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n-3 Fatty acids from fish or fish-oil supplements, but not α -linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review¹⁻³

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ABSTRACT

Studies on the relation between dietary n-3 fatty acids (FAs) and cardiovascular disease vary in quality, and the results are inconsistent. A systematic review of the literature on the effects of n-3 FAs (consumed as fish or fish oils rich in eicosapentaenoic acid and docosahexaenoic acid or as α -linolenic acid) on cardiovascular disease outcomes and adverse events was conducted. Studies from MEDLINE and other sources that were of ≥ 1 y in duration and that reported estimates of fish or n-3 FA intakes and cardiovascular disease outcomes were included. Secondary prevention was addressed in 14 randomized controlled trials (RCTs) of fish-oil supplements or of diets high in n-3 FAs and in 1 prospective cohort study. Most trials reported that fish oil significantly reduced all-cause mortality, myocardial infarction, cardiac and sudden death, or stroke. Primary prevention of cardiovascular disease was reported in 1 RCT, in 25 prospective cohort studies, and in 7 case-control studies. No significant effect on overall deaths was reported in 3 RCTs that evaluated the effects of fish oil in patients with implantable cardioverter defibrillators. Most cohort studies reported that fish consumption was associated with lower rates of all-cause mortality and adverse cardiac outcomes. The effects on stroke were inconsistent. Evidence suggests that increased consumption of n-3 FAs from fish or fish-oil supplements, but not of α -linolenic acid, reduces the rates of all-cause mortality, cardiac and sudden death, and possibly stroke. The evidence for the benefits of fish oil is stronger in secondary- than in primary-prevention settings. Adverse effects appear to be minor. *Am J Clin Nutr* 2006;84:5-17.

KEY WORDS n-3 Fatty acids, eicosapentaenoic acid, docosahexaenoic acid, fish oil, linolenic acid, cardiovascular disease, adverse events, systematic review

INTRODUCTION

Since the first cross-cultural epidemiologic studies conducted in the 1970s (1, 2), evidence has been accumulating regarding the role of n-3 fatty acids (FAs) in the prevention and management of cardiovascular disease (CVD). Evidence from observational

studies, randomized controlled trials (RCTs), and clinical, animal, and in vitro studies suggests that increased intakes of very-long-chain n-3 FAs found in fatty fish or fish-oil supplements may reduce the risk of CVD.

The n-3 FAs of particular interest for the prevention of CVD include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These very-long-chain n-3 FAs are found predominantly in fish and fish oils. α -Linolenic acid (ALA), a plant-derived n-3 FA and a precursor to EPA and DHA, is also of interest for CVD prevention. It is found in certain vegetable oils (eg, flaxseed, canola, and soybean) and walnuts.

The postulated biological mechanisms underlying the relation between n-3 FAs and the prevention of CVD include decreased arrhythmias, lower triacylglycerol concentrations, lower blood pressure, and decreased platelet aggregation (3-6). However, in many of the studies that examined these mechanisms, the n-3 FA intakes were high, and the effects were small and limited to subsets of the population or were not consistently observed (7-10). It is unresolved whether all forms of n-3 FAs have similar biological activity in vivo and similar effects on CVD risk. Notably, most studies suggest that humans convert <5% of ALA to EPA or DHA (11).

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We performed an evidence-based review of the health effects of n-3 FAs on clinical CVD outcomes in humans. Included are data from RCTs and observational studies that investigated the effect of fish consumption or dietary supplementation of n-3 FAs on CVD outcomes.

METHODS

Literature search

We conducted comprehensive searches of the medical literature from 1966 to July 2005 in 6 databases: MEDLINE, PreMEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, Biological Abstracts, and Commonwealth Agricultural Bureau of Health. We also consulted domain experts and examined references of retrieved articles to identify additional studies. Search terms for n-3 FAs included the specific FAs, fish and other marine oils, and the specific plant oils flaxseed, linseed, rapeseed, canola, soy, walnut, mustard seed, butternut, and pumpkin seed.

Eligibility criteria

We included English-language studies that reported original data on the effect of any type of n-3 FA intake in human adults on all-cause mortality and the following clinical CVD outcomes: cardiac death, sudden death, myocardial infarction (MI), and stroke. Both primary-prevention (general population without a history of CVD) and secondary-prevention (patients with a history of CVD) studies were included. Because of distinct differences in the population, we separately analyzed the results of studies that evaluated the effect of fish oils in patients with implantable cardioverter defibrillators (ICDs). We accepted RCTs and prospective cohort studies that followed patients for ≥ 1 y and case-control studies that reported intakes of n-3 FAs or fish. Supplementation with >6 g n-3 FAs/d (12–18 large capsules) was not considered to be a practical daily dose; thus, these studies were excluded. Also excluded were case-control and cohort studies based on n-3 FA biomarkers that did not include estimates of dietary intakes.

Selection of studies for adverse events and drug interactions

For the purpose of reviewing adverse events and drug interactions, we reviewed prospective human trials analyzed for either CVD clinical outcomes or risk factors. We included studies of any duration or dosage. We also reviewed prospective and retrospective studies that evaluated potential interactions between n-3 FAs and commonly used drugs.

Data extraction and synthesis

We extracted information about the study design, population demographics, background diet, intervention or exposure, and CVD outcomes. For the RCTs, we compared the relative risks of CVD outcomes between n-3 FA intervention and controls. For the prospective cohort studies, we extracted data on the estimates of fish or fish-oil consumption and the associated effect. Observational studies typically report estimated fish or n-3 FA intakes as quantiles (eg, quartiles or quintiles). The higher intakes were compared with the lowest intakes and were reported as odds ratios or risk ratios for the clinical outcome of interest. Because different studies used different quantiles in their analyses, we translated the

results into a qualitative scale to facilitate interpretation and comparison across studies. Meta-analyses were not performed because of the heterogeneity of the study designs, background diets, endpoint definitions, and baseline fish or n-3 FA intakes.

Grading methodologic quality of studies

We evaluated each trial and study against design-specific quality criteria and appraised the methodologic quality of the studies using a 3-category summary quality grade (12). This scheme defines an approach to assessing study quality that is applicable to each type of study design (ie, RCT, cohort study, or case-control study). The categories or summary quality grades are defined as follows: grade A, results are valid without obvious major bias; grade B, study is susceptible to some bias that is unlikely to invalidate the results; and grade C, significant bias is present that may invalidate the results. An assigned grade is applicable only within a specific study-design category.

For all studies, assessment of quality was based on the methods for estimating the amount of n-3 FAs consumed, the adequacy of reporting of background diets in the comparison groups, the method of ascertainment of CVD outcomes, and the consistency of calculations in the reporting of results. Additional criteria used to assess the quality of RCTs included adequacy of concealment of random allocation, blinding, and dropout rates; for case-control studies, the appropriateness of cases and control subjects was used to assess quality.

RESULTS

We screened 8039 abstracts and evaluated 842 full text articles for potentially relevant data. We identified 46 unique eligible studies on CVD outcomes, including 14 RCTs, 25 prospective cohort studies, and 7 case-control studies. We also reviewed 395 clinical studies for potentially relevant human data on adverse events associated with n-3 FA consumption. The results of secondary-prevention studies are presented first, an analysis of 3 RCTs of patients with an ICD is presented second, and the primary-prevention studies are presented last.

Secondary-prevention studies

Eleven RCTs, none of which enrolled patients with ICDs, studied a total of 19 403 patients with prior CVD and reported relevant CVD outcomes (13–24). These included 6 trials of n-3 FA supplements (**Table 1**) and 5 diet or dietary-advice trials (**Table 2**). These trials lasted between 1 and 5 y. In addition, one 5-y prospective cohort study assessed the association of fish consumption with CVD outcomes in 415 subjects (28).

Supplement trials

Six RCTs evaluated EPA or EPA+DHA supplements in dosages ranging from 0.27 to 4.8 g/d (**Table 1** and **Figure 1**) (13–19); 5 were of grade A or grade B methodologic quality. The largest trial followed 11 324 patients with recent MI for 3.5 y in Italy and reported that 0.85 g EPA+DHA/d compared with a usual case-control group significantly reduced the relative risk of all-cause mortality, cardiac death, and sudden death by 21%, 35%, and 45%, respectively (13, 14). However, there was a nonsignificant increase in strokes (relative risk = 1.2).

A Norwegian trial randomly assigned 300 patients who had survived a recent MI to receive either 3.4 g EPA+DHA/d or a



TABLE 1
Secondary prevention: randomized controlled trials of the effect of n-3 fatty acid supplements on cardiovascular disease outcomes¹

Reference	n-3 Fatty acid		Control		All-cause mortality		Cardiac death		Sudden death		Nonfatal MI		All strokes		Quality ²
	n	Type and dosage	n	Type and dosage	Control group event rate	RR (95% CI)	Control group event rate	RR (95% CI)	Control group event rate	RR (95% CI)	Control group event rate	RR (95% CI)	Control group event rate	RR (95% CI)	
					%		%			%		%		%	
EPA + DHA Marchioli et al, 2002, Italy (13,14)	5665	EPA+DHA: 0.85 g/d	5658	Usual care	9.8	0.79 ³ (0.66, 0.93)	5.4	0.65 ³ (0.51, 0.82)	2.7	0.55 ³ (0.39, 0.77)	4.1	0.91 (0.70, 1.2)	1.4	1.2 (0.81, 1.9)	B
Nilsen et al, 2001, Norway (15)	150	EPA+DHA: 3.4 g/d	150	Corn oil: 3.4 g/d	7.3	1.0 (0.45, 2.2)	5.3	1.0 (0.39, 2.6)	—	—	10	1.4 (0.75, 2.6)	—	—	B
von Schacky et al, 1999, Germany (16)	112	EPA+DHA: 3.4 g/d × 3 mo, 1.7 g/d × 21 mo	111	Equivalent dose of mixed fatty acids (nonmarine n-3)	1.8	0.5 (0.05, 5.4)	0.9	(0.01, 8.0)	—	—	2.7	0.33 (0.03, 3.1)	2.7	0.33 (0.03, 3.1)	A
Leng et al, 1998, Scotland (17)	60	EPA: 0.27 g/d	60	Sunflower seed oil: 3 g/d	5.0	1.0 (0.21, 4.8)	—	—	—	—	6.7	0.75 (0.18, 3.2)	1.7	3.0 (0.32, 28) (nonfatal)	A
Sacks et al, 1995, US (18)	31	EPA+DHA: 4.8 g/d	28	Olive oil	3.6	0.3 (0.01, 7.1)	3.6	0.3 (0.01, 7.1)	—	—	7.1	0.45 (0.04, 4.7)	0	2.7 (0.12, 64)	B
ALA vs EPA + DHA Singh et al, 1997, India (19)	120	Mustard oil ALA: 2.9 g/d	118	Non-oil placebo	—	—	22	0.61 (0.34, 1.1)	6.6	0.25 (0.05, 1.1)	25	0.59 ³ (0.35, 1.0)	—	—	C
	122	EPA+DHA (2:1): 1.8 g/d	118	Non-oil placebo	—	—	22	0.52 (0.29, 0.95)	6.6	0.24 (0.05, 1.1)	25	0.52 (0.3, 0.9)	—	—	C

¹ EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ALA, α-linolenic acid; RR, relative risk; MI, myocardial infarction, US, United States.

² Grade A: results valid without obvious major bias; grade B: susceptible to bias that is unlikely to invalidate results; grade C: significant bias that may invalidate results.

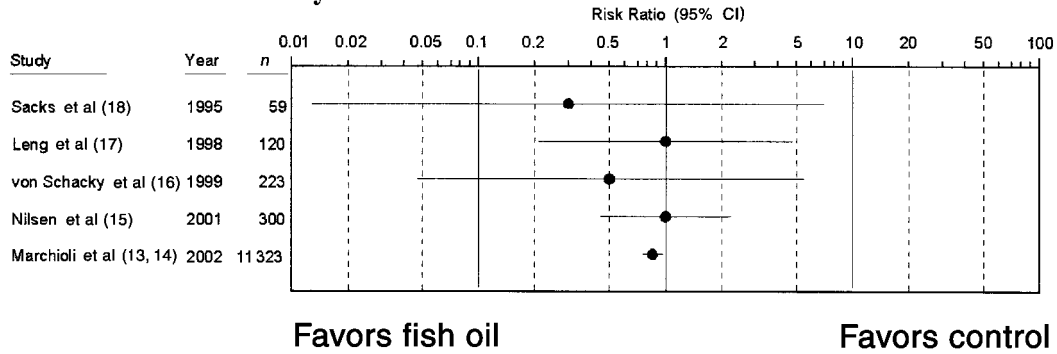
³ Adjusted for main confounders as reported in article.

TABLE 2
Secondary prevention: randomized controlled trials of the effect of an n-3 fatty acid diet or dietary advice to consume fish on cardiovascular disease outcomes¹

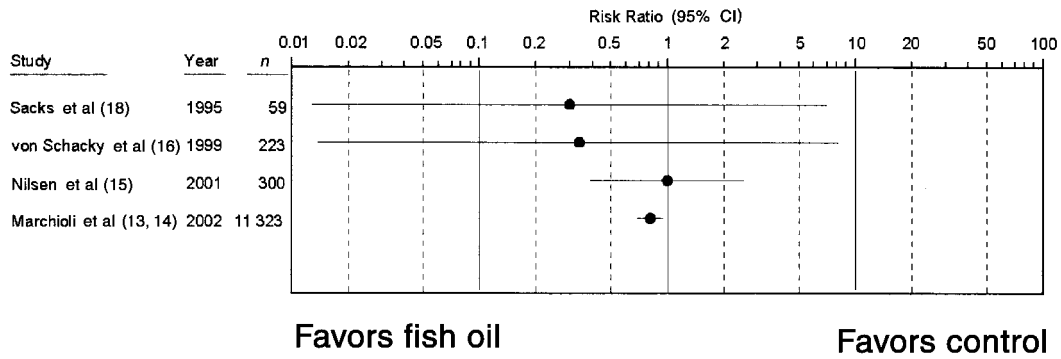
Reference	Intervention		No intervention		All-cause mortality		Cardiac death		Sudden death		Nonfatal MI		All strokes	
	n	Estimated n-3 fatty acid intake (g/d)	n	Estimated n-3 fatty acid intake (g/d)	Control group event rate	RR (95% CI)	Control group event rate	RR (95% CI)	Control group event rate	RR (95% CI)	Control group event rate	RR (95% CI)	Control group event rate	RR (95% CI)
ALA Singh et al, 2002, India (20) ²	499	Indo Mediterranean diet (ALA:1.8 g/d)	501	ALA: 0.8 g/d	8.0	0.63 (0.38, 1.04)	—	ND	3.2	0.38 (0.15, 0.95)	8.6	0.49 (0.30, 0.81)	2.6	0.54 (0.22, 1.3)
de Lorgeril et al, 1999, France (21)	302	Cretan Mediterranean diet (ALA: 1.9 g/d)	303	Prudent diet (ALA: 0.67 g/d) ⁵	7.9	0.44 (0.21, 0.94) ⁶	6.3	0.35 (0.15, 0.83) ⁶	2.6	0.06 (0.003, 1.02)	8.3	0.32 (0.15, 0.70)	1.3	0.11 (0.01, 2.1)
Bemelmans et al, 2002, Netherlands (22) Fish advice	109	ALA: 6.3 g/d	157	ALA: 1.0 g/d	0.6	4.3 (0.46, 41)	—	ND	—	ND	2.5	0.16 (0.01, 2.9)	1.3	0.29 (0.01, 5.9)
Burr et al, 2003, UK (23)	1571	EPA+DHA: 1.07 g/d ⁷	1543	EPA+DHA: 0.28 g/d ⁸	16	1.15 (0.86, 1.36) ⁸	9	1.3 (1.0, 1.58) ⁸	3	1.5 (1.1, 2.2) ⁸	—	ND	—	ND
Burr et al, 1989, UK (24)	1015	EPA+DHA: 0.86 g/d ⁷	1018	EPA+DHA: 0.21 g/d ⁸	13	0.73 (0.56, 0.93)	11	0.67 (0.51, 0.89)	—	ND	3.2	1.5 (0.97, 2.3)	—	ND

¹ EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ALA, α-linolenic acid; RR, relative risk; MI, myocardial infarction; UK, United Kingdom; ND, no data.
² Grade A: results valid without obvious major bias; grade B: susceptible to bias that is unlikely to invalidate results; grade C: significant bias that may invalidate results.
³ The results of this study were recently criticized; see text for details (25–27).
⁴ ALA accounted for 0.84% of energy and was calculated from daily nutrient intakes recorded on the final visit in 144 unselected consecutive experimental patients.
⁵ ALA accounted for 0.29% of energy and was calculated from daily nutrient intakes recorded on the final visit in 83 unselected consecutive control patients.
⁶ Adjusted for main confounders, as reported in article.
⁷ Only the EPA intake was estimated. Assuming that oily fish contains a ratio of DHA to EPA of ≈60:40, the estimated EPA+DHA intake would have been as indicated.
⁸ Values are hazard ratios.

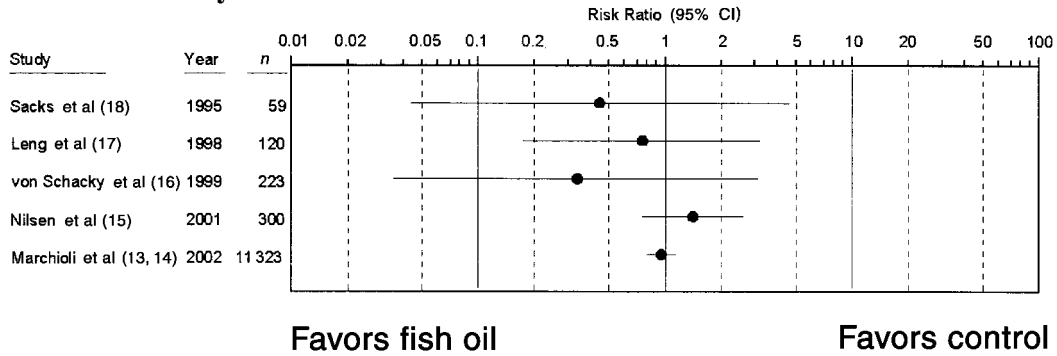
All-cause mortality



Cardiac death



Nonfatal myocardial infarction



Stroke

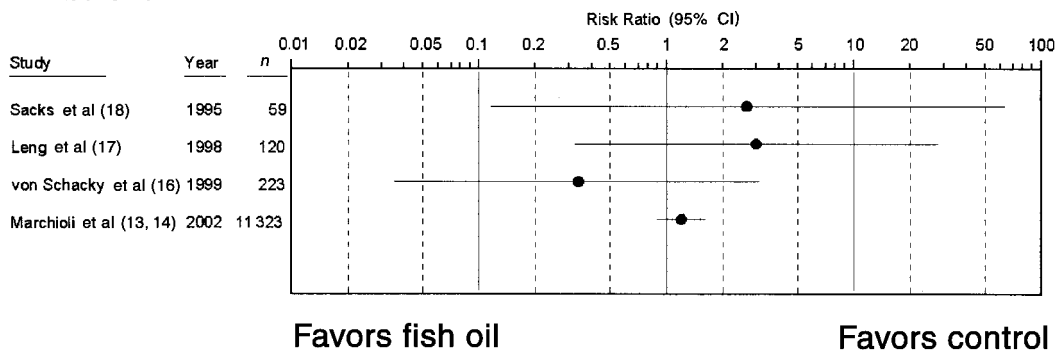


FIGURE 1. Randomized controlled trials of fish-oil supplementation for secondary prevention of cardiovascular disease outcomes. (Trials of patients with an implantation cardioverter defibrillator were excluded.) Four clinical outcomes are shown: all-cause mortality, cardiac death, nonfatal myocardial infarction, and stroke. Only trials with grade A or grade B methodologic quality are included. Meta-analyses of the trials were not conducted because of the heterogeneity of the study designs and settings.

corn oil placebo and followed them for 1.5 y. This trial reported no beneficial effect of $n-3$ FAs on any CVD outcome (15). In a follow-up analysis, the authors suggested that the lack of an effect may have been attributable to the high background $n-3$ FA intake (29).

Another fish-oil-supplement trial, conducted by von Schacky et al (16), followed 223 patients, half of whom had a history of MI. Fewer CVD events were reported among the patients who took 1.7 g fish oil/d, although the reduction in the event rate was not statistically significant. Two other small fish-oil-supplement trials reported nonsignificant beneficial trends on peripheral arterial disease (17) and cardiovascular disease (18) outcomes.

The sixth RCT of fish-oil supplements was a 3-arm study by Singh et al (19) that compared mustard seed oil (containing ALA), fish oil, and non-oil placebo. This 1-y trial was conducted in 360 patients hospitalized for suspected acute MI in India. Both oil supplements reduced total CVD outcomes, but only the effects of the fish oil were statistically significant. This trial, however, had limitations: inadequate randomization concealment, the use of a non-oil placebo, extremely high reported event rates, and many calculation errors in the published results. In addition, the scientific credibility of this study and of another dietary-advice study by the same authors (20) was recently questioned (25–27).

Diet or dietary-advice trials

Five diet or dietary-advice trials—from India, Great Britain, France, and the Netherlands (Table 2)—were reviewed (20–24). Two (20, 21) of these trials involved major dietary restructuring with shifts in overall dietary patterns. In 2 other trials, the patients were advised to increase their intake of oily fish to 200–400 g/wk (23, 24). Three studies assessed the effects of increased intakes of ALA, which were estimated to be between 1.8 and 6.3 g/d (20–22). No firm conclusions regarding the effects of either ALA or the marine $n-3$ FA could be reached from these trials. In general, all of the diet and dietary-advice trials were limited by the use of too many dietary variables and the inability to precisely determine the intake of $n-3$ FAs, especially of ALA. These trials also had other methodologic problems, including an uncertainty regarding the CVD history of the participants (20, 23), inadequate or faulty descriptions of the random allocation process, or the lack of blinding (20, 23, 24). The validity of one trial (noted above) was recently questioned (25–27). Four of the 5 diet or dietary-advice trials had a methodologic quality rating of grade C.

Two of the ALA dietary trials reported significant reductions or trends toward lower rates of all-cause mortality, cardiac and sudden death, or nonfatal MI (20, 21), whereas the third trial reported a nonsignificant increase in the risk of all-cause mortality (22), which was very low in both groups.

The 2 dietary-advice trials that recommended increased fish intake emanated from a single team of investigators in Great Britain (23, 24). The first trial, which was conducted in 1989, followed 2033 men recovering from MI for 2 y and reported a beneficial effect of advice to increase intakes of oily fish (eg, EPA+DHA) on all-cause mortality, cardiac death, and fatal MI (24). The second trial, which was published in 2003, followed 3114 patients with stable angina, 50% of whom had a history of MI. This study followed patients for 3–9 y via a national mortality database and reported that advice to eat more oily fish resulted in a nonsignificant increase in the risk of all-cause mortality and cardiac death and a significant increase in the risk of sudden death (hazard ratio = 1.54), particularly in the group that

was subrandomly assigned to receive fish-oil capsules (23). An explanation for these widely discrepant findings is not clear.

Cohort study

One prospective cohort study from Finland followed 415 patients with coronary artery disease for 5 y and compared those who consumed fish with those who consumed no fish. The study reported a significant decrease in all-cause mortality (RR = 0.37) in patients who consumed >57 g fish/d (28).

Patients with implantable cardioverter defibrillators

Three RCTs, 2 from the US and 1 from Europe, evaluated the effect of fish-oil consumption in patients with an ICD (Table 3) (30–32). These trials lasted from 1 to 2 y. The study by Raitt et al (30) included 200 patients with an ICD and a recent episode of sustained ventricular tachycardia or ventricular fibrillation (VF/VT). The participants were randomly assigned to receive either 1.8 g/d fish oil or placebo and were followed for 2 y. There was no significant decrease in total mortality between those who received fish oil and those who received placebo, but there was a shorter time to the first episode of ICD therapy for VT/VF and an increase ($P < 0.001$) in recurrent VT/VF events in the patients who received fish oil.

Leaf et al (31) randomly assigned 402 patients to receive either 2.6 g/d fish oil or olive oil and followed them for 12 mo. No difference in overall deaths was observed between groups. For those assigned to receive the fish-oil supplement, in contrast with the findings of Raitt et al (30), there was a trend toward a prolonged time to first VT or VF or death from any cause (risk reduction of 28%; $P = 0.057$).

The results of the Study on Omega-3 Fatty Acids and Ventricular Arrhythmia (SOFA) (32) is yet unpublished. The investigators reported at the European Society of Cardiology Congress in September 2005 “a small beneficial effect” in 546 patients with an ICD who were randomly assigned to receive either 2 g/d of fish oil or sunflower oil and were followed for 12 mo. There was no significant difference in the combined outcome of VT/VF or death from any cause between those who received fish oil and those who received placebo (70% compared with 67%, respectively). In a subgroup of 324 patients with a prior MI, there was a nonsignificant trend in the combined outcome of VT/VF or death from any cause in those who received fish oil compared with those who received placebo (71% compared with 63%, respectively).

Primary-prevention studies

One RCT (33), 25 prospective cohort studies (34–67), and 7 case-control studies (48, 68–73) reported outcomes in study populations with no history of CVD. (Tables 4, 5, 6, and 7) These studies were conducted in the United States, Europe, China, and Japan. Almost all of these studies estimated fish or fish-oil intakes; only 3 estimated ALA intakes (35, 38, 47). The methodologic quality of these observational studies was grade A or B. Most of the cohort studies used food-frequency questionnaires to assess dietary intake, used standard definitions of CVD outcomes, had several thousand subjects each, and lasted from 4 to 30 y.

The single primary-prevention RCT of $n-3$ FA supplementation by Natvig et al (33) included 13 578 subjects between 50 and 59 y of age who were randomly assigned to receive 10 mL flaxseed oil (5.5 g ALA/d) or sunflower seed oil (0.14 g ALA/d) for 1 y. This trial was conducted in Norway >30 y ago and

TABLE 3

Randomized controlled trials of the effects of n-3 fatty acid supplements on cardiovascular disease outcomes in patients with implantable cardioverter defibrillators¹

Reference	n-3 Fatty acid		Control		Duration	All-cause mortality		Cardiac death		Sudden death		Quality ²
	n	Type and dosage	n	Type and dosage		Control group event rate	RR (95% CI)	Control group event rate	RR (95% CI)	Control group event rate	RR (95% CI)	
Raitt et al, 2005, US (30)	100	EPA+DHA: 1.8 g/d	100	Olive oil	2	10	0.4 (0.13, 1.23)	5	0.4 (0.08, 2.01)	0	5 (0.24, 103)	B
Leaf et al, 2005, US (31)	200	EPA+DHA: 2.6 g/d	202	Olive oil: 4 g/d	1	5.9	1.09 (0.51, 2.34)	4.5	1.01 (0.41, 2.49)	—	ND	B
Brouwer et al, 2005, Europe (32) ³	278	EPA+DHA: 2 g/d	278	Sunflower oil: 3.4 g/d	1	—	1.04 (0.93, 1.17) ⁴	—	ND	—	ND	—

¹ EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; RR, relative risk; US, United States.

² Grade A: results valid without obvious major bias; grade B: susceptible to bias that is unlikely to invalidate results; grade C: significant bias that may invalidate results.

³ Full report unavailable; results presented only at a meeting.

⁴ Combination of all-cause deaths or ventricular tachycardia and ventricular fibrillation events.

reported a very-low CVD event rate (<1%) in the control group and no significant cardiovascular benefit of ALA supplementation. Background fish and fish-oil intake was already quite high in this population, which possibly limited any potential benefit of additional ALA.

Most of the large cohort studies reviewed, which involved >340 000 participants in total, reported significant reductions after multivariate adjustment in one or more of the CVD outcomes of interest (34, 36, 38, 39, 41, 43, 44, 50, 51, 59, 60, 62, 63).

All-cause mortality

Three large prospective cohort studies that comprised >53 000 participants from China, Japan, and the United States (39, 43, 44) provided data on fish-oil intake and reported significant reductions in all-cause mortality. In a cohort study conducted by Folsom and Demissie of 41 836 US women initially free of heart disease, the estimated marine n-3 FA intake was not associated with total mortality (38). However, the authors reported in a secondary analysis that ALA intake was modestly inversely associated with total mortality after multivariate adjustment.

Eleven prospective cohort studies provided data on the effect of fish consumption and the estimate of all-cause mortality. Eight of these studies reported no reduction in all-cause mortality (38, 39, 53–55, 58, 64, 67). In contrast, 3 studies—the Physicians' Health Study (35), a large cohort study ($n = 63\ 000$ men) from China (43), and a subset of 5103 diabetic women in the Nurses' Health Study (74)—reported associations between increased fish consumption and reduced mortality.

Cardiac death

Two prospective cohort studies (40, 44) reported data on n-3 FA consumption and cardiac death. A 6-y cohort study from Finland by Pietinen et al (40) of 21 930 middle-aged men who smoked found no association of cardiac death with either ALA or

EPA+DHA intake. Cardiac death was defined in the Finnish study as International Classification of Disease (revision 9) codes 410–414, which specifies coronary heart disease (CHD) as the underlying cause of death. The Multiple Risk Factor Intervention Trial (MRFIT), which followed 12 866 middle-aged men at high risk of CHD for 10.5 y, found no association between ALA intake and risk of cardiac death, whereas the highest quintile of EPA+DHA intake was associated with a 40% lower risk (44). In MRFIT, cardiac death is defined as deaths due to MI within 30 d of symptoms or hospitalization, sudden death, congestive heart failure due to CHD, or death during hospitalization for surgery for CHD or from attendant complications (45).

There were 15 cohort studies that reported data on fish consumption and cardiac death, 4 of which showed a statistically significant reduction in fatal and total coronary heart disease with higher fish consumption (35, 42, 60, 64). Eight cohort studies (36, 38, 42, 43, 50, 59, 62, 65) showed some protective benefit, and 4 showed none (52, 55, 57, 58). The Cardiovascular Health Study by Mozaffarian (60), which followed 3910 older subjects for 9.3 y, found that a statistically significant lower risk of total ischemic heart disease associated specifically with higher intakes of oily fish (ie, tuna and other nonfried fish). Of note, in this study, trends for increased cardiac events were observed with increasing consumption of fried fish or fish sandwiches.

Sudden death

Two prospective cohort studies and 1 case-control study reported data on sudden death (41, 48, 64). The Physicians' Health Study followed 20 551 men for 11 y and reported an ≈50% lower relative risk even in participants who ate fish only once a month (>0.3 g/mo n-3 FA) (41). The case-control study of 827 subjects conducted in the United States by Siscovick et al (48) reported a significant decrease in sudden death with increasing fish intake and fish-oil consumption. Another prospective cohort study, the Chicago Western Electric Study, followed 1822 men

TABLE 4Cohort studies on the association of estimates of n-3 fatty acid consumption with clinical outcomes in the general population¹

Reference	n	Duration	Dietary assessment	Type and intake	Clinical outcomes ²					Quality ³
					All-cause mortality	Cardiac death	Sudden death	MI	Stroke	
Iso et al, 2001, US (34)	79 839	14	FFQ	EPA+DHA: 0.08–0.48 g/d					+	A
Hu et al, 2002, US (35)	84 688	16	FFQ	EPA+DHA: 0.03–0.24 g/d				++		A
Ascherio et al, 1995, US (36)	44 895	6	FFQ	EPA+DHA: <0.1 to >0.42 g/d				+		A
He et al, 2002, US (37)	43 671	12	FFQ	EPA+DHA: <0.05 to >0.6 g/d					++	A
Folsom and Demissie, 2004, US (38)	41 836	14	FFQ	ALA: 0.96–1.21 g/d	0					A
Nagata et al, 2002, Japan (39)	29 079	7	FFQ	EPA+DHA: 0.33–1.6 g/d	++					A
Pietinen et al, 1997, Finland (40)	21 930	6.1	FFQ	EPA+DHA: 0.2–0.8 g/d		0				A
Morris et al, 1995, US (41)	21 185	4	FFQ	EPA+DHA: <0.5 to >2.3 g/wk				–	0	A
Albert et al, 1998, US (42)	20 551	11	FFQ	EPA+DHA: <0.3 to >7.4 g/mo			++			A
Yuan et al, 2001, China (43)	18 244	12	FFQ	EPA+DHA: <0.27 to >1.1 g/wk	++			++	+	A
Dolecek, 1992, US (44)	6250	10.5	Multiple 24-h dietary recall	EPA+DHA: 0–0.66 g/d	++	++				A
Seino et al, 1997, Japan (46)	2283	15.5	FFQ	n-3 FA: 1.8–3.2 g/d					–	B
Oomen et al, 2001, Holland (47)	67	20	CCD	ALA: <0.45 to >0.58 of energy				–		B

¹ EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ALA, α -linolenic acid; US, United States; FFQ, food-frequency questionnaire; CCD, cross-check dietary history; MI, myocardial infarction.

² ++, Clinically meaningful benefit shown: statistically significant trend of benefit for the quantile estimates of n-3 fatty acid or fish consumption; at least one-half of the quantile estimates of n-3 fatty acid or fish intake reported statistically significant beneficial effects [ie, a reduction in relative risk (RR) of $\geq 10\%$ (RR < 0.9)], and no quantile indicated a statistically significant adverse outcome. +, clinically meaningful beneficial trend exists but is not conclusive: a borderline significant ($0.10 > P > 0.05$) trend of benefit for the quantile estimates of n-3 fatty acid or fish intake; nonsignificant but potentially clinically meaningful effect (RR < 0.9) in at least one-half of the quantile estimates, and no quantile indicated a statistically significant adverse outcome. 0, clinically meaningful effect not shown or is unlikely: study reported clinically unimportant differences between low and no n-3 fatty acid or fish intake with various higher n-3 fatty acid or fish intakes. Most quantiles of estimates of n-3 fatty acid or fish intake reported a relative difference from the reference (ie, $1.1 > RR > 0.9$) of <10%. –, harmful effect shown or is likely: a positive association ($P < 0.10$) between quantile estimates of n-3 fatty acid or fish intake and increased risk. Several quantile estimates reported an RR >1.1.

³ Grade A: results valid without obvious major bias; grade B: susceptible to bias that is unlikely to invalidate results; grade C: significant bias that may invalidate results.

for 30 y and provided data on fish consumption and also found an association between higher fish consumption and lower rates of sudden death (64).

Myocardial infarction

Five cohort studies and 1 case-control study reported data on both fatal and non-fatal MI. Three of these large cohort studies—the Nurses' Health Study (35), the Health Professionals Follow-Up Study (36)—and a study from China by Yuan et al (43)—as well as 1 case-control study by Tavani et al of 148 802 participants from Italy (68) showed benefits of FA intake. Among 84 688 female nurses, higher EPA+DHA intakes were associated with a lower risk of nonfatal MI, ie, a 31% lower risk in the highest compared with the lowest quintile of intake. On the other hand, neither the Physicians' Health Study nor the Zutphen Elderly Study (which followed 667 Dutch elderly men free of coronary artery disease for 10 y)

reported reductions in the risk of MI with increasing intakes of EPA+DHA or fish (41, 47).

Nine prospective cohort studies and 4 case-control studies reported data on fish consumption and MI. Four of the 9 cohort studies (35, 36, 43, 64) and 1 case-control study (68) showed a statistically significant reduction in CHD, whereas 3 cohort studies (38, 42, 52) and 1 case-control study (72) found no such reduction in risk.

Stroke

Five prospective cohort studies and 1 case-control study provided data on estimates of n-3 FA intake and stroke. These studies included the large US cohorts of the Nurses' Health Study (34) and of the Health Professionals Follow-Up Study (37). Only the latter study, which followed 43 671 men free of CVD for 12 y, reported a significant reduction in ischemic strokes at all fish-oil intakes above the lowest quintile (37). The Nurses' Health Study

TABLE 5

Case-control studies of the association of estimates of n-3 fatty acid consumption with clinical outcomes in the general population¹

Reference	n	Dietary assessment	EPA+DHA intake	Clinical outcome ²					Quality ³
				All-cause mortality	Cardiac death	Sudden death	MI	Stroke	
Tavani et al, 2001, Italy (68)	975	FFQ	<0.81 to >1.28 g/wk				++		B
Caicoya, 2002, Spain (69)	913	FFQ	<0.12 to >0.66 g/d					-	A
Siscovick et al, 1995, US (48)	827	FFQ	0-13.7 g/mo			++			A

¹ EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; MI, myocardial infarction; FFQ, food-frequency questionnaire; US, United States.² See Table 4, footnote 2, for explanation.³ Grade A: results valid without obvious major bias; grade B: susceptible to bias that is unlikely to invalidate results; grade C: significant bias that may invalidate results.

found a nonsignificant trend of decreased strokes with increasing fish-oil intake (34).

Twelve prospective cohort studies and 1 case-control study provided data on fish consumption and stroke. Three large cohort studies (37, 51, 61) showed a statistically significant reduction in stroke, particularly ischemic stroke. The Health Professionals Follow-Up Study reported a significant reduction in ischemic strokes with any level of fish consumption (37). The Hiroshima/Nagasaki Life Span Study, which followed 30 827 male and female survivors of the atomic bomb in Japan, found that those in the highest tertile of fish consumption had a lower risk of death from stroke than did those in the lowest tertile (51). In the Cardiovascular Health Study by Mozaffarian et al (61), increased consumption of tuna or other nonfried fish was associated with a decrease in total stroke and ischemic stroke. In contrast, increased consumption of fried fish and fish sandwiches was associated with an increased risk of stroke. There was no association with hemorrhagic stroke in either of the latter 2 studies. Three cohort studies and 1 case-control study (34, 47, 56, 66) found a nonsignificant trend of decreased strokes with increasing fish consumption. An additional 5 cohort studies provided no evidence to support the hypothesis that fish consumption reduces the risk of stroke.

Adverse events

None of the RCTs reviewed were specifically designed to determine whether the use of lipid-lowering agents or diabetes medications altered the efficacy of n-3 FAs in reducing CVD outcomes. Likewise, none of the cohort studies specifically adjusted for CVD risk factor medications.

Of the 395 articles reviewed, 247 provided no information on adverse events; 2 additional articles were rejected because they were duplicate publications. Of the remaining 148 studies, 71 reported ≥ 1 adverse event. Most of these studies evaluated only a few dozen subjects, typically for <6 mo. The categorization and reporting of adverse events varied greatly across studies. Only one study (17) explicitly defined serious adverse events based on the scale developed by the World Health Organization. For example, some studies combined all nausea and vomiting, whereas others limited reporting to "mild" to "severe" gastrointestinal (GI) disturbance. No definitions for clinical bleeding or headache were given. The GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico)-Prevenzione trial,

which enrolled 11 324 subjects and had a follow-up duration of 3.5 y, reported a 29% dropout rate, with <5% of patients noting side effects as the cause for discontinuation (14). Similar dropout and side effect rates were observed in the vitamin E arm. The GISSI trial accounted for almost one-third of all GI complaints (in both the n-3 FA treatment and control arms) reported by all studies as well as almost all the withdrawals due to adverse events. When grouped together, these common GI symptoms occurred at rates of $\approx 4\%$ at dosages <3 g/d and increased to $\approx 20\%$ at a dosage of 4 g/d. Nine studies involving 2612 patients addressed the risk of clinically significant bleeding episodes (75-83). Four of the 9 studies reported no bleeding in either arm; (75-78) in the remaining 5 studies, no consistent association was found between the dosage of n-3 FAs and the risk of bleeding, even though the patients in some of these studies were taking aspirin or warfarin (79-83).

Concern has recently been raised about the possibility that ALA may increase the risk for prostate cancer. A meta-analysis of trials examining the relation between higher intakes or blood concentrations of ALA and prostate cancer risk reported a significant 70% increase in risk (84). Further studies are clearly needed to determine whether this is a spurious or real relation.

DISCUSSION

Overall, the data from the secondary- and primary-prevention studies support the hypothesis that consumption of very-long-chain n-3 FAs from fish and fish-oil supplements reduces all-cause mortality, cardiac and sudden death, and stroke. This conclusion agrees with 2 recent meta-analyses by He et al (85, 86), which were more limited in scope than the current review, and with the results of a 1999 ecologic study by Zhang et al (87). The latter showed a significant association between fish consumption and total mortality across 36 countries on the basis of data from the Food and Agriculture Organization and the World Health Organization. However, this conclusion is not applicable to the population of patients with an ICD, in whom 3 recent RCTs found inconsistent antiarrhythmic effects and no significant overall effect on mortality.

The evidence appears strong for a beneficial effect of very-long-chain n-3 FA intakes on CVD risk in secondary, but not in primary, prevention because data from RCTs are available for the

TABLE 6Cohort studies of the association of estimates of fish consumption with clinical outcomes in the general population¹

Reference	n	Duration	Dietary assessment	Fish consumption (amount or frequency)	Clinical outcomes ²					Quality ³
					All-cause mortality	Cardiac death	Sudden death	MI	Stroke	
Kinjo et al, 1999, Japan (49)	223 170	15	1-page questionnaire	>1 to >4/wk					0	C
Iso et al, 2001, US (34)	79 839	14	FFQ	<1/mo to >5/wk					+	A
Hu et al, 2002, US (35)	84 688	16	FFQ	<1/mo to >5/wk		++		++		A
Ascherlo et al, 1995, US (36)	44 895	6	FFQ	<1/mo to >6/wk		+		++		A
He et al, 2002, US (37)	43 671	12	FFQ	<1/mo to >5/wk					++	A
Egeland et al, 2001, Norway (50)	42 612	7	Dietary questionnaire	Cod liver oil user or not		+				C
Folsom and Demissie 2004, US (38)	41 836	14	FFQ	<0.5 to >2.5/wk	0	+		0	0	A
Sauvaet et al, 2003, Japan (51)	30 827	16	FFQ	0–7/wk					++	A
Nagata et al, 2002, Japan (39)	29 079	7	FFQ	37–158 g/d	0					A
Fraser et al, 1997, US (52)	26 743	6	FFQ	0 to >1/wk		0		0		B
Fraser and Shavlik, 1997, US (53) ⁴	603	12	FFQ		0					
Morris et al 1995, US (41)	21 185	4	FFQ	<1/mo to >5/wk					–	A
Albert et al, 1998, US (42)	20 551	11	FFQ	<1/mo to >5/wk	++	+	++	0		A
Yuan et al, 2001, China (43)	18 244	12	FFQ	<50 to ≥200 g/wk	++			++	0	A
Mann et al 1997, UK (54)	10 802	13.3	FFQ	0 to >1/wk	0	–				B
Nakamura et al, 2005, Japan (55)	8879	19	FFQ	0 to >2/d	0	0			0	A
Gillum et al, 1996, US (56)	5192	12	FFQ + 24-h dietary recall	0 to >1/wk					+	B
Gillum et al, 2000, US (57)	8825	18.8	FFQ + 24-h dietary recall		+	0				B
Osler et al, 2003, Denmark (58)	8497	18	FFQ	<1/mo to >2/wk	–	0				B
Rodriguez et al, 1996, US (59)	8006	23	Dietary questionnaire	<2/wk to >2/wk		+				C
Mozaffarian et al, 2003, US (60)	3910	9.3	FFQ	<1/mo to >3/wk		++		+		A
Mozaffarian et al, 2005, US (61)	4775	12	FFQ	<1/mo to >3/wk (fried-fish/sandwich)		–		–		
				<1/mo to >3/wk (fried-fish/sandwich)					++	A
				<1/mo to >3/wk (fried-fish/sandwich)					–	
Oomen et al 2000, Finland, Italy, Holland (62)	2738	20	CCD	1 to >40 g/d		+				A
Orencia et al, 1996, US (63)	1847	30	FFQ/24-h dietary recall	0 to ≥35 g/d					0	A
Daviglus et al, 1997, US (64)	1822	30		0 to ≥35 g/d	0	++	+	++		A
Kromhout et al, 1985, Holland (65)	852	20	CCD	0–45 g/d		+				B
Keli et al 1994, Holland (66)	872	15	CCD	<20 to ≥20 g/d					+	B
Kromhout et al 1995, Holland (67)	272	17	CCD	Fish eater or not	0	+				C

¹ MI, myocardial infarction; CCD, cross-check dietary history; FFQ, food-frequency questionnaire; US, United States; UK, United Kingdom.² See Table 4, footnote 2, for explanation.³ Grade A: results valid without obvious major bias; grade B: susceptible to bias that is unlikely to invalidate results; grade C: significant bias that may invalidate results.⁴ The subjects were aged >84 y.

former, but less evidence exists to support a beneficial effect on MI. There is no high-quality evidence to support a beneficial effect of ALA.

GI symptoms associated with fish-oil or ALA supplementation are the most commonly reported adverse events. Most GI symptoms were reported at dosages >3 g/d for EPA+DHA.

TABLE 7

Case-control studies of the association of estimates of fish consumption with clinical outcomes in the general population¹

Reference	n	Dietary assessment	Fish consumption (amount or frequency)	Clinical outcomes ²					Quality ³
				All-cause mortality	Cardiac death	Sudden death	MI	Stroke	
Sasazuki et al, 2001, Japan (70)	1846	FFQ	<2 to >4/wk				+		B
Rastogi et al, 2004, India (71)	1050	FFQ	No intake vs any intake				+		C
Tavani et al 2001, Italy (68)	975	FFQ	<1 to ≥2/wk				++		B
Gramenzi et al 1990, Italy (72)	936	FFQ	<1 to >1 portion/wk				0		C
Caicoya 2002, Spain (69)	913	FFQ	0 to >91 g/d					+	A
Siscovick et al 1995, US (48)	827	FFQ	0-13.7 g/mo			++			A
Martinez-Gonzalez et al, 2002, Spain (73)	234	FFQ	<80 to >142 g/d				+		B

¹ FFQ, food-frequency questionnaire; MI, myocardial infarction; US, United States.² See Table 4, footnote 2, for explanation.³ Grade A: results valid without obvious major bias; grade B: susceptible to bias that is unlikely to invalidate results; grade C: significant bias that may invalidate results.


Because current recommendations do not exceed 1 g/d for secondary prevention of CVD risk (88), it is unlikely that GI symptoms are a limiting factor for the implementation of this intervention. Overall, adverse events related to the consumption of fish-oil or ALA supplements appear to be minor and bothersome but not clinically relevant.

The totality of the data available on the effect of n-3 FAs on CVD outcomes suffered from many limitations that make drawing firm conclusions difficult. The evidence to support cardiovascular benefits from n-3 FA intakes in the secondary-prevention population came mostly from one large RCT. All the evidence for beneficial effects in the general population is derived from cohort studies. Data in women are limited, but the results are similar to those in men. Also, the studies analyzed were heterogeneous with regard to the methods of estimating fish or n-3 FA intakes, background diets, background risks for heart disease, settings, and the methods of reporting results. Different species of fish contain different amounts of EPA and DHA, and it is difficult to derive an accurate assessment of n-3 FA intakes from food frequency questionnaires. The method of food preparation, often not specified, may affect the n-3 FA content (89) or add saturated and *trans* fatty acids, which can affect the outcome variables (60).

Data on the effects of ALA on CVD outcomes are limited and typically of poor quality. Only one comparative trial of ALA and fish oil was identified, and serious concerns were recently raised about the validity of these data (25-27). Dose-response effects of n-3 FAs on CVD outcomes are inconsistent: no direct comparisons of doses in fish-oil- or ALA-supplement trials have been attempted. Only 2 cohort studies reported outcome data using the ratio of n-6 to n-3 FAs, but these data were presented in inconsistent formats and with different degrees of quality, which makes the interpretation difficult. In addition, such a ratio may provide little useful information without knowledge of the absolute intakes of linoleic acid, ALA, EPA, and DHA. Finally, data on the effects and associations of n-3 FAs with CVD outcomes in different subpopulations are limited.

Future research needs to address all of these lingering issues. Well-designed large RCTs that assess the effects of EPA and

DHA on CVD outcomes with long follow-up periods are needed, especially in the general population. There are currently 2 primary-prevention RCTs in progress, the results of which are expected before 2008: the JELIS trial, which randomly assigned patients taking statin drugs for hypercholesterolemia to receive either 1800 mg/d EPA or placebo (90), and the ASCEND trial, which is evaluating the effect of aspirin and n-3 FAs on CVD outcomes in a 2 × 2 factorial study in 10 000 patients with diabetes mellitus and free of known CVD (Internet: <http://www.clinicaltrials.gov/ct/show/NCT00135226>; accessed 7 November 2005). A secondary-prevention trial is also underway: the SU.FOL.OM3 trial, which randomly assigned patients who already experienced a coronary or cerebrovascular event to receive 600 mg/d EPA+DHA + 5-methyltetrahydrofolate + vitamins B-6 and B-12 or placebo (91). Future trials should not only attempt to confirm the findings of the GISSI trial in countries with different background dietary habits and risk but should also explore the various underlying mechanisms. Such studies should adequately assess background diet and fish consumption, particularly the species of fish and the methods of fish cooking and preparation. Attempts should also be made to determine the effect of higher fish intakes on the displacement of other foods from the diet, specifically meat and cheese, which are high in saturated fat.

Evidence suggests that increased consumption of n-3 FAs from fish or fish-oil supplements, but not of ALA, reduces the rates of all-cause mortality, cardiac and sudden death, and possibly stroke. Evidence of the benefits of fish oil is stronger in secondary- than in primary-prevention settings. However, no benefits of FA supplementation were seen in patients with an ICD, and adverse effects appear to be minor. 

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