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Volume 4: Effects of Eicosapentanoic Acid and Docosahexanoic Acid on Mortality Across Diverse Settings: Systematic Review and Meta-Analysis of Randomized Trials and Prospective Cohorts

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The information in this report is intended to help health care decisionmakers—patients and clinicians, health system leaders, and policymakers, among others—make well-informed decisions and thereby improve the quality of health care services. This report is not intended to be a substitute for the application of clinical judgment. Anyone who makes decisions concerning the provision of clinical care should consider this report in the same way as any medical reference and in conjunction with all other pertinent information, i.e., in the context of available resources and circumstances presented by individual patients.

This report may be used, in whole or in part, as the basis for development of clinical practice guidelines and other quality enhancement tools, or as a basis for reimbursement and coverage policies. ODS, AHRQ, or the U.S. Department of Health and Human Services endorsement of such derivative products may not be stated or implied.

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Preface

The Agency for Healthcare Research and Quality (AHRQ), through its Evidence-based Practice Centers (EPCs), sponsors the development of evidence reports and technology assessments to assist public- and private-sector organizations in their efforts to improve the quality of health care in the United States. The Office of Dietary Supplements of the National Institutes of Health requested and provided funding for this report.

The reports and assessments provide organizations with comprehensive, science-based information on common, costly medical conditions and new health care technologies and strategies. The EPCs systematically review the relevant scientific literature on topics assigned to them by AHRQ and conduct additional analyses when appropriate prior to developing their reports and assessments.

To bring the broadest range of experts into the development of evidence reports and health technology assessments, AHRQ encourages the EPCs to form partnerships and enter into collaborations with other medical and research organizations. The EPCs work with these partner organizations to ensure that the evidence reports and technology assessments they produce will become building blocks for health care quality improvement projects throughout the Nation. The reports undergo peer review and public comment prior to their release as a final report.

AHRQ expects that the EPC evidence reports and technology assessments will inform individual health plans, providers, and purchasers as well as the health care system as a whole by providing important information to help improve health care quality.

We welcome comments on this evidence report. They may be sent by mail to the Task Order Officer named below at: Agency for Healthcare Research and Quality, 540 Gaither Road, Rockville, MD 20850, or by e-mail to epc@ahrq.hhs.gov.

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Nutritional Systematic Reviews

The medical and clinical communities have effectively used systematic reviews to develop clinical and public health practice guidelines, set research agendas, and develop scientific consensus statements. However, the use of systematic reviews in nutrition applications is more recent and limited. The Office of Dietary Supplements (ODS) at the National Institutes of Health (NIH) has been proactive and developed and evidence-based review program using the EPC program established by AHRQ, as part of a congressional mandate to review the current scientific evidence on the efficacy and safety of dietary supplements and identify research needs (http://ods.od.nih.gov/Research/Evidence-Based_Review_Program.aspx). To date, this program has sponsored 17 evidence reports on a range of supplement-related topics including B vitamins, ephedra, multivitamin/mineral supplements, omega-3 fatty acids, soy, and vitamin D. ODS is currently sponsoring an augmentation of the vitamin D report published in August 2007 to provide relevant information for a pending Institute of Medicine review of the current Dietary Reference Intakes for vitamin D and calcium. The completed ODS-sponsored evidence reports have resulted in numerous associated publications in scientific journals, have formed the basis for an NIH-sponsored state-of-the-science conference, and have been used to assist in setting research agendas.

To facilitate a better understanding of the challenges involved in conducting nutrition-related systematic reviews and in integrating these reviews with nutrition applications for which such reviews have not been previously used, ODS has sponsored the development of a series of technical reports via the EPC Program. The purpose of these reports was to: (1) identify the challenges, advantages, and limitations of conducting nutrition-based systematic reviews; (2) work with a panel of experts to explore approaches for integrating systematic reviews into processes associated with the derivation of nutrient intake reference values; (3) identify the breadth and quality of currently available nutrition-related systematic reviews against generally accepted quality guidelines within the contexts of the unique needs for nutrition topics; and (4) critically explore the consistencies and inconsistencies in results between observational and intervention studies and evaluate how the formulation of research questions may have contributed to these discrepancies.

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The authors' responsibilities were as follows:

- Trikalinos designed the research.
- Lee, Moorthy, Yu, and Chung conducted the research.
- Trikalinos and Lee performed statistical analysis.
- Trikalinos wrote the paper, which was critically commented on by all other authors.
- Trikalinos had primary responsibility for final content.
- All authors read and approved the final manuscript.

Effects of Eicosapentanoic Acid and Docosahexanoic Acid on Mortality Across Diverse Settings: Systematic Review and Meta-Analysis of Randomized Trials and Prospective Cohorts

Structured Abstract

Background: Eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) intake may protect from cardiovascular or all-cause mortality.

Objective: To synthesize evidence from randomized controlled trials (RCTs) and large prospective cohorts on the effects of EPA and DHA on cardiac, cardiovascular, or all-cause mortality.

Design: We conducted a systematic review with random effects meta-analysis and mixed effects dose-response meta-regression. Included were RCTs of EPA and DHA supplementation (>4 weeks of intervention, <6 grams per day) and large prospective cohorts (>1000 people, >3 years of followup) quantifying DHA or EPA intake.

Results: In RCTs, the summary relative risks for all-cause mortality (17 trials, 51,264 patients) and cardiovascular mortality (14 trials, 48,500 patients) were 0.95 (95% confidence interval, CI: 0.89, 1.01) and 0.89 (95% CI, 0.83, 0.96), respectively, with no evidence for heterogeneity. The effect of DHA and EPA was not significantly associated with population or study characteristics or supplement dose. In dose-response meta-regressions, mean EPA and DHA intake up to 0.20 grams daily was associated with decreased risk of cardiac, cardiovascular, or sudden cardiac death (odds ratio 0.64 per 0.20 grams average daily intake, 95% CI: 0.46, 0.89—data from 7 cohorts, 123,122 participants), with no significant change in risk (positive or negative) at higher mean intakes. Dose-response analyses were not statistically significant for other intake thresholds or alternative mortality definitions.

Conclusions: The maximal positive effect of EPA and DHA appears to plateau at a mean daily intake of 0.20 grams. There is no evidence that the effect of EPA and DHA on mortality phenotypes differs across populations and settings.

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Introduction

Since the seminal observation that Greenland Eskimos have lower cardiovascular disease mortality than genetically related Danes,[1,2]adequate intake of fish or the fatty acids unique to fish, eicosopentanoic acid (EPA, 20:5n-3) and docosexaenoic acid (DHA, 22:6n-3), have been recommended to decrease risk.[3,4] Potential mechanisms for reduced cardiovascular risk included anti-inflammatory, anti-thrombotic, antihypertensive, hypo-triglyceridemic and antiarrhythmic properties.[5] A large body of clinical research is available to address issues related to defining a potential association or a causative link between EPA and DHA intake and clinical cardiovascular outcomes. Nonetheless, the results of several dozen prospective cohort studies and randomized trials,[5-30] and several systematic reviews and meta-analyses generated from this body of work are inconsistent.[31-40]

In a previous systematic review we provided a qualitative synthesis of evidence from prospective cohorts and RCTs on the relationship between fish consumption and EPA and DHA intakes with cardiovascular clinical outcomes.[36,40] While there was evidence that higher consumption of fish was associated with favorable clinical outcomes in cohorts, the corresponding data on EPA and DHA intakes was equivocal. On the basis of available data others concluded that for daily intakes of (combined) EPA and DHA in excess of 0.30 to 0.50 grams, the impact of EPA and DHA on cardiovascular mortality reaches a plateau, and that this could explain the different conclusions based on the data from various cohorts, or cohorts and RCTs.[4] From this perspective, and in analogy to observations from clinical medicine,[41-43] prospective cohorts and RCTs offer complementary information on the association of interest, as they refer to different intake (dose) levels and periods of observations.

We performed a systematic review and meta-analysis of evidence from prospective cohorts and RCTs on the relationship of EPA and DHA intakes with cardiovascular or all-cause mortality. Our aim was to assess the apparent congruence of these two types of study designs and to describe how randomized intervention trials and observational studies compare in their target populations, outcome definitions and results, with the intent to defining the dose-response relationships of EPA and DHA intakes to cardiovascular and all-cause mortality across diverse settings and wide ranges of intake levels.

Subject and Methods

Literature Search

We included all eligible prospective cohorts and randomized trials that were identified in our previous comprehensive systematic review, which was based on literature searches of MEDLINE and the Cochrane Central Register of Controlled trials through July 2005 (with no lower date limit).[36,40] In addition, we updated this search from 2005 to May 2011 and supplemented the results with perusal of bibliographies from other systematic reviews. The exact search strategy is listed in the Systematic Review Protocol that accompanies this submission. Briefly, we used search terms for long chain polyunsaturated n-3 fatty acids (EPA and DHA) and fish oils, as well as terms related to cardiovascular disease. Searches were limited to English language publications and to humans.

Eligibility Criteria

We included randomized controlled trials (with at least 4 weeks of intervention duration and less than 6 grams per day of EPA and DHA supplementation) and prospective cohorts (with at least 3 years of follow-up and at least 1000 participants) reporting on the association between EPA or DHA intake and various definitions of mortality, namely cardiac, cardiovascular, or all-cause mortality. For RCTs, the interventions of interest were EPA, DHA or fish oil (defining fish oils as EPA and DHA). For the prospective cohorts, our exposure of interest was dietary intake levels of EPA and DHA either as fish or supplements. Studies containing information on fish intake without reporting the amount of EPA and DHA or interventions that involved alpha-linolenic acid (ALA) only were excluded. Eligible were studies on human subjects with or without a history of cardiovascular disease (secondary or primary prevention settings, respectively). Two methodologists evaluated potentially relevant articles that met the eligibility criteria. Any disagreements were resolved by consensus or by a third arbitrator when consensus could not be reached.

Data Extraction and Synthesis

Each study was extracted by a single investigator using piloted data-extraction forms. We did not contact primary study authors, and relied on the information reported in the publications. Data extraction included the following items: study design, population characteristics (country, mean age, gender distribution), type and intake or dosage of EPA and DHA, comparison groups (placebo or comparative control, when applicable), sample size, and outcomes of interest. We also extracted information on items related to methodological quality and on funding sources (described in a following paragraph). We accepted the definition of cardiac and cardiovascular mortality that was used in the primary studies. Typically, cardiac mortality was death ascribed to coronary heart disease or sudden cardiac death. Cardiovascular mortality also included vascular deaths (e.g., fatal strokes).

For RCTs we extracted number of events in the intervention and control groups. For prospective cohorts we recorded risk or hazard ratios for each quantile-category (i.e., tertiles, quartiles, or quintiles) of estimated DHA or EPA intake compared with a reference group. We also classified the adjustments performed in each prospective cohort into eight categories: demographic (i.e., age, marital status, race, gender, area); socio-economic (i.e., education, occupation); anthropometric (i.e., body mass index); health (i.e., cardiovascular disease, history of hypertension, diabetes, hypercholesterolemia or blood pressure); life style (i.e., smoking, alcohol consumption); nutrients (i.e., dietary intakes of cholesterol, saturated fat, omega-6 polyunsaturated fatty acids); energy (i.e., total energy intake), and other factors (i.e., treatment assignment).

Grading Methodological Quality

We recorded the following items that have been proposed as indicative of methodological quality of RCTs: adequate description of randomization mode, use of double blinding (for participants and outcome assessors), proportion of dropouts, using valid methods to assess baseline intake, and discrepancies in the reporting of results.[44] For prospective cohorts we assessed the following nine items: unbiased selection of the cohort; large sample size (more than 10,000 subjects); adequate description of the cohort; use of validated dietary assessment method; reporting of methods to estimate long chain omega-3 fatty acid intake; use of validated methods

for ascertaining clinical outcomes; adequately long follow-up (at least 5 years); more than 80 percent completeness of follow-up; and analyses adjusting for energy.[44]

Evidence Synthesis for Randomized Controlled Trials

We calculated summary relative risks by random effects meta-analysis using the DerSimonian and Laird model.[45] We tested for between-study heterogeneity with the *Q* statistic (considered significant at the 0.10 level) and quantified its extent with the I^2 statistic[46] I^2 ranges between 0 and 100 percent and expresses the proportion of between study variance that is attributable to heterogeneity rather than chance.

We further investigated potential associations of the treatment effect with study-level variables in subgroup analyses and random effects meta-regressions. Specifically, we examined country of origin (United States versus other; countries with high background intakes of EPA and DHA such as Japan and Norway versus other), randomization mode (adequately described versus not), double-blinding (used versus not used or not described), funding (any industry funding versus not), excessive drop-out rates (using 20 percent as threshold), supplementation dose (as a continuous variable, per 0.20 grams per day), and type of population (classified in three groups). The three population groups were: patients with history of coronary artery disease or myocardial infarction; patients with intermittent claudication or hypercholesterolemia; and patients with implantable cardioverter defibrillators.

Evidence Synthesis for Prospective Cohorts

For each cohort study, we calculated statistics to perform dose-response meta-regressions. We back-calculated the "effective counts" of events in each category of EPA and DHA intake based on the pertinent adjusted log odds ratios (versus a reference intake category), their variance, and the total number of participants per intake category.[47] The effective counts of events are such that when used in a logistic regression with the intake categories as the sole predictors they result in the same log odds ratios (coefficients), variances and covariances as those from the original adjusted model. If one started from the results of an unadjusted model, the effective counts would be identical to the actual counts of events.

The mean intake value per category of intake level is also needed for dose-response meta-regressions. When it was not reported, we selected the midpoint between intake category thresholds, and for the lowest and highest intake categories we imputed a mean intake 20 percent lower for the lowest quintile threshold or 20 percent higher for the highest quintile threshold, respectively. This method yielded very similar results to fitting a normal density over intake percentiles in two examples, and was retained for simplicity.

We then evaluated the association between cardiac or all-cause mortality and mean intake levels across all studies in meta-regression models. We performed two sets of analyses: We used a fixed intercept–fixed slope model, which is essentially a logistic regression with indicator variables for different studies. This analysis ignores any heterogeneity in the strength of the dose-response association (slopes) across studies, and is analogous to a fixed effects meta-analysis. We relaxed this assumption by also fitting a logistic mixed effects meta-regression (with fixed intercepts and random slopes), which explicitly models between-study variability in the strength of the dose-response relationship.

Further, we examined several different dose response relationships: a linear dose-response model and models that allowed different associations for mean intake levels above or below 0.20, 0.30, 0.40 or 0.50 grams per day (using a piecewise linear spline with a knot at the

corresponding threshold). The latter splined analyses explicitly address the claim that the effects of EPA and DHA plateau at a low intake value.[4] The above thresholds are in the same range as the intake of 0.30-0.50 grams of EPA and DHA per day currently recommended by the American Heart Association.[3] We also performed meta-regressions using quality items as covariates interacting with the average EPA and DHA intake level (dose), to assess for relationships between the dose-response coefficients and study-level quality items.

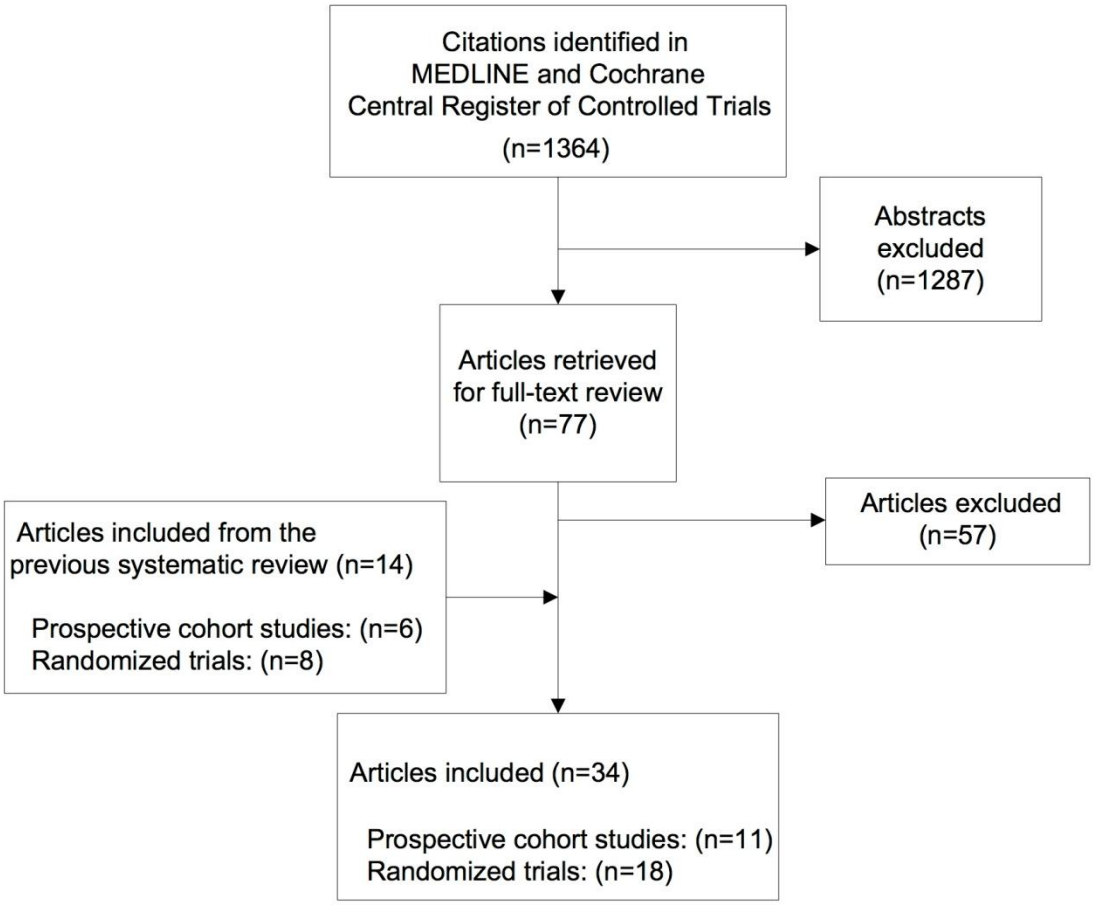
Software

Analyses were performed in Stata SE (version 11, Stata Corporation, College Station, Texas, U.S.) and Meta-Analyst (version 3 beta, Boston, U.S.).[48] Unless otherwise stated, all tests are two-tailed and considered significant when $p<0.05$.

Results

The updated searches returned 1,364 abstracts, of which 77 were retrieved and reviewed in full text (Figure 1). After excluding overlapping publications and together with the studies identified in our previous report, 18 independent RCTs (described in 23 publications[5-20,49-55] and 11 prospective cohorts[21-30,56] were eligible.

Figure 1. Literature search and selection



Randomized Controlled Trials

Of the 18 eligible trials, 13 were conducted in Europe, three in the United States, one in Japan and one in India (Table 1). Twelve trials enrolled patients with a history of heart disease[6,7,9,11-15,17-19,50,54,55] (Group 1), three enrolled patients with claudication[10] or hypercholesterolemia[20,49] (Group 2), and three included patients with implantable defibrillators (Group 3).[5,8,16] The majority included predominantly male patients with mean ages in the early

sixties. No trial adequately described the distribution of EPA or DHA intake at baseline, but two trials reported average serum EPA and DHA measurements suggesting low mean intake at baseline (e.g., corresponding to less than one fish per week).[8,20] In the intervention arms, supplementation included purified EPA and DHA (ethyl esters), EPA alone, or fish oil. In the RCTs daily supplement doses ranged from approximately 0.27 to 6 grams. Control arms received a variety of non-marine oils (Table 1).

Table 1. Randomized controlled trials of the effects of long chain n-3 fatty acid supplements on mortality phenotypes

Study (Year), Country (Reference)	Mean age [y] (males [%])	Population	Baseline EPA +DHA	Intervention		Control		Quality items:				
				N	Description	N	Description	A	B	C	D	E
Group 1 (patients with history of coronary artery disease or myocardial infarction)												
von Schacky (1999), Germany (19)	59 (76)	CAD	ND	111	EPA+DHA (3.4 g/d for 3 mo, then 1.7 g/d for 21 mo)[1]	112	Equivalent dose of mixed FA; no marine n-3 FA	Y	Y	Y	N	Y
Sacks (1995), United States (17)	62 (93)	CAD	ND	31	EPA+DHA (6 g/d)[2]	28	Olive oil (6 g/d)	Y	Y	Y	Y	Y
Johansen (1999), Norway (6)	60 (78)	CAD patients undergoing PTCA	26% taking fish oil	250	EPA+DHA (5.1 g/d)	250	Placebo	Y	Y	Y	Y	Y
Calo (2005),Italy (9)	66 (85)	Patients undergoing CABG	ND	79	EPA+DHA (1:2 ratio, 0.85 g/d)	81	Placebo	Y	N	N	N	Y
Durrington (2001), United Kingdom (7)	59 (73)	CHD	ND	30	EPA (44%) +DHA (36%) from n-3 FA (4g/d)+ simvastatin	29	Corn oil (4g/d) + simvastatin	Y	Y	N	N	Y
Nilsen (2001), Norway (11)	64 (80)	Recent MI	ND	150	EPA+DHA (1:2 ratio, from 4 g/d n-3 FA)	150	Corn oil (4 g/d)	Y	N	N	N	Y
GISSI-Prevenzione[3] (2002), Italy (12-14)	60 (85)	Recent MI	≤1fish/ week (73% of patients)	5665	EPA+DHA (1:2 ratio, 0.85 g/d) ± vitamin E (0.3 g/d)	5658	Vitamin E (0.3 g/d) or no supplement	Y	N	Y	N	Y
GISSI-HF (2008), Italy (15;51;52)	67 (78)	Heart failure	ND	3494	EPA+DHA (1:2 ratio, 0.85 g/d)	3481	Placebo	Y	Y	N	N	Y
Singh[4] (1997), India (18)	49 (93)	Recent MI	ND	122	EPA+DHA (1.8 g/d)[5]	118	Aluminum hydroxide (0.1 g/d)	Y	Y	N	N	N

Table 1. Randomized controlled trials of the effects of long chain n-3 fatty acid supplements on mortality phenotypes (continued)

Study (Year), Country (Reference)	Mean age [y] (males [%])	Population	Baseline EPA +DHA	Intervention		Control		Quality items:				
				N	Description	N	Description	A	B	C	D	E
Rauch (2010), Germany (54)	64 (74)	Recent MI	Fish intake significantly increased during study in both groups	1940	1 g/d n-3-acid ethyl esters-90 (460 mg EPA; 380 mg DHA)	1911	Placebo olive oil (1g/d)	Y	Y	Y	N	Y
Galan (2010), France (55)	61 (80)	Recent CHD	ND	1253	400 mg EPA + 200 mg DHA	1248	Placebo	Y	Y	Y	N	Y
Kromhout (2010) Netherlands (50)	69 (78)7	MI in past 10 years	EPA + DHA: 125 mg/day	2404	Margarine (0.4 g/day of EPA-DHA in ratio of 3:2 w/ or w/o 2 g of ALA)	2433	Placebo margarine (w/ or w/o 2 g of ALA)	Y	Y	Y	N	Y
Group 2 (patients with intermittent claudication or hypercholesterolemia)												
Leng (1998), Scotland (10)	66 (68)	Claudication	ND	60	EPA (0.27 g/d)	60	Sunflower seed oil (3 g/d)	Y	Y	Y	N	Y
JELIS (2007), Japan (20;53)	61 (32)	Hypercholesterolemia	Plasma EPA: 2.9 mol%	9326	EPA (1.8 g/d) + statins	9319	statins	Y	N	N	N	Y
Einvik (2010) Norway (49)	70 (100)7	Hypercholesterolemia	EPA 1.4% of FA intake; DHA 1.1% of FA intake	282	2.4 g n-3 PUFA / day8 (w/ or w/o diet counselling)	281	Corn oil (w/ or w/o diet counselling)	Y	Y	Y	N	Y
Group 3 (patients with implantable cardioverter defibrillators)												
Brouwer (2006), Europe[6] (8)	62 (84)	Patients with ICD	Serum EPA 1.1% of cholesteryl esters	273	Fish oil (2 g/d) of fish oil [9]	273	High-oleic acid sunflower oil (2 g/d)	Y	Y	N	N	Y
Leaf (2005), United States (5)	66 (83)	Patients with ICD	EPA+DHA 3.5% of FA intake	200	EPA+DHA (2.6 g/d) from n-3 FA (4g/d)	202	Olive oil (4 g/d)	Y	Y	N	N	Y
Raitt (2005), United States (16)	63 (86)	Patients with ICD	≤1 fatty fish meal/week.	100	EPA+DHA (1.3g/d) from fish oil (1.8 g/d)	100	Placebo (olive oil: 73% oleic acid, 12% palmitic acid)	Y	N	Y	N	Y

For quality items, A= randomization mode, B=double blinding, C= less than 20% dropouts, D= valid diet assessment for assessing background exposure, E= Report without internal inconsistencies

Abbreviations: EPA = eicosapentaenoic acid; CAD = coronary artery disease; CHD = coronary heart disease; DHA = docosahexaenoic acid; ICD = Implantable cardioverter defibrillators; MI = myocardial infarction; GISSI-HF or -Prevenzione, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico –Heart Failure or –Prevenzione; JELIS = Japan EPA Lipid Intervention Study; mo, months; FA, fatty acids; RR, relative risk

1 Fish oil concentrate (55% EPA+DHA) 6 grams/day for 3 months and 3 grams/day for 21 months

2 EPA 2.88 grams/day, DHA 1.92 grams/day and other (mainly DHA) 1.2 grams/day

3 Data on GISSI-Prevenzione were extracted from three publications. Main result data are from Marchioli, et al., 2002 and 2005.(12;13) Macchia, et al., 2005(14) reports on a subgroup with progressive impairment of left ventricular systolic function – which was used in sensitivity analyses.

4 This study was highly criticized (see text)(70;71)

5 EPA 1.08 grams/day and DHA 0.72 grams/day

6 Poland, Germany, the Netherlands, United Kingdom, Czech Republic, Belgium, Austria, Switzerland

7 For baseline characteristics, a weighted mean of intervention and placebo groups is reported.

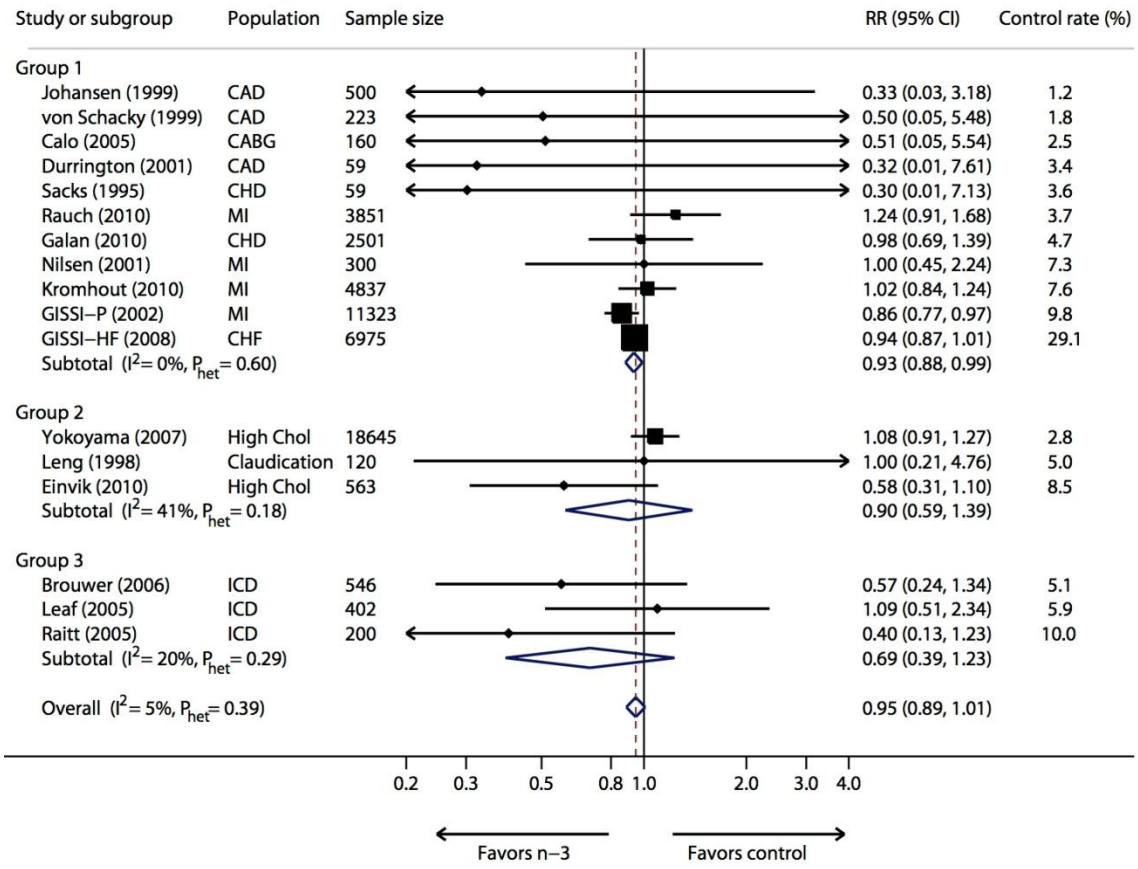
8 2.4 grams n-3 fatty acids (49% EPA, 35% DHA, 3.5 mg tocopherols/gram) taken as 2 capsules twice a day.

9 EPA (464 mg) + DHA (335 mg) + other n-3 FA (162 mg)

The median RCT sample size was 451 (25th percentile 200, 75th percentile 3,851), with three trials having a sample size in excess of 5000. With the exception of two trials that were 28 days[9] and 6 months[6] in duration, the majority of trials ranged from 1 to 5 years. All RCTs reported details on mode of randomization. Blinding of both participants and outcome assessors was reported in 12 RCTs.[5-8,10,15,17-19,50,54,55] Loss to follow up was reported as less than 20 percent in 10 trials (citations).[6,10,12-14,16,17,19,50,54,55] No RCT provided an estimate of mean dietary EPA and DHA intakes at baseline, although two report using a validated dietary instrument to assess participants' baseline intake.[6,17] Finally, the publication by Singh, et al.,[18] contained a number of internal discrepancies, suggesting suboptimal reporting, analyses, and study conduct, or the possibility of fraud by the first author.[57] Because this publication was never retracted, we included the results in the main analyses and excluded the results from the subgroup analyses.

Figure 2 shows the meta-analysis of 17 RCTs (51,264 participants) with data on all-cause mortality. The summary random effects risk ratio was 0.95 (95% confidence interval, CI: 0.89, 1.01), with little evidence for between-study heterogeneity. Three trials contributed 72 percent (n=36,943) of the patients in the meta-analysis, and their summary relative risk was 0.94 (95% CI 0.85, 1.04; Table 2). A borderline statistically significant effect was observed in several subgroup analyses: in the 11 RCTs in patients with a history of coronary artery disease (Group 1; relative risk 0.93 with 95% CI 0.8, 0.99), the subgroup of six RCTs in which less than 20 percent of patients were lost to follow-up (relative risk 0.85 with 95% CI 0.76, 0.95), and the 13 studies conducted in countries with lower average intakes of EPA and DHA than Japan and Norway (relative risk 0.93, 95% CI 0.88, 0.99). Effects across subgroups did not differ beyond what was expected by chance (meta-regression analyses, (Table 2). There was no evidence for an association between treatment effect and supplement dose (relative risk changed by a factor of 1.00 per 0.20 grams per day increase in supplement dose, 95% CI 0.97, 1.03).

Figure 2. Randomized controlled trials of the effect of EPA and DHA supplementation on all-cause mortality



Group 1 includes studies in patients with history of coronary artery disease or myocardial infarction; Group 2 includes studies in patients with intermittent claudication or hypercholesterolemia; and Group 3 includes studies in patients with implantable cardioverter defibrillators.
CAD: coronary artery disease; CABG: coronary artery bypass grafting; CHF: chronic heart failure; High Chol: hypercholesterolemia; GISSI-P: GISSI-Prevenzione trial; ICD: implantable cardiac defibrillators; MI: myocardial infarction

Table 2. Results of subgroup and sensitivity analyses in randomized trials

	All-cause mortality				Cardiac mortality			
	Studies (Patients)	**RR (95% CI)**	**Meta-regression (*P* value)**	**Heterogeneity *P* value (I^2 %)**	**Studies (Patients)**	**RR (95% CI)**	**Meta-regression (*P* value)**	**Heterogeneity *P* value (I^2 %)**
All studies	17 (51264)	0.95 (0.89, 1.01)	–	0.39 (5)	14 (48500)	0.89 (0.83, 0.96)*	–	0.63 (0)
Subgroup analyses								
Randomization mode clear								
Yes	17 (51264)	0.95 (0.89, 1.01)	–	0.39 (5)	14 (48500)	0.89 (0.83, 0.96)*	–	0.63 (0)
No	–	–		–	–	–		–
Double-blinding								
Yes	12 (20636)	0.95 (0.89, 1.02)	0.74	0.59 (0)	10 (18032)	0.91 (0.84, 0.99)*	0.24	0.53 (0)
No	5 (30628)	0.93 (0.77, 1.13)		0.12 (45)	4 (30468)	0.82 (0.70, 0.95)*		0.75 (0)
Drop-out rates <20%								
Yes	11 (38839)	0.97 (0.91, 1.03)	0.09	0.45 (0)	10 (36418)	0.92 (0.84, 0.99)*	0.15	0.65 (0)
No	6 (12425)	0.85 (0.76, 0.95)*		0.69 (0)	4 (12082)	0.79 (0.68, 0.93)*		0.65 (0)
Population group[1]								
Group 1	11 (30788)	0.93 (0.88, 0.99)*	0.41	0.60 (0)	9 (28144)	0.89 (0.83, 0.96)*	0.62	0.49 (0)
Group 2	3 (19328)	0.90 (0.59, 1.39)		0.18 (41)	2 (19208)	0.86 (0.55, 1.34)		0.47 (0)
Group 3	3 (1148)	0.69 (0.39, 1.23)		0.29 (20)	3 (1148)	0.64 (0.35, 1.18)		0.41 (0)
Industry funding								
Yes	11 (42744)	0.94 (0.84, 1.04)	0.57	0.14 (33)	10 (42521)	0.89 (0.83, 0.96)*	0.92	0.69 (0)
No	6 (8520)	1.01 (0.85, 1.19)		0.93 (0)	4 (5979)	0.80 (0.54, 1.19)		0.23 (30)
Performed in the United States								
Yes	3 (661)	0.72 (0.34, 1.53)	0.5	0.29 (19)	3 (661)	0.76 (0.36, 1.64)	0.74	0.52 (0)
No	14 (50603)	0.95 (0.89, 1.01)		0.37 (7)	11 (47839)	0.89 (0.83, 0.96)*		0.50 (0)

Table 2. Results of subgroup and sensitivity analyses in randomized trials (continued)

	All-cause mortality				Cardiac mortality			
	Studies (Patients)	RR (95% CI)	Meta-regression (*P* value)	Heterogeneity *P* value (I^2 %)	Studies (Patients)	RR (95% CI)	Meta-regression (*P* value)	Heterogeneity *P* value (I^2 %)
Performed in Countries With Background Intake								
Yes	4 (20008)	0.91 (0.64, 1.29)	0.4	0.23 (31)	4 (20008)	0.85 (0.57, 1.27)	0.92	0.73 (0)
No	13 (31256)	0.93 (0.88, 0.99)*		0.52 (0)	10 (28492)	0.88 (0.80, 0.96)*		0.39 (5)
Study size								
Exclude 3 largest studies [2]	14 (14321)	0.99 (0.86, 1.13)	0.75	0.57 (0)	11 (11557)	0.83 (0.67, 1.02)	0.56	0.62 (0)
Only 3 largest studies	3 (36943)	0.94 (0.85, 1.04)		0.09 (59)	3 (36943)	0.89 (0.82, 0.98)*		0.33 (9)
Internal inconsistencies								
Yes	–	–	–	–	1 (240)	0.52 (0.28, 0.95)*	0.11	–
No	–	–		–	13 (48260)	0.90 (0.83, 0.97)*		0.81 (0)

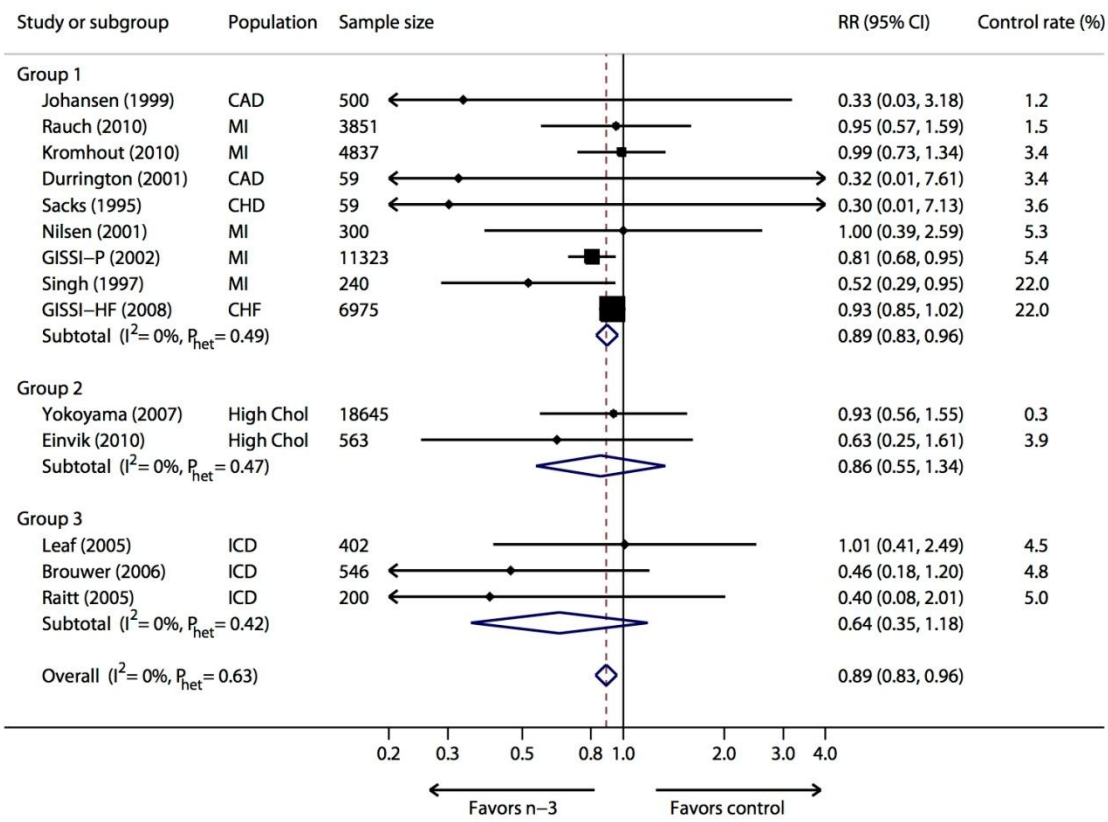
* $p<0.05$

1 Group 1: patients with coronary artery diseases, undergoing coronary artery bypass surgery or with recent myocardial infarction; Group 2: patients with intermittent claudication or hypercholesterolemia; Group 3: patients with implantable cardioverter defibrillators.

2 GISSI-Prevenzione,(12-14) GISSI-Heart Failure(15;51;52) and JELIS.(20;53)

In the meta-analysis of 14 trials (48,500 patients) with data on cardiac mortality, the relative risk was 0.89 (95% CI, 0.83, 0.96) favoring supplementation (Figure 3). There was no evidence of between-study heterogeneity based on Cochran's *Q* and the I^2 statistic. The point estimates of the relative risks were similar across subgroups (Table 2). In subgroup analyses the relative risks of cardiac mortality remained statistically significant in several subgroups, including trials performed in countries with presumed lower background intakes, and trials in patients with a history of coronary artery disease (Group 1). Excluding the trial by Singh, et al.,[18] that had internal inconsistencies and a large effect size (relative risk 0.50), did not affect the summary estimate. No differences in the effects in each subgroup in meta-regression analyses were statistically significant (Table 2). Again, there the treatment effect was not associated with supplement dose beyond what would be expected by chance (relative risk changed by a factor of 0.99 per 0.20 grams per day increase in supplement dose, 95% CI 0.95, 1.02).

Figure 3. Randomized controlled trials of the effect of EPA and DHA supplementation on cardiac mortality



Group 1 includes studies in patients with history of coronary artery disease or myocardial infarction; Group 2 includes studies in patients with intermittent claudication or hypercholesterolemia; and Group 3 includes studies in patients with implantable cardioverter defibrillators.
CAD: coronary artery disease; CABG: coronary artery bypass grafting; CHF: chronic heart failure; High Chol: hypercholesterolemia; GISSI-P: GISSI-Prevenzione trial; ICD: implantable cardiac defibrillators; MI: myocardial infarction

Prospective Cohort Studies

Of the 11 eligible prospective cohort studies, two[23,28] that did not report sufficient data were excluded from quantitative analyses (Table 3). All nine remaining studies included participants without prior history of cardiovascular disease. EPA and DHA intake was estimated from fish consumption using frequency questionnaires in six studies and dietary history questionnaires in two studies. Mean or median daily intakes ranged from 0.04 to approximately 0.90 grams, the highest intake corresponding to a Japanese cohort.[27] The higher intake category (percentile) threshold was slightly under 0.30 grams per day in cohorts from China[22] and the United States.[25]

Table 3. Prospective cohort studies (general population) on the effects of EPA and DHA consumption on mortality phenotypes

Author, Year, Country (Reference)	Sample size	Males (%)	Age mean (range), [y]	Dietary assessment	EPA, DHA intake (g/d) [1]	Duration (y)	Outcome (ascertainment)	Adjustments	Quality assessment								
									A	B	C	D	E	F	G	H	I
Studies reporting on cardiac or cardiovascular death																	
Pietinen et al, 1997, Finland (21)	21930	100	NR (50-69)	FFQ	0.4	6.1	Coronary death from central population register, death certificates	DEM, SES, ANTHRO, HEALTH, LIFE, NUTRIENT, ENERGY, OTHER	Y	Y	Y	Y	Y	Y	Y	Y	Y
De Goede et al, 2010, Netherlands (56)	21342	45	42 (20-65)	FFQ	0.1	11.3	CHD death from registry	DEM, ENERGY, LIFE, SES, NUTRIENT	Y	Y	Y	Y	Y	Y	Y	Y	Y
Yuan et al, 2001, China (22)	18244	100	56 (45-64)	FFQ	0.1	12	Acute MI death from death certificates	DEM, SES, ANTHRO, HEALTH, LIFE, ENERGY	Y	Y	Y	Y	Y	Y	Y	Y	Y
Kamphuis et al, 2006, Netherlands (24)	2574	100	NR(70-90)	Dietary history method	0.1	10	CVD death from general practitioners, hospital registries	DEM, SES, ANTHRO , HEALTH, LIFE, ENERGY	Y	N	N	N	Y	Y	Y	NR	Y

Table 3. Prospective cohort studies (general population) on the effects of EPA and DHA consumption on mortality phenotypes (continued)

Author, Year, Country (Reference)	Sample size	Males (%)	Age mean (range), [y]	Dietary assessment	EPA, DHA intake (g/d) [1]	Duration (y)	Outcome (ascertainment)	Adjustments	Quality assessment								
									A	B	C	D	E	F	G	H	I
Studies reporting on cardiac, cardiovascular or sudden cardiac death																	
Iso et al, 2006, Japan (27)	33262	48	NR (40-59)	FFQ	0.9	10	Sudden cardiac death (ND)	DEM, SES, ANTHRO, HEALTH, LIFE, NUTRIENT, ENERGY	Y	Y	Y	Y	Y	Y	Y	Y	Y
Albert et al, 1998, United States (25)	20551	100	NR (40-84)	FFQ	0.3	11	Sudden death from medical records, reports from next of kin	DEM, ANTHRO, HEALTH, LIFE, NUTRIENT, OTHER	Y	Y	Y	Y	Y	Y	Y	Y	Y
Studies reporting on all-cause deaths																	
Folsom et al. 2004, United States (29)	41835	0	54 (55-69)	FFQ	0.1	14	All-cause deaths from questionnaires, death records, national death index	DEM, SES, ANTHRO, HEALTH, LIFE, NUTRIENT, ENERGY	Y	Y	N	Y	Y	Y	Y	Y	Y
Nagata et al, 2002, Japan (30)	29080	46	55 (35-NR)	FFQ	0.8	7	All-cause deaths (ND)	DEM, ANTHRO, HEALTH, LIFE, ENERGY	Y	Y	N	Y	Y	Y	Y	Y	Y

Table 3. Prospective cohort studies (general population) on the effects of EPA and DHA consumption on mortality phenotypes (continued)

Author, Year, Country (Reference)	Sample size	Males (%)	Age mean (range), [y]	Dietary assessment		EPA, DHA intake (g/d) [1]	Duration (y)		Outcome (ascertainment)	Adjustments	Quality assessment								
											A	B	C	D	E	F	G	H	I
Studies that do not report sufficient data for meta-regression																			
Dolecek et al., 1992, United States (23)			6250	100	(35-57)	Multiple 24-hr dietary recall	0.04	10.5	All-cause and CHD mortality (National Death Index, death certificates)	DEM, HEALTH, LIFE	Y	Y	Y	NR	Y	Y	Y	Y	N
Kaushik et al, 2008, Australia (28)			2683	56	49	FFQ	ND	10	CHD death	DEM, SES, ANTHRO, HEALTH, LIFE, ENERGY	Y	Y	Y	N	N	NR	Y	Y	Y

Abbreviations: FA = Fatty acids; FFQ = Food frequency questionnaire; US = United States; BMI = Body mass index; CHD = Coronary heart disease; HDL= High density lipoprotein; DEM = Demographics; SES = Socio-economic status; ANTHRO = Anthropometric data; HEALTH = health or disease conditions; LIFE = lifestyle variables such as smoking, alcohol consumption, or physical activity; NUTRIENT = other nutrient intakes; NR, Not reported

For quality assessment: A, unbiased cohort selection; B, large sample size (>10000), C, adequate description of participants, D: validated dietary assessment method, E: Quantification of the type and amount of long chain n-3 fatty acid intakes, F: adequate method to ascertain clinical outcome, G: long follow-up period (at least 5 years), H: completeness of data throughout follow-up (at least for 80 percent of participants), I: Multivariate analyses adjusted for energy or nutritional variables

1 Mean or median intake of EPA and DHA

All prospective cohorts described unbiased selection of subjects and had follow-up periods ranging from 6.1 to 21.5 years (Table 3). Eight out of 11 cohorts enrolled at least 10,000 participants. At the end of the observation period survival status was available for at least 80 percent of participants for all except one study.[24] All except one study[24] reported using a validated method to assess EPA and DHA intake and survival status, and performed multivariate analyses adjusting for potential confounders. The outcome of interest was cardiac or cardiovascular mortality in 5 studies (6 meta-regression entries as Jarvinen, et al.,[26] reported separate data per sex), sudden cardiac death in two, and all cause mortality in the remaining two.

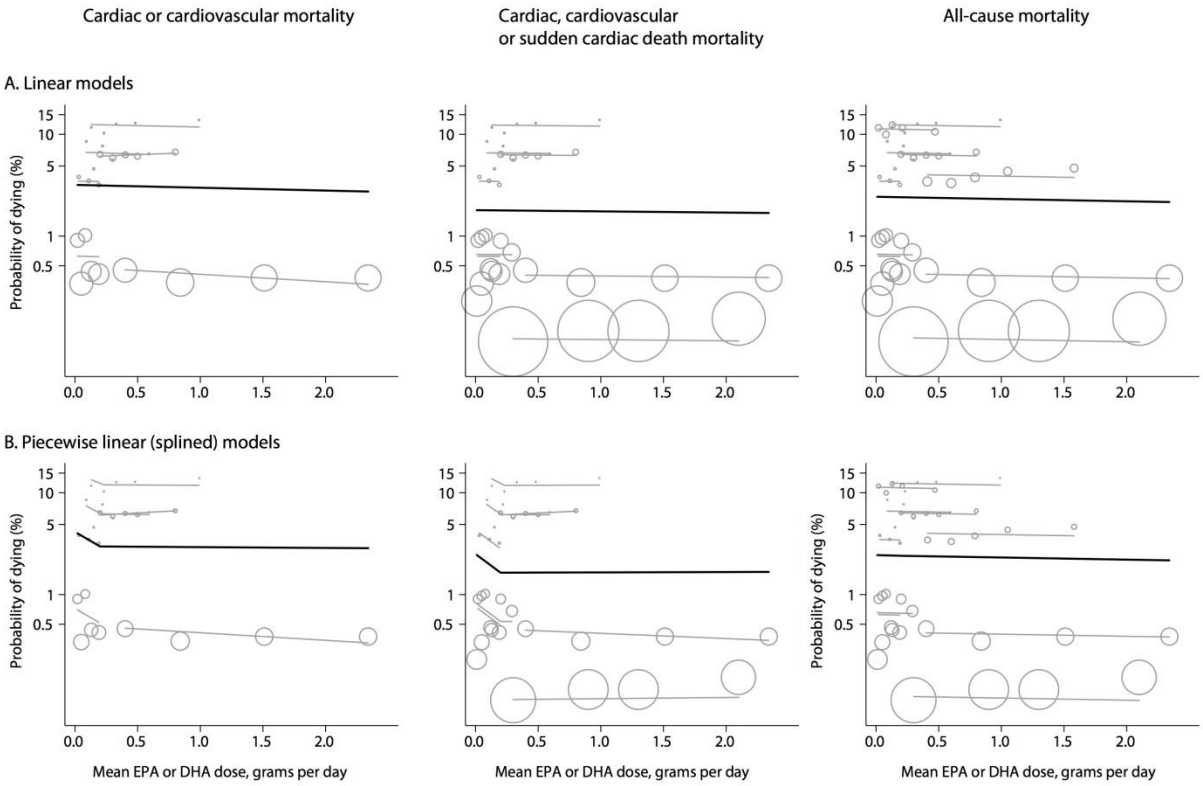
Table 4 shows the results of linear and piecewise linear (splined) dose-response meta-regression analyses for increasingly broader definitions for mortality: cardiac or cardiovascular (left most panel, 6 meta-regression entries); cardiac, cardiovascular or sudden cardiac death (middle panel, 8 meta-regression entries); and all-cause (rightmost panel, 10 meta-regression entries). Overall, in meta-regression analyses (Figure 4) based on adjusted data (main analyses) we identified a statistically significant association between increasing mean EPA or DHA intake up to 0.20 grams per day and decreased risk of cardiac, cardiovascular or sudden cardiac death (odds ratio 0.64 per 0.20 grams average daily intake, 95% CI: 0.45 to 0.89), with no change in the risk at higher mean intakes where the odds ratio is 1.00 (Table 4; p=0.03 for joint testing of both mean intake variables, above or below the spline threshold). Associations in the same direction were not statistically significant for other combinations of definitions of mortality and thresholds for linear splines. In meta-regressions using unadjusted data the risk of any death increased with higher mean EPA or DHA intakes beyond what would be expected by chance (Table 4); however, this may be indicative of confounding. Overall, there is no evidence for substantial heterogeneity in the strength of the association across studies, as the estimated standard deviation of the study-specific slopes is very small ($<10^{-5}$) in all analyses.

Table 4. Dose-response meta-regression analyses in prospective cohort studies

Definition of mortality (references) Strata (Participants)	Threshold (grams/day)	Relative OR (95% CI) per 0.20 g/day in mean intake			
		Relative ORadj	Pintake, adj	Relative ORunadj	Pintake, unadj
Cardiac or cardiovascular mortality (21;22;24;26;56) 6 (69309)	— (linear)	0.99 (0.96, 1.03)	0.753	1.01 (0.98, 1.05)	0.423
	≤0.20	0.70 (0.48, 1.02)	0.184	0.69 (0.49, 0.97)	0.066
	>0.20	1.00 (0.97, 1.04)		1.02 (0.99, 1.06)	
	≤0.30	0.93 (0.77, 1.13)	0.752	0.89 (0.74, 1.06)	0.242
	>0.30	1.00 (0.96, 1.04)		1.03 (0.99, 1.07)	
	≤0.40	1.03 (0.91, 1.15)	0.813	0.98 (0.88, 1.10)	0.626
	>0.40	0.99 (0.94, 1.03)		1.02 (0.98, 1.07)	
	≤0.50	1.03 (0.95, 1.13)	0.569	1.00 (0.93, 1.09)	0.701
	>0.50	0.98 (0.93, 1.03)		1.02 (0.97, 1.07)	
Cardiac, cardiovascular or sudden cardiac death mortality (21;22;24-27;56) 8 (123122)	— (linear)	0.99 (0.96, 1.03)	0.749	1.02 (0.99, 1.05)	0.196
	≤0.20	0.64 (0.46, 0.89)*	0.030	0.95 (0.73, 1.25)	0.383
	>0.20	1.00 (0.97, 1.04)		1.02 (0.99, 1.06)	
	≤0.30	0.88 (0.74, 1.05)	0.357	0.97 (0.83, 1.14)	0.350
	>0.30	1.01 (0.97, 1.05)		1.03 (0.99, 1.06)	
	≤0.40	1.00 (0.89, 1.11)	0.950	1.01 (0.91, 1.13)	0.427
	>0.40	0.99 (0.95, 1.04)		1.02 (0.98, 1.07)	
	≤0.50	1.02 (0.94, 1.10)	0.815	1.02 (0.94, 1.10)	0.431
	>0.50	0.98 (0.94, 1.03)		1.02 (0.98, 1.07)	
All-cause mortality (21;22;24-27;29;30;56) 10 (194037)	— (linear)	0.99 (0.97, 1.01)	0.311	1.03 (1.01, 1.05)	0.003
	≤0.20	0.98 (0.89, 1.08)	0.593	0.97 (0.89, 1.06)	0.004
	>0.20	0.99 (0.97, 1.01)		1.04 (1.01, 1.06)	
	≤0.30	0.98 (0.92, 1.05)	0.581	0.96 (0.90, 1.02)	<0.001
	>0.30	0.99 (0.97, 1.01)		1.04 (1.02, 1.07)	
	≤0.40	0.99 (0.94, 1.04)	0.597	0.97 (0.93, 1.01)	<0.001
	>0.40	0.99 (0.96, 1.01)		1.05 (1.03, 1.07)	
	≤0.50	0.99 (0.95, 1.03)	0.599	0.98 (0.95, 1.02)	<0.001
	>0.50	0.99 (0.96, 1.02)		1.06 (1.03, 1.08)	

Jarvinen et al. (26) reported separate data per sex, and has been entered as two strata (two meta-regression entries) in the analyses. For each mortality phenotype definition we performed a linear and four piecewise linear spline meta-regressions. In the splined dose response analyses we allowed separate linear dose-response relationships below or above a threshold of 0.20 through 0.50 grams of mean EPA and DHA intake per day. In each row, the first number is the slope for average mean intakes up to the threshold (0.20 to 0.50 grams per day); the second number is the slope for average intakes in excess of the threshold. Relative OR_{adj} (OR_{unadj}): Relative odds ratio per 0.20 grams per day of higher mean intake using adjusted data (using unadjusted data); $P_{intake,\ adj}$ ($P_{intake,\ adj}$): p-value for the association of mean EPA or DHA daily intake with the probability of dying (for splined models this comes from joint testing of both mean intake variables, above or below the spline threshold)

Figure 4. Meta-regression analyses



The three columns display three alternative definitions of mortality. The upper row (A) shows dose-response meta-regressions assuming a linear dose response relationship throughout the range of mean intakes of EPA and DHA. The lower row (B) shows corresponding meta-regressions assuming different linear relationships with mean dose above or below a threshold of 0.20 grams per day. See Table 4 for results using alternative thresholds.

Finally, there were no statistically significant interactions between quality items and the mean EPA and DHA intake in any of the above analyses (all p-values for the dose-response-by-quality item interaction effects were above 0.55).

Discussion

Principal Findings

In RCTs, supplementation with EPA and DHA reduced mortality, primarily in patients with a history of cardiovascular disease or with risk factors for the disease. Meta-regressions of data from prospective cohort studies suggested evidence of an association between higher EPA or DHA mean intake and lower risk of cardiac mortality phenotypes, up to a threshold of 0.20 grams per day. Higher intakes of EPA and DHA were not related with mortality. The conclusions drawn from the analysis of the available prospective cohort and randomized trial data were, for the most part, similar, despite differences in populations, settings, mean EPA and DHA intake, and duration of follow-up. The levels of EPA and DHA associated with lowest mortality risk, either cardiovascular or all-cause, are consistent with the current dietary guidance of the 2010 Dietary Guidelines for Americans[58] and 2006 American Heart Association Diet and Lifestyle Recommendations to consume at least two fish meals per week.[59.]

Concordance Between Randomized Trials and Prospective Cohorts

The hypothesis that the effect of long chain n-3 fatty acids on cardiovascular clinical outcomes plateaus beyond a minimum average daily intake of approximately 0.30-0.50 grams is partly based on ecological observations in Japanese populations, where high average fish intakes are not associated with cardiovascular or overall mortality.[4] At the same time, the risk of cardiovascular mortality is lower in Japan than Western countries such as the United States. Noteworthy, the mean daily EPA and DHA intake in Japan is approximately 0.80-0.90 grams per day[27,30] versus 0.04-0.13 grams per day in the United States.[23,29] These data suggest that supplemental EPA or DHA above a minimum threshold is not efficacious. If anything, there are concerns for environmental contaminants such as mercury[60] at high fish intakes.

We identified agreement in the actual magnitude of the summary effects between RCTs and prospective cohorts using formal meta-analysis and meta-regression methods. The summary dose-response odds ratio for cardiac, cardiovascular or sudden cardiac death in prospective cohort studies was 0.64 to 0.88 per 0.20 grams per day of mean intake (using thresholds of 0.20 to 0.30 grams per day for the floor effect, respectively) and the relative risk from RCTs was 0.89 (for supplement doses that are well in excess of 0.20 grams per day). If the background EPA and DHA intake in RCTs is relatively small, the summary effect of EPA and DHA supplementation appears to correspond to that observed up to the threshold of 0.20 grams per day in prospective cohorts. Although no data were reported for background diets in RCTs, most are from western countries where the mean EPA or DHA intake is generally lower than in Japan or in Scandinavian countries.[27,30] Further, the treatment effect in RCTs was not significantly associated with supplementation dose, perhaps because supplement doses in all RCTs were well above the plateau threshold.

A strength of this systematic review and meta-analysis is its detailed modelling of adjusted data from observational studies. Most prior meta-analyses of observational data are limited to a synthesis of unadjusted odds ratios across extreme intake categories (e.g., of highest versus lowest intake), thereby omitting useful information from the intermediate categories and including potentially confounded results. Other studies[4] attempted to utilize all available data, but did not account for the correlations between the intake-group-specific odds ratios, or the uncertainty that accompanies these odds ratios. The importance of using adjusted data in dose-

response meta-regressions is evident by comparing the results with meta-regressions based on unadjusted data: the former suggest that the risk of cardiac death decreases up to a mean EPA and DHA intake of 0.20 grams per day, and does not change in higher mean intakes, consistent with the aforementioned epidemiological observations. In contrast, the latter suggest an effect in the opposite direction, i.e. increased risk of mortality at higher mean intakes of EPA or DHA. A possible explanation is that unadjusted data are subject to confounding within each study.

Our approach has several limitations. Our analyses are based on systematic reviews of published evidence, and therefore may be susceptible to selection biases, publication bias[61-63] and outcome reporting bias in particular.[64-66] When such biases operate, positive results may be overrepresented among the analysable published data, resulting in summary effects that systematically deviate from the null. We focused on large prospective cohorts (with at least 1000 participants and at least 3 years of follow-up) assuming the results from larger studies are less susceptible to such biases – although we have no empirical data to support this assumption. At the same time, larger studies may not have as rigorous assessment of habitual intake levels as smaller studies, and their results may be subject to larger measurement error compared to smaller experimental studies. While random measurement error is expected to attenuate any associations, larger sample size increases statistical power. For these reasons, it is unclear whether limiting analyses to larger studies biases the summary estimates away from their ~~"tr~~ue" values.

There is substantial diversity in study characteristics, both among RCTs and among observational studies. However, these differences did not translate to systematic differences in the effect size across studies; if anything, we found suggestive evidence of concordance in the summary results of RCTs and prospective cohorts, despite their clinical and methodological diversity. Insufficient data were available to perform analyses by ethnic descent or gender, so it is unclear whether these factors are effect modifiers. Lastly, especially for analyses in prospective cohorts, we cannot rule of the possibility that higher fish intake displaces other foods, e.g., meat and dairy, that are major contributors of dietary saturated fat and that it is the lower intakes of saturated fat that mainly contribute to lower rates of cardiovascular mortality. Likewise, we cannot rule out the possibility that higher EPA and DHA intake is a marker for a healthier dietary pattern, e.g., more vegetables and fruits or whole grains.

EPA and DHA are examples of nutrients whose dietary reference intake values should be set taking into account the dose-response relationships with the risk of outcomes related to chronic diseases. Thus they are subject to different considerations compared with other nutrients whose reference intakes are set to prevent adverse health outcomes associated with a deficient status.[44,63] Information on threshold effects for chronic disease is important in considering reference intake values. Our analyses are consistent with the hypothesis that the beneficial effect of EPA and DHA on mortality reaches a plateau after a mean intake threshold of approximately 0.20 to 0.30 grams per day. Ideally, one would refine this threshold by analysing individual participant data with suitable methodologies (e.g., using isotonic regression modelling,[67] and would examine several other outcomes as well. We believe that a meta-analysis of individual participant data would be much more informative than yet another primary study. Notwithstanding ongoing (the VITAL trial[68] on vitamin D and EPA and DHA supplementation) or recently completed study (the OMEGA trial[69] that was completed in 2008 and whose results are still pending), it is at best unclear whether further RCTs and prospective cohort studies are necessary to assess the effects of EPA or DHA on mortality phenotypes.

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