Original Investigation

ω-3 Polyunsaturated Fatty Acid Biomarkers and Coronary Heart Disease
Pooling Project of 19 Cohort Studies

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IMPORTANCE The role of ω-3 polyunsaturated fatty acids for primary prevention of coronary heart disease (CHD) remains controversial. Most prior longitudinal studies evaluated self-reported consumption rather than biomarkers.

OBJECTIVE To evaluate biomarkers of seafood-derived eicosapentaenoic acid (EPA; 20:5ω-3), docosapentaenoic acid (DPA; 22:5ω-3), and docosahexaenoic acid (DHA; 22:6ω-3) and plant-derived α-linolenic acid (ALA; 18:3ω-3) for incident CHD.

DATA SOURCES A global consortium of 19 studies identified by November 2014.

STUDY SELECTION Available prospective (cohort, nested case-control) or retrospective studies with circulating or tissue ω-3 biomarkers and ascertained CHD.

DATA EXTRACTION AND SYNTHESIS Each study conducted standardized, individual-level analysis using harmonized models, exposures, outcomes, and covariates. Findings were centrally pooled using random-effects meta-analysis. Heterogeneity was examined by age, sex, race, diabetes, statins, aspirin, ω-6 levels, and *FADS* desaturase genes.

MAIN OUTCOMES AND MEASURES Incident total CHD, fatal CHD, and nonfatal myocardial infarction (MI).

RESULTS The 19 studies comprised 16 countries, 45 637 unique individuals, and 7973 total CHD, 2781 fatal CHD, and 7157 nonfatal MI events, with ω-3 measures in total plasma, phospholipids, cholesterol esters, and adipose tissue. Median age at baseline was 59 years (range, 18-97 years), and 28 660 (62.8%) were male. In continuous (per 1-SD increase) multivariable-adjusted analyses, the ω-3 biomarkers ALA, DPA, and DHA were associated with a lower risk of fatal CHD, with relative risks (RRs) of 0.91 (95% CI, 0.84-0.98) for ALA, 0.90 (95% CI, 0.85-0.96) for DPA, and 0.90 (95% CI, 0.84-0.96) for DHA. Although DPA was associated with a lower risk of total CHD (RR, 0.94; 95% CI, 0.90-0.99), ALA (RR, 1.00; 95% CI, 0.95-1.05), EPA (RR, 0.94; 95% CI, 0.87-1.02), and DHA (RR, 0.95; 95% CI, 0.91-1.00) were not. Significant associations with nonfatal MI were not evident. Associations appeared generally stronger in phospholipids and total plasma. Restricted cubic splines did not identify evidence of nonlinearity in dose responses.

CONCLUSIONS AND RELEVANCE On the basis of available studies of free-living populations globally, biomarker concentrations of seafood and plant-derived ω-3 fatty acids are associated with a modestly lower incidence of fatal CHD.

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A review[1] of experimental studies and randomized clinical trials revealed a protective effect of ω-3 polyunsaturated fatty acids (PUFAs) on coronary heart disease (CHD) risk pathways and clinical risk factors. However, key controversies remain. First, randomized clinical trials using fish oil supplements have found mixed effects on CHD events.[2-8] However, most trials provided supplements for a few years or less and were conducted in patients with preexisting CHD or at high risk for CHD and taking multiple cardiovascular drugs. Furthermore, the background dietary intake in these trials was not usually measured, and many participants may have had adequate intake from diet alone. Thus, their generalizability and relevance for long-term effects of dietary ω-3 PUFAs for primary CHD prevention are uncertain. Second, although a review[9] of several prior observational studies found inverse associations between seafood-derived ω-3 PUFAs and CHD death, potential effects on nonfatal myocardial infarction (MI) or total CHD are less clear. This review[9] additionally found that most studies also relied on self-reported dietary questionnaires, which may produce errors or bias in recall. A previous review[1] also found that a handful of studies have measured objective circulating or tissue ω-3 PUFA levels, but such findings could be limited by publication bias. In addition, prior individual biomarker studies were generally underpowered to explore potentially relevant differences in effects depending on underlying participant characteristics, medication use, or genetic variation. Thus, uncertainties remain about the effects of ω-3 PUFAs on CHD.

Most studies[10,11] have evaluated combined intakes or biomarker levels of long-chain ω-3 PUFAs. However, individual ω-3 PUFAs, including eicosapentaenoic acid (EPA; 20:5ω-3), docosapentaenoic acid (DPA; 22:5ω-3), and docosahexaenoic acid (DHA; 22:6ω-3), may have shared and complementary effects.[10] Similarly, most prior research[11] has focused on seafood-derived long-chain ω-3 PUFAs, and potential CHD effects of plant-derived α-linolenic acid (ALA; 18:3ω-3) are far less understood.

To address each of these important questions, we developed a global consortium of available studies with circulating or tissue ω-3 biomarkers and ascertained incident CHD. Our aims were to evaluate the associations of individual seafood- and plant-derived ω-3 PUFAs with incident total CHD, fatal CHD, and nonfatal MI. We also explored dose response and potential heterogeneity in effects according to key underlying participant characteristics, including medication use. Our primary hypothesis was that biomarkers of long-chain ω-3 PUFAs would be associated with a lower risk of incident fatal CHD but not nonfatal MI.

Key Points

Question Are seafood and plant-derived ω-3 fats related to first coronary heart disease (CHD) events?

Findings In a consortium of 19 studies, biomarkers of seafood and plant-derived ω-3 fats were associated with a significantly lower risk of fatal CHD. In contrast, associations with nonfatal myocardial infarction were generally less robust.

Meaning Blood biomarkers of seafood and plant-derived omega-3 fats are linked to a modestly lower risk of dying from heart attacks. Consumption of ω-3–rich foods should be encouraged.

Methods

Consortium Formation

The Fatty Acids and Outcomes Research Consortium (FORCe) is an extension of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Fatty Acids Working Group,[12,13] originally developed to investigate effects of genetic variation on biomarker fatty acid levels. Using direct expert contacts and by reviewing the literature, we identified large studies that had measured circulating or tissue biomarkers of ω-3 PUFAs in general populations and had ascertained incident CHD. We focused on prospective (cohort, nested case-control) studies; retrospective studies were included if fatty acids were measured in adipose tissue at the time of the first event, given the stability of adipose measures. Studies were asked to join the consortium and participate in standardized pooled analyses of biomarker fatty acids and clinical events. Of 20 identified studies by November 2014, only 1 study[14] declined to join the consortium. All cohort participants gave written informed consent according to their local guidelines. All studies received approval from ethical oversight committees. Data were deidentified.

Analysis Plan

A standardized analysis protocol was developed and provided to each participating study, including harmonized definitions of populations, exposures, outcomes, covariates, effect modifiers, and methods for pooling across studies. These specific methods are detailed below. An experienced analyst from each study performed the analysis for that study using individual-level data and provided all results to the lead investigator (L.C.D.G.) in standardized electronic forms.

Population

To understand the potential effects on primary prevention and minimize reverse causation, participants from each study were excluded if they had a prior history of MI, angina, coronary revascularization, or stroke. All remaining participants with measured ω-3 PUFA biomarker levels were included in the analyses.

Fatty Acid Ascertainment

Fatty acid biomarker concentrations were assessed in study-specific lipid compartments, including total plasma, erythrocyte and plasma phospholipids, cholesterol esters, triglycerides, and adipose tissue, with all reported as weight percentage of total fatty acids. Details of fatty acid measurement methods for each study are provided in the eMethods in the Supplement.

Assessment of CHD

We evaluated total incident CHD, defined as fatal or nonfatal MI, CHD death, or sudden cardiac death; nonfatal MI, generally defined as chest pain with abnormal cardiac enzyme concentrations or serial electrocardiogram changes; and fatal CHD,

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defined as fatal MI, CHD death, or sudden cardiac death. Details of the methods for assessing and defining these outcomes in each study are provided in eTable 1 in the Supplement. We excluded soft CHD end points (eg, angina, revascularization) to minimize bias in ascertainment of these events.

Covariates

Covariates were standardized across studies based on prespecified, harmonized definitions and categorizations, including age (continuous), sex (male, female), race (white, black, and other study-specific subgroups, if available), clinical center (study-specific categories, if applicable), body mass index (BMI [calculated as the weight in kilograms divided by height in meters squared], continuous), educational level (less than high school graduate, high school graduate, some college or vocational school, or college graduate), smoking (current, former, or never or current vs not current, if former not assessed), physical activity (quartiles of metabolic equivalents or, if metabolic equivalents unavailable, 4 categories of physical or leisure activity as defined in the study), alcohol intake (none, 1-6 drinks per week, 1-2 drinks per day, or >2 drinks per day; with 14 g of alcohol equaling 1 standard drink), diabetes mellitus (yes or no, defined as treatment with oral hypoglycemic agents, insulin, or by using fasting glucose levels, or study-specific definitions otherwise), treated hypertension (yes or no, defined as treatment with antihypertensive drugs or as diagnosed hypertension otherwise), treated hypercholesterolemia (yes or no, defined as treatment with lipid-lowering drugs or as diagnosed hypercholesterolemia otherwise), regular aspirin use (yes or no, categorized as ≥3 times per week), and biomarker concentrations of ω-6 PUFA linoleic acid (LA; 18:2ω-6), arachidonic acid (AA; 20:4ω-6), and total trans fatty acids (each continuous). For categorical variables, missing covariate data were included as an indicator category.

Statistical Analysis and Pooling

Standardized study-specific analyses were performed for each study using individual-level data. Because of variability in the total number of fatty acids assessed across the different assays (12-58 fatty acids) and to permit comparison and pooling of findings across the different biomarker compartments, each fatty acid was analyzed continuously per 1-SD increase.

For prospective cohorts, Cox proportional hazards regression models estimated the hazard ratio for incident total CHD, nonfatal MI, and fatal CHD, with follow-up from the date of biomarker measurement to date of event, end of follow-up, loss to follow-up, or death, whichever occurred first. For prospective and retrospective matched case-control studies, conditional logistic regression was used to estimate the hazard ratio (if matched on time to event) or odds ratio. All analyses used robust SEs.

For pooled meta-analyses, hazard ratios and odds ratios were considered to approximate relative risks (RRs). Meta-analysis was performed using random effects models using the method of DerSimonian and Laird.[15] Results were first pooled separately for each biomarker compartment and then pooled together. If more than 1 biomarker had been measured in a given study, main results included the biomarker that might best represent diet: for long-chain ω-3 fatty acids, total plasma or serum measures were used, and for ALA, adipose tissue measures were used in the main results.[16]

To assess potential noncontinuous associations, we also evaluated study-specific quintiles for each biomarker compartment, performing meta-analysis within each quintile and comparing each quintile-specific pooled result to the first quintile. To statistically test potential nonlinear dose responses, we performed multivariate meta-regression, modeling restricted cubic splines.[17] These analyses evaluated total plasma and phospholipids; cholesterol esters and adipose tissue fatty acids were not evaluated using splines because of fewer numbers of studies in these compartments.

Overall heterogeneity was assessed using the I^2 statistic, with heterogeneity considered low if the I^2 was less than 35%. Heterogeneity by prespecified subgroups was evaluated by pooling individual study results based on defined strata, including age (<60 or ≥60 years), sex (male or female), race/ethnicity (white, black, Hispanic, or Chinese), LA and AA biomarker concentrations (less than or greater than or equal to the median value in each study), type 2 diabetes (yes or no), statin use (yes or no), regular aspirin use (yes or no), year of biomarker sampling (before 2000 or 2000 or later), and (for ALA) EPA, DPA, and DHA biomarker concentration (less than or greater than or equal to the median value in each study). Effect estimates in each study-specific strata were pooled, and the statistical significance of differences between subgroups of potential sources of heterogeneity was assessed using meta-regression. In a meta-analysis[13] of 7 studies with available genetic data, we also examined potential interaction by variants in *FADS* (OMIM 208150) desaturase genes (rs174546, rs968567), pooling study-specific estimates using an additive genetic model (eMethods in the Supplement). Within each study, interaction terms for each single-nucleotide polymorphism (SNP) were constructed as a cross-product term of the ω-3 PUFA biomarker (continuous) by the SNP (ordinal: 0, 1, or 2 T alleles), including the main effects in the model; these interaction terms were pooled using meta-analysis.

Meta-analyses were performed using STATA statistical software, version 12 (StataCorp). We considered 2-tailed $P < .05$ to be statistically significant.

Sensitivity Analyses

Several sensitivity analyses were performed. We also performed continuous meta-analysis using absolute (percentage of fatty acids), rather than study-specific, units. To minimize potential reverse causation attributable to preexisting subclinical disease, we excluded cases identified in the first 2 years after biomarker sampling. To minimize exposure misclassification attributable to within-person changes in biomarker levels over time, we censored participants after the first 6 years of follow-up. We performed additional analyses limited to prospective studies only and those without self-reported CHD events.

Results

Characteristics of Studies and Participants

The 19 studies[18-35] included 45 637 unique participants from 16 countries, including the United States, Australia, Costa Rica,

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Table 1. Baseline Characteristics of 19 Studies and 45 637 Participants With Biomarker Measures of ω-3 Fatty Acids[a]

Study	Country	Study Design	Age, Mean, y	Sex, % Male	Biomarker Compartment	Year of Blood Sampling	Fatty Acids Assessed	Coronary Heart Disease Outcomes
ARIC[18]	United States	PC	54	48	Plasma phospholipid	1987-1989	ALA, DPA, EPA, DHA	Total CHD
Costa Rican adults study[19]	Costa Rica	RCC	59	72	Adipose tissue	1995-2004	ALA, DPA, EPA, DHA	Nonfatal MI
CHS[20]	United States	PC	74	40	Plasma phospholipid	1992-1993	ALA, DPA, EPA, DHA	Total CHD, nonfatal MI, fatal CHD
EPIC-Norfolk[21]	United Kingdom	PCC	63	49	Plasma phospholipid	2001-2004	ALA, DPA, EPA, DHA	Total CHD, nonfatal MI, fatal CHD
EURAMIC[22]	Finland, Norway, Scotland, Ireland, Germany, Switzerland, Spain, Israel, and Russia	RCC	54	100	Adipose tissue	1991-1992	ALA, DPA, DHA	Nonfatal MI
HPFS[23]	United States	PCC	64	100	Total plasma, erythrocyte phospholipid	1994 1994	ALA, DPA, EPA, DHA	Total CHD, nonfatal MI, fatal CHD
InCHIANTI[24]	Italy	PCC	65	45	Total plasma	1998-2000	ALA, EPA, DHA	Total CHD
KIHD[25]	Finland	PC	52	100	Total plasma	1991-1992	ALA, DPA, EPA, DHA	Total CHD, nonfatal MI, fatal CHD
MCCS[26]	Australia	PC	56	49	Plasma phospholipid	1990-1994	ALA, DPA, EPA, DHA	Fatal CHD
MESA[27]	United States	PC	62	47	Plasma phospholipid	2000-2002	ALA, DPA, EPA, DHA	Total CHD, nonfatal MI, fatal CHD
NSHDS I[28]	Sweden	PCC	55	79	Plasma phospholipid	1995	ALA, DPA, EPA, DHA	Total CHD
NSHDS II[29]	Sweden	PCC	55	62	Plasma phospholipid	2007	ALA, DPA, EPA, DHA	Total CHD, nonfatal MI, fatal CHD
NHS I[30]	United States	PCC	60	0	Total plasma, erythrocyte phospholipid	1989-1990 1989-1990	ALA, DPA, EPA, DHA	Total CHD, nonfatal MI, fatal CHD
PHS[31]	United States	PCC	69	100	Erythrocyte phospholipid	1995-2000	ALA, DPA, EPA, DHA	Total CHD, nonfatal MI, fatal CHD
SHHEC[32]	Scotland	PC	49	52	Adipose tissue	1984-1986	DPA, DHA	Total CHD, nonfatal MI, fatal CHD
SCHS[33]	Singapore	PCC	66	65	Total plasma	1994-2005	ALA, EPA, DHA	Total CHD, nonfatal MI, fatal CHD
3C Study[34]	France	PC	75	39	Total plasma	1999-2000	ALA, DPA, EPA, DHA	Total CHD
ULSAM 50[35]	Sweden	PC	50	100	Cholesterol esters	1970	ALA, EPA, DHA	Total CHD, nonfatal MI, fatal CHD
ULSAM 70[35]	Sweden	PC	71	100	Adipose tissue	1990	ALA, DPA, EPA, DHA	Total CHD, nonfatal MI, fatal CHD

Abbreviations: ALA, α-linolenic acid; ARIC, Atherosclerosis Risk in Communities; CHD, coronary heart disease; CHS, Cardiovascular Health Study; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; EPIC-Norfolk, European Prospective Investigation of Cancer (Norfolk); EURAMIC, European Study on Antioxidants, Myocardial Infarction and Cancer; HPFS, Health Professionals Follow-up Study; InCHIANTI, Invecchiare in Chianti; KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study; MCCS, Melbourne Collaborative Cohort Study; MESA, Multi-Ethnic Study of Atherosclerosis; MI, myocardial infarction; NSHDS, Northern Sweden Health and Disease Study; NHS, Nurses' Health Study; PC, prospective cohort; PCC, prospective nested case-control; PHS, Physicians' Health Study; RCC, retrospective case-control; SHHEC, Scottish Heart Health Extended Cohort; SCHS, Singapore Chinese Health Study; ULSAM, Uppsala Longitudinal Study of Adult Men; 3C Study, Three-City study.

[a] Characteristics described at time of fatty acid biomarker measurement.

Finland, France, Germany, Ireland, Israel, Italy, Norway, Singapore, the Soviet Union, Spain, Sweden, Switzerland, and the United Kingdom (**Table 1** and eMethods in the Supplement). Most were prospective cohort[18,20,25-27,32,34,35] (n = 10 studies) or prospective nested case-control[21,23,24,28-31,33] (n = 7) studies; 2 were retrospective case-control studies[19,22] that used adipose tissue biomarkers. Biomarker types included phospholipids (plasma or erythrocyte) (n = 10), total plasma (n = 6), adipose tissue (n = 4), and cholesterol esters (n = 1); 2 studies (Nurses' Health Study [NHS][30] I and Health Professionals Follow-up Study [HPFS][23]) measured total plasma and erythrocyte phospholipids. As expected, ω-3 PUFA concentrations and distributions varied across biomarker types and study assays (eTable 2 and eTable 3 in the Supplement), with coefficients of variation less than 10% for most fatty acids and biomarkers (eMethods in the Supplement). For example, median ALA concentrations were generally higher in adipose (0.72% of total fatty acids) than in circulating biomarkers (0.20% of total fatty acids), whereas EPA, DPA, and DHA concentrations were generally higher in phospholipids than in other compartments, consistent with prior reports.[16,36]

Median age at baseline was 59 years (range, 18-97 years), and 28 660 participants (62.8%) were male (eTable 4 in the Supplement). During a median 10 years of follow-up (range, 1.3-42

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years), 7973 incident CHD events, 7157 nonfatal MIs, and 2781 fatal CHD events occurred. Because not all studies ascertained every exposure and outcome (Table 1), cases of nonfatal MI and fatal CHD do not sum to the number of total CHD cases. Medication use varied across studies; by design, all participants in the analysis were free of prevalent CHD or stroke (eTable 5 in the Supplement). Most participants were white, but the Cardiovascular Health Study (CHS)[20] and Multi-Ethnic Study of Atherosclerosis (MESA)[27] included relatively higher proportions of African American individuals (12.3% and 24.7%, respectively); MESA and the Costa-Rican Case-Control Study of Myocardial Infarction (Costa Rican adults study)[19] included Hispanic individuals (24.9% and 100%, respectively), and MESA[27] and the Singapore Chinese Health Study (SCHS)[33] included Chinese participants (25.0% and 100%, respectively). Across all studies, the median BMI was 26, and generally up to 30% of participants were current smokers, except for higher rates in the Scottish Heart Health Extended Cohort[32] (44.6%) and Uppsala Longitudinal Study of Adult Men[35] 50 (51.0%). Alcohol intake was modest, with most participants consuming up to 1 drink per day. Fish oil supplementation was infrequently assessed across studies; use was low (0%-4% of participants) in 5 of 6 studies with these data (eTable 6 in the Supplement); only the European Prospective Investigation of Cancer (Norfolk) (EPIC-Norfolk)[21] had a higher prevalence (33%).

EPA, DPA, and DHA for Incident CHD

In continuous (per 1-SD increase) multivariable-adjusted analyses, each ω-3 PUFA was associated with an approximately 9% lower risk of fatal CHD, with RRs of 0.91 (95% CI, 0.82-1.00) for EPA, 0.90 (95% CI, 0.85-0.96) for DPA, and 0.90 (95% CI, 0.84-0.96) for DHA (**Figure 1** and **Figure 2**). There was moderate heterogeneity in this association for EPA and DHA (I^2 = 37% for both) and low heterogeneity for DPA and DHA (I^2 = 0% and 31%, respectively) (Figure 1 and Figure 2). The sum of EPA, DPA, and DHA was associated with an 11% lower risk of fatal CHD (eFigure 1 in the Supplement). By contrast, none of the long-chain ω-3 PUFAs were significantly associated with nonfatal MI (**Table 2**). We found that DPA, but not EPA or DHA, was associated with a significantly lower risk of total CHD, with RRs of 0.94 (95% CI, 0.90-0.99), 0.94 (95% CI, 0.87-1.02), and 0.95 (95% CI, 0.91-1.00), respectively (Table 2).

Some differences were observed when EPA, DPA, and DHA were evaluated across quintiles (eTable 7 in the Supplement). Upper quintiles of EPA and DHA were associated with a lower risk of nonfatal MI (quintile 5 vs quintile 1 comparison: RR, 0.71 [95% CI, 0.56-0.90] vs 0.87 [95% CI, 0.78-0.97]). Top quintiles of DPA and DHA were associated with a lower risk of fatal CHD (quintile 5 vs quintile 1 comparison: RR, 0.76 [95% CI, 0.65-0.90] vs 0.77 [95% CI, 0.64-0.89]) (eTable 7 in the Supplement). Across biomarker types, inverse associations were generally stronger in the phospholipid and total plasma compartments (Table 2); heterogeneity decreased when adipose tissue and cholesterol ester estimates were excluded from meta-analyses.

ALA and Incident CHD

In continuous analysis, ALA was associated with a 9% lower risk of fatal CHD (RR, 0.91 [95% CI, 0.84-0.98]) (Figure 1) but not total CHD or nonfatal MI. For ALA, no single biomarker compartment consistently had stronger associations across outcomes.

Restricted Cubic Spline Analysis

Restricted cubic splines did not identify evidence of nonlinear associations of any of the ω-3 PUFA biomarkers and outcomes in total plasma or phospholipids (*P* for nonlinearity > .05 for each) (eFigure 2 in the Supplement).

Effect Modification

No significant differences in associations of ω-3 PUFA biomarkers with incident CHD events were observed by age, sex, ω-6 PUFA (LA or AA) concentrations, type 2 diabetes status, statin use, regular aspirin use, year of biomarker sampling, or (for ALA) EPA, DPA, and DHA concentrations (*P* for heterogeneity > .05 for each) (eTable 8 in the Supplement). In a post hoc meta-regression, we observed no significant differences in associations by median length of follow-up (<10 or ≥10 years) (*P* for heterogeneity > .05 for each). Effect modification was suggested by ethnicity. Compared with white individuals, ALA was associated with a significantly lower risk of nonfatal MI among African American individuals (*P* for heterogeneity = .001). For EPA, ALA was associated with a significantly lower risk of total CHD and nonfatal MI among Chinese participants (*P* for heterogeneity = .02 and .01, respectively). Among 7 studies[10,17,19,33-35] with SNP data (eTable 9 in the Supplement), no significant interaction was identified by *FADS* desaturase gene variants (eTable 10 in the Supplement).

Sensitivity Analyses

Compared with the main findings, no appreciable differences were observed after excluding cases identified in the first 2 years after biomarker sampling, censoring participants at the first 6 years of follow-up, excluding retrospective studies, or excluding studies with self-reported events (eTable 11 in the Supplement). For 2 studies[23,30] that analyzed phospholipids in addition to total plasma (NHS I and HPFS), results were similar when phospholipid results from these studies were used in place of total plasma in the meta-analysis.

Discussion

In this consortium which pooled individual-level harmonized analyses from 19 studies, including 45 637 unique participants and nearly 8000 first CHD events in 16 countries, the ω-3 biomarkers ALA, DPA, and DHA were associated with a modestly lower risk of fatal CHD. The magnitude of observed effect sizes for fatal CHD (approximately 9% per 1-SD increase) are consistent with findings for cardiac death from a meta-analysis[8] of trials. In contrast, associations with nonfatal MI were generally less robust. Across these diverse studies, findings were consistent by age, sex, ω-6 PUFA LA and AA levels, year of biomarker sampling, presence or absence of diabetes, statin use, and aspirin use. By contrast, effect modification by ethnicity was observed for select exposure-outcome pairings. Separate analyses by biomarker compartment provided further insights, with

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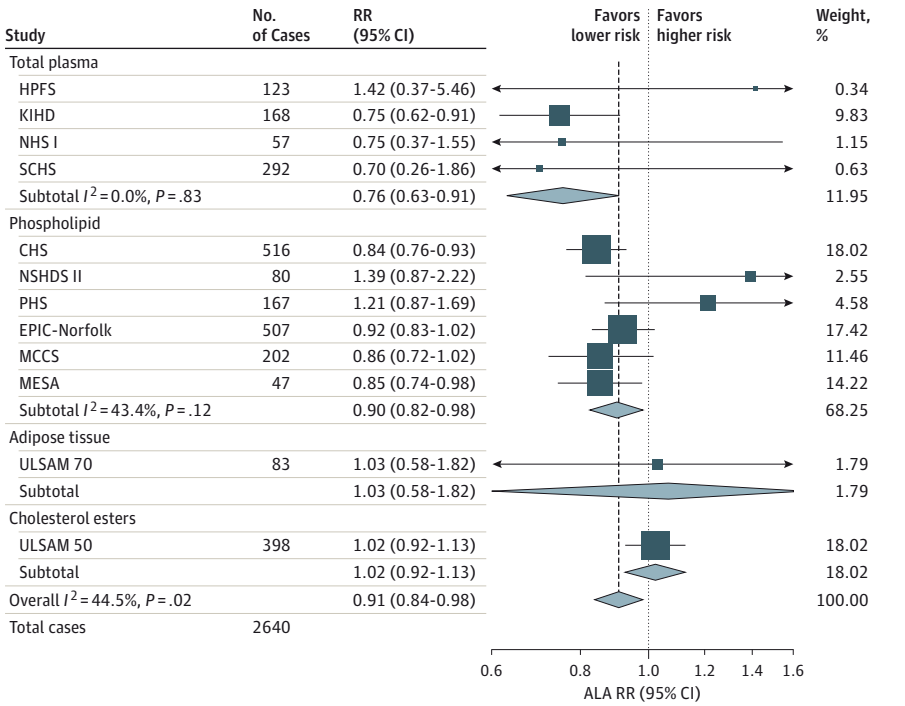
Figure 1. Relative Risk (RR) of Fatal Coronary Heart Disease (CHD) per 1-SD Increase in the Biomarkers α-Linolenic Acid (ALA; 18:3ω-3) and Eicosapentaenoic Acid (EPA; 20:5ω-3)



Estimates were pooled using random effects meta-analysis. CHS indicates Cardiovascular Health Study[20]; EPIC-Norfolk, European Prospective Investigation of Cancer (Norfolk)[21]; HPFS, Health Professionals Follow-up Study[23]; KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study[25]; MCCS, Melbourne Collaborative Cohort Study[26]; MESA, Multi-Ethnic Study of Atherosclerosis[27]; NHS, Nurses' Health Study[30]; NSHDS, Northern Sweden Health and Disease Study[28,29]; PHS, Physician's Health Study[31]; SCHS, Singapore Chinese Health Study[33]; and ULSAM, Uppsala Longitudinal Study of Adult Men.[35]

generally stronger inverse associations in phospholipids and total plasma for EPA, DPA, and DHA. This investigation provides the most comprehensive estimates to date of the associations between seafood and plant-based ω-3 PUFAs, assessed using biomarkers, and primary incidence of CHD in generally healthy, free-living populations around the world.

In randomized clinical trials, long-chain ω-3 PUFAs benefit multiple major cardiovascular risk factors, including triglyceride levels, blood pressure, heart rate, heart rate variability, endothelial function, and myocardial oxygen demand.[1,10] Compared with many other single nutrients, these demonstrated physiologic benefits provide strong biologic plausibil-

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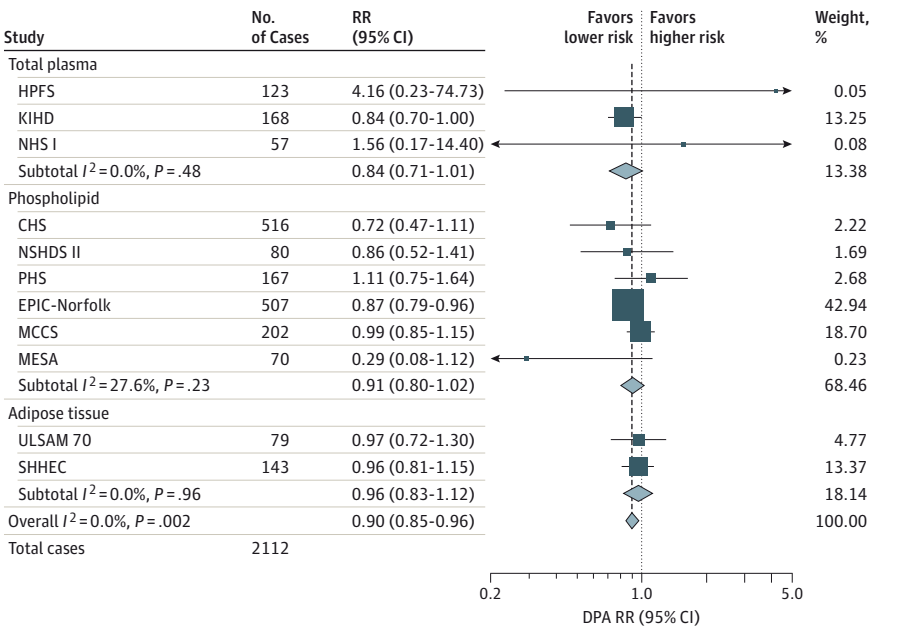
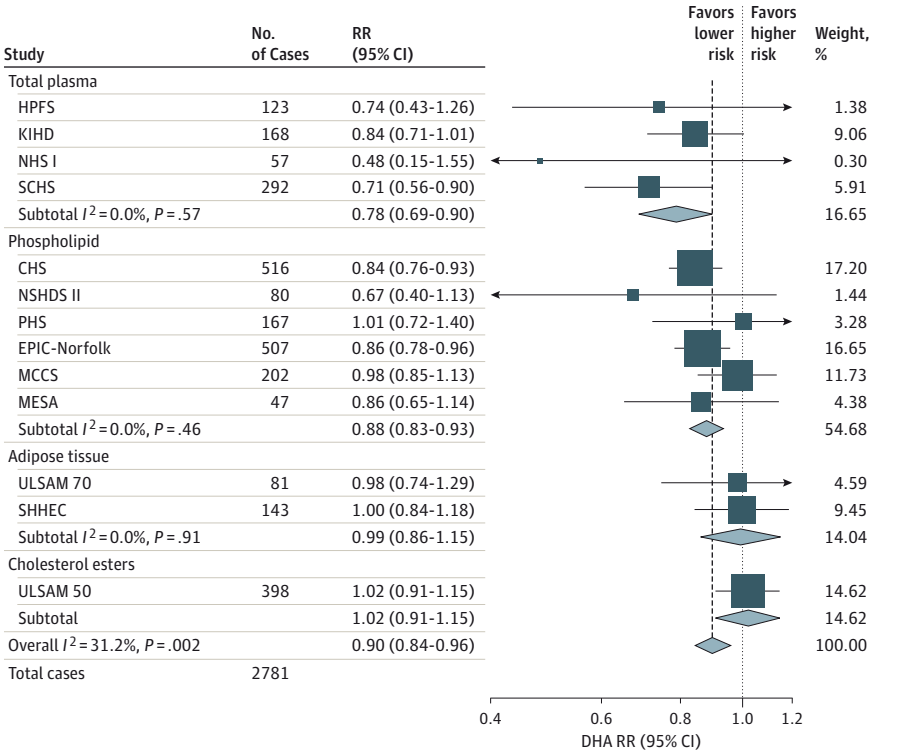
Figure 2. Relative Risk (RR) of Fatal Coronary Heart Disease (CHD) per 1-SD Increase in the Biomarkers Docosapentaenoic Acid (DPA; 22:5ω-3) and Docosahexaenoic Acid (DHA; 22:6ω-3)





Estimates were pooled using random effects meta-analysis. CHS indicates Cardiovascular Health Study[20]; EPIC-Norfolk, European Prospective Investigation of Cancer (Norfolk)[21]; HPFS, Health Professionals Follow-up Study[23]; KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study[25]; MCCS, Melbourne Collaborative Cohort Study[26]; MESA, Multi-Ethnic Study of Atherosclerosis[27]; NHS, Nurses' Health Study[30]; NSHDS, Northern Sweden Health and Disease Study[28,29]; PHS, Physician's Health Study[31]; SCHS, Singapore Chinese Health Study[33]; SHHEC, Scottish Heart Health Extended Chart[32]; and ULSAM, Uppsala Longitudinal Study of Adult Men.[35]

ity to support an effect on clinical events. Both EPA and DHA are also precursors to bioactive lipid metabolites, including specialized proresolving mediators[36] and cytochrome P450–generated monoepoxides,[37] that could contribute to lower CHD risk. Our findings are consistent with prior experimental evidence that long-chain ω-3 PUFAs may have membrane stabilizing actions in the setting of ischemia-induced ventricular fibrillation[1,38] and observational evidence indicating that benefits of fish consumption are most related to arrhythmic events[39] and fatal CHD.[40]

Use of biomarkers allowed separate investigation of each ω-3 PUFA. Of interest, EPA, DPA, and DHA were similarly associated with lower risk of fatal CHD. Biomarker levels of these fatty acids are only moderately interrelated (eg, r = 0.43, 0.51,

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Table 2. Pooled RRs of Total CHD, Nonfatal MI, and Fatal CHD per 1-SD Increase in ALA, EPA, DHA, and DPA[a]

	Total CHD			Nonfatal MI			Fatal CHD		
Exposure and Biomarker	No. of Cases[b]	No. of Studies	RR (95% CI)	No. of Cases	No. of Studies	RR (95% CI)	No. of Cases	No. of Studies	RR (95% CI)
ALA									
Total plasma	2286	6	1.05 (0.91-1.20)	1578	4	1.07 (0.87-1.31)	640	4	0.76 (0.63-0.91)
Phospholipids	3719	7	0.98 (0.92-1.06)	1790	5	0.95 (0.87-1.03)	1519	6	0.90 (0.82-0.98)
Adipose tissue	206	1	1.09 (0.74-1.62)	2385	3	0.71 (0.26-1.95)	83	1	1.03 (0.58-1.82)
Cholesterol esters	749	1	1.00 (0.92-1.08)	364	1	0.90 (0.80-1.01)	398	1	1.02 (0.92-1.13)
Overall	6960	15	1.00 (0.95-1.05)	6117	13	0.95 (0.87-1.05)	2640	12	0.91 (0.84-0.98)
EPA									
Total plasma	2194	6	0.93 (0.97-1.11)	1499	4	0.89 (0.69-1.14)	627	4	0.80 (0.51-1.25)
Phospholipids	3703	7	0.89 (0.81-0.99)	1790	5	0.91 (0.79-1.04)	1519	6	0.85 (0.74-0.97)
Adipose tissue	181	1	1.19 (1.05-1.33)	1734	2	0.85 (0.67-1.09)	74	1	1.08 (0.86-1.37)
Cholesterol esters	749	1	0.97 (0.88-1.07)	364	1	0.95 (0.83-1.09)	398	1	0.97 (0.83-1.14)
Overall	6827	15	0.94 (0.87-1.02)	5387	12	0.92 (0.83-1.01)	2613	12	0.91 (0.82-1.00)
DPA									
Total plasma	1412	4	0.93 (0.84-1.02)	1111	3	0.94 (0.81-1.08)	348	3	0.84 (0.71-1.01)
Phospholipids	3703	7	0.92 (0.86-0.97)	1767	5	0.95 (0.86-1.06)	1542	6	0.91 (0.80-1.02)
Adipose tissue	1092	2	1.01 (0.94-1.08)	3137	4	0.98 (0.88-1.11)	222	2	0.96 (0.83-1.12)
Cholesterol esters	NA	NA	NA	NA	NA	NA	NA	NA	NA
Overall	6207	13	0.94 (0.90-0.99)	6015	12	0.97 (0.93-1.02)	2112	11	0.90 (0.85-0.96)
DHA									
Total plasma	2425	6	0.91 (0.82-1.02)	1866	4	0.91 (0.84-0.98)	640	4	0.78 (0.69-0.90)
Phospholipids	3703	7	0.93 (0.89-0.97)	1790	5	0.97 (0.91-1.03)	1519	6	0.88 (0.83-0.93)
Adipose tissue	1096	2	1.05 (0.95-1.16)	3137	4	0.89 (0.68-1.18)	224	2	0.99 (0.86-1.15)
Cholesterol esters	749	1	1.01 (0.93-1.09)	364	1	1.03 (0.81-1.15)	398	1	1.02 (0.91-1.15)
Overall	7973	16	0.95 (0.91-1.00)	7157	14	0.96 (0.92-1.01)	2781	13	0.90 (0.84-0.96)

Abbreviations: ALA, α-linolenic acid; CHD, coronary heart disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; MI, myocardial infarction; NA, not applicable; RR, relative risk.

[a] Continuous estimates were pooled using random effects meta-analysis.

[b] Because not all studies ascertained every exposure and outcome (Table 1), cases of nonfatal MI and fatal CHD do not sum to the number of total CHD cases.

and 0.13 for EPA and DHA, EPA and DPA, and DPA and DHA, respectively).[41] Thus, observed associations for any 1 of these fatty acids are unlikely to be fully explained by the others. Compared with EPA and DHA, comparatively less is known about the molecular and physiologic effects of DPA; a previous study[10] suggests that DPA may inhibit ex vivo collagen-stimulated platelet aggregation, thromboxane production, and cyclooxygenase 1 activity. In addition, whereas circulating and tissue levels of EPA and DHA are strongly influenced by dietary seafood consumption, DPA concentrations appear to be mainly derived from endogenous elongation of EPA; interconversion between DPA and DHA is very limited.[10]

Given diverse global dietary sources, the effects of ALA on CHD are of particular interest. However, a meta-analysis[11] of prior reports of dietary and biomarker ALA and CHD risk revealed inconsistency, perhaps owing to methodologic and analytic differences in these investigations. A key strength of our analysis was the prespecified, harmonized analytic plan using individual-level data within each study, which allowed consistent assessment of how ALA relates to CHD. In addition, the inclusion of 19 studies minimized the effect of publication bias, wherein studies with positive findings are more likely reported. Compared with prior work,[42] we also took advantage of a far larger number of events (eg, >4-fold larger for fatal CHD)

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and observed a similarly lower risk of fatal CHD for ALA as for long-chain ω-3 PUFAs. Mechanistically, these findings are supported by effects of ALA on thrombosis, inflammation, arrhythmia, and endothelial function.[43-47] Our findings, combined with relative affordability, global accessibility, and sustainability of ALA,[48] support the potential importance of ALA for improving global cardiovascular health.

The large number of cases in our analysis and inclusion of multiple ethnic subgroups allowed us to explore potential effect modification in ω-3 PUFAs and CHD outcome associations by race/ethnicity. The stronger effects among African American and Chinese individuals for select ω-3–outcome pairings could be attributable to differences in consumption patterns, true biologic diversity in pathophysiologic pathways, or chance. For instance, preparation methods among Chinese populations could differ from that among white populations, with raw or steamed fish in the former population vs more deep fried fish in the latter. Differential pathophysiologic pathways for cardiometabolic risk have also been documented among Asian vs white populations; for example, Asian individuals develop type 2 diabetes at a much lower BMI than white individuals,[49,50] and at least 3 meta-analyses[51-53] have reported a protective effect of seafood-derived ω-3 fatty acids on incident diabetes among Asian individuals, but not white individuals. Finally, our findings of effect modification by race could be attributable to chance; few studies were available with African American (n = 2 studies: CHS and MESA) and Chinese subgroups (n = 2 studies: SCHS and MESA).[27,33] Our results highlight the need for further work to better understand differences in associations by race for ω-3 fatty acids and incident CHD phenotypes.

Randomized clinical trials of fish oil supplements have found mixed effects, although overall pooled findings indicate a benefit in lowering risk of cardiac death,[8] consistent with our findings. Our investigation tests unique and separate questions from these trials attributable to differences in the ω-3 PUFA source (dietary in our study vs supplements in trials), population (primary prevention vs secondary prevention or high risk), and duration (habitual intake vs short-term supplementation). In cohort-harmonized, stratified analyses, we found little evidence of effect modification by statin or aspirin use, which have been hypothesized to reduce benefits of ω-3 fatty acids.[54] We also found little evidence of effect modification by circulating ω-6 PUFAs (LA or AA), which have been hypothesized but never found in humans to reduce cardiovascular benefits of ω-3 PUFAs.[55] Altogether, our findings suggest that seafood and plant-derived ω-3 PUFAs are beneficial for fatal CHD prevention across diverse population subgroups.

Our stratification by biomarker type provides new insights on which lipid compartments may most influence CHD. Adipose tissue has been suggested for measurement of fatty acids because it reflects long-term dietary intake (approximately 1 year).[56] However, EPA and DHA are more highly concentrated in phospholipids than other compartments, and phospholipids also respond rapidly to dietary changes[57,58] and may best reflect effects on membrane receptors.[1] The best compartment for assessing ALA has also been unclear, with possible advantages to adipose tissue[16] but relatively little prior evaluation of other compartments. Our findings suggest generally stronger inverse associations with CHD for EPA, DPA, and DHA in phospholipids and total plasma, although fewer estimates and cases were available for adipose and cholesterol esters compartments, decreasing precision. Our results support use of phospholipids or total plasma for long-chain ω-3 PUFA exposure and a need for further investigation of optimal biomarkers for assessing ALA.

Our analysis has several important strengths. Use of biomarkers provided measures of exposure free of recall error and allowed separate evaluation of different ω-3 PUFAs and different circulating and tissue lipid compartments. We used standardized definitions and modeling for the populations, exposures, outcomes, covariates, effect modifiers, and analysis, reducing heterogeneity and potential investigator bias. Most studies[18-25,27-35] used centralized adjudication processes or registry linkage rather than self-report alone in ascertaining events, reducing information bias. Findings were similar in several sensitivity analyses, suggesting the robustness of results to varying assumptions. Our inclusion of all available studies substantially reduces the potential for publication bias. The studies included both sexes, multiple races, and a range of socioeconomic statuses across 16 countries, increasing generalizability.

Potential limitations should be considered. Relatively few studies[19,22,32,35] were available for some lipid compartments (eg, adipose, cholesterol esters), limiting inference for these specific compartments. All cohorts assessed ω-3 PUFA exposure once at baseline, and changes over time would attenuate findings toward the null, causing underestimation of associations. Reduced statistical power in quintile analyses and spline analyses made it difficult to ascertain the specific shape of dose responses. Because we analyzed observational studies, biomarkers and risk factors were not evenly distributed by randomization of study participants; hence, unmeasured or residual confounding cannot be excluded, and ω-3 biomarker measures may in part be markers of a long-term healthy lifestyle. However, our findings were independent of a range of major cardiovascular risk factors, and the relative specificity for fatal CHD and known biologic effects of ω-3 PUFAs also argue against residual confounding as the sole explanation for our findings. Because our findings reflect habitual ω-3 exposure for primary CHD prevention, our results do not imply that fish oil supplementation will necessarily reduce CHD events, particularly in the context of short-term treatment or in those with preexisting CHD. Awareness of ω-3 PUFAs, laboratory ascertainment methods, and medical treatment of hypertension, hypercholesterolemia, diabetes, angina, and MI have changed over time and could contribute to heterogeneity of results; however, we observed no significant differences in the results for studies assaying ω-3 PUFA levels before vs after the year 2000.

Conclusions

Our pooled collaboration of global studies using biomarkers of ω-3 PUFAs in participants without prevalent CHD reveals that habitual consumption of seafood and plant-based ω-3 PUFAs is associated with a modestly lower risk of fatal CHD.

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Study concept and design: All authors.
Acquisition, analysis, or interpretation of data: Del Gobbo conducted meta-analyses and data analysis for CHS. Aslibekyan performed analysis for the Costa-Rican adults study. Marklund performed analysis for ULSAM 50 and 70. Virtanen performed analysis for KIHD. Wennberg performed analysis for NSHDS I and II. Yakoob performed analysis for NHS I. Chiuve performed analysis for HPFS. dela Cruz performed analysis for MCCS. Frazier-Wood performed analysis for MESA. Guallar performed analysis for EURAMIC. Matsumoto performed analysis for PHS. Prem performed analysis for SCHS. Samieri performed analysis for the 3C study. Tanaka performed analysis for InCHIANTI. Wu performed analysis for SHHECS. Zhou performed analysis for ARIC. Imamura performed spline analyses. All authors interpreted data.
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REFERENCES

1. Mozaffarian D, Wu JHY. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *J Am Coll Cardiol*. 2011;58(20):2047-2067.

2. Bucher HC, Hengstler P, Schindler C, Meier G. N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. *Am J Med*. 2002;112(4):298-304.

3. Hooper L, Thompson RL, Harrison RA, et al. Omega 3 fatty acids for prevention and treatment of cardiovascular disease. *Cochrane Database Syst Rev*. 2004;(4):CD003177.

4. León H, Shibata MC, Sivakumaran S, Dorgan M, Chatterley T, Tsuyuki RT. Effect of fish oil on arrhythmias and mortality: systematic review. *BMJ*. 2008;337:a2931. doi:10.1136/bmj.a2931.

5. Marik PE, Varon J. Omega-3 dietary supplements and the risk of cardiovascular events: a systematic review. *Clin Cardiol*. 2009;32(7):365-372.

6. Trikalinos TA, Lee J, Moorthy D, et al. *Effects of Eicosapentanoic Acid and Docosahexanoic Acid on Mortality Across Diverse Settings: Systematic Review and Meta-analysis of Randomized Trials and Prospective Cohorts*. Rockville, MD: Agency for Healthcare Research and Quality; 2012.

7. Kwak SM, Myung SK, Lee YJ, Seo HG; Korean Meta-analysis Study Group. Efficacy of ω-3 fatty acid supplements (eicosapentaenoic acid and docosahexaenoic acid) in the secondary prevention of cardiovascular disease: a meta-analysis of randomized, double-blind, placebo-controlled trials. *Arch Intern Med*. 2012;172(9):686-694.

8. Rizos EC, Ntzani EE, Bika E, Kostapanos MS, Elisaf MS. Association between ω-3 fatty acid supplementation and risk of major cardiovascular disease events: a systematic review and meta-analysis. *JAMA*. 2012;308(10):1024-1033.

9. Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health: evaluating the risks and the benefits. *JAMA*. 2006;296(15):1885-1899.

10. Mozaffarian D, Wu JH. (n-3) fatty acids and cardiovascular health: are effects of EPA and DHA shared or complementary? *J Nutr*. 2012;142(3):614S-625S.

11. Pan A, Chen M, Chowdhury R, et al. α-Linolenic acid and risk of cardiovascular disease: a systematic review and meta-analysis. *Am J Clin Nutr*. 2012;96(6):1262-1273.

12. Psaty BM, O'Donnell CJ, Gudnason V, et al; CHARGE Consortium. Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium: design of prospective meta-analyses of genome-wide association studies from 5 cohorts. *Circ Cardiovasc Genet*. 2009;2(1):73-80.

13. Lemaitre RN, Tanaka T, Tang W, et al. Genetic loci associated with plasma phospholipid n-3 fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. *PLoS Genet*. 2011;7(7):e1002193. doi:10.1371/journal.pgen.1002193.

14. Dawber TR, Meadors GF, Moore FE Jr. Epidemiological approaches to heart disease: the Framingham Study. *Am J Public Health Nations Health*. 1951;41(3):279-281.

15. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. 1986;7(3):177-188.

16. Baylin A, Kim MK, Donovan-Palmer A, et al. Fasting whole blood as a biomarker of essential fatty acid intake in epidemiologic studies: comparison with adipose tissue and plasma. *Am J Epidemiol*. 2005;162(4):373-381.

17. Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *Am J Epidemiol*. 1992;135(11):1301-1309.

18. ARIC Investigators. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. *Am J Epidemiol*. 1989;129(4):687-702.

19. Campos H, Bailey SM, Gussak LS, Siles X, Ordovas JM, Schaefer EJ. Relations of body habitus, fitness level, and cardiovascular risk factors including lipoproteins and apolipoproteins in a rural and urban Costa Rican population. *Arterioscler Thromb*. 1991;11(4):1077-1088.

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20. Fried LP, Borhani NO, Enright P, et al. The Cardiovascular Health Study: design and rationale. *Ann Epidemiol*. 1991;1(3):263-276.

21. Day N, Oakes S, Luben R, et al. EPIC-Norfolk: study design and characteristics of the cohort: European Prospective Investigation of Cancer. *Br J Cancer*. 1999;80(1)(suppl 1):95-103.

22. Kardinaal AF, van 't Veer P, Kokl FJ, et al. EURAMIC Study: antioxidants, myocardial infarction and breast cancer: design and main hypotheses. *Eur J Clin Nutr*. 1993;47(2)(suppl 2):S64-S72.

23. Rimm EB, Giovannucci EL, Willett WC, et al. Prospective study of alcohol consumption and risk of coronary disease in men. *Lancet*. 1991;338(8765):464-468.

24. Bartali B, Benvenuti E, Corsi AM, et al. Changes in anthropometric measures in men and women across the life-span: findings from the InCHIANTI study. *Soz Praventivmed*. 2002;47(5):336-348.

25. Vanharanta M, Voutilainen S, Lakka TA, van der Lee M, Adlercreutz H, Salonen JT. Reduced risk of acute coronary events at high levels of mammalian lignan, enterolactone: a prospective population-based cohort study. *Lancet*. 1999;354:2112-2115.

26. Giles GG, English DR. The Melbourne Collaborative Cohort Study. *IARC Sci Publ*. 2002;156:69-70.

27. Bild DE, Bluemke DA, Burke GL, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. *Am J Epidemiol*. 2002;156(9):871-881.

28. Norberg M, Wall S, Boman K, Weinehall L. The Västerbotten Intervention Programme: background, design and implications. Global Health Action website 2010. http://www.globalhealthaction.net/index.php/gha/article/view/4643/5514. Accessed May 30, 2016.

29. Löwel H, Döring A, Schneider A, Heier M, Thorand B, Meisinger C; MONICA/KORA Study Group. The MONICA Augsburg surveys—basis for prospective cohort studies. *Gesundheitswesen*. 2005;67(1)(suppl 1):S13-S18.

30. Willett WC, Stampfer MJ, Colditz GA, Rosner BA, Hennekens CH, Speizer FE. Dietary fat and the risk of breast cancer. *N Engl J Med*. 1987;316(1):22-28.

31. Christen WG, Gaziano JM, Hennekens CH. Design of Physicians' Health Study II—a randomized trial of beta-carotene, vitamins E and C, and multivitamins, in prevention of cancer, cardiovascular disease, and eye disease, and review of results of completed trials. *Ann Epidemiol*. 2000;10(2):125-134.

32. Tunstall-Pedoe H, Woodward M, Tavendale R, A'Brook R, McCluskey MK. Comparison of the prediction by 27 different factors of coronary heart disease and death in men and women of the Scottish Heart Health Study: cohort study. *BMJ*. 1997;315(7110):722-729.

33. Hankin JH, Stram DO, Arakawa K, et al. Singapore Chinese Health Study: development, validation, and calibration of the quantitative food frequency questionnaire. *Nutr Cancer*. 2001;39(2):187-195.

34. 3C Study Group. Vascular factors and risk of dementia: design of the Three-City Study and baseline characteristics of the study population. *Neuroepidemiology*. 2003;22(6):316-325.

35. Hedstrand H. Results and observations from the Uppsala project [in Swedish]. *Lakartidningen*. 1972;69(41):4653.

36. Serhan CN. Novel lipid mediators and resolution mechanisms in acute inflammation: to resolve or not? *Am J Pathol*. 2010;177(4):1576-1591.

37. Arnold C, Konkel A, Fischer R, Schunck WH. Cytochrome P450-dependent metabolism of ω-6 and ω-3 long-chain polyunsaturated fatty acids. *Pharmacol Rep*. 2010;62(3):536-547.

38. Siscovick DS, Lemaitre RN, Mozaffarian D. The fish story: a diet-heart hypothesis with clinical implications: n-3 polyunsaturated fatty acids, myocardial vulnerability, and sudden death. *Circulation*. 2003;107(21):2632-2634.

39. Siscovick DS, Raghunathan TE, King I, et al. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *JAMA*. 1995;274(17):1363-1367.

40. Zheng J, Huang T, Yu Y, Hu X, Yang B, Li D. Fish consumption and CHD mortality: an updated meta-analysis of seventeen cohort studies. *Public Health Nutr*. 2012;15(4):725-737.

41. Mozaffarian D, Lemaitre RN, King IB, et al. Plasma phospholipid long-chain ω-3 fatty acids and total and cause-specific mortality in older adults: the Cardiovascular Health Study. *Ann Intern Med*. 2013;158(7):515-525.

42. Chowdhury R, Warnakula S, Kunutsor S, et al. Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. *Ann Intern Med*. 2014;160(6):398-406.

43. Holy EW, Forestier M, Richter EK, et al. Dietary α-linolenic acid inhibits arterial thrombus formation, tissue factor expression, and platelet activation. *Arterioscler Thromb Vasc Biol*. 2011;31(8):1772-1780.

44. Rallidis LS, Paschos G, Liakos GK, Velissaridou AH, Anastasiadis G, Zampelas A. Dietary α-linolenic acid decreases C-reactive protein, serum amyloid A and interleukin-6 in dyslipidaemic patients. *Atherosclerosis*. 2003;167(2):237-242.

45. McLennan PL, Dallimore JA. Dietary canola oil modifies myocardial fatty acids and inhibits cardiac arrhythmias in rats. *J Nutr*. 1995;125(4):1003-1009.

46. Billman GE, Kang JX, Leaf A. Prevention of sudden cardiac death by dietary pure ω-3 polyunsaturated fatty acids in dogs. *Circulation*. 1999;99(18):2452-2457.

47. Thies F, Miles EA, Nebe-von-Caron G, et al. Influence of dietary supplementation with long-chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy adults. *Lipids*. 2001;36(11):1183-1193.

48. Pauly D, Christensen V, Guénette S, et al. Towards sustainability in world fisheries. *Nature*. 2002;418(6898):689-695.

49. Chiu M, Austin PC, Manuel DG, Shah BR, Tu JV. Deriving ethnic-specific BMI cutoff points for assessing diabetes risk. *Diabetes Care*. 2011;34(8):1741-1748.

50. Wildman RP, Gu D, Reynolds K, Duan X, He J. Appropriate body mass index and waist circumference cutoffs for categorization of overweight and central adiposity among Chinese adults. *Am J Clin Nutr*. 2004;80(5):1129-1136.

51. Zheng JS, Huang T, Yang J, Fu YQ, Li D. Marine N-3 polyunsaturated fatty acids are inversely associated with risk of type 2 diabetes in Asians: a systematic review and meta-analysis. *PLoS One*. 2012;7(9):e44525. doi:10.1371/journal.pone.0044525.

52. Wallin A, Di Giuseppe D, Orsini N, Patel PS, Forouhi NG, Wolk A. Fish consumption, dietary long-chain n-3 fatty acids, and risk of type 2 diabetes: systematic review and meta-analysis of prospective studies. *Diabetes Care*. 2012;35(4):918-929.

53. Wu JHY, Micha R, Imamura F, et al. Omega-3 fatty acids and incident type 2 diabetes: a systematic review and meta-analysis. *Br J Nutr*. 2012;107(suppl 2):S214-S227.

54. Wu JHY, Mozaffarian D. ω-3 Fatty acids, atherosclerosis progression and cardiovascular outcomes in recent trials: new pieces in a complex puzzle. *Heart*. 2014;100(7):530-533.

55. Harris WS, Mozaffarian D, Rimm E, et al. Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. *Circulation*. 2009;119(6):902-907.

56. Dayton S, Hashimoto S, Dixon W, Pearce ML. Composition of lipids in human serum and adipose tissue during prolonged feeding of a diet high in unsaturated fat. *J Lipid Res*. 1966;7(1):103-111.

57. Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am J Clin Nutr*. 2006;83(6)(suppl):1467S-1476S.

58. Conquer JA, Holub BJ. Effect of supplementation with different doses of DHA on the levels of circulating DHA as non-esterified fatty acid in subjects of Asian Indian background. *J Lipid Res*. 1998;39(2):286-292.

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