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ORIGINAL ARTICLE

A Meta-Analysis of Randomized Controlled Trials and Prospective Cohort Studies of Eicosapentaenoic and Docosahexaenoic Long-Chain Omega-3 Fatty Acids and Coronary Heart Disease Risk

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Abstract

Objective: To conduct meta-analyses of randomized controlled trials (RCTs) to estimate the effect of eicosapentaenoic and docosahexaenoic acid (EPA+DHA) on coronary heart disease (CHD), and to conduct meta-analyses of prospective cohort studies to estimate the association between EPA+DHA intake and CHD risk.

Methods: A systematic literature search of Ovid/Medline, PubMed, Embase, and the Cochrane Library from January 1, 1947, to November 2, 2015, was conducted; 18 RCTs and 16 prospective cohort studies examining EPA+DHA from foods or supplements and CHD, including myocardial infarction, sudden cardiac death, coronary death, and angina, were identified. Random-effects meta-analysis models were used to generate summary relative risk estimates (SRREs) and 95% CIs. Heterogeneity was examined in subgroup and sensitivity analyses and by meta-regression. Dose-response was evaluated in stratified dose or intake analyses. Publication bias assessments were performed.

Results: Among RCTs, there was a nonstatistically significant reduction in CHD risk with EPA+DHA provision (SRRE=0.94; 95% CI, 0.85-1.05). Subgroup analyses of data from RCTs indicated a statistically significant CHD risk reduction with EPA+DHA provision among higher-risk populations, including participants with elevated triglyceride levels (SRRE=0.84; 95% CI, 0.72-0.98) and elevated low-density lipoprotein cholesterol (SRRE=0.86; 95% CI, 0.76-0.98). Meta-analysis of data from prospective cohort studies resulted in a statistically significant SRRE of 0.82 (95% CI, 0.74-0.92) for higher intakes of EPA+DHA and risk of any CHD event.

Conclusion: Results indicate that EPA+DHA may be associated with reducing CHD risk, with a greater benefit observed among higher-risk populations in RCTs.

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Guidance from the American College of Cardiology/American Heart Association Task Force and other major health organizations, agencies, and public health groups recommend dietary patterns that include fish and/or greater intakes of fish or the omega-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for heart health.[1,2] As the available literature on n-3 LCPUFA intake and coronary heart disease (CHD) risk increases, with some mixed results reported, comprehensive systematic reviews and meta-analyses that evaluate the scientific evidence from both clinical and observational study designs are needed. Therefore, the objective of our study was to perform a comprehensive meta-analysis of randomized controlled trials (RCTs) to estimate the effect of EPA+DHA on CHD, and

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to conduct a comprehensive meta-analysis of prospective cohort studies to estimate the association between EPA+DHA intake and CHD risk. Additional objectives included examining the effects of dose, as well as the effects of EPA+DHA on specific outcomes (eg, myocardial infarction) and among higher-risk populations (eg, those with elevated triglyceride levels) using subgroup analyses and meta-regression.

METHODS

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (see Supplemental Figure 1, available online at http://www.mayoclinicproceedings.org) for this systematic review and meta-analysis.[3]

Literature Search and Study Selection

Comprehensive literature searches using the PubMed, Ovid/Medline, and Embase databases were conducted. The Cochrane Library was also reviewed. Literature searches, which covered studies published from January 1, 1947, through November 2, 2015, were designed to identify RCTs and prospective cohort studies that examined EPA+DHA and CHD outcomes. The full Ovid Medline search strategy is included as Supplemental Material (see Supplemental Figure 2, available online at http://www.mayoclinicproceedings.org). Level I screening included review of all titles and/or abstracts. Supplementary literature searches included examining the reference lists of all relevant studies, published meta-analyses, and the report published by the Agency for Healthcare Research and Quality Technical Review in 2012.[4] In addition, previously published reviews were scrutinized to identify pertinent studies that may not have been captured in our electronic searches.[5-10] Full-text publications of all studies not eliminated at level I were retrieved for complete review at level II screening. All search results were screened by 2 individuals, with minor differences resolved by discussion and consultation with a third researcher.

Included studies were required to report 1 or more of the following CHD outcomes: myocardial infarction (fatal or nonfatal), angina, sudden cardiac death, coronary death, and CHD incidence (for prospective cohort studies). Study populations included nonhospitalized adults (>18 y) with and without CHD but otherwise free of significant non–CHD-related disease pathologies. Studies were required to report hazard ratios (HR) or rate ratios (RR) of outcomes and measures of variance (ie, 95% CIs), or data were required to be available to calculate such measures. Additional information on the inclusion criteria, data extraction methods, and evaluation of study quality are available in Supplemental Figure 3 (available online at http://www.mayoclinicproceedings.org).

For the RCTs, a composite variable, "any CHD event," was created and defined as the combination of fatal or nonfatal myocardial infarction (MI), coronary death, sudden cardiac death, and angina. Fatal CHD events included fatal MI, coronary death, and sudden cardiac death. Nonfatal CHD events included nonfatal MI and angina. Coronary death included all fatal events minus sudden cardiac death. Individual events, that is, sudden cardiac death, were also analyzed separately. For the prospective cohort studies, "any CHD event" was defined as the combination of fatal or nonfatal MI, CHD incidence, coronary death, sudden cardiac death, and angina; fatal CHD events included fatal MI, coronary death, and sudden cardiac death; and nonfatal CHD events included angina, CHD incidence, and nonfatal MI.

Statistical Analyses

Primary statistical analyses using meta-analysis methodology were based on comparing rates of total CHD events, as well as specific CHD event outcomes between the EPA+DHA group and the control group. If studies did not report RRs, the absolute rate of CHD events was calculated for each group and then compared to produce an RR and 95% CI. Random-effects meta-analysis models were used to generate summary relative risk estimates (SRREs) and 95% CIs. Summary associations were interpreted as statistically significant (ie, $P<.05$) if the 95% CIs did not include the null value of 1.0 in their range. The study weights were equal to the inverse of the variance of each study's effect estimate according to the methodology developed by DerSimonian and Laird.[11] If data for specific mutually exclusive CHD events, but not CHD overall, were reported in the same

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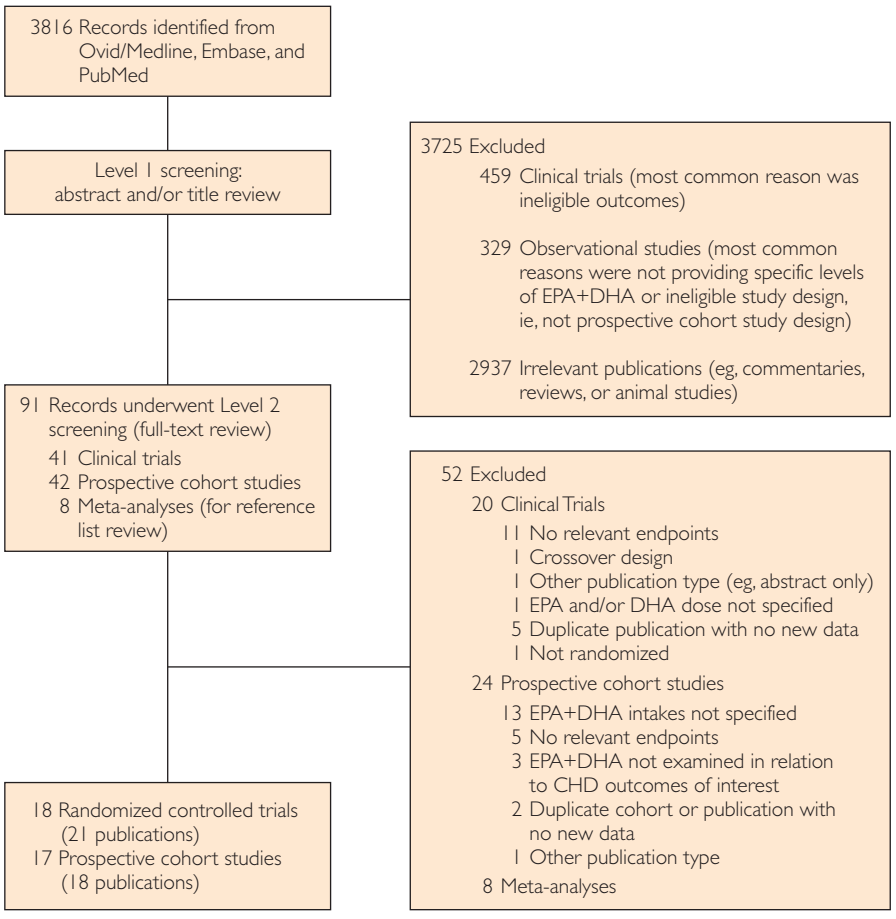

FIGURE 1. Flow diagram of literature search and study selection. DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid.

study, results data from that study were combined using a fixed effects model to produce a single risk estimate for total CHD. For the RCTs, meta-analysis models were generated for overall study population analyses as well as for subgroup-specific analyses. Stratified dose meta-analyses based on levels above and below 1 g/d were conducted. In addition, meta-regression analyses based on increasing EPA+DHA dose and CHD risk were performed. Meta-regression was also used to evaluate the impact of study quality on observed summary associations. Given the larger sample size for "any CHD event" compared with specific CHD events, such as fatal MI, a greater number of subgroup-specific and sensitivity analyses were possible for this category. The subgroup analyses were conducted to identify potential sources of between-study variation and to estimate the effect of EPA+DHA for specific subpopulations and study characteristics. To determine the influence that each individual study (RCT or prospective cohort study) had on the overall summary effect in the primary meta-analysis models, one-study removed sensitivity analyses, whereby the meta-analysis is conducted multiple times with a single study removed, were undertaken.

Statistical heterogeneity was assessed using Cochran's Q, which tests for between-study statistical variation. A Cochran's Q *P* value of .10 or less in a specific meta-analysis model is an indication of statistically significant heterogeneity of the intervention effects across the RCTs or the associations across the

TABLE 1. Characteristics of Randomized Controlled Trials[a]

Reference, year	Country	Study name	Duration (y)	n[b]	Intervention regimen			Control
					Intervention type	Dose (g/d)[c]	EPA+DHA (g/d)	
Primary prevention								
Roncaglioni et al,[28] 2013[d]	Italy	Risk and Prevention Study	5	12,513	Ethyl esters	1	0.85	Olive oil
Yokoyama et al,[31] 2007	Japan	JELIS (subgroup with no CHD)	5	14,981	Ethyl esters	1.84	1.80	Control (no supplement)[e]
Mixed prevention[f]								
Bosch et al,[32] 2012	40 countries[g]	ORIGIN	7	12,611	Ethyl esters	1	0.84	Olive oil
Brouwer et al,[13] 2006	8 countries[h]	SOFA	1	546	Fish oil	2	0.80	Sunflower oil
Einvik et al,[16] 2010	Norway	DOIT	3	563	Fish oil	4	2.02	Corn oil
Galan et al,[17] 2010	France	SU.FOL.OM4	5	2501	Fish oil	1	0.60	Gelatin
Ishikawa et al,[18] 2010[i]	Japan	JELIS (PAD subgroup)	5	223	Ethyl esters	1.84	1.80	Control (no supplement)[e]
Leaf et al,[22] 2005	United States	—	1	402	Ethyl esters	4	2.60	Olive oil
Macchia et al,[24] 2013	Argentina	FORWARD	3	586	Ethyl esters	1	0.88	Olive oil
Raitt et al,[26] 2005	United States	—	2	200	Fish oil	1.8	1.30	Olive oil
Yokoyama et al,[31] 2007	Japan	JELIS	5	18,645	Ethyl esters	1.84	1.80	Control (no supplement)[e]
Secondary prevention								
Burr et al,[14] 1989	UK	DART	2	2,033	Fatty fish and/or fish oil	41 (fish)/3 (oil)	0.75	Balanced diet advice
Burr et al,[15] 2003	UK	—	2	3,114	Fatty fish and/or fish oil	41 (fish)/3 (oil)	0.75	Balanced diet advice
Johansen et al,[19] 1999	Norway	CART	0.5	500	Ethyl esters	6	5.04	Corn oil
Kromhout et al,[20] 2010	The Netherlands	Alpha Omega Trial	3	4,837	EPA+DHA enriched margarine	23.8	0.38	Oleic acid margarine
Kromhout et al,[21] 2011[j]	The Netherlands	Alpha Omega Trial (type 2 diabetes subgroup)	3	511	EPA+DHA enriched margarine	23.8	0.38	Oleic acid margarine

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TABLE 1. Continued

Reference, year	Country	Study name	Duration (y)	n[b]	Intervention regimen			Control
					Intervention type	Dose (g/d)[c]	EPA+DHA (g/d)	
Secondary prevention, continued								
Macchia et al,[23] 2005[k]	Italy	GISSI (LVSD subgroup)	4	4,324	Ethyl esters	1	0.88	Control (no supplement)[e]
Marchioli et al,[25] 2001	Italy	GISSI	3.5	11,323	Ethyl esters	1	0.88	Control (no supplement)[e]
Nilsen et al,[33] 2001	Norway	—	2	300	Ethyl ester	4	3.46	Corn oil
Rauch et al,[27] 2010	Germany	OMEGA trial	1	3,851	Ethyl esters	1	0.84	Olive oil
Singh et al,[29] 1997	India	IEIS-4	1	240[l]	Fish oil	6	1.80	Aluminum hydroxide
Von Schacky et al,[30] 1999	Germany	SICMO	2	223	Fish oil	3	0.94	Oil mixture without marine n-3 fatty acids
Yokoyama et al,[31] 2007	Japan	JELIS (CHD subgroup)	5	3,664	Ethyl esters	1.84	1.80	Control (no supplement)[e]

[a]CART = Coronary Angioplasty Restenosis Trial; CHD = coronary heart disease; DART = Diet and Reinfarction Trial; DHA = docosahexaenoic acid; DOIT = Diet and Omega-3 Intervention Trial; EPA = eicosapentaenoic acid; FORWARD, Randomized Trial to Assess Efficacy of PUFA for the Maintenance of Sinus Rhythm in Persistent Atrial Fibrillation Fish Oil Research with omega-3 for Atrial fibrillation Recurrence Delaying; GISSI = Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardio; IEIS-4 = Indian Experiment of Infarct Survival; JELIS = Japan EPA Lipid Intervention Study; LVSD = left ventricular systolic dysfunction; NR = not reported; ORIGIN = Outcome Reduction with an Initial Glargine Intervention; PAD = peripheral arterial disease; PUFA = polyunsaturated fatty acid; SICMO = Study on Prevention of Coronary Atherosclerosis by Intervention with Marine Omega-3 fatty acids; SOFA = Study on Omega-3 Fatty Acids and Ventricular Arrhythmia; SU.FOL.OM4 = Supplémentation en Folates et Omega-3.

[b]Represents the number of subjects initially enrolled in the study.

[c]Dose of entire fish oil supplement or food.

[d]Study includes a population with no history of myocardial infarctions, but 12% with angina at baseline.

[e]Control arm did not receive a placebo.

[f]Mixed prevention trials include studies where some but not all participants have CHD at baseline.

[g]Forty countries from Asia, Australia, Europe, North American, Africa (South Africa), and South America.

[h]Countries include Poland, Germany, the Netherlands, the United Kingdom, Czech Republic, Belgium, Austria, and Switzerland.

[i]Subgroup publication of JELIS trial, consulted for additional trial information only, subgroup data not used in meta-analysis.

[j]Subgroup publication of Alpha Omega trial, consulted for additional trial information only, subgroup data not used in meta-analysis.

[k]Subgroup publication of GISSI trial, consulted for additional trial information only, subgroup data not used in meta-analysis.

[l]Represents the number of subjects in final analysis (number of subjects enrolled was not reported).

TABLE 2. Characteristics of the Prospective Cohort Studies[a]

Reference, year	Country	Cohort	Follow-up (y)	Baseline CHD (%)	n	Highest vs lowest EPA+DHA intake categories[b]	RR (95% CI)[c]
Albert et al,[34] 1998	United States	PHS	11	0	20,551 (M)	≥0.25 vs <0.09 g/d	SCD: 0.43 (0.20-0.93)
Amiano et al,[35] 2014	Spain	Spanish EPIC	13	0	15,444 (M)	>0.34 vs <0.08 g/d EPA	Coronary events: 1.18 (0.90-1.56)
						≥0.62 vs ≤0.19 g/d DHA	Coronary events: 1.08 (0.83-1.42)
					25,647 (F)	>0.22 vs <0.05 g/d EPA	Coronary events: 0.71 (0.40-1.25)
						≥0.41 vs ≤0.12 g/d DHA	Coronary events: 0.79 (0.44-1.39)
Ascherio et al,[37] 1995	United States	HPFS[d]	6	0	44,895 (M)	0.58 vs 0.07 median g/d	Total MI: 1.09 (0.88-1.35)
Bergkvist et al,[36] 2015	Sweden	SMC	12	0	33,446 (F)	5.18 vs 1.48 median g/d	Total MI: 0.74 (0.52-1.06)
Chiuve et al,[38] 2012	United States	NHS I[d]	30	2	91,981 (F)	0.51 vs 0.05% of total fat	SCD: 0.50 (0.35-0.70)
de Goede et al,[39] 2010	The Netherlands	MORGEN	11.3	0	21342 (M+F)	>0.19 vs <0.06 g/d	Coronary death: 0.51 (0.27-0.94) Fatal MI: 0.38 (0.19-0.77) Nonfatal MI: 1.07 (0.74-1.54)
Hu et al,[40] 2003	United States	NHS I[d]	16	0	84,688 (F)	0.24 vs 0.03 median % of total kcal	Coronary events: 0.69 (0.57-0.84) Coronary death: 0.62 (0.44-0.88) Nonfatal MI: 0.73 (0.57-0.93)
Iso et al,[41] 2006	Japan	JPHC	11	0	41,578 (M+F)	2.1 vs 0.3 median g/d	Total MI: 0.43 (0.24-0.78) SCD: 1.24 (0.39-3.98) Fatal events: 1.54 (0.60-3.99) Nonfatal MI: 0.33 (0.17-0.63)
Jarvinen et al,[43] 2006	Finland	FMC	21.5	0	2,775 (M)	0.99 vs 0.13 mean g/d	Coronary death: 0.96 (0.68-1.38)
					2,445 (F)	0.59 vs 0.09 mean g/d	Coronary death: 0.73 (0.44-1.19)
Joensen et al,[44] 2010	Denmark	DDCHCS	7.6	0	24,786 (M)	>1.08 vs. ≤0.39 g/d	Any CHD event (angina+MI): 0.81 (0.64-1.04)
					29,017 (F)	>1.03 vs. ≤0.38 g/d	Any CHD event (angina+MI): 0.97 (0.62-1.52)
Koh et al,[42] 2015	Singapore	SCHS	19	4.1	60,299 (M+F)	0.46 vs 0.19 mean g/d	Coronary death: 0.86 (0.77-0.96)
Manger et al,[45] 2010	Norway	WENBIT	4.8	100	2,412 (M+F)	2.64 vs 0.58 mean g/d	Coronary death: 1.33 (0.67-2.62) Total MI: 1.05 (0.72-1.52) Coronary events: 0.95 (0.69-1.31)
Miyagawa et al,[51] 2014	Japan	NIPPON DATA80	24	0	9,190 (M+F)	1.72 vs 0.42 mean g/d	Coronary death: 0.82 (0.53-1.29)
Mozaffarian et al,[46] 2005	United States	HPFS[d]	14	0	45,722 (M)	≥0.25 vs <0.25 g/d	SCD: 0.52 (0.34-0.79) Nonfatal MI: 1.16 (0.99-1.36) Coronary events: 1.05 (0.92-1.19)
Pietinen et al,[47] 1997	Finland	ATBC	6.1	0	21,930 (M)	0.8 vs 0.2 median g/d	Coronary events: 1.15 (0.97-1.35) Coronary death: 1.24 (0.97-1.58)
Streppel et al,[48] 2008	The Netherlands	Zutphen Study	40	0	1,373 (M)	>0.25 vs 0 g/d	Fatal events (SCD+coronary death): 0.65 (0.40-1.06) SCD: 0.68 (0.23-2.02)
Takata et al,[50] 2013	China	SMHS+SWHS	13	6.2	134,296 (M+F)	0.22 vs 0.01 median g/d	Coronary death: 0.79 (0.57-1.09)

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TABLE 2. Continued

Reference, year	Country	Cohort	Follow-up (y)	Baseline CHD (%)	n	Highest vs lowest EPA+DHA intake categories[b]	RR (95% CI)[c]
Yuan et al,[49] 2001	China	Shanghai Cohort Study	12	0	18,244 (M)	≥0.15 vs <0.04 g/d	Fatal MI: 0.43 (0.23-0.81) CHD death (other than MI): 0.71 (0.32-1.57)

[a]ACS = acute coronary syndrome; ATBC = Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CHD = coronary heart disease; DDCHCS = Danish Diet, Cancer and Health Cohort Study; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; EPIC = European Prospective Investigation into Cancer and Nutrition; F = female; FMC = Finnish Mobile Clinic; JPHC = Japan Public Health Center–Based Study Cohort I; HPFS = Health Professionals Follow-up Study; M = male; MI = myocardial infarction; MORGEN = The Monitoring Project on Risk Factors for Chronic Diseases; NIPPON DATA80 = National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged, 1980; NHS I = Nurses' Health Study I; PHS = Physicians Health Study; RR = relative risk; SCD = sudden cardiac death; SCHS = Singapore Chinese Healthy Study; SMC = Swedish Mammography Cohort; SMHS = Shanghai Men's Health Study; SWHS = Shanghai Women's Health Study; WENBIT = Western Norway B Vitamin Intervention Trial.

[b]All studies assessed dietary intake via food frequency questionnaires, with the exception of Streppel et al,[48] who used a cross-check dietary history method.

[c]RRs and 95% CIs included in at least 1 of the meta-analyses or combined using a fixed effects model to produce a single risk estimate for "any CHD event" or other composite outcome are shown.

[d]When a series of publications from the same cohort were available, data from only the most recent publication were used in the meta-analysis except in cases in which earlier publications provided estimates for unique outcomes not presented in the most recent publication. In these cases, outcome data from the earlier publication(s) were also included in the meta-analysis. This approach resulted in only 17 prospective cohort studies in the any CHD event model as Mozaffarian et al[46] was used in place of Ascherio et al[37] because Ascherio et al[37] reported only total MI vs Mozaffarian et al,[46] which reported more recent data including collective coronary events for the HPFS cohort. Ascherio et al,[37] however, was used in the statistical model for total MI because these authors provided this outcome for the HPFS cohort, but Mozaffarian et al[46] did not.

prospective cohort studies. This *P* value for heterogeneity is not a significance test for the relationship between the exposure (ie, EPA+DHA) and the outcome (eg, CHD). In addition, the I^2 statistic, which indicates the percentage of variation attributable to between-study heterogeneity, is shown in the forest plot figures for the primary analyses. The presence of publication bias was assessed visually by examining a funnel plot measuring the standard error as a function of effect size, as well as statistically by using Egger's regression method.[12] All statistical analyses were performed using Comprehensive Meta-Analysis (version 3.2.00089; Biostat).

RESULTS

Descriptive Study Characteristics

A flow diagram of the literature search and study selection is shown in Figure 1; 18 RCTs (21 publications)[13-33] and 16 prospective cohort studies (18 publications)[34-51] were included in the meta-analysis. Studies excluded after full-text review are listed in Supplemental Table 1 (available online at http://www.mayoclinicproceedings.org). The main study characteristics of the RCTs and prospective cohort studies are summarized in Table 1 and Table 2, respectively. Approximately 93,000 subjects were included in the meta-analysis of RCT data and 732,000 subjects were included in the meta-analysis of prospective cohort studies.

Meta-Analyses Results

Results from meta-analyses of RCTs and prospective cohort studies are reported in Tables 3 and 4, with the primary meta-analytic findings summarized in Figure 2. Results from additional subgroup and meta-regression analyses are also presented in Supplemental Table 2 (available online at http://www.mayoclinicproceedings.org).

In the overall meta-analysis of RCT data, EPA+DHA provision was associated with a nonstatistically significant SRRE of 0.94 (95% CI, 0.85-1.05) for any CHD event (Figure 2A). Statistical heterogeneity in this RCT model (*P*=.07) was explained in part by differences in several study characteristics, including baseline triglyceride and low-density lipoprotein cholesterol. Participants

with elevated triglyceride levels (≥150 mg/dL) (SRRE=0.84; 95% CI, 0.72-0.98) (Figure 2A) and elevated low-density lipoprotein cholesterol (SRRE=0.86; 95% CI, 0.76-0.98) (Figure 2C) experienced statistically significant reduced CHD events, respectively. Furthermore, higher dose (above 1 g/d of EPA+DHA) had a stronger impact among those with elevated triglyceride levels (SRRE=0.75; 95% CI, 0.64-0.89) compared with trials of less than 1 g/d of EPA+DHA (SRRE=0.93; 95% CI, 0.82-1.07).

The SRRE for any CHD event was 0.83 in the subgroup analysis of RCTs administering 1 g/d or more of EPA+DHA, but this finding was not statistically significant (95% CI, 0.61-1.14) (see Supplemental Figure 4, available online at http://www.mayoclinicproceedings.org). Meta-regression did not produce a continuous dose-response effect when including data for less than 1 g/d of EPA+DHA with all other dose groups (see Supplemental Figure 5, available online at http://www.mayoclinicproceedings.org). Participants in RCTs who received EPA+DHA were less likely (SRRE=0.81; 95% CI, 0.65-1.00) to have a coronary death event compared with those who received a placebo; the effect was not modified by dose level but the summary association was stronger among secondary prevention studies (Table 3). No apparent effect modification was found by prevention status (primary, secondary, or mixed), prevalence of diabetes medication use (Table 3), or in subgroup analyses by duration of follow-up, use of an implantable cardioverter defibrillator, or hypertensive status (Supplemental Table 2) among participants in RCTs.

TABLE 3. Randomized Controlled Trials—Summary of Meta-Analysis Results: EPA+DHA and CHD End Points[a,b]

Model	Studies (n)	SRRE	Lower 95% CI	Upper 95% CI	Cochran's Q Heterogeneity[c] test
Any CHD event—All RCTs[13-17,19,20,22,24-33]	18	0.94	0.85	1.05	*P*=.07
Any CHD event—Primary prevention[28,31]	2	0.92	0.80	1.05	*P*=.41
Any CHD event—Secondary prevention[14,15,19,20,25,27,29-31,33]	10	0.92	0.76	1.11	*P*=.03
Any CHD event—0 <1 g[13-15,17,20,24,25,27,28,30,32]	11	0.99	0.91	1.07	*P*=.34
Any CHD event—1+ g[16,19,22,26,29,31,33]	7	0.83	0.61	1.14	*P*=.23
Any CHD event—Triglycerides <150[17,20,32,33]	4	1.04	0.96	1.13	*P*=.74
Any CHD event—Triglycerides 150+[d,16,25,28-31]	6	0.84	0.72	0.98	*P*=.21
Any CHD event—LDL <130[17,20,32]	3	1.03	0.95	1.12	*P*=.83
Any CHD event—LDL 130+[d,16,25,28,30,31]	5	0.86	0.76	0.98	*P*=.30
Any CHD event—<25% of population taking diabetes medication[13-16,19,20,24-26,31,33]	11	0.93	0.80	1.09	*P*=.12
Any Fatal CHD event—0 <1 g[14,15,20,24,25,27,28,32]	7	0.97	0.81	1.17	*P*=.003
Any fatal CHD event —1+ g[16,19,22,26,29-31]	7	0.89	0.58	1.37	*P*=.68
Any nonfatal CHD event—0 <1 g[13,14,17,25,30,32]	6	0.97	0.80	1.19	*P*=.06
Any nonfatal CHD event—1+ g[29,31,33]	3	0.80	0.59	1.10	*P*=.25
Coronary death—All RCTs[d,14,19,25,28,31]	5	0.81	0.65	1.00	*P*=.17
Coronary death—Primary prevention[28,31]	2	1.09	0.81	1.46	*P*=.98
Coronary death—Secondary prevention[d,14,19,25,31]	4	0.80	0.64	0.99	*P*=.20

[a]CHD = coronary heart disease; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; LDL = low-density lipoprotein; RCT = randomized controlled trial; SRRE = summary relative risk estimate.

[b]"Any CHD event" includes fatal and nonfatal MI, coronary death, sudden cardiac death, and angina. "Coronary death" includes fatal MI, death from other acute or subacute forms of CHD, or death from chronic CHD.

[c]Statistical heterogeneity was assessed using Cochran's Q, which tests for between-study statistical variation. In a conventional meta-analysis, a Cochran's Q *P* value of .10 or less is an indication of statistically significant heterogeneity. It is noteworthy that this *P* value is only an indication that statistical variability may be present in a specific meta-analysis model, and this *P* value is not a significance test for the relationship between the exposure (ie, EPA+DHA) and the outcome (eg, CHD).

[d]Indicates statistical significance per SRRE and corresponding CI. Summary associations were interpreted as statistically significant if the 95% CIs did not include the null value of 1.0 in their range.

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TABLE 4. Prospective Cohort Studies—Summary of Meta-Analysis Results: EPA+DHA and CHD End Points[a,b]

Model[c]	Studies (n)	SRRE	Lower 95% CI	Upper 95% CI	Cochran's Q Heterogeneity[c] test
Any CHD event[d,34-36,38-51]	17	0.82	0.74	0.92	*P*<.001
Fatal events[d,34,38-43,45-51]	14	0.77	0.66	0.90	*P*<.001
Nonfatal events[39-41,46]	4	0.81	0.55	1.19	*P*<.001
Coronary death[d,37,39-43,45,47-51]	9	0.82	0.69	0.98	*P*=.01
Coronary events[35,40,45-47]	5	0.96	0.81	1.14	*P*=.001
Total MI[36,37,39,41,45]	5	0.85	0.66	1.10	*P*=.03
Nonfatal MI[39-41,46]	4	0.81	0.55	1.19	*P*<.001
Sudden cardiac death[d,34,38,41,46,48]	5	0.53	0.41	0.67	*P*=.62

[a]CHD = coronary heart disease; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; MI = myocardial infarction; SRRE = summary relative risk estimate.

[b]"Any CHD event" includes fatal and nonfatal MI, coronary death, sudden cardiac death, and angina. "Coronary death" includes fatal MI, death from other acute or subacute forms of CHD, or death from chronic CHD.

[c]Statistical heterogeneity was assessed using Cochran's Q, which tests for between-study statistical variation. In a conventional meta-analysis, a Cochran's Q *P* value of .10 or less is an indication of statistically significant heterogeneity. It is noteworthy that this *P* value is only an indication that statistical variability may be present in a specific meta-analysis model, and this *P* value is not a significance test for the relationship between the exposure (ie, EPA+DHA) and the outcome (eg, CHD).

[d]Indicates statistical significance per SRRE and corresponding CI. Summary associations were interpreted as statistically significant if the 95% CIs did not include the null value of 1.0 in their range.

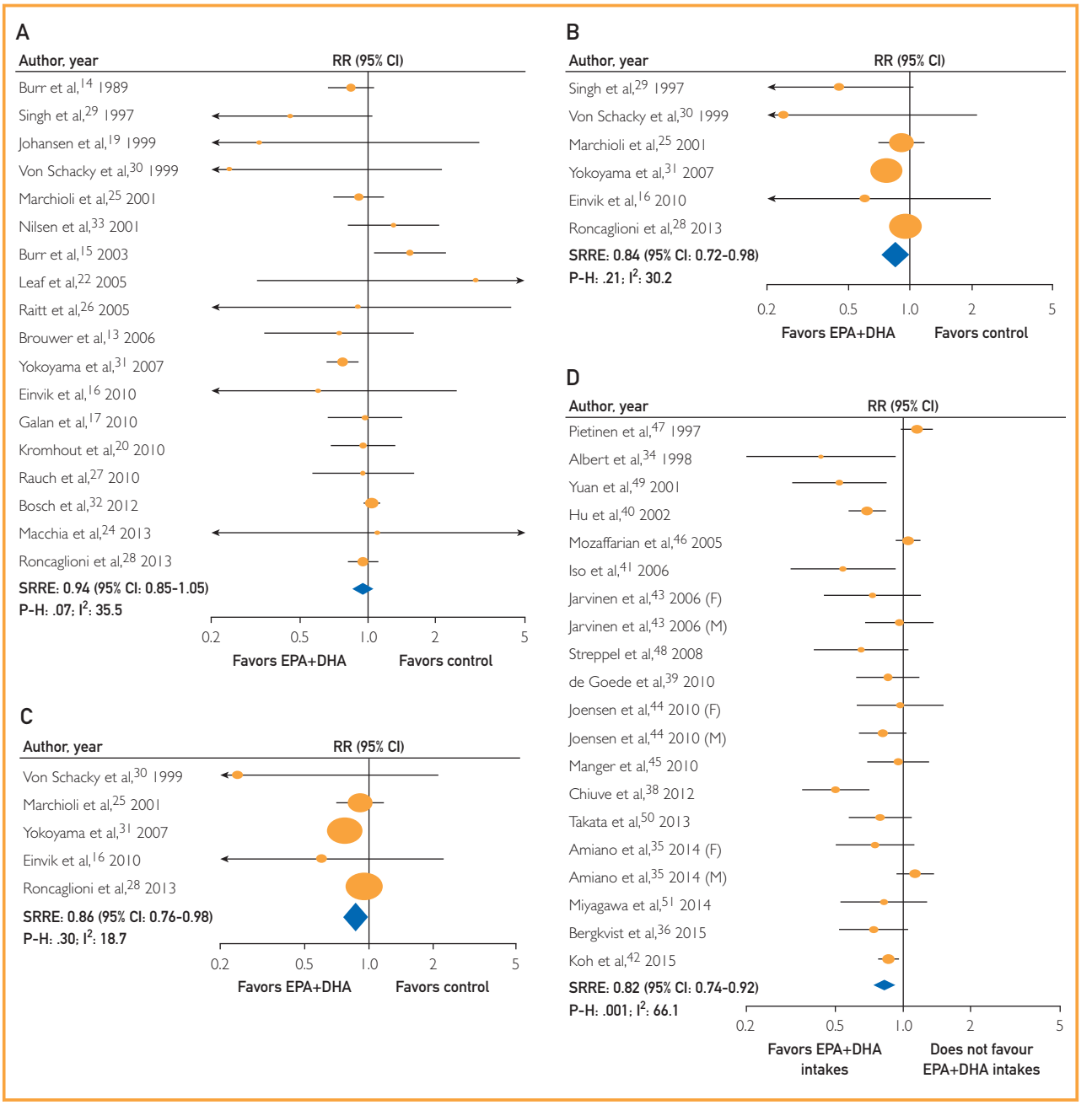
Visual examination of funnel plots and statistical testing of data from the RCTs revealed no apparent publication bias (see Supplemental Figure 6, available online at http://www.mayoclinicproceedings.org). In the overall analysis of any CHD event, removal of any single RCT did not modify appreciably the summary association (range of SRRE based on 1 study removed at a time, 0.92-0.98) (see Supplemental Figure 7, available online at http://www.mayoclinicproceedings.org) or alter the level of statistical significance. The Cochrane Bias Assessment score for each RCT (see Supplemental Figure 8, available online at http://www.mayoclinicproceedings.org) was modeled as a linear variable. Meta-regression of the log risk ratio of the Cochrane Bias Assessment score on CHD risk did not result in a statistically significant quality response trend (beta coefficient=0.01; *P*=.64) (see Supplemental Figure 9, available online at http://www.mayoclinicproceedings.org).

Statistically significant inverse associations were observed among prospective cohort studies for any CHD event (SRRE=0.82; 95% CI, 0.74-0.92) (Figure 2D), fatal CHD events (SRRE=0.77; 95% CI, 0.66-0.90), coronary death (SRRE=0.82; 95% CI, 0.69-0.98), and sudden cardiac death (SRRE=0.53; 95% CI, 0.41-0.67), with nonstatistically significant inverse associations found for nonfatal CHD events, coronary events, and nonfatal MI (Table 4). Between-study statistical variation was observed in all prospective cohort models (indicated by the *P* values for heterogeneity), except for sudden cardiac death (*P*=.62; Table 4). However, of note, the direction of association was similar for the large majority of prospective cohort studies (ie, most individual study's RRs were below 1.0; Table 4). The funnel plot revealed slight asymmetry around the effect size but the potential for publication bias was inconsequential (see Supplemental Figure 10, available online at http://www.mayoclinicproceedings.org). The number of Newcastle-Ottawa Score stars for each prospective cohort study (see Supplemental Table 3, available online at http://www.mayoclinicproceedings.org) was modeled as a linear variable. Meta-regression of the log risk ratio of the Newcastle-Ottawa Score stars on CHD risk did not result in a statistically significant quality response trend (beta coefficient=0.04; *P*=.50) (see Supplemental Figure 11, available online at http://www.mayoclinicproceedings.org). Furthermore, removal of any single prospective cohort study did not modify the summary association for any CHD event (range of SRRE based on 1 study removed at a time, 0.80-0.85; data not shown) or alter the precision of the CIs.

DISCUSSION

To our knowledge, this is the most comprehensive quantitative assessment of the relationship between EPA+DHA supplementation and intake and CHD risk to date. Our inclusion criteria were specific for CHD, which distinguishes our findings from those of other meta-analyses that included a mixture of vascular as well as less well defined coronary outcomes. Collectively, the SRREs for RCT data were relatively consistent across all types of analyses with many statistically significant inverse effects that were supported by inverse associations between EPA+DHA intake and all coronary outcomes across the prospective cohort studies.

A well-documented effect of n-3 LCPUFA supplementation is the reduction in serum triglyceride levels in subjects with hypertriglyceridemia.[52] Although large-scale RCTs examining



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the effect of lowering triglyceride levels among those with hypertriglyceridemia are underway (eg, Outcomes Study to Assess STatin Residual Risk Reduction with EpaNova in HiGh CV Risk PatienTs with Hypertriglyceridemia [STRENGTH], see clintrials.gov), results from currently available prospective cohort studies indicate that elevated triglyceride levels are associated with increasing CHD risk.[53] Our results indicate that EPA+DHA provision reduced CHD risk among subjects with triglyceride levels of 150 mg/dL or more in RCTs but not among those with triglyceride levels within normal limits. Similarly, a CHD risk reduction benefit of n-3 LCPUFA provision was found among RCT subjects with low-density lipoprotein (LDL) cholesterol levels of 130 mg/dL or more but not for those with LDL cholesterol levels of less than 130 mg/dL. Although past meta-analyses have shown that n-3 LCPUFA administration may increase LDL cholesterol levels (particularly in patients with very high triglyceride levels),[52,54] the beneficial effect of n-3 LCPUFA on CHD seen in subjects with higher LDL cholesterol levels in this analysis may reflect the redistribution of LDL cholesterol to larger, less atherogenic LDL particles that has been reported following n-3 LCPUFA supplementation in a number of RCTs.[55,56] These findings are particularly relevant for the management of CHD risk in the general US population because 25% of Americans older than 20 years are estimated to have triglyceride levels of 150 mg/dL[57] or more and 27% of Americans aged between 40 and 74 years have LDL cholesterol levels of 130 mg/dL or more.[58] Blood pressure is another well-documented CHD risk factor impacted favorably by n-3 LCPUFA administration.[59]

Our findings are relatively consistent with previous meta-analyses from the last 10 years for which 10% to 30% decreased risks of cardiac/coronary death have been observed with provision or greater intakes of n-3 LCPUFA.[5-9,60-62] In a meta-analysis of prospective cohort studies, Chowdhury et al[63] reported a statistically significant 13% (RR=0.87; 95% CI, 0.78-0.97) CHD risk reduction with greater n-3 LCPUFA intake and a statistically significant 25% (RR=0.75; 95% CI, 0.62-0.89) CHD risk reduction with higher circulating EPA+DHA levels. Casula et al[61] conducted a meta-analysis of RCTs in which at least 1 g/d of n-3 LCPUFA was administered, and reported statistically significant inverse effects for cardiac death (RR=0.68; 95% CI, 0.56-0.83), sudden death (RR=0.67; 95% CI, 0.52-0.87), and MI (RR=0.75; 95% CI, 0.63-0.88). In our subgroup analysis of RCTs in which at least 1 g/d of EPA+DHA was administered, reduced risks of most coronary outcomes, including any CHD event, MI, nonfatal MI, coronary death,

FIGURE 2. Forest plots derived from random-effects meta-analysis models depicting the effect of EPA+DHA on any CHD event in RCTs among all subjects (A), among subjects with baseline triglyceride levels of more than 150 mg/dL (B), and among subjects with baseline low-density lipoprotein cholesterol levels of more than 130 mg/dL (C), and the association between EPA+DHA intake and any CHD event in prospective cohort studies (D). Circles represent the RR within the individual studies; 95% CIs are represented by horizontal lines. Circle size is proportional to the weight of each study. Diamonds represent the SRRE. Summary associations were interpreted as statistically significant if the 95% CIs did not include the null value of 1.0 in their range. Any CHD event includes fatal or nonfatal MI, coronary death, sudden cardiac death, and angina, as well as CHD incidence in prospective cohort studies. If a study did not report a variable for total CHD events, specific events were combined to create a composite CHD variable. When a series of publications from the same prospective cohort study were available, data only from the most recent publication were used in the meta-analysis except in cases in which earlier publications provided estimates for unique outcomes not presented in the most recent publication. In these cases, outcome data from the earlier publication(s) were also included in the meta-analysis. This approach resulted in only 17 prospective cohort studies in the any CHD event model as Mozaffarian et al[46] was used in place of Ascherio et al[37] because Ascherio et al[37] reported only total MI vs Mozaffarian et al,[46] which reported more recent data including collective coronary events for the HPFS cohort. Ascherio et al,[37] however, was used in the statistical model for total MI because these authors provided this outcome for the HPFS cohort, but Mozaffarian et al did not. CHD = coronary heart disease; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; HPFS = Health Professionals Follow-up Study; MI = myocardial infarction; P-H = *P* value for heterogeneity in statistical model; RCT = randomized controlled trial; RR = relative risk; SRRE = summary relative risk estimate. Statistical heterogeneity was assessed using Cochran's Q, which tests for between-study statistical variation. In a conventional meta-analysis, a Cochran's Q *P* value of .10 or less is an indication of statistically significant heterogeneity. It is noteworthy that this *P* value is only an indication that statistical variability may be present in a specific meta-analysis model, and this *P* value is not a significance test for the relationship between the exposure (ie, EPA+DHA) and the outcome (eg, CHD).

and angina, were observed, although most were not statistically significant, with the exception of nonfatal MI (SRRE=0.71; 95% CI, 0.53-0.97).

Findings from our meta-analysis models of RCTs were supported by strong and consistent, statistically significant inverse associations in meta-analyses of prospective cohort studies. These results expand upon findings from a previous meta-analysis of prospective cohort studies that examined fish consumption and CHD mortality; Zheng et al[64] reported RRs of 0.79 (95% CI, 0.67-0.92) and 0.83 (95% CI, 0.68-1.01) for 2 to 4 servings of fish/wk and 5+ servings of fish/wk, respectively, and CHD. Stronger inverse associations for n-3 LCPUFA and CHD in observational studies (including prospective cohort studies) compared with RCTs have also been reported in other reviews and meta-analyses.[65,66] There are many design and methodological differences between RCTs and prospective cohort studies. More than 50% of cardiac deaths occur among individuals without diagnosed heart disease, and large prospective cohort studies are able to evaluate populations that are healthy at baseline and free of the changes in dietary habits and medications that result from disease diagnosis.[67] Moreover, it is more feasible and economical to evaluate longer follow-up periods using a prospective cohort study design. Prospective cohort studies are typically longer in duration than RCTs, and dietary intake data collected in these studies may be more representative of life-long eating habits. Cardiovascular benefits have been observed at lower EPA+DHA intake levels in studies of longer duration.[46] Most RCTs, in comparison, are shorter in duration and evaluate subjects with established CHD or who are at high risk to maximize power and minimize cost.[67] However, a foremost advantage of RCTs is the ostensibly greater ability to control for confounding through random allocation of exposure. Findings from prospective cohort studies and RCTs are both important, and results from prospective cohort studies may provide guidance for first-line treatments for the prevention of CHD.[67] Despite methodological differences, our analyses by study type (ie, RCTs and prospective cohort studies) should be viewed as complementary, offering a comprehensive summary of the state-of-the-epidemiologic science on EPA+DHA provision and intake.

As expected when summarizing and analyzing data from the peer-reviewed literature, some potential limitations and sources of variability should be noted. In the current meta-analysis, the individual RCTs differed in terms of CHD prevalence at baseline, the EPA+DHA dosage provided, follow-up duration, and the methods of patient selection and randomization. The benefit of n-3 LCPUFA intake is thought to accrue over time; however, RCTs of longer duration may suffer from poorer compliance with dietary supplementation. For example, at the end of 5 years in the study by Roncaglioni et al,[28] almost 1 in 5 participants in the n-3 LCPUFA group had discontinued supplementation. Our method of data extraction was designed to specifically address CHD outcomes. Despite these differences, our sensitivity analyses did not indicate that these factors contributed meaningfully to differing patterns of summary effects. The variable use of terminology specific to CHD outcomes, or a lack of specificity required to discern CHD from broader cardiovascular disease outcomes, may have resulted in the exclusion of some publications. All but 1 study[31] provided EPA+DHA in combination as opposed to either independently; therefore, more RCTs are needed to fully evaluate the relationship between EPA and DHA, alone or in combination, for reducing CHD outcomes. Many of the RCTs lack statistical power to detect an effect because of relatively small sample sizes and/or few observed events due to the increased survival rate associated with current standards of care.[68] However, an inherent methodological strength in meta-analyses is that combining data from similar studies enhances the power to detect a statistically significant difference between groups, given that the evidence base of studies reflects the true nature of associations. Finally, most RCTs did not measure baseline intake of EPA+DHA from the diet nor track EPA+DHA intake from sources other than that supplemented during the course of study, thus making it impossible to determine whether background dietary EPA+DHA intake affected the relationship between supplemental EPA+DHA and CHD. Prospective cohort studies in nutritional epidemiology suffer

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from several methodological limitations, namely, the potential for inaccurate ascertainment and classification of exposure, and uncontrolled and residual confounding. Analyses in the prospective cohort studies included in the present meta-analyses were based on self-reported dietary intakes, although the CHD outcomes were based on clinical reporting and validation. These limitations notwithstanding, summary associations across the prospective cohort study meta-analyses were remarkably consistent, showing a benefit for CHD outcomes.

Heart disease morbidity and mortality is the foremost public health burden in the United States[69] and many countries worldwide.[70] Poor diet is a leading cause of CHD burden and one of the leading risk factors related to disability-adjusted life-years.[69] Because a diet low in seafood omega-3 fatty acids is reported as a contributor to ischemic heart disease disability-adjusted life-years and is considered a dietary risk factor with potentially significant effects on mortality worldwide,[71,72] authoritative bodies recommend intake of EPA+DHA for heart and overall health.[2,73,74]

CONCLUSION

Our comprehensive meta-analysis of data from RCTs and prospective cohort studies supports this recommendation. Although not statistically significant, a 6% reduced risk of any CHD event was observed among RCTs, a finding supported by a statistically significant 18% reduced risk of CHD among the prospective cohort studies. From a clinical perspective, our results indicate that EPA+DHA may be associated with reducing CHD risk to a greater extent in populations with elevated triglyceride levels or LDL cholesterol, which are risk factors that impact a significant portion of the general adult population in the United States. Additional RCTs with more homogeneous exposure and outcome classifications with longer follow-up periods may continue to provide a better understanding of the promising beneficial relationship between EPA+DHA and CHD risk.

SUPPLEMENTAL ONLINE MATERIAL

Supplemental material can be found online at http://www.mayoclinicproceedings.org. Supplemental material attached to journal articles has not been edited, and the authors take responsibility for the accuracy of all data.

Abbreviations and Acronyms: **CHD** = coronary heart disease; **DHA** = docosahexaenoic acid; **EPA** = eicosapentaenoic acid; **HR** = hazard ratio; **LDL** = low-density lipoprotein; **MI** = myocardial infarction; **n-3 LCPUFA** = omega-3 long-chain polyunsaturated fatty acids; **RCT** = randomized controlled trial; **RR** = relative risk as represented by rate ratios and hazard ratios; **SRRE** = summary relative risk estimate

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