Serum fatty acids and the risk of coronary heart disease. *Am J Epidemiol* 1995; 142: 469–476.
12. Burr ML, Gilbert JF, Holliday RM, et al. Effects of changes in fat, fish, and fiber intakes on death and myocardial infarction: diet and myocardial infarction (DART). *Lancet* 1989; 2: 757–761.
13. de Lorgeril M, Renaud S, Mamelle N, et al. Mediterranean alpha-linolenic acid rich diet in secondary prevention of coronary heart disease. *Lancet* 1994; 343: 1454–1459.
14. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI)-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* 1999; 354: 447–455 [published correction: *Lancet* 2001; 357: 642.
15. Marchioli R, Barzi F, Bomba E, et al.; 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 Miocardico (GISSI)-Prevenzione. *Circulation*. 2002; 105: 1897–1903.
16. Macchia A, Levantesi G, Franzosi MG, et al.; GISSI Prevenzione Investigators. Left ventricular systolic dysfunction, total mortality, and sudden death in patients with myocardial infarction treated with n-3 polyunsaturated fatty acids. *Eur J Heart Fail* 2005; 7: 904–909.

17. Ness AR, Hughes J, Elwood PC, et al. The long-term effect of dietary advice in men with coronary disease: follow-up of the diet and reinfarction trial (DART) *Eur J Clin Nutr* 2002; 56: 512–518.
18. 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.
19. von Schacky C, Harris WS. Cardiovascular benefits of  $\omega$ -3 fatty acids. *Cardiovasc Res* 2007; 73: 310–315.
20. Nilsen DW, Albrektsen G, Landmark K, et al. Effects of a high-dose concentrate of n-3 fatty acids or corn oil introduced early after an acute myocardial infarction on serum triacylglycerol and HDL cholesterol. *Am J Clin Nutr* 2001; 74: 50–56.
21. Yokoyama M, Origasa H, Matsuzaki M, et al., for the Japan EPA lipid intervention study (JELIS) Investigators. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open label, blinded endpoint analysis *Lancet* 2007; 369: 1090–1098.
22. FAO/WHO. Fats and oils in human nutrition report of a joint expert consultation. Food and Agriculture Organization of the United Nations and the World Health Organization. FAO Food Nutr Pap 1994; 57: 1–147.
23. Tavazzi L, Maggioni AP, Marchioli R, et al. GISSI-HF Investigators. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. *Lancet* 2008; 372: 1223–1230.
24. Tavazzi L, Maggioni AP, Marchioli R, et al. GISSI-HF Investigators. Effect of rosuvastatin in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. *Lancet* 2008; 372: 1231–1239.
25. 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: 1363–1367.
26. Calo 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* 2005; 45: 1723–1728.
27. Leaf A, Albert CM, Josephson M, et al. Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. *Circulation* 2005; 112: 2762–2768.
28. 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* 2005; 293: 2884–2891.
29. Brouwer IA, Zock PL, Camm AJ, et al. 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. *J Am Med Assoc* 2006; 295: 2613–2619.
30. Jacobson TA. Beyond Lipids: The Role of  $\omega$ -3 Fatty Acids from Fish Oil in the prevention of Coronary Heart Disease. *Curr Atheroscler Rep* 2007; 9: 145–153.
31. Bucher HC, Hengstler P, Schindler C, et al.  $\omega$ -3 fatty acids polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. *Am J Med* 2002; 112: 298–304.
32. Balk E, Chung M, Lichtenstein A, et al.; for the Tufts-New England Medical Center Evidence-Based Practice Center, Boston, MA: Effects of omega--3 Fatty Acids On Cardiovascular Risk Factors And Intermediate Markers Of Cardiovascular Disease: Summary, Evidence Report/Technology Assessment No. 93. Rockville, MD: Agency for Healthcare Research and Quality; March 2004. AHRQ Publication No. 04-E010-2.
33. Hooper L, Thompson RL, Harrison RA, et al. omega- 3 fatty acids for prevention and treatment of cardiovascular disease. *Cochrane Database Syst Rev* 2004; 4: CD003177.
34. He K, Song Y, Daviglius ML, et al. Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. *Circulation* 2004; 109: 2705–2711.
35. Studer M, Briel M, Leimenstoll B, et al. Effect of different antilipidemic agents and diets on mortality (a systematic review). *Arch Intern Med* 2005; 165: 725–730.
36. Mozaffarian D, Geelen A, Brouwer IA, et al. Effect of fish oil on heart rate in humans (a meta-analysis of randomized controlled trials). *Circulation* 2005; 112: 1945–1955.
37. Hooper L, Thompson RL, Harrison RA, et al. Risks and benefits of  $\omega$ -3 fats for mortality, cardiovascular disease, and cancer: a systematic review. *Br Med J* 2006; 332: 752–760.
38. MacLean CH, Newberry SJ, Mojica WA, et al. Effects of  $\omega$ -3 fatty acids on cancer risk: a systematic review. *J Am Med Assoc* 2006; 295: 403–415.
39. Mozaffarian D, Rimm EB: Fish intake, contaminants, and human health: evaluating the risks and the benefits. *J Am Med Assoc* 2006; 296: 1885–1899.
40. Brouwer IA, Raitt MH, Dullemeyer C, et al. Effect of fish oil on ventricular tachyarrhythmia in three studies in patients with implantable cardioverter defibrillators. *Eur Heart J* 2009; 30: 820–826.
41. Harris WS.  $\omega$ -3 fatty acids and cardiovascular disease: a case for  $\omega$ -3 index as a new risk factor. *Pharmacol Res* 2007; 55: 217–223.
42. Di Minno G, Tufano A, Garofano T, Di Minno MND. Polyunsaturated fatty Acids, thrombosis and vascular disease. *Patophysiol Haemost Thromb* 2002; 32: 361–364.
43. Huikuri HV, Castellanos A, Myerburg RJ. Sudden death due to cardiac arrhythmias. *N Engl J Med* 2001; 345: 1473–1482.
44. Pepe S, McLennan PL. Cardiac membrane fatty acid composition modulates myocardial oxygen consumption and postischemic recovery of contractile function. *Circulation* 2002; 105: 2303–2308.
45. O'Keefe JH Jr, Abuissa H, Sastre A, et al. 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* 2006; 97: 1127–1130.
46. Abuissa A, O'Keefe JH Jr, Harris WS, et al. Autonomic function,  $\omega$ -3, and cardiovascular risk. *Chest* 2005; 127: 1088–1091.
47. Leaf A, Kang JX, Xiao YF, et al. n-3 Fatty acids in the prevention of cardiac arrhythmias. *Lipids* 1999; 34 (Suppl): S187–S189.
48. Hallaq H, Smith TW, Leaf A. Modulation of dihydropyridine-sensitive calcium channels in heart cells by fish oil fatty acids. *Proc Natl Acad Sci USA* 1992; 89: 1760–1764.
49. Xiao YF, Ke Q, Wang SY, et al. Single point mutations affect fatty acid block of human myocardial sodium channel  $\alpha$  subunit Na<sup>+</sup> channels. *Proc Natl Acad Sci USA* 2001; 98: 3606–3611.
50. Anand RG, Alkadri M, Lavie CJ, et al. The role of fish oil in arrhythmia prevention. *J Cardiopulm Rehabil Prev* 2008; 28: 2–8.
51. Harris WS, Gonzales M, Laney N, et al. Effects of  $\omega$ -3 fatty acids on heart rate in cardiac transplant recipients. *Am J Cardiol*. 2006 Nov 15; 98(10): 1393–1395.
52. Cerbone AM, Cirillo F, Coppola A, et al. Persistent impairment of platelet aggregation following cessation of a short course dietary supplementation of moderate amounts of  $\omega$ -3 fatty acid ethyl esters. *Thromb Haemost* 1999; 82: 128–133.
53. Gaidiano G, Ghigo D, Schena M, et al. Na<sup>+</sup>/K<sup>+</sup> exchange activation mediates the lipopolysaccharide-induced proliferation of human lymphocytes and is impaired in malignant B-chronic lymphocytic leukemia lymphocyte. *J Immunol* 1989; 142: 913–918.
54. Resnik LM, Gupta RK, Sosa RE, et al. Intracellular pH in human and experimental hypertension. *Proc Natl Acad Sci USA* 1987; 84: 7663–7667.
55. Lee JH, O'Keefe JH, Lavie C J, et al.  $\Omega$ -3 Fatty Acids for Cardioprotection *Mayo Clin. Proc* 2008; 83: 324–332.
56. Ventura HO, Milani RV, Lavie CJ, et al. Cyclosporine-induced hypertension: efficacy of  $\omega$ -3 fatty acids in patients after cardiac transplantation. *Circulation* 1993; 88: II281-II285.
57. 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: 477–485.
58. Geleijnse JM, Giltay EJ, Grobbee DE, et al. Blood pressure response to fish oil supplementation: meta-regression analysis of randomized trials. *J Hypertens* 2002; 20: 1493–1499.
59. Ueshima H, Stamler J, Elliott P, et al, INTERMAP Research Group. Food  $\omega$ -3 fatty acid intake of individuals (total, linolenic acid, long-chain) and their blood pressure: INTERMAP study. *Hypertension* 2007; 50: 313–319.
60. Wada M, Delong CJ, Hong YH, et al. Enzymes and receptors of prostaglandin pathways with arachidonic acid-derived versus eicosapentaenoic acid derived substrates and products. *J Biol Chem* 2007; 282: 22254–22266.
61. Itoh M, Suganami T, Satoh N, et al. Increased adiponectin secretion by highly purified eicosapentaenoic acid in rodent models of obesity and human obese subjects. *Arterioscler Thromb Vasc Biol* 2007; 27: 1918–1925.
62. Mehra MR, Lavie CJ, Ventura HO, et al. Fish oils produce antiinflammatory effects and improve body weight in severe heart failure. *J Heart Lung Transplant* 2006; 25: 834–838.
63. Din JN, Harding SA, Valerio CJ, et al. Dietary intervention with oil rich fish reduces platelet-monocyte aggregation in man. *Atherosclerosis* 2007; prepub online doi: 10.1016/j.atherosclerosis.2007.04.047.
64. Schmidt EB, Pedersen JO, Varming K, et al. n-3 fatty acids and leukocyte chemotaxis: effects in hyperlipidemia and dose-response studies in healthy men. *Arterioscler Thromb* 1991; 11: 429–435.

65. Calder PC. Polyunsaturated fatty acids and inflammation. *Prostaglandins Leukot Essent Fatty Acids* 2006; 75: 197–202.
66. Shi Y, Zhang P, Zhang L, et al. Role of lipoprotein-associated phospholipase A2 in leukocyte activation and inflammatory responses. *Atherosclerosis* 2007; 191: 54–62.
67. Zhao G, Etherton TD, Martin KR, et al. Dietary  $\alpha$ -linolenic acid inhibits proinflammatory cytokine production by peripheral blood mononuclear cells in hypercholesterolemic subjects. *Am J Clin Nutr* 2007; 85: 385–391.
68. He K, Liu K, Daviglius ML, et al. Associations of dietary long-chain n-3 polyunsaturated fatty acids and fish with biomarkers of inflammation and endothelial activation (from the Multi-Ethnic Study of Atherosclerosis [MESA]). *Am J Cardiol* 2009; 103: 1238–1243.
69. Madsen T, Schmidt EB, Christensen JH. The effect of n-3 fatty acids on C-reactive protein levels in patients with chronic renal failure. *J Ren Nutr* 2007; 17: 258–263.
70. Lee KW, Blann AD, Lip GY. Effects of omega-3 polyunsaturated fatty acids on plasma indices of thrombogenesis and inflammation in patients post-myocardial infarction. *Thromb Res* 2006; 118: 305–312.
71. Hansen J, Grimsgaard S, Nordoy A, et al. Dietary supplementation with highly purified eicosapentaenoic acid and docosahexaenoic acid does not influence PAI-1 activity. *Thromb Res* 2000; 98: 123–132.
72. Davidson MH, Stein EA, Bayes HE, et al. COMBination of prescription  $\Omega$ -3 with Simvastatin (COMBOS) Investigators. Efficacy and tolerability of adding prescription  $\omega$ -3 fatty acids 4 g/d to simvastatin 40 mg/d in hypertriglyceridemic patients: an 8-week, randomized, double-blind, placebo controlled study. *Clin Ther* 2007; 29: 1354–1367.
73. Harris WS. n-3 fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr* 1997; 65 (5) (Suppl): 1645s–1654s.
74. Harris WS, Ginsberg HN, Arunakul N, et al. Safety and efficacy of Omacor in severe hypertriglyceridemia. *J Cardiovas Risk* 1997; 4: 385–391.
75. Pedersen MW, Koenig W, Christensen JH, et al. The effect of marine n-3 fatty acids in different doses on plasma concentrations of Lp-PLA2 in healthy adults. *Eur J Nutr* 2009; 48: 1–5.
76. 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: 477–485.
77. Weintraub HS. Identifying the vulnerable patient with rupture-prone plaque. *Am J Cardiol* 2008; 101: 3F–10F.
78. Miller M. Is hypertriglyceridaemia an independent risk factor for coronary heart disease? *Eur Heart J* 1998; 19: H18–H22.
79. Manninen V, Tenkanen L, Koskinen P, et al. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study. Implications for treatment. *Circulation* 1992; 85: 37–45.
80. Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta analysis of population-based prospective studies. *J Cardiovasc Risk* 1996; 3: 213–299.
81. Nordestgaard BG, Benn M, Schnohr P, et al. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *J Am Med Assoc* 2007; 298: 299–308.
82. British Cardiac Society; British Hypertension Society; Diabetes UK; HEART UK; Primary Care Cardiovascular Society; Stroke Association. JBS 2: Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice. *Heart* 2005; 91 (Suppl 5): v1–v5.
83. Rubins HB, Robins SJ, Collins D, et al; Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. *N Engl J Med* 1999; 341: 410–418.
84. The BIP Study Group. Secondary prevention by raising HDL cholesterol and reducing triglycerides in patients with coronary artery disease: the Bezafibrate Infarction Prevention (BIP) Study. *Circulation* 2000; 102: 21–27.
85. Meade T, Zuhrie R, Cook C, et al; MRC General Practice Research Framework, Bezafibrate in men with lower extremity arterial disease: randomised controlled trial. *Br Med J* 2002; 325: 1139–1141.
86. Keech A, Simes RJ, Barter P, et al; FIELD study investigators. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet* 2005; 366: 1849–1861.
87. Stampfer MJ, Krauss RM, Ma J, et al. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. *J Am Med Assoc* 1996; 276: 882–888.
88. Jeppesen J, Hein MO, Suadicani P, et al. Triglyceride concentration and ischaemic heart disease. An eight-year follow-up in the Copenhagen male study. *Circulation* 1998; 97: 1029–1036.
89. Crepaldi G. Origin and development of the Metabolic Syndrome. In: *The Metabolic Syndrome at the beginning of the XXIst century. A genetic and molecular approach*. Elsevier Madrid, 2005. pp. 5–12.
90. Martín de Santa Olalla L, Sánchez Muniz FJ, Vaquero MP. N-3 fatty acids in glucose metabolism and insulin sensitivity. *Nutr Hosp* 2009; 24: 113–127.
91. Sirtori CR, Paoletti R, Mancini M, et al. N-3 fatty acids do not lead to an increased diabetic risk in patients with hyperlipidemia and abnormal glucose tolerance. Italian Fish Oil Multicenter Study. *Am J Clin Nutr* 1997; 65: 1874–1881.
92. Ramel A, Martínéz A, Kiely M, et al. Beneficial effects of long-chain n-3 fatty acids included in an energy-restricted diet on insulin resistance in overweight and obese European young adults. *Diabetologia* 2008; 51: 1261–1268.
93. Tarantino G, Saldalamacchia G, Conca P, et al. Non-alcoholic fatty liver disease: further expression of the metabolic syndrome. *J Gastroenterol Hepatol* 2007; 22: 293–303.
94. Bellentani S, Marino M. Epidemiology and natural history of non-alcoholic fatty liver disease (NAFLD). *Ann Hepatol* 2009; 8 (Suppl 1): S4–8.
95. Hamaguchi M, Kojima T, Takeda N, et al. Nonalcoholic fatty liver disease is a novel predictor of cardiovascular disease. *World J Gastroenterol* 2007; 10: 1579–1584.
96. Choi SY, Kim D, Kim HJ, et al. The relation between non-alcoholic fatty liver disease and the risk of coronary heart disease in Koreans. *Am J Gastroenterol* 2009; 104: 1953–1960.
97. Masterton GS, Plevris JN, Hayes PC. Review article: omega-3 fatty acids – a promising novel therapy for non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2010; 31: 679–692.
98. Oliver MF, Opie LH. Effects of glucose and fatty acids on myocardial ischemia and arrhythmias. *Lancet* 1994; 343: 155–158.
99. Ommen SR, Odell KA, Stanton MS. Atrial arrhythmias after cardiothoracic surgery. *N Engl J Med* 1997; 336: 1429–1434.
100. Billman GE, Kang JX, Leaf A. Prevention of sudden cardiac death by dietary pure omega-3 polyunsaturated fatty acids in dogs. *Circulation* 1999; 99: 2452–2457.
101. Breslow JL.  $\omega$ -3 fatty acids and cardiovascular disease *Am J Clin Nutr* 2006; 83 (Suppl): 1477S–1482S.