n−3 Fatty acids and cardiovascular disease[1–4]

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ABSTRACT

The results of prospective cohort studies indicate that consuming fish or fish oil containing the n−3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is associated with decreased cardiovascular death, whereas consumption of the vegetable oil–derived n−3 fatty acid α-linolenic acid is not as effective. Randomized control trials (RCTs) in the context of secondary prevention also indicate that the consumption of EPA plus DHA is protective at doses <1 g/d. The therapeutic effect appears to be due to suppression of fatal arrhythmias rather than stabilization of atherosclerotic plaques. At doses >3 g/d, EPA plus DHA can improve cardiovascular disease risk factors, including decreasing plasma triacylglycerols, blood pressure, platelet aggregation, and inflammation, while improving vascular reactivity. Mainly on the basis of the results of RCTs, the American Heart Association recommends that everyone eat oily fish twice per week and that those with coronary heart disease eat 1 g/d of EPA plus DHA from oily fish or supplements. Directions for future research include *1*) RCTs to confirm the initial trials showing that EPA plus DHA decreases cardiovascular death and additional studies to determine whether this effect is due to EPA, DHA, or the combination; the dosage of the effective components; and whether the mechanism of action in humans is prevention of fatal arrhythmias. *2*) Clinical studies to determine whether the reduction in cardiovascular disease risk factors is due to EPA, DHA, or the combination and the dosage of the effective components. *3*) Clinical studies to determine whether vegetable oil–derived α-linolenic acid added to a diet enriched in n−6 fatty acids can effectively substitute for fish oil–derived EPA plus DHA. *Am J Clin Nutr* 2006; 83(suppl):1477S–82S.

KEY WORDS Coronary artery disease, n−3 fatty acids, fish oil, docosahexaenoic acid, eicosapentaenoic acid, arrhythmia

OVERVIEW OF EXISTING FINDINGS

Pioneering studies in Greenland Eskimos almost 30 y ago suggested that ingestion of n−3 fatty acids conveys protection from cardiovascular diseases (1). These initial observations set off a flurry of epidemiologic studies. Of 14 prospective cohort studies, n−3 fatty acids were found to be beneficial in 12 (2–13) and to have no effect in 2 (14, 15). In a recent meta-analysis of 11 prospective cohort studies (encompassing 222 364 persons with an average of 11.8 y of follow-up), compared with persons who never ate fish or ate fish less than once per month, the risk ratio for coronary heart disease mortality was 0.85 (95% CI: 0.76, 0.96) for fish consumption once per week, 0.77 (95% CI: 0.66, 0.89) for 2–4 times/wk, and 0.62 (95% CI: 0.46, 0.82) for ≥5 times/wk (16). The authors of this study calculated that each 20-g/d increase in fish intake was associated with a 7% lower risk of coronary heart disease mortality (*P* for trend: 0.03).

Several nested case-control studies have associated decreased plasma phospholipid DHA, red blood cell membrane n−3 fatty acid concentrations, and whole blood long-chain n−3 fatty acid concentrations with increased risk of coronary heart disease. 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 coronary heart disease between December 1973 and February 1976 and 94 matched controls who did not (17). In a multivariate model controlled for the ratio of HDL to LDL cholesterol, the concentration of DHA was inversely associated with coronary heart disease risk with an odds ratio of 0.57 (95% CI: 0.36, 0.90). In a Seattle-based study, red blood cell membrane n−3 fatty acids were compared between 82 primary cardiac arrest victims attended by paramedics between 1988 and 1994 and 108 controls from the community matched for age and sex (18). Compared with a red blood cell membrane n−3 fatty acid concentration of 3.3% of total fatty acids (the mean of the lowest quartile), a red blood cell membrane n−3 fatty acid concentration of 5.0% (the mean of the third quartile) was associated with a 70% reduction in the risk of primary cardiac arrest (odds ratio: 0.3; 95% CI: 0.2, 0.6). In the Physicians' Health Study, whole blood long-chain n−3 fatty acid concentrations were measured in 94 men in whom sudden death occurred as the first manifestation of cardiovascular disease and 184 matched controls (19). Compared with men with baseline blood concentrations of long-chain n−3 fatty acids in the lowest quartile, the relative risk of sudden death for men in the third quartile of whole blood long-chain n−3 fatty acid concentrations was 0.28 (95% CI: 0.09, 0.87) and that for men in the fourth quartile was 0.19 (95% CI: 0.05, 0.71) (*P* for trend: 0.004).

In support of the epidemiologic studies, which can only indicate associations, the hypothesis that ingestion of n−3 fatty acids protects individuals from cardiovascular disease has been tested in a few randomized control trials (RCTs). In the DART trial, 2033 men who had recovered from myocardial infarction were

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randomly assigned to receive dietary advice in 1 of 3 categories: reduce fat intake and increase the ratio of polyunsaturated to saturated fats, increase fatty fish intake, and increase cereal fiber intake (20). There was also a no diet advice group. Subjects advised to eat fatty fish had a significant 29% reduction in 2-y all-cause mortality compared with the no diet advice group. Subjects advised either to reduce fat intake or to increase cereal fiber intake had no significant change in mortality. None of the 3 dietary groups had a significant change in the incidence of reinfarction plus death from ischemic heart disease. The investigators concluded that "a modest intake of fatty fish (2 or 3 portions per week) may reduce mortality in men who have recovered from myocardial infarction." This corresponds to an additional 500–800 mg/d of n−3 fatty acids as EPA plus DHA. In the group randomly assigned to increase its intake of oily fish, a subgroup chose to ingest a fish oil capsule containing 450 mg of EPA plus DHA daily. In this subgroup, total mortality was lowered by 56% and coronary heart disease mortality was lowered by 62%, which strongly suggests that the benefit of fish consumption was in the fish oil fraction.

In the Indian Experiment of Infarct Survival Trial, patients admitted with suspected myocardial infarction received fish oil capsules (1.8 g/d; EPA plus DHA), mustard oil [20 g/d; 2.9 g α-linolenic acid (ALA)], or placebo (≈120 patients per group) (21). After 1 y, total cardiac events were significantly lower in the fish oil and mustard oil groups than in the placebo group (24.5%, 28%, and 34.7%, respectively, $P < 0.01$). Total cardiac deaths were less in the fish oil group than in the placebo group (11.4% compared with 22.0%; $P < 0.05$) with no significant benefit in the mustard oil group.

The GISSI-Prevention trial represents the largest RCT (22). This was a multicenter, open-label study in which 11 324 patients surviving recent (≤3 mo) myocardial infarction were enrolled between October 1993 and September 1995 and randomly assigned to receive one gelatin capsule of n−3 fatty acids (≈850 mg/d as EPA plus DHA ethyl esters in a ratio of EPA to DHA of 2:1), 300 mg vitamin E, the combination, or neither (≈2830 subjects per treatment group). After 3.5 y, the n−3 fatty acid–supplemented and unsupplemented groups were compared. The primary endpoint of combined efficacy, all-cause death plus nonfatal myocardial infarction and stroke, indicated a risk ratio of 0.90 (95% CI: 0.82, 0.99) in favor of treatment. The most favorable risk ratios (RRs) were for cardiovascular death [RR = 0.83 (95% CI: 0.71, 0.97)] and especially sudden death [RR = 0.74 (95% CI: 0.58, 0.93)]. Treatment had no significant effect on nonfatal cardiovascular events [RR = 0.98 (95% CI: 0.83, 1.15)]. Vitamin E treatment had no significant effect. Thus, RCTs done in the setting of secondary prevention have indicated a benefit of fish oil consumption at doses <1 g/d of EPA plus DHA.

RCTs of n−3 fatty acids to treat other related aspects of coronary heart disease have also been reported. Patients presenting for coronary angiography ($n = 223$) given EPA plus DHA at a dosage of 3 g/d for 3 mo followed by 1.5 g/d for 21 mo had less progression and more regression of preexisting lesions ($P = 0.04$) than did those given placebo (23). In patients undergoing coronary artery bypass grafting, those given 3.4 g/d of EPA plus DHA had vein graft occlusion rates of 27% compared with 33% in controls ($P = 0.03$) (24). In patients undergoing percutaneous transluminal coronary angioplasty, however, 2 large trials providing 5-7 g/d of EPA plus DHA failed to find a beneficial effect (25, 26).

Finally, the issue of whether the plant-derived n−3 fatty acid, ALA (as opposed to fish oil– or algal oil–derived EPA plus DHA), is beneficial has been examined. Although the mustard oil arm of the Indian Experiment of Infarct Survival Trial (21) and the Lyon Heart Trial (27, 28) suggested benefit, the much larger Norwegian Vegetable Oil Experiment (29) and the Mediterranean α-Linolenic Enriched Groningen Dietary Intervention (MARGARIN) Study (30) did not. A recent meta-analysis of all RCTs investigating the benefit of n−3 fatty acids (including ALA, EPA, and DHA) in coronary heart disease indicated overall benefit with risk ratios for nonfatal myocardial infarction of 0.8, fatal myocardial infarction of 0.7, and sudden death of 0.7 (31).

MECHANISTIC EXPLANATIONS

Several mechanisms have been proposed to explain how EPA plus DHA might beneficially influence cardiovascular disease (32). These include preventing arrhythmias (33), lowering plasma triacylglycerols (34, 35), decreasing blood pressure (36), decreasing platelet aggregation (37, 38), improving vascular reactivity (39, 40), and decreasing inflammation (41).

Epidemiologic studies provide support for an antiarrhythmic mechanism of action (33). In the GISSI-Prevention trial, fish oil consumption was associated with reductions in cardiac mortality, with the greatest benefit in the first 9 mo after myocardial infarction, but no reduction in nonfatal myocardial infarction (42). The relatively immediate benefit and the strongest effect on sudden coronary death suggest that fish oils act to prevent fatal arrhythmias (31). In contrast, the benefits of statins in secondary prevention trials are not apparent for 1–2 y and are more strongly for prevention of nonfatal coronary heart disease events rather than cardiac mortality (43). Statin therapy acts principally to lower LDL cholesterol, which in turn slows plaque progression and stabilizes plaque morphology, although many other nonlipid-lowering roles for statins have been proposed (44). Therefore, it is reasonable to conclude from the different time courses and major endpoints of their effects that fish oils and statins act mainly by different mechanisms.

Animal studies also support the potential antiarrhythmic action of fish oils, which have been shown to reduce ventricular fibrillation in rat (45), nonhuman primate (46), and dog (47) models. In vitro cell culture studies also support this mechanism of action (33). Leaf et al (33) showed that adding EPA or DHA to cultured neonatal rat cardiomyocytes slows the beating rate and inhibits the induction of tachyarrhythmias by extracellular calcium, ouabain, isoproterenol, lysophosphatidylcholine and acylcarnitine, thromboxane, and the calcium ionophore A23187. In electrophysiologic studies, these investigators showed that these fish oils inhibit the fast, voltage-dependent sodium and the L-type calcium currents.

The following hypothesis has been offered to explain the putative antiarrhythmic action of fish oils (33). After injury, including ischemia, cardiomyocytes are partially depolarized and can more easily generate action potentials, which if they occur during a vulnerable period of the cardiac electrical cycle can initiate arrhythmias. However, in the presence of fish oils, there is a voltage dependent shift to more hyperpolarized potentials, primarily as a result of inhibition of the fast, voltage-dependent sodium current. This effectively closes the sodium channel and prevents it from contributing to the generation of an action potential in the injured, partially depolarized cardiomyocytes. The

epidemiologic, animal, and in vitro cardiomyocyte studies present a strong case that the long-chain n−3 fatty acids act mainly through prevention of fatal arrhythmias.

Several recent clinical studies have appeared testing the hypothesis that long-chain n−3 fatty acids are antiarrhythmic in humans. In a population-based cohort of 4815 subjects aged ≥65 y, usual dietary intake assessed at baseline in 1989–1990 was compared with atrial fibrillation incidence over 12 y of follow-up on the basis of hospital discharge records and annual electrocardiograms (48). During this period, 980 cases occurred. In a multivariate analysis, consumption of tuna or other broiled or baked fish was associated with a 28% reduced incidence in those ingesting these foods 1–4 times/wk [odds ratio (OR) = 0.72 (95% CI: 0.58, 0.91), $P = 0.005$] and a 31% reduced incidence in those ingesting these foods ≥5 times/wk [OR = 0.69 (95% CI: 0.52, 0.91), $P = 0.008$] compared with <1 time per month (P for trend: 0.004).

In the National Heart, Lung, and Blood Institute Family Heart Study of 3642 subjects, there was an inverse association between ALA intake and the EKG-derived QTrr and JTrr parameters used to define abnormally prolonged repolarization (49). The OR for the highest versus the lowest ALA intake was 0.59 (95% CI: 0.44, 0.77) for QTrr and 0.59 (95% CI: 0.40, 0.87) for JTrr. In another study, 84 patients with ≥1440 premature ventricular contractions in a 24-h period were randomly assigned to receive 1.5 g EPA plus DHA/d or placebo (50). EPA plus DHA did not significantly decrease the number of premature ventricular contractions, although there was a trend, whereas there was a significant 2.1-beats/min decrease in heart rate. Beats/min is a significant predictor of sudden death and the change induced by EPA plus DHA might lower the risk.

In yet another study, 160 patients were prospectively randomly assigned to 2 g EPA plus DHA/d or placebo for 5 d before elective coronary artery bypass surgery until discharge from the hospital (51). Postoperative atrial fibrillation developed in 15.2% of the EPA plus DHA–treated and 33.3% of the placebo-treated groups ($P = 0.013$) and was also associated with a shorter hospital stay. Finally, EPA plus DHA (1.8 g/d; 72% n−3 fatty acids) was given to subjects with implantable cardioverter defibrillators who had had a recent episode of ventricular arrhythmia (52). Compared with the placebo group, there was no increase in the time to first episode of ventricular tachycardia or ventricular fibrillation in the treatment group, and the opposite may have occurred. The explanation for the difference between this result and the one expected (on the basis of the GISSI and DART trials) might be due to the differing populations selected for study (subjects with recent arrhythmia in contrast with recent myocardial infarction) or to the differing primary endpoints (time to first episode of ventricular tachycardia or fibrillation in contrast with sudden cardiac death).

In summary, it appears that n−3 fatty acids may decrease atrial fibrillation and heart rate and counter prolonged repolarization but have not yet been shown to decrease perhaps the most relevant clinical conditions of ventricular tachycardia or fibrillation. Additional studies are still warranted to examine the hypothesis that long-chain n−3 fatty acids are protective in humans through their antiarrhythmic actions.

EPA plus DHA has also been shown to favorably affect cardiovascular disease risk factors. At a dose of ≈4 g/d of EPA plus DHA, triacylglycerols decrease by 25–30%, LDL cholesterol increases 5–10%, and HDL cholesterol increases 1–3% (34). Equivalent to slightly lower doses of EPA plus DHA also reduce postprandial hypertriglyceridemia (53–55). Doses of 3–5 g/d of EPA plus DHA have been used to treat marked hypertriglyceridemia (>750 mg/dL) (34, 56–58). At doses of between 3 and 5.6 g/d, EPA plus DHA has been shown to reduce blood pressure in hypertensive persons by up to 5.5/3.5 mm Hg (36, 59–61). Doses of EPA plus DHA >3 g/d have also been shown to decrease platelet aggregation (37, 38), improve vascular reactivity (39, 40), and decrease inflammation (41). Thus, the effects of EPA plus DHA on cardiovascular disease risk factors appear to be at doses considerably higher than those shown to be effective in the DART and GISSI-Prevention trials. This suggests that the favorable effects of EPA and DHA in altering cardiovascular disease risk factors may be less important than other mechanisms, such as effects on arrhythmias.

RECOMMENDATIONS FROM EXISTING DATA

In 2002 the American Heart Association revised its recommendations for dietary intake of n−3 fatty acids (62). The following were recommended: *1*) Individuals without documented coronary heart disease were advised to eat fish (preferably oily) twice per week plus oils and foods rich in ALA (flaxseed, canola, soy, walnuts). This comes to ≈500 mg/d compared with current intake of <100 mg/d of n−3 fatty acids. *2*) Individuals with documented coronary heart disease were counseled to eat 1 g/d of EPA plus DHA, preferably from oily fish, but could take EPA plus DHA supplements in consultation with a physician. *3*) Individuals with hypertriglyceridemia could ingest 2–4 g/d of EPA plus DHA under a physician's care.

Many international organizations have made recommendations to increase the intake of EPA plus DHA, and these are summarized by the International Society for the Study of Fatty Acids and Lipids (Internet: http://www.issfal.org.uk/Recommendations.htm). In general, these recommendations are for ≈200 mg/d of EPA plus DHA for all adults. The United States has also issued a Dietary Reference Intake for n−3 fatty acids (Internet: http://www.iom.edu/Object.File/Master/7/300/0.pdf).

DIRECTIONS FOR FUTURE RESEARCH

1) Randomized controlled trials

Only 3 RCTs of EPA plus DHA in the prevention of coronary heart disease have been conducted, all in the setting of secondary prevention. The DART and GISSI-Prevention trials both involved large numbers of patients. In the DART trial, patients were randomly assigned to different dietary advice groups (20), whereas in the Gissi-Prevention trial, patients were randomly assigned to receive an EPA plus DHA supplement or placebo on an open-label basis (22). The Indian Experiment of Infarct Survival Trial is the only randomized double-blind placebo-controlled trial, but was rather small with only ≈120 patients treated with EPA plus DHA (21). Therefore, at this point, public health recommendations to ingest fish oils to prevent or treat coronary heart disease are based on rather thin evidence, and more RCTs are needed. The highest priority would seem to be replication of the GISSI-Prevention study. In this study, the treatment group experienced significant reductions in total mortality by 3 mo, sudden death by 4 mo, and cardiovascular, cardiac, and coronary deaths by 6–8 mo (42). Therefore, as suggested by

Grundy (63), for maximum efficiency, the next trial should focus on the 9-mo post–myocardial infarction period with the major endpoint of cardiac mortality, rather than longer term secondary prevention with nonfatal endpoints or primary prevention. Also, if possible, subsequent trials should attempt to document whether the mechanism of the fish oil effect is antiarrhythmic. The study should be double-blinded with subjects ingesting EPA plus DHA (≈1 g/d), EPA or DHA alone (≈0.5–1 g/d), or placebo.

2) EPA versus DHA

The studies of the effects of n−3 fatty acids on cardiovascular disease risk factors have involved doses of >3 g/d given as supplements of EPA plus DHA. Side effects of n−3 fatty acids are dose dependent; at >3 g/d, gastrointestinal upset, clinical bleeding, fishy aftertaste, worsening hyperglycemia, and increased LDL cholesterol are all more problematic than at doses of 1–3 g/d, which are in turn are more problematic than doses <1 g/d (62, 64). Therefore, it is of some interest to determine the efficacy versus side-effect profile of EPA and DHA when used separately. If efficacy resides in EPA or DHA or if both are efficacious but their effects are not additive and side effects are proportional to total n−3 fatty acid ingestion, it would be possible to use a lower dose of either EPA or DHA to modulate cardiovascular disease risk factors while minimizing side effects. These types of studies can be carried out on large numbers of outpatients being treated for hypertriglyceridemia with increasing doses of EPA, DHA, EPA plus DHA, and placebo who are carefully monitored for compliance. A similar study could be done on many fewer patients in a metabolic ward setting. Algal oils containing DHA but not EPA are available for testing, but the opposite is not true. This would necessitate using EPA and DHA esters for a direct comparison study. In fact, 2 studies have done just that. Grimsgaard et al (65, 66) randomly assigned 234 healthy men aged 36–56 y to 7 wk of dietary supplementation of 4 g/d of either EPA, DHA, or corn oil. Compared with concentrations at baseline, triacylglycerol concentrations in the EPA and DHA groups decreased 21% and 26%, respectively ($P < 0.0001$ for each). In the EPA group, cholesterol decreased 0.15 mmol/L ($P = 0.02$) and apolipoprotein A-I decreased 4 mg/dL ($P = 0.0003$), whereas in the DHA group, HDL cholesterol increased 0.06 mmol/L ($P = 0.0002$). The EPA and DHA groups had no significant changes in blood pressure. In the EPA group, mean heart rate increased 1.9 beats/min ($P = 0.04$); whereas in the DHA group, it decreased 2.2 beats/min ($P = 0.006$).

In other studies, Beilin's group randomly assigned 59 patients with type 2 diabetes who were being treated for hypertension, including men and postmenopausal women aged 40–75 y, to 6 wk of dietary supplementation with 4 g/d of either EPA or DHA or 6 g/d of olive oil (67–69). Compared with concentrations at baseline, in the EPA and DHA groups, fasting glucose concentrations increased 1.40 mmol/L and 0.98 mmol/L, respectively ($P = 0.002$ for each), but EPA and DHA had no significant effects on glycated hemoglobin, fasting insulin, C-peptide, insulin sensitivity or secretion, or blood pressure. In the EPA and DHA groups, triacylglycerols decreased 19% and 15%, respectively ($P = 0.022$ for each), but there were no significant effects on total, LDL, or HDL cholesterol. EPA and DHA did raise HDL_2 cholesterol 16% and 12%, respectively ($P = 0.026$ and $P = 0.05$), and EPA decreased HDL_3 cholesterol 11% ($P = 0.026$). There was no significant effect of EPA on vascular reactivity; however, DHA improved forearm blood flow in response to acetylcholine infusion and co-infusion of acetylcholine and L-NG-monomethylarginine ($P = 0.04$ for both), enhanced dilatory responses to sodium nitroprusside ($P < 0.0001$), and attenuated constrictor responses to norepinephrine ($P = 0.017$). EPA and DHA supplementation were also tested for effects on collagen- and PAF-stimulated platelet aggregation; collagen-stimulated thromboxane release; plasma concentrations of tissue-type plasminogen activator, plasminogen activator inhibitor 1, von Willebrand factor, and p-selectin; and flow-mediated and glyceryl-trinitrate-mediated brachial artery dilatation. The only significant results were for DHA, which reduced collagen aggregation of platelets and collagen stimulation of thromboxane release.

3) ALA versus EPA plus DHA

In the food chain, some higher plants make ALA but only cold water algae make EPA and DHA, which are in turn ingested by fish and incorporated into fish oil (70, 71). Although humans cannot synthesize the n−3 double bond, we do have the elongases and desaturases needed to convert ALA to EPA and DHA. However, this is an inefficient process, due in part to the large and increasing amounts of n−6 fatty acids in the diet, which compete for the same enzymes. Before 1940, the dietary ratio of n−6 to n−3 fatty acids was ≈4; since then, a profusion of n−6-rich, n−3-poor vegetable oils have entered the diet and the ratio has risen to between 10 and 20. Research is now needed to determine how much ALA in the diet would be required to make sufficient EPA and DHA given the current dietary ratio of n−6 to n−3 fatty acids. If the amount is higher than practicable, public health measures will have to entail either decreasing n−6 intake sufficiently to allow conversion to occur or direct ingestion of preformed EPA and DHA from fish or algal sources.

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