Original Investigation

Effect of Long-Chain ω-3 Fatty Acids and Lutein + Zeaxanthin Supplements on Cardiovascular Outcomes

Results of the Age-Related Eye Disease Study 2 (AREDS2) Randomized Clinical Trial

Writing Group for the AREDS2 Research Group

IMPORTANCE Dietary supplements have been proposed as a mechanism to improve health and prevent disease.

OBJECTIVE To determine if supplementing diet with long-chain ω-3 polyunsaturated fatty acids or with macular xanthophylls results in a reduced rate of cardiovascular disease (CVD).

DESIGN, SETTING, AND PARTICIPANTS The Cardiovascular Outcome Study (COS) was an ancillary study of the Age-Related Eye Disease Study 2 (AREDS2), a factorial-designed randomized clinical trial of 4203 participants recruited from 82 US academic and community ophthalmology clinics, who were followed up for a median of 4.8 years. Individuals were eligible to participate if they were between the ages of 50 and 85 years, had intermediate or advanced age-related macular degeneration in 1 eye, and were willing to be randomized. Participants with stable, existing CVD (>12 months since initial event) were eligible to participate. Participants, staff, and outcome assessors were masked to intervention.

INTERVENTIONS Daily supplementation with long-chain ω-3 polyunsaturated fatty acids (350-mg docosahexaenoic acid [DHA] + 650-mg eicosapentaenoic acid [EPA]), macular xanthophylls (10-mg lutein + 2-mg zeaxanthin), combination of the two, or matching placebos. These treatments were added to background therapy of the AREDS vitamin and mineral formulation for macular degeneration.

MAIN OUTCOMES AND MEASURES A composite outcome of myocardial infarction, stroke, and cardiovascular death with 4 prespecified secondary combinations of the primary outcome with hospitalized heart failure, revascularization, or unstable angina.

RESULTS Study participants were primarily white, married, and highly educated, with a median age at baseline of 74 years. A total of 602 cardiovascular events were adjudicated, and 459 were found to meet 1 of the study definitions for a CVD outcome. In intention-to-treat analysis, no reduction in the risk of CVD or secondary CVD outcomes was seen for the DHA + EPA (primary outcome: hazard ratio [HR], 0.95; 95% CI, 0.78-1.17) or lutein + zeaxanthin (primary outcome: HR, 0.94; 95% CI, 0.77-1.15) groups. No differences in adverse events or serious adverse event were seen by treatment group. The sample size was sufficient to detect a 25% reduction in CVD events with 80% power.

CONCLUSIONS AND RELEVANCE Dietary supplementation of long-chain ω-3 polyunsaturated fatty acids or macular xanthophylls in addition to daily intake of minerals and vitamins did not reduce the risk of CVD in elderly participants with age-related macular degeneration.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT00345176

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JAMA Intern Med. 2014;174(5):763-771. doi:10.1001/jamainternmed.2014.328
Published online March 17, 2014.

Long-chain ω-3 polyunsaturated fatty acids have been investigated for a potential to reduce heart disease. Attention has focused mostly on the marine long-chain ω-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—polyunsaturated fatty acids found in many species of fatty fish such as Atlantic salmon and trout.[1] Putative mechanisms for beneficial cardiovascular effects for long-chain ω-3 polyunsaturated fatty acids include reduction of inflammation, platelet aggregation, blood pressure, and risk for cardiac dysrhythmias.[2] Clinical trials have tested changes in dietary ω-3 fatty acid and the use of supplements. Diet studies have demonstrated that increased fish intake reduced rates of all-cause mortality, cardiac death, and myocardial infarction.[3,4] However, the evidence for ω-3 fatty acid supplements is inconsistent, with 2 recent large trials of DHA plus EPA showing no reduction in CVD events.[5,6]

The carotenoids lutein and zeaxanthin represent the 2 forms of dietary xanthophylls found in the human macula. Although the data supporting the impact of lutein and zeaxanthin on cardiovascular disease (CVD) are not as substantial as that seen for the ω-3 fatty acids, smaller studies point to a beneficial impact including lower levels of carotid atherosclerosis among individuals with higher lutein levels.[7]

The Age-Related Eye Disease Study 2 (AREDS2) is a randomized clinical trial designed primarily to determine the impact of ω-3 fatty acids and the macular xanthophylls on progression to advanced age-related macular degeneration. As part of the AREDS2 clinical trial, cardiovascular outcomes were prospectively obtained. This article reports on the cardiovascular outcomes by main treatment effects of ω-3 fatty acids and macular xanthophylls.

Methods

Study Design and Participant Eligibility

Multiple institutional review boards approved the AREDS2 research protocol (for a full list of institutions and institutional review boards, see eAppendix 2 in the Supplement), and all participants provided written informed consent. The AREDS2 is a 2 × 2 factorial-designed randomized clinical trial. The full design is described elsewhere.[8] Briefly, participants were eligible to participate in AREDS2 if they were between the ages of 50 and 85 years, had retinal findings consistent with intermediate or advanced age-related macular degeneration in 1 eye, and were willing to be randomized. Individuals with existing CVD were eligible if their condition was believed to be stable and the initial event occurred more than 12 months prior to randomization. All study participants participated in a run-in period of 30 days, during which they received the AREDS supplements. Individuals who consumed 75% of the supplements during the run-in period were eligible for randomization. Randomization was conducted using a random block design, implemented by the data coordinating center via the AREDS2 Advantage Electronic Data Capture system (AdvantageEDC), and stratified by clinical center and age-related macular degeneration status. Participants were enrolled by clinical center. Participants and cardiovascular adjudicators were masked to allocation assignment.

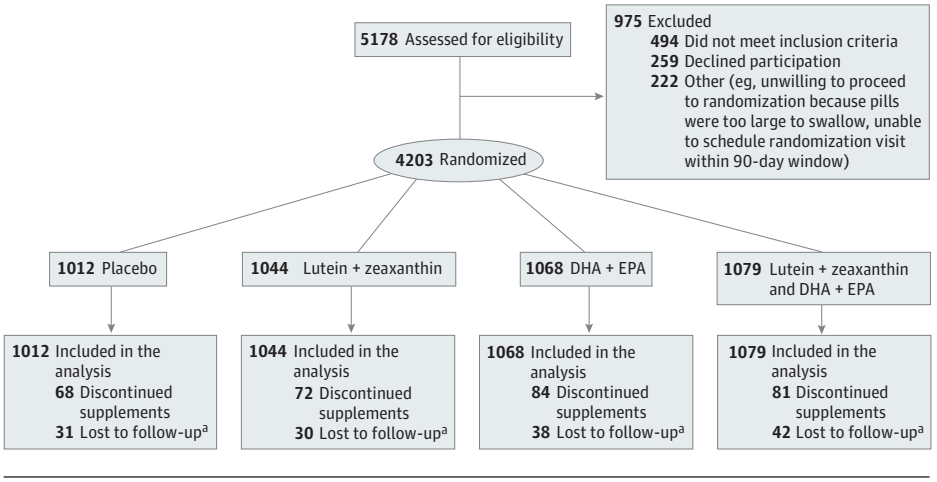
The study intervention consisted of daily supplementation with long-chain ω-3 polyunsaturated fatty acids (350-mg DHA + 650-mg EPA), macular xanthophylls (10-mg lutein + 2-mg zeaxanthin), combination of the two, or matching placebos. These treatments were added to background therapy with the AREDS formulation of ascorbic acid (500 mg), vitamin E (*dl*-alpha tocopherol acetate, 400 IU), beta carotene (15 mg), and zinc (80-mg zinc oxide) with copper (2-mg cupric oxide), a treatment recommended for patients with this severity of age-related macular degeneration.[9] Eligible participants could also agree to participate in a second randomization of various formulations of the AREDS supplement, including the elimination of beta carotene and reducing the dose of zinc to 25 mg. Eighty-two clinical sites participated in the study and recruited 4203 participants between October 17, 2006, and September 30, 2008. Participants were seen in the clinic annually and received telephone calls every 6 months from clinical coordinators. Adherence to study medication was assessed annually through a pill count. At baseline, participants completed the Harvard Dietary Assessment to determine baseline consumption of the 2 nutrient groups under comparison, and serum samples were obtained from 545 participants in selected clinics to evaluate serum levels of the nutrients studied at baseline and at 1, 3, and 5 years. At annual visits and during telephone calls, participants were queried about adverse events and cardiovascular events of interests.

Cardiovascular Outcome Study Procedures

The Cardiovascular Outcomes Study (COS) is a prospective ancillary study of the parent AREDS2. Prior to the start of the study, a group of 5 physicians (B.B.W., A.G.B., C.B.E., J.H., J.R.) with expertise in adjudicating cardiovascular and neurological events for clinical trials established the cardiovascular outcomes of interest, determined the criteria for each outcome, and agreed to adjudicate events reported by participants (see eAppendix 3 in the Supplement). Every 6 months, AREDS2 participants were asked if they had been hospitalized or had undergone specific outpatient procedures since the last AREDS2 contact. Eligible cardiovascular events included the following: myocardial infarction; hospitalized acute coronary syndrome; coronary artery bypass surgery; hospitalized congestive heart failure; unexpected (sudden) death; resuscitated cardiac arrest; cardiac angioplasty or stent; implantable cardioverter-defibrillator; transient ischemic attacks; ischemic stroke; hemorrhagic stroke; and carotid artery stent, angioplasty, or endarterectomy.

Medical records were collected, centrally, by the COS collection unit based at the University of Virginia using information from a case report completed by the local clinical sites. The collection unit staff and all adjudicators were masked to study intervention assignment. After appropriate informed consent, study team members at the University of Virginia obtained medical records for the event, collated the relevant records, deidentified personal information, screened records to determine if the records included a potential event, and sent them to the AREDS2 coordinating center. On receipt, the co-

Figure 1. Age-Related Eye Disease Study 2 (AREDS2) CONSORT Diagram for Cardiovascular Outcome Study



Treatments were given at the following doses: 350-mg docosahexaenoic acid (DHA) + 650-mg eicosapentaenoic acid (EPA); 10-mg lutein + 2-mg zeaxanthin.
[a]Participants included to point when lost to follow-up.

ordinating center randomly assigned the event to a study adjudicator. The study adjudicators made the final determination of these study end points through review of the medical records and applying the end point criterion defined a priori. All adjudicators were masked to study assignment. All cerebrovascular-related events were assigned to the neurologist, and 10% of these events were readjudicated by the same neurologist to assess consistency. Ten percent of cardiovascular events were randomly assigned to 2 cardiologists for quality assurance. In the quality assurance subsample, if the 2 final adjudicators disagreed, the event was reviewed again by the full group of adjudicators and classified by consensus or vote. Study adjudicators had the option to request that the full group review the case if the adjudicator was unsure of study end point classification.

Cardiovascular Study Statistical Procedures

The primary end point for the cardiovascular ancillary study was a composite outcome of time to the first event in a category: CVD mortality (ie, sudden death; death due to myocardial infarction, heart failure, or stroke) and CVD morbidity (ie, myocardial infarction; stroke; unstable angina; coronary and carotid revascularization; hospitalized congestive heart failure; resuscitated cardiac arrest). The following 4 composite secondary end points were also designated prior to the start:

1. Myocardial infarction, stroke, or CVD death
2. Myocardial infarction, stroke, or CVD death PLUS unstable angina
3. Myocardial infarction, stroke, or CVD death PLUS hospitalized congestive heart failure
4. Myocardial infarction, stroke, or CVD death PLUS revascularization procedures

Statistical analyses were conducted to estimate the main effects of DHA + EPA vs no DHA + EPA, and lutein + zeaxanthin vs no lutein + zeaxanthin separately on CVD morbidity and mortality. Sample size was based on the estimated effect of the supplements on the eye outcomes. Analyses of the primary and secondary outcomes were conducted according to intention-to-treat principles. Adjusted hazard ratios (HRs) and their 95% confidence intervals are presented. Kaplan-Meier survival (event-free) curves by treatment were estimated for the primary outcome. The assumptions for proportional hazards models were tested and met for all outcomes. Comparisons of the primary outcome between DHA + EPA and lutein + zeaxanthin main effects on 5 prespecified subgroups (baseline DHA or lutein intake; CVD history; hypertension history; high cholesterol history; medication for CVD) were conducted. Effects of DHA + EPA vs no DHA + EPA, and lutein + zeaxanthin vs no lutein + zeaxanthin separately on total mortality were estimated. All analyses were conducted using SAS version 9.2 (SAS Institute Inc).

Participants were queried about any adverse events at each contact semiannually. Adverse events were MedDRA (Medical Dictionary for Regulatory Activities) coded centrally and reported by system organ class. Comparisons by intervention group of counts by system organ class were evaluated by χ^2 tests.

Results

A total of 5178 potential participants were assessed for eligibility into the study and 975 were excluded, resulting in 4203 participants randomized to 1 of 4 primary study arms. The median length of follow-up was 4.8 years. Of the randomized participants, 141 (3%) were lost to follow-up and 368 (9%) died during the course of the study. Distributions were similar across all treatment groups. A complete flow of participants is shown in **Figure 1**.

Baseline characteristics for the main treatment effect groups of DHA + EPA and lutein + zeaxanthin are given in **Table 1**. The median age at baseline was 74 years (range, 50-85 years). The study participants were primarily white, non-Hispanic, married, and highly educated. Approximately 19% had a history of CVD; 44% reported taking a statin medication; and 14% reported taking any type of medication for congestive heart failure, CVD, or cerebrovascular disease. Overall, baseline characteristics were balanced between the 2

Table 1. Baseline Characteristics by Treatment[a]

Characteristic	DHA + EPA[b]	No DHA + EPA	Lutein + Zeaxanthin[c]	No Lutein + Zeaxanthin
Age, median (IQR), y	74.6 (11.1)	74.0 (11.1)	74.6 (11.2)	74.1 (10.9)
White race	2071 (96.5)	1987 (96.6)	2063 (97.2)	1995 (95.9)
Female sex	1244 (57.9)	1143 (55.6)	1236 (58.2)	1151 (55.3)
Hispanic ethnicity	56 (2.6)	27 (1.3)	46 (2.2)	37 (1.8)
Education				
≤Grade 11	141 (6.6)	133 (6.5)	135 (6.4)	139 (6.7)
High school graduate	549 (25.6)	514 (25.0)	516 (24.3)	547 (26.3)
Some college or Associate's degree	609 (28.4)	532 (25.9)	572 (26.9)	569 (27.4)
Bachelor's degree	384 (17.9)	399 (19.4)	428 (20.2)	355 (17.1)
Postgraduate work	423 (19.7)	441 (21.4)	430 (20.3)	434 (20.9)
Refused to answer	41 (1.9)	37 (1.8)	42 (2.0)	36 (1.7)
Marital status				
Married	1367 (63.7)	1385 (67.4)	1372 (64.6)	1380 (66.3)
Divorced	223 (10.4)	206 (10.0)	216 (10.2)	213 (10.2)
Widowed	466 (21.7)	383 (18.6)	456 (21.5)	393 (18.9)
Never married	91 (4.2)	82 (4.0)	79 (3.7)	94 (4.5)
Medical history				
CHF, CHD, angina, MI, or stroke	405 (18.9)	400 (19.5)	396 (18.7)	409 (19.7)
CHF	79 (3.7)	68 (3.3)	73 (3.4)	74 (3.6)
CHD	198 (9.2)	207 (10.1)	202 (9.5)	203 (9.8)
Angina	92 (4.3)	106 (5.2)	90 (4.2)	108 (5.2)
MI	142 (6.6)	151 (7.3)	137 (6.5)	156 (7.5)
Stroke	108 (5.0)	103 (5.0)	112 (5.3)	99 (4.8)
Diabetes	291 (13.6)	255 (12.4)	264 (12.4)	282 (13.6)
Former/current smoker	1202 (56.0)	1177 (57.2)	1160 (54.6)	1219 (58.6)

Abbreviations: CHD, coronary heart disease; CHF, congestive heart failure; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IQR, interquartile range; MI, myocardial infarction.

[a] Data are given as number (percentage) of participants unless otherwise specified.

[b] Dose: 350-mg DHA + 650-mg EPA.

[c] Dose: 10-mg lutein + 2-mg zeaxanthin.

comparisons groups (DHA + EPA vs no DHA + EPA; lutein + zeaxanthin vs no lutein + zeaxanthin).

Approximately 7% of participants permanently stopped taking their study medications at some time during the study but continued to be followed up. Approximately 80% of the participants in each treatment group took the study medications at least 75% of the time, as assessed by pill count. The median serum levels of lutein in 545 participants randomized to lutein + zeaxanthin in selected clinical sites increased by 190% to 210% at years 1, 3, and 5 from baseline, while those randomized to no lutein + zeaxanthin showed little change. Similarly, participants randomized to DHA + EPA demonstrated a 30% to 40% increase in median serum DHA level and a 90% to 120% increase in median serum EPA level during the study.

A total of 694 potential cardiovascular events were reported by 507 participants. Medical records could not be obtained on 77 reported events (41 in the DHA + EPA group vs 36 in the no DHA + EPA group; 44 in the lutein + zeaxanthin group vs 33 in the no lutein + zeaxanthin group; no statistically significant difference), leaving 617 events for screening by the COS collection unit. Of the 617 screened events, on preliminary review, 147 (24% [82 in the DHA + EPA group vs 65 in the no DHA + EPA group; 70 in the lutein + zeaxanthin group vs 77 in the no lutein + zeaxanthin group; no statistically significant difference]) were found to not include a potential AREDS2 cardiovascular event, leaving a total of 470 events that were sent to the adjudication team. A sensitivity analysis to evaluate the impact of not obtaining medical records was performed using all 547 events (470 adjudicated events plus the 77 events that were not adjudicated), with similar results (data not shown).

Within the DHA + EPA analysis, the incidence rate per 100 person-years for the primary CVD outcome was 1.94 for the DHA + EPA group and 2.04 in the no DHA + EPA group. The unadjusted HR analyses show no significant effect for either the primary outcomes (HR, 0.95; 95% CI, 0.78-1.17; **Figure 2**A) or any of the prespecified secondary composite outcomes (**Table 2**). A summary of adjudicated events is provided in eTable 1 in the Supplement. Similar results were seen when all 547 events (470 adjudicated plus 77 events with no medical records) were included in the analysis (see eFigure 1 in the Supplement). When the 5 prespecified subgroups were examined (**Figure 3**A), a significant protective effect was only seen among those without a baseline history of hypertension (HR, 0.66; 95% CI, 0.46-0.96) with a similar trend seen among those without a baseline history of CVD (HR, 0.81; 95% CI, 0.62-1.06) and among those without a self-reported history of elevated cholesterol level (HR, 0.82; 95% CI, 0.58-1.17). There was a significant interaction between DHA + EPA and history of CVD (P = .04) and history of hypertension (P = .02). We conducted an exploratory analysis among those participants who provided serum samples at baseline (DHA + EPA, n = 214; no DHA + EPA, n = 218) and found no significant difference in

event rates in either the high triglyceride level group (≥200 mg/dL [to convert to millimoles per liter, multiply by 0.0113]) or the normal triglyceride level group, although the number of events in each triglyceride level was very low (9 events in the high triglyceride level group and 31 events in the normal triglyceride level group).

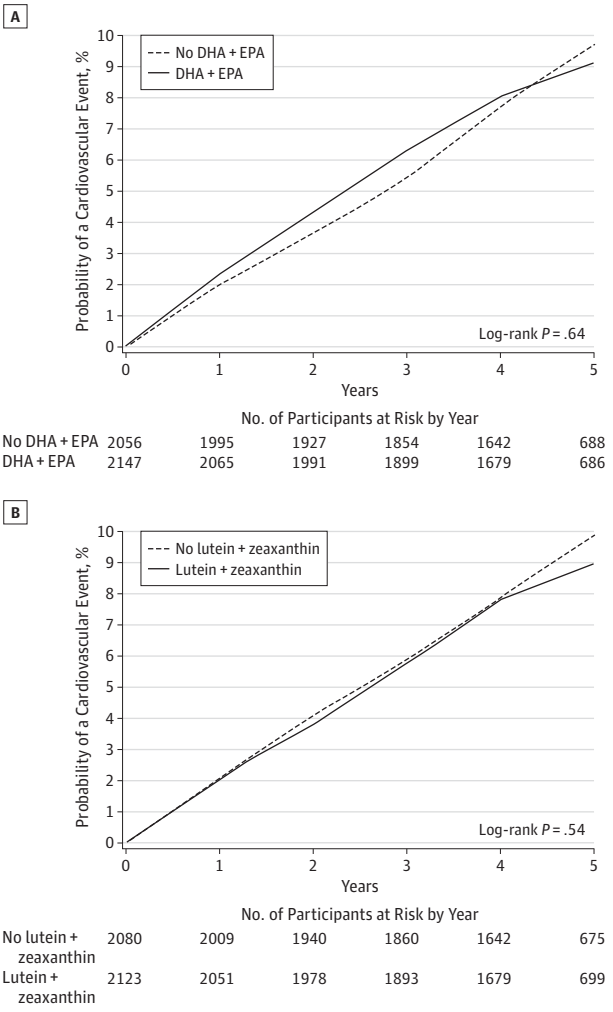
Similar findings were seen for the lutein + zeaxanthin comparison. The incidence rate per 100 person-years for the primary CVD outcome was 1.92 for the lutein + zeaxanthin group and 2.05 for the no lutein + zeaxanthin group. The HR for lutein + zeaxanthin vs no lutein + zeaxanthin group was not statistically significant (for the primary composite CVD outcome, HR, 0.94; 95% CI, 0.77-1.15; Figure 2B). The prespecified secondary outcomes had similar null results, as given in **Table 3**. No variation was seen by subgroups (Figure 3B) or when all events were included (see eFigure 2 in the Supplement). A summary of adjudicated events is provided in the eTable 2 in the Supplement. Among individual outcomes, there were more nonfatal and fatal strokes in the lutein + zeaxanthin group (55 [2.59%] vs 34 [1.63%] in the no lutein + zeaxanthin group; P = .03), although there were fewer coronary revascularizations in the lutein + zeaxanthin group (45 [2.12%] vs 68 [3.27%] in the no lutein + zeaxanthin group; P = .03).

We also compared total mortality for each treatment comparison and found no significant effect. Within the DHA + EPA comparison, 200 individuals in the active treatment group died during the study compared with 168 in the placebo group (HR, 1.16; 95% CI, 0.94-1.42), while in the lutein + zeaxanthin treatment group, 191 participants died compared with 177 in the placebo group (HR, 1.06; 95% CI, 0.87-1.31). Similar HRs were seen when adjusting for age, sex, history of CVD, smoking status, and race.

In exploratory analyses, we compared the event rate in those receiving both lutein + zeaxanthin and DHA + EPA (n = 1079) with those randomized to receive no lutein + zeaxanthin and no DHA + EPA (n = 1012) and found no significant effect of receiving both supplements compared with not receiving either for our primary outcome (HR, 0.89; 95% CI, 0.67-1.19) or for any of the prespecified secondary outcomes (data not shown).

Serious adverse events are summarized in **Table 4** by DHA + EPA and lutein + zeaxanthin main effects and MedDRA system organ class. Ninety percent of participants reported at least 1 adverse event in each of the treatment groups, and 47% reported at least 1 serious adverse event. During the course of the study, 1 unexpected serious adverse event occurred that required a Medwatch form to be reported. The serious adverse event involved a 76-year-old white woman who was randomized to the EPA + DHA and lutein + zeaxanthin as well as the AREDS-type supplement. She presented to the hospital for repetitive vomiting and was hospitalized owing to hyponatremia. Her serum sodium level improved during hospitalization. The investigator considered this event possibly related to the study supplements.

Figure 2. Time to First Cardiovascular Disease (CVD) Mortality/Morbidity Event



A, Time to first CVD mortality/morbidity event by docosahexaenoic acid plus eicosapentaenoic acid (DHA + EPA) and no DHA + EPA (adjudicated events only). B, Time to first CVD mortality/morbidity event by lutein + zeaxanthin vs no lutein + zeaxanthin (adjudicated events only). Treatments were given at the following doses: 350-mg DHA + 650-mg EPA; 10-mg lutein + 2-mg zeaxanthin.

Discussion

In this randomized clinical trial of 4203 older adults with prevalent macular degeneration testing long-chain ω-3 polyunsaturated fatty acids (DHA and EPA) and xanthophylls (lutein and zeaxanthin), we found no statistically significant benefit of either supplement on CVD events or CVD mortality. There was a numerical but not statistically significant difference toward heterogeneity in effects in the participants receiving ω-3 fatty acids, with those having no history of CVD or at low risk for development of the disease (no CVD, no hypertension, no elevated cholesterol level) showing potential benefit and those with a history of CVD or at higher risk of CVD showing no effect. However, the number of events for these subgroups was small, the confidence interval was wide, and the lack of isolated, individual risk factors may not provide a complete picture of an individual's total cardiovascular risk. Until further confirmation is provided by additional studies, these findings should be interpreted cautiously. No such findings were seen among the participants in the lutein + zeaxanthin study.

Table 2. Outcomes for DHA + EPA and No DHA + EPA

Primary Outcome	DHA + EPA[a] No. (%)[b]	DHA + EPA[a] Event Rate (per 100 Person-years)	No DHA + EPA No. (%)[b]	No DHA + EPA Event Rate (per 100 Person-years)	Unadjusted HR (95% CI), DHA + EPA vs No DHA + EPA
CVD mortality and CVD morbidity[c]	183 (9)	1.94	187 (9)	2.04	0.95 (0.78-1.17)
Secondary outcomes					
MI/stroke/CVD death	90 (4)	0.93	88 (4)	0.94	0.99 (0.74-1.33)
MI/stroke/CVD death/unstable angina	116 (5)	1.21	113 (5)	1.21	1.00 (0.77-1.29)
MI/stroke/CVD death/hospital CHF	145 (7)	1.52	137 (7)	1.48	1.03 (0.81-1.30)
MI/stroke/CVD death/ revascularization procedures	94 (4)	0.97	99 (5)	1.06	0.92 (0.69-1.22)

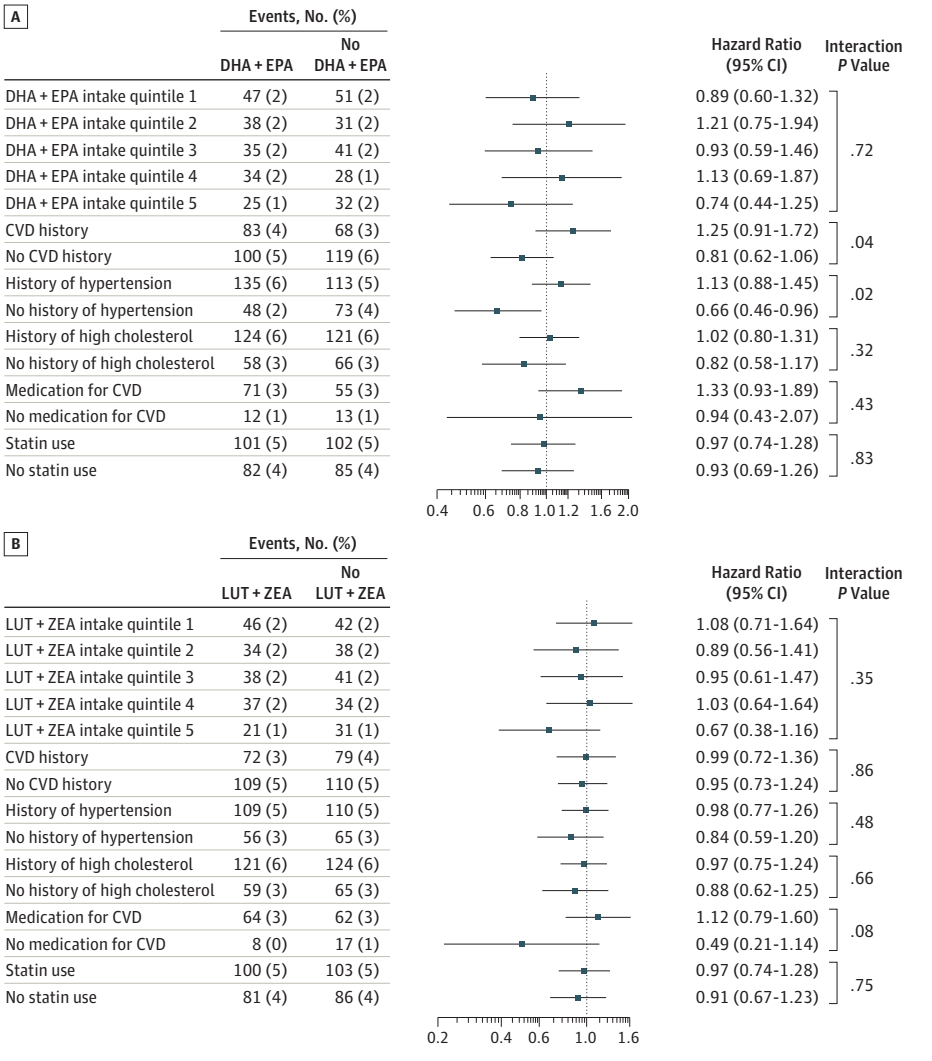
Abbreviations: CHF, congestive heart failure; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HR, hazard ratio; MI, myocardial infarction.

[a] Dose: 350-mg DHA + 650-mg EPA.

[b] Percentage of total number of participants randomized to that intervention group.

[c] Cardiovascular disease mortality (sudden death; death due to MI, heart failure, or stroke); CVD morbidity (MI; stroke; unstable angina; coronary and carotid revascularization; hospitalized CHF; resuscitated cardiac arrest).

Figure 3. Comparison of the Main Effects



A, Comparison of the main effects of docosahexaenoic acid plus eicosapentaenoic acid (DHA + EPA) vs no DHA + EPA stratified by cardiovascular medical history and dietary intake of DHA + EPA. B, Comparison of the main effects of lutein + zeaxanthin (LUT + ZEA) vs no LUT + ZEA stratified by cardiovascular medical history and dietary intake of LUT + ZEA. CVD indicates cardiovascular disease. Treatments were given at the following doses: 350-mg DHA + 650-mg EPA; 10-mg LUT + 2-mg ZEA.

Table 3. Outcomes for Lutein + Zeaxanthin and No Lutein + Zeaxanthin

Primary Outcome	Lutein + Zeaxanthin[a] No. (%)[b]	Lutein + Zeaxanthin[a] Event Rate (per 100 Person-years)	No Lutein + Zeaxanthin No. (%)[b]	No Lutein + Zeaxanthin Event Rate (per 100 Person-years)	Unadjusted HR (95% CI)
CVD mortality and CVD morbidity[c]	181 (9)	1.92	189 (9)	2.05	0.94 (0.77-1.15)
Secondary outcomes					1.24 (0.92-1.66)
MI/stroke/CVD death	99 (5)	1.03	79 (4)	0.83	
MI/stroke/CVD death/ unstable angina	119 (6)	1.24	110 (5)	1.17	1.07 (0.82-1.38)
MI/stroke/CVD death/ hospital CHF	140 (7)	1.47	142 (7)	1.52	0.97 (0.77-1.22)
MI/stroke/CVD death/ revascularization procedures	104 (5)	1.08	89 (4)	0.94	1.15 (0.87-1.52)

Abbreviations: CHF, congestive heart failure; CVD, cardiovascular disease; HR, hazard ratio; MI, myocardial infarction.

[a] Dose: 10-mg lutein + 2-mg zeaxanthin.

[b] Percentage of total number of participants randomized to that intervention group.

[c] Cardiovascular disease mortality (sudden death; death due to MI, heart failure, or stroke); CVD morbidity (MI; stroke; unstable angina; coronary and carotid revascularization; hospitalized CHF; resuscitated cardiac arrest).

Table 4. Serious Adverse Event Summary by MedDRA System Organ Class and Treatment[a]

MedDRA System Organ Class	Participants, No. (%) DHA + EPA[b] (n = 2147)	No DHA + EPA (n = 2056)	Lutein + Zeaxanthin[c] (n = 2123)	No Lutein + Zeaxanthin (n = 2080)
Participants with at least 1 adverse event	1024 (47.7)	963 (46.8)	1003 (47.2)	984 (47.3)
Cardiac disorders	222 (10.3)	206 (10.0)	213 (10.0)	215 (10.3)
Gastrointestinal disorders	119 (5.5)	145 (7.1)	130 (6.1)	134 (6.4)
General disorders and administration site conditions	97 (4.5)	104 (5.1)	96 (4.5)	105 (5.0)
Infections and infestations	202 (9.4)	192 (9.3)	201 (9.5)	193 (9.3)
Injury, poisoning, and procedural complications	140 (6.5)	138 (6.7)	128 (6.0)	150 (7.2)
Musculoskeletal and connective tissue disorders	156 (7.3)	140 (6.8)	159 (7.5)	137 (6.6)
Neoplasms benign, malignant, and unspecified	175 (8.2)	168 (8.2)	180 (8.5)	163 (7.8)
Nervous system disorders	145 (6.8)	140 (6.8)	147 (6.9)	138 (6.6)
Renal and urinary disorders	49 (2.3)	44 (2.1)	50 (2.4)	43 (2.1)
Reproductive system and breast disorders	23 (1.1)	17 (0.8)	19 (0.9)	21 (1.0)
Respiratory, thoracic, and mediastinal disorders	83 (3.9)	87 (4.2)	89 (4.2)	81 (3.9)
Surgical and medical procedures	59 (2.7)	61 (3.0)	61 (2.9)	59 (2.8)
Vascular disorders	84 (3.9)	81 (3.9)	82 (3.9)	83 (4.0)

Abbreviations:
DHA, docosahexaenoic acid;
EPA, eicosapentaenoic acid;
MedDRA, Medical Dictionary for Regulatory Activities.

[a] No statistical differences were seen. Multiple occurrences of adverse events under the same system organ class in the same participant is not included.

[b] Dose: 350-mg DHA + 650-mg EPA.

[c] Dose: 10-mg lutein + 2-mg zeaxanthin.

Support for the hypothesis that long-chain ω-3 polyunsaturated fatty acids may reduce CVD from epidemiologic studies has been mixed. The Chicago Western Electric Study[10] and Nurses' Health Study[11] reported an inverse association between fish and ω-3 fatty acid intake and CHD events in men and women, respectively. However, this association was not confirmed in Health Professionals' Follow-up Study or the Physicians' Health Study.[12,13] Interest in ω-3 fatty acid supplementation was stimulated by the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI) Prevention study, which found a reduction in sudden death with marine oils,[14] but this early observation has not been confirmed by recent clinical trials.[15-17] A recent meta-analysis of diet and supplementation studies found no reduction in cardiovascular events with long-chain ω-3 polyunsaturated fatty acids.[18]

The evidence connecting lutein and zeaxanthin levels with CVD is similarly mixed. Mean serum lutein and zeaxanthin concentrations were lower in CVD cases compared with controls in the Atherosclerosis Risk in Communities Study.[19] However, no such association was observed in the Physician's Health Study.[20] Similarly, dietary carotenoid consumption was not associated with CHD risk in the Nurses' Health Study.[21] To our knowledge, this is one of the first clinical trials testing the impact of lutein + zeaxanthin on CVD. We found no indication of a beneficial effect at this dose, reducing enthusiasm for this intervention for CVD prevention.

Treatment with DHA + EPA appeared to be well-tolerated without evidence of adverse effects on individual CVD outcomes, including deaths. Treatment with lutein + zeaxanthin was also well tolerated, although there was a potential signal for an increased stroke risk. However, given the multiple comparisons, replication in another study would be needed to determine the clinical implication of this finding.

The many strengths of this cardiovascular study include the following: a high retention rate; standardized, placebo-controlled dosing; a systematic query for cardiovascular outcomes; and the use of rigorous adjudication. Despite these strengths, the study has some weaknesses that should be mentioned. The COS was an ancillary study to the AREDS2 eye study, which was powered to detect a change in macular degeneration. This resulted in a sample size that was small to detect a biologically plausible change in rates of myocardial infarction and cardiovascular deaths, the area in which the strongest evidence for benefit exists. To achieve 80% power,

a 25% reduction in the incidence would have been needed given the number of participants enrolled and the high percentage (91%) of participants with no CVD event in the no DHA + EPA group. This magnitude of an effect has only been seen in trial with an outcome of mortality and only in those focused on increasing ω-3 fatty acid consumption through dietary change. To overcome the relatively small sample size, a broader range of cardiovascular outcomes for the primary outcome with more narrow outcomes examined in secondary analysis. The dose of either the long-chain ω-3 polyunsaturated fatty acids or xanthophylls may have been inadequate to affect large-vessel disease as seen in CVD outcomes. However, a substudy testing actual levels of DHA + EPA and lutein + zeaxanthin among those receiving active intervention showed a substantial increase after starting the intervention, indicating a dose at least large enough to change detectable serum levels. Finally, the timing of the administration of the supplements may have been too late to affect outcomes. The AREDS2 participants were older, and many had existing CVD or elevated risk for CVD. We cannot exclude a beneficial effect from starting supplementation earlier in life.

Conclusions

We found no significant benefit among older individuals treated with either ω-3 supplements or with a combination of lutein + zeaxanthin. Our results are consistent with a growing body of evidence from clinical trials that have found little CVD benefit from moderate levels of dietary supplementation.

ARTICLE INFORMATION

Accepted for Publication: January 8, 2014.

Published Online: March 17, 2014. doi:10.1001/jamainternmed.2014.328.

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Obtained funding: Fine, Chew.
Administrative, technical, or material support: Bonds, Worrall, Hsia, Fine.
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AREDS2 Research Group: The AREDS2 Research Group members are listed in eAppendix 1 in the Supplement.

Conflict of Interest Disclosures: Dr Worrall is an Associate Editor of the journal *Neurology*. Dr Hsia is employed by and owns stock in AstraZeneca. Dr Robinson has received institutional research grants from Amarin, Amgen, Daiichi-Sankyo, Esperion, Genentech/Hoffmann La Roche, Glaxo-Smith Kline, Merck. No other disclosures are reported.

Funding/Support: The AREDS2 study is supported by the intramural program funds and contracts from the National Eye Institute/National Institutes of Health, Department of Health and Human Services (contract No. HHS-N-260-2005-00007-C and ADB contract No. N01-EY-5-0007). Funds were generously contributed to these contracts by the following National Institutes of Health (NIH): Office of Dietary Supplements, National Center for Complementary and Alternative Medicine, National Institute on Aging, National Heart, Lung, and Blood Institute, and National Institute of Neurological Disorders and Stroke.

Role of the Sponsors: Drs Bonds, Fine, and Chew are employed by the NIH. The individuals at NIH who made the funding decisions were not the same as who designed or wrote the manuscript. Drs Bonds, Fine, and Chew had a direct role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication; however, no other individual at NIH was involved in these roles.

Additional Information: The full study protocol is available at http://www.areds2.org.

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Invited Commentary

ω-3 Fatty Acids and Lutein + Zeaxanthin Supplementation for the Prevention of Cardiovascular Disease

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The truth is rarely pure and never simple.
Mark Twain

ω-3 Polyunsaturated fatty acid supplements are increasingly used for the prevention of cardiovascular disease (CVD) either under a physician's prescription or as over-the-counter products. They currently support a thriving industry with 2011 market sales of $25.42 billion, which grows by more than 15% annually.[1] The main effect of ω-3 supplementation is the reduction of triglyceride (TG) levels, whereas additional suggested mechanisms for CVD protection are the prevention of serious arrhythmias, decrease of platelet aggregation, stabilization of atherosclerotic plaque, and reduction of blood pressure.[2] Their effect on TG levels is dose dependent, and doses close to 4 g daily can decrease TG level by up to 25%. Although high TG levels have been traditionally considered a secondary target for CVD prevention, they are not incorporated in cardiovascular risk assessment tools. Thus, not surprisingly, the current guidance on ω-3 supplementation for CVD protection is controversial. The latest US guidelines focus on safety of nonstatin treatment and claim that in cases when nonstatin agents must be used, those with reported clinical benefit should be preferred.[3] The Food and Drug Administration has approved their administration only as triglyceride-lowering agents for overt hypertriglyceridemia,[4] whereas the European Medicines Agency has additionally approved ω-3 supplementation as an adjuvant treatment after myocardial infarction.[5]

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And that, along with Mark Twain's quote on the properties of truth, brings us to the report from the Writing Group for the Age-Related Eye Disease Study 2 (AREDS2) Research Group[6] in this issue of *JAMA Internal Medicine*. The AREDS2 is a large multicenter, factorial-designed, randomized clinical trial (RCT) of 4203 subjects with age-related macular degeneration, who were followed-up for a median of 4.8 years. In this elegant, well-designed study, participants were randomized to daily supplementation with ω-3 (350-mg docosahexaenoic [DHA] + 650-mg eicosapentaenoic [EPA]), macular xanthophylls (10-mg lutein + 2-mg zeaxanthin), combination of the two, or matching placebo on top of minerals and vitamins used for age-related macular degeneration. In the intention-to-treat analysis, no reduction in the risk of the composite outcomes of CVD mortality (ie, sudden death, fatal myocardial infarction, heart failure, or stroke) and CVD morbidity (ie, myocardial infarction, stroke, unstable angina, coronary and carotid revascularization, hospitalized heart failure, resuscitated cardiac arrest) was observed in the DHA + EPA or lutein + zeaxanthin supplementation groups. Supplementation with either ω-3 or macular xanthophylls was well tolerated and safe.

So, why does yet another RCT fail to show a clear cardioprotective benefit for ω-3? One will argue that the study was underpowered; with the attained sample size, the trial had 80% power to detect a fairly substantial clinical benefit (25% instead of a more prudent and realistic 10%-15%) for CVD events, even after the consideration of the composite outcomes of cardiovascular mortality and morbidity. Although the outcome adjudication was blinded, the open-label design of the study should be also acknowledged. Furthermore, there is a lack of information on the baseline and follow-up TG levels, and a potentially varying ω-3 effect in high compared with low TG level subgroups had not been studied. Finally, the study included a mixed high-risk and low-risk CVD population (20% of the subjects had established CVD).

How do these findings fit into the (re)considerations about the design and conduct of future trials on ω-3 supplementation? After many years of randomized evidence accumulated on various outcomes across a variety of diseases, ω-3 supplementation still fails to find its place in everyday clinical practice. With more than 2000 PubMed-indexed clinical trials (more than 600 published after 2010), almost 200 systematic reviews and meta-analyses, and more than 700 registered