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Lutein + Zeaxanthin and Omega-3 Fatty Acids for Age-Related Macular Degeneration

The Age-Related Eye Disease Study 2 (AREDS2) Randomized Clinical Trial

The Age-Related Eye Disease Study 2 (AREDS2) Research Group*

Importance Oral supplementation with the Age-Related Eye Disease Study (AREDS) formulation (antioxidant vitamins C and E, beta carotene, and zinc) has been shown to reduce the risk of progression to advanced age-related macular degeneration (AMD). Observational data suggest that increased dietary intake of lutein + zeaxanthin (carotenoids), omega-3 long-chain polyunsaturated fatty acids (docosahexaenoic acid [DHA] + eicosapentaenoic acid [EPA]), or both might further reduce this risk.

Objectives To determine whether adding lutein + zeaxanthin, DHA + EPA, or both to the AREDS formulation decreases the risk of developing advanced AMD and to evaluate the effect of eliminating beta carotene, lowering zinc doses, or both in the AREDS formulation.

Design, Setting, and Participants The Age-Related Eye Disease Study 2 (AREDS2), a multicenter, randomized, double-masked, placebo-controlled phase 3 study with a 2 × 2 factorial design, conducted in 2006-2012 and enrolling 4203 participants aged 50 to 85 years at risk for progression to advanced AMD with bilateral large drusen or large drusen in 1 eye and advanced AMD in the fellow eye.

Interventions Participants were randomized to receive lutein (10 mg) + zeaxanthin (2 mg), DHA (350 mg) + EPA (650 mg), lutein + zeaxanthin and DHA + EPA, or placebo. All participants were also asked to take the original AREDS formulation or accept a secondary randomization to 4 variations of the AREDS formulation, including elimination of beta carotene, lowering of zinc dose, or both.

Main Outcomes and Measures Development of advanced AMD. The unit of analyses used was by eye.

Results Median follow-up was 5 years, with 1940 study eyes (1608 participants) progressing to advanced AMD. Kaplan-Meier probabilities of progression to advanced AMD by 5 years were 31% (493 eyes [406 participants]) for placebo, 29% (468 eyes [399 participants]) for lutein + zeaxanthin, 31% (507 eyes [416 participants]) for DHA + EPA, and 30% (472 eyes [387 participants]) for lutein + zeaxanthin and DHA + EPA. Comparison with placebo in the primary analyses demonstrated no statistically significant reduction in progression to advanced AMD (hazard ratio [HR], 0.90 [98.7% CI, 0.76-1.07]; $P = .12$ for lutein + zeaxanthin; 0.97 [98.7% CI, 0.82-1.16]; $P = .70$ for DHA + EPA; 0.89 [98.7% CI, 0.75-1.06]; $P = .10$ for lutein + zeaxanthin and DHA + EPA). There was no apparent effect of beta carotene elimination or lower-dose zinc on progression to advanced AMD. More lung cancers were noted in the beta carotene vs no beta carotene group (23 [2.0%] vs 11 [0.9%], nominal $P = .04$), mostly in former smokers.

Conclusions and Relevance Addition of lutein + zeaxanthin, DHA + EPA, or both to the AREDS formulation in primary analyses did not further reduce risk of progression to advanced AMD. However, because of potential increased incidence of lung cancer in former smokers, lutein + zeaxanthin could be an appropriate carotenoid substitute in the AREDS formulation.

Trial Registration clinicaltrials.gov Identifier: NCT00345176

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Age-Related Macular Degeneration (AMD), the leading cause of blindness in the developed world, accounts for more than 50% of all blindness in the United States.[1] In 2004, it was estimated that 8 million individuals had intermediate AMD, defined as bilateral drusen, and approximately 2 million had advanced AMD, either neovascular AMD or geographic atrophy.[2] Although intraocular drugs that inhibit vascular endothelial growth factor are currently available for treatment of neovascular AMD,[3] no effective therapies are proven for atrophic AMD. Without more effective ways of slowing progression, the number of persons with advanced AMD is expected to double over the next 20 years, resulting in increasing socioeconomic burden.[2]

The Age-Related Eye Disease Study (AREDS) demonstrated that daily oral supplementation with antioxidant vitamins and minerals reduced the risk of developing advanced AMD by 25% at 5 years.[4] Animal studies[5-7] and epidemiologic studies provide a rationale for examining the potential effects of other nutrients on the development of advanced AMD. Observational studies suggest that higher dietary intake of lutein + zeaxanthin, omega-3 long-chain polyunsaturated fatty acids (docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]), or both are associated with a decreased risk of de-

Author Video Interview available at www.jama.com.

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veloping advanced AMD.[8-11] Lutein and zeaxanthin are the main components of the macular pigment, DHA is a major structural component of the retina, and EPA may play a role as a precursor to signaling molecules with potential to influence retinal function, providing biological bases for testing these nutrients. AREDS2 was designed to test whether adding lutein + zeaxanthin, DHA + EPA, or lutein + zeaxanthin and DHA + EPA to the AREDS formulation might further reduce the risk of progression to advanced AMD. A secondary goal was to test the effects of eliminating beta carotene and reducing zinc dose in the AREDS formulation.

METHODS

Study Population

The details of the study design have been reported previously.[12] Between October 2006 and September 2008, we enrolled 4203 participants in 82 clinical sites. Enrollment was restricted to people between the ages of 50 and 85 years at high risk of progression to advanced AMD with either bilateral large drusen or large drusen in 1 eye and advanced AMD in the fellow eye. Inclusion and exclusion criteria have been published.[12] In brief, participants were required to consent to follow-up of at least 5 years. Likelihood of adherence to the study regimen was evaluated during a run-in phase using study placebo and the AREDS formulation. Participants were eligible for randomization if they took at least 75% of the run-in supplements and agreed to stop the use of other supplements containing lutein, zeaxanthin, DHA, EPA, vitamin C, vitamin E, beta carotene, zinc, or copper. They could not have other ocular diseases such as high myopia, glaucoma, clinically significant diabetic retinopathy (10 or more microaneurysms or retinal hemorrhages), and other diseases that might confound the assessment of the ocular outcome measurements. Eyes that had undergone cataract surgery at least 3 months prior to enrollment were eligible; eyes that had undergone other intraocular surgeries were not included. Persons with systemic diseases, including oxalate kidney stones, Wilson disease, hemochromatosis, lung cancer, or other diseases associated with poor 5-year survival, were excluded. Institutional review boards approved the AREDS2 research protocol, and all participants provided written informed consent.

This study, supported by the National Institutes of Health (NIH), was required to gather information on race/ethnicity. Using guidelines from the *NIH Health Policy on Reporting Race and Ethnicity Data*: *Subjects in Clinical Research*, self-reported race and ethnicity of the AREDS2 participants were collected with 2 ethnic categories (Hispanic or Latino, not Hispanic or Latino) and 5 racial categories (American Indian or Alaska Native, Asian, black or African American, Native Hawaiian or other Pacific Islander, white). Participants were able to select more than 1 racial category.

Interventions

AREDS2 was a randomized, double-masked, placebo-controlled, 2 × 2 factorial trial evaluating the risks and benefits of adding lutein (10 mg) + zeaxanthin (2 mg), DHA (350 mg) + EPA (650 mg), or both to the AREDS formulation, which consisted of vitamin C (500 mg), vitamin E (400 international units), beta carotene (15 mg), zinc (80 mg as zinc oxide), and copper (2 mg as cupric oxide) for the treatment of progression to advanced AMD (FIGURE 1). Previous dose-ranging studies provided support for the doses used.[13,14] Study participants were randomly assigned to take 1 of the following study supplements daily: placebo (because participants assigned to the "placebo" group also received the AREDS supplement, either within or outside of the secondary randomization, there was no true placebo group); lutein + zeaxanthin; DHA + EPA; or lutein + zeaxanthin and DHA + EPA. These components were donated by DSM Nutritional Products Inc. Lutein + zeaxanthin was supplied as water-soluble triglyceride beadlets and DHA + EPA in ethyl ester form as ROPUFA 75 n-33 EE.

Because AREDS2 enrolled participants who were at high risk for developing advanced AMD, all were recommended to take AREDS supplements daily. A second-tier randomization was conducted to evaluate the effect of eliminating beta carotene and lowering the zinc doses in the original AREDS formulation (Figure 1). Beta carotene had been used in this formulation because lutein and zeaxanthin were not commercially available at the start of AREDS. Because beta carotene may increase the risk of lung cancer in cigarette smokers,[15,16] for AREDS2 we tested a version of the AREDS formulation without beta carotene.

A zinc dose of 80 mg was used in the original AREDS formulation because it was the dose used in an earlier trial suggesting efficacy.[17] We tested the AREDS formulation with a lower dose of zinc (25 mg) because data suggested that this may be the maximal level absorbed.[18] Participants who consented to the optional secondary randomization were randomly assigned to receive either the original AREDS formulation (vitamin C [500 mg], vitamin E [400 IU], beta carotene [15 mg], zinc [80 mg, as zinc oxide], and copper [2 mg, as cupric oxide]), AREDS formulation without beta carotene, AREDS formulation with lower zinc dose (25 mg), or AREDS formulation with no beta carotene and lower zinc dose. Persons who did not consent to this secondary randomization but who agreed to take the original AREDS supplements, provided they were not current or former smokers within the past year, were allowed to participate in the study. In summary, the interventions used in this study consisted of nearly all participants taking 1 of the 4 variations of the AREDS formulation with lutein + zeaxanthin, DHA + EPA, lutein + zeaxanthin and DHA + EPA, or placebo. Participants and study personnel were masked to treatment assignment in both randomizations.

Follow-up and Adherence

Follow-up study visits were scheduled annually; follow-up also included telephone contact 3 months after

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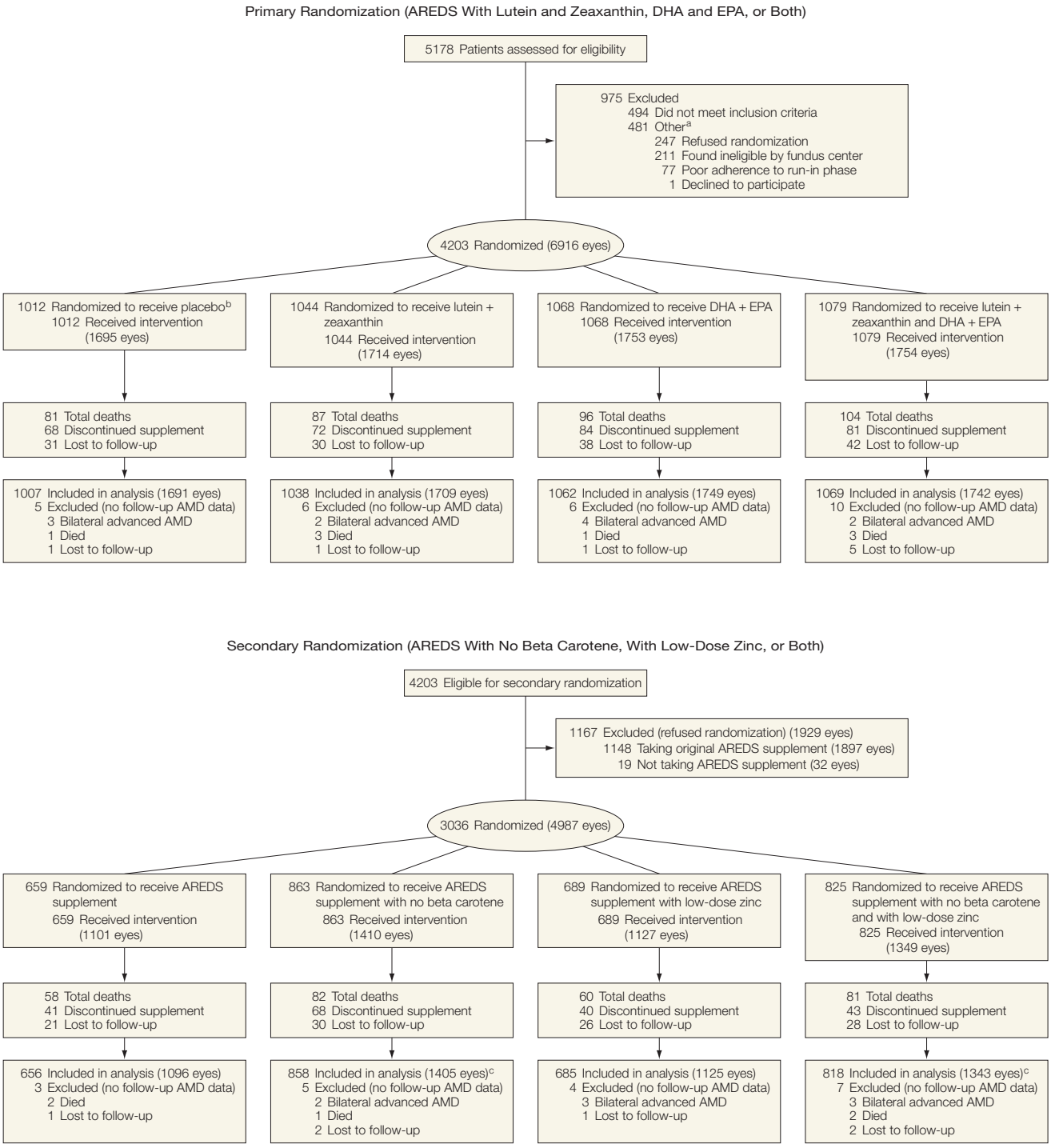
Figure 1. Study Flow and Randomization



The original Age-Related Eye Disease Study (AREDS) supplement comprised vitamin C (500 mg), vitamin E (400 IU), beta carotene (15 mg), zinc (80 mg, as zinc oxide), and copper (2 mg, as cupric oxide). DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid.

[a] Patients could be excluded for more than 1 reason.

[b] The participants assigned to the placebo group were also given the AREDS supplement either within or outside of the secondary randomization for the 4 variations of the AREDS supplements; thus, there is no true placebo group.

[c] Smokers were not randomized to groups receiving beta carotene (n = 181, AREDS with no beta carotene; n = 166, AREDS with no beta carotene and with low-dose zinc).

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randomization and subsequent telephone contacts at 6 months between study visits. At each study visit, participants received a comprehensive eye examination, including best-corrected visual acuity using a standardized protocol.[19] Certified photographers obtained stereoscopic fundus photographs of the macula and optic nerve. Masked graders at a central reading center used a standard protocol to assess the photographs.[20]

Adherence to the treatment regimen was assessed by pill count at each annual visit. In 545 participants from a subset of centers, blood was drawn to measure serum levels of lipids, lutein + zeaxanthin, fat-soluble vitamins, zinc, and copper at baseline and at years 1, 3, and 5. Information on AMD treatment and adverse effects was collected by telephone and at annual visits.

Outcome Measures

The primary outcome was the development of advanced AMD, defined as central geographic atrophy or retinal features of choroidal neovascularization detected on central grading of the stereoscopic fundus photographs or a history of treatment for advanced AMD after study enrollment. Secondary outcomes included progression to moderate vision loss (≥3 lines) from baseline or treatment for choroidal neovascularization. Treatment for choroidal neovascularization was included in the outcome because treatment might maintain visual acuity in an eye with advanced AMD. Safety outcomes included serious adverse events and mortality.

Statistical Analyses

A previous report detailed the power calculation.[12] Assumptions were based on the AREDS 5-year progression rates to advanced AMD and the expected 25% reduction in development of advanced AMD with use of the AREDS supplements. Assuming a 15% loss to follow-up, a sample size of 4000 participants was estimated to provide at least 90% power to detect a 25% reduction in the progression to advanced AMD, comparing the placebo group with each treatment group using an α level of .013, Bonferroni-adjusted for 3 treatments vs placebo comparisons. For all secondary analyses, including main-effects and subgroup analyses, we used an α level of .05 without adjustment for multiple comparisons. All analyses were conducted following the intention-to-treat principle.

The unit of analysis for ophthalmic outcomes was by eye. The primary efficacy outcome, time to progression to advanced AMD, was assessed using a Cox proportional hazards model incorporating the method of Wei et al for obtaining robust variance estimates that allows for dependence among multiple event times (1 or 2 study eyes).[21] The models were adjusted for baseline AMD status, with and without stratification by the secondary interventions. Participants lost to follow-up or who died during the course of the study were censored at the time of last contact. Hazard ratios (HRs) and 98.7% CIs of the 3 active treatment groups compared with the placebo group (primary analyses) were computed. Secondary efficacy variables and subgroup analyses were analyzed in the same fashion as the primary efficacy outcome but with 95% CIs. All analyses were conducted using SAS version 9.2 (SAS Institute Inc).

RESULTS

A total of 4203 participants were enrolled, with a mean age of 73.1 (SD, 7.7) years. Baseline characteristics of the AREDS2 cohort were comparable across the 4 treatment groups in the primary randomization (**Table 1**). The AREDS2 participants were 4058 (96%) white and 2388 (57%) women. Approximately 2724 (65%) of AREDS2 participants had bilateral large drusen and 1468 (35%) had advanced AMD in 1 eye and large drusen in the fellow eye. Eleven participants (0.3%) who had bilateral advanced AMD were excluded from the analyses of AMD progression.

A total of 3036 participants (72.2%) agreed to the secondary randomization evaluating the modifications to the AREDS supplements. Of the remaining participants, 1148 (98.4%) chose to take the original commercial AREDS formulation (Figure 1). A comparison of the participants who chose the secondary randomization with those who refused it showed that women (1680 [55%] women vs 708 [61%] for men; $P = .002$) and participants with higher educational level (589 [19%] for postgraduate education vs 275 [24%] for bachelor's degree or less; $P < .001$) were less likely to participate in the secondary randomization (eTable 1, available at http://www.jama.com). As expected, participants who were smokers were more likely to participate in the second randomization (277 [9%] vs 5 [0.4%]; $P < .001$) because they could enroll in the study only if they consented to randomization to study groups not receiving beta carotene. **Table 2** reports the distribution of randomization of the variations of the AREDS formulation as well as participants who chose to take the AREDS formulation within each of the treatment groups in the primary randomization.

Of the 4203 randomized participants, 141 (3%) were lost to follow-up and 368 (9%) died during the course of the study. Distributions were similar across the 4 treatment groups. Participants underwent follow-up for a median of 4.9 years (interquartile ranges, 4.3 and 5.1 years). Two hundred ninety (7%) of the participants in the primary study cohort and 182 (6%) of those in the secondary study cohort permanently stopped their study medications at some time during the study but continued in follow-up. These percentages were similar across treatment groups for both randomizations. Some participants reported taking lutein + zeaxanthin (132 [3%]) or DHA + EPA (469 [11%]) on their own. In the primary randomization, 3420 (84%) of the participants in each treatment group took at least 75% the study medications; in the secondary randomization, 2431 (83%) of the participants in each group took at least 75% of the variations of the AREDS supplements, as assessed by pill count. For those participants who chose to take the original AREDS supplements outside of the secondary randomization, 989

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(87%) took at least 75% of the AREDS supplements.

Serum Levels of Study Nutrients

The baseline serum levels of study nutrients were balanced across the treatment groups; the levels achieved during the study are reported in eTables 2-4. The median serum levels of lutein in participants randomized to receive lutein increased by 190% to 210% at years 1, 3, and 5 from baseline, whereas participants randomized to receive placebo showed little change. Participants randomized to receive 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 (eTable 2). At year 5, serum levels of lutein in those randomized to receive lutein + zeaxanthin and AREDS formulation with beta carotene (39.1 [SD, 18.7] μg/dL) were lower than in those randomized to receive lutein + zeaxanthin and AREDS formulation without beta carotene (46.9 [SD, 20.3] μg/dL) ($P = .02$).

Compared with general population participants 60 years or older sampled in the National Health and Nutrition Examination Survey 2005-2006, AREDS2 participants had significantly higher serum levels of lutein and zeaxanthin and of DHA and EPA (eTable 5).

Dietary Levels of Lutein + Zeaxanthin and DHA + EPA

Baseline dietary intake of the study nutrients, including those in the AREDS supplements, was balanced across treatment groups. AREDS2 participants had a dietary intake of lutein + zeaxanthin similar to that in participants in the Women's Health Study of health professionals, based on the Harvard Semi-Quantitative Assessment Food Frequency Questionnaire.[22] Both populations are highly educated and well nourished, not accounting for supplements.[23] In a report that evaluated the carotenoid intake of 18 cohorts, the median level of dietary intake of lutein + zeaxanthin in the AREDS2 participants was exceeded in only 2 of these study cohorts, suggesting that the

Table 1. Baseline Characteristics by Treatment Group in the Age-Related Eye Disease Study 2

	Primary Randomized Treatment, No. (%)			
Baseline Characteristics	Placebo (n = 1012)	Lutein + Zeaxanthin (n = 1044)	DHA + EPA (n = 1068)	Lutein + Zeaxanthin and DHA + EPA (n = 1079)
Race				
White	973 (96.1)	1014 (97.1)	1022 (95.7)	1049 (97.2)
Black or African American	13 (1.3)	8 (0.8)	20 (1.9)	13 (1.2)
Other/mixed race	26 (2.6)	22 (2.1)	26 (2.4)	17 (1.6)
Hispanic ethnicity	13 (1.3)	14 (1.3)	24 (2.2)	32 (3.0)
Age at randomization, median (IQR), y	74 (68-79)	74 (68-79)	74 (68-79)	75 (68-79)
Women	548 (54.2)	596 (57.0)	603 (56.5)	641 (59.4)
Education[a]				
Grade 11 or less	61 (6.1)	72 (7.0)	78 (7.4)	63 (6.0)
High school graduate	268 (27.0)	246 (24.0)	279 (26.6)	270 (25.6)
Some college or associate's degree	255 (25.6)	277 (27.0)	314 (29.9)	295 (27.9)
Bachelor's degree	191 (19.2)	208 (20.3)	164 (15.6)	220 (20.8)
Postgraduate work	219 (22.0)	222 (21.7)	215 (20.5)	208 (19.7)
AMD status				
Bilateral large drusen	686 (67.8)	672 (64.4)	689 (64.5)	677 (62.7)
Advanced AMD 1 eye, large drusen fellow eye	323 (31.9)	370 (35.4)	375 (35.1)	400 (37.1)
Bilateral advanced AMD	3 (0.3)	2 (0.2)	4 (0.4)	2 (0.2)
Lens status				
Phakic bilateral	716 (70.8)	709 (67.9)	730 (68.4)	721 (66.8)
Pseudophakic/aphakic in 1 eye	59 (5.8)	78 (7.5)	73 (6.8)	73 (6.8)
Pseudophakic/aphakic in both eyes	237 (23.4)	257 (24.6)	265 (24.8)	285 (26.4)
Diabetes	137 (13.5)	118 (11.3)	145 (13.6)	146 (13.5)
Medication use				
Multivitamin	890 (87.9)	920 (88.1)	954 (89.3)	964 (89.3)
Cholesterol-lowering drug	457 (45.2)	459 (44.0)	471 (44.1)	479 (44.4)
NSAID	122 (12.1)	106 (10.2)	113 (10.6)	115 (10.7)
Acetaminophen	95 (9.4)	84 (8.0)	106 (9.9)	104 (9.6)
Aspirin	495 (48.9)	504 (48.3)	526 (49.3)	532 (49.3)
Smoking status				
Never	410 (40.5)	469 (44.9)	451 (42.2)	494 (45.8)
Former	529 (52.3)	510 (48.0)	548 (51.3)	519 (48.1)
Current	73 (7.2)	74 (7.1)	69 (6.5)	66 (6.1)
Dietary intakes				
DHA, g/d				
Median	0.13	0.12	0.12	0.13
Quintile 1	0.06	0.06	0.06	0.06
Quintile 5	0.23	0.22	0.23	0.23
EPA, g/d				
Median	0.07	0.07	0.07	0.07
Quintile 1	0.03	0.03	0.03	0.03
Quintile 5	0.15	0.14	0.15	0.15
Lutein + zeaxanthin, μg/d				
Median	2725	2590	2553	2572
Quintile 1, range	121-1403	109-1388	154-1428	43-1419
Quintile 5, range	4608-38 110	4740-34 398	4554-21 513	4492-39 790

Abbreviations: AMD, age-related macular degeneration; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IQR, interquartile range; NSAID, nonsteroidal anti-inflammatory drug.
[a] Seventy-eight refused to answer.

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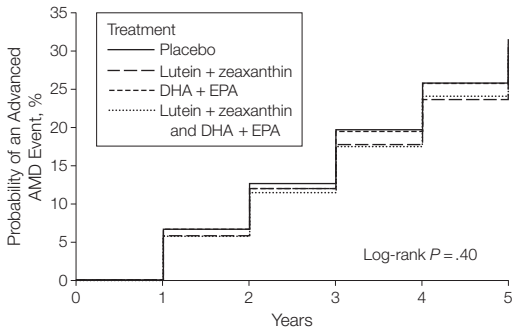
Table 2. AREDS2 Participants' Assignments of Primary Randomization to Lutein + Zeaxanthin, DHA + EPA, or Lutein + Zeaxanthin and DHA + EPA, With Subsequent Randomization of Variations of AREDS Supplement or the Nonrandomized Use of AREDS Supplement[a]

Random Assignment	No. (%)			
	Placebo (n = 1012)[b]	Lutein + Zeaxanthin (n = 1044)	DHA + EPA (n = 1068)	Lutein + Zeaxanthin and DHA + EPA (n = 1079)
AREDS supplement				
Original supplement	168 (16.6)	169 (16.2)	147 (13.8)	175 (16.2)
With no beta carotene	201 (19.9)	200 (19.2)	231 (21.6)	231 (21.4)
With low-dose zinc	184 (18.2)	162 (15.5)	179 (16.8)	164 (15.2)
With low-dose zinc and no beta carotene	190 (18.8)	207 (19.8)	201 (18.8)	227 (21.0)
Nonrandomized cohort				
No AREDS supplement	4 (0.3)	8 (0.8)	5 (0.5)	2 (0.2)
Not randomized to AREDS (taking original AREDS supplement)	265 (26.2)	298 (28.5)	305 (28.6)	280 (25.9)

Abbreviations: AREDS, Age-Related Eye Disease Study; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.
[a] AREDS supplement: vitamin C (500 mg), vitamin E (400 IU), beta carotene (15 mg), zinc (80 mg, as zinc oxide), and copper (2 mg, as cupric oxide).
[b] Placebo was not true placebo, because participants either were given nonrandomized AREDS supplement or were randomly assigned to receive 1 of the 4 variations of AREDS supplement in the secondary randomization.

Figure 2. Probability of Eyes of AREDS2 Participants Developing Advanced Age-Related Macular Degeneration in the Primary Analyses



No. of eyes at risk						
Placebo	1691	1647	1509	1377	1243	900
Lutein + zeaxanthin	1709	1673	1535	1405	1284	938
DHA + EPA	1749	1702	1549	1420	1260	897
Lutein + zeaxanthin and DHA + EPA	1742	1683	1542	1415	1281	923

AREDS indicates Age-Related Eye Disease Study; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

AREDS2 participants are relatively well nourished.[24]

Progression to Advanced AMD

Primary Randomization. A total of 1608 participants had experienced at least 1 advanced AMD event by the end of the study (1940 events in 6891 study eyes). The Kaplan-Meier probabilities of progression to advanced AMD by 5 years were 31% (493 eyes [406 participants]) for placebo, 29% (468 eyes [399 participants]) for lutein + zeaxanthin, 31% (507 eyes [416 participants]) for DHA + EPA, and 30% (472 eyes [387 participants]) for lutein + zeaxanthin and DHA + EPA (FIGURE 2). In the primary analyses, comparisons with placebo demonstrated no statistically significant reductions in progression to advanced AMD (HR, 0.90 [98.7% CI, 0.76-1.07]; $P = .12$) for lutein + zeaxanthin; HR, 0.97 [98.7% CI, 0.82-1.16]; $P = .70$ for DHA + EPA; and HR, 0.89 [98.7% CI, 0.75-1.06]; $P = .10$ for lutein + zeaxanthin and DHA + EPA) (FIGURE 3). These analyses were performed with stratification by the secondary interventions. The analyses conducted without the stratification showed essentially similar results.

Main Effects of Primary Randomization. The analyses of the main effects of the 2 × 2 randomization are considered exploratory secondary analyses. The results presented were also analyzed with stratification by the secondary interventions. Comparison of DHA + EPA vs no DHA + EPA showed an HR of 0.98 (95% CI, 0.89-1.08; $P = .74$) for progression to advanced AMD. The HR for lutein + zeaxanthin vs no lutein + zeaxanthin was 0.91 (95% CI, 0.82-1.00; $P = .05$) for progression to advanced AMD (FIGURE 4). The P value for the interaction of lutein + zeaxanthin and DHA + EPA was $P = .80$. The results from analyses without the stratification by secondary interventions were very similar to these results.

Secondary Randomization. The exploratory analyses of the secondary randomization were restricted to participants randomized to the 4 variations of the AREDS supplements. The secondary randomization analyses showed that lowering zinc dose and eliminating beta carotene had no statistically significant effect on progression to advanced AMD (HR, 1.06 [95% CI, 0.95-1.19]; $P = .32$ and HR, 1.07 [95% CI, 0.94-1.20]; $P = .31$, respectively) (Figure 4).

Subgroup Analyses

We conducted further exploratory analyses of the main effect of lutein + zeaxanthin by stratifying by quintiles of dietary lutein + zeaxanthin intake to examine whether supplementation may have relatively different treatment effects within subgroup of dietary

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intake. For persons in the lowest quintile, comparison of lutein + zeaxanthin vs no lutein + zeaxanthin resulted in an HR of 0.74 (95% CI, 0.59-0.94; $P = .01$) for progression to advanced AMD. For participants in the highest quintile of lutein + zeaxanthin intake the corresponding HR was 0.90 (95% CI, 0.71-1.15; $P = .41$), with the results for remaining quintiles similar to that of the highest quintile (FIGURE 5). The interaction term for treatment and quintile groups was $P = .47$. Additional analyses stratified by tertiles, quartiles, and deciles (eFigure 1) showed similar results, with the lowest stratum demonstrating a protective effect compared with the remaining strata.

Subgroup analyses, not prespecified, were conducted to evaluate the effects of lutein + zeaxanthin on the 2 forms of advanced AMD. The HRs were 0.89 (95% CI, 0.79-1.00]; $P = .05$) for neovascular AMD and 0.92 (95% CI, 0.78-1.07]; $P = .27$) for central geographic atrophy (FIGURE 6).

To investigate relative treatment effects, we also conducted a post hoc subgroup analysis comparing participants assigned to receive lutein + zeaxanthin and the AREDS formulation without beta carotene (310 of 1114 eyes) with participants assigned to no lutein + zeaxanthin and the original AREDS formulation with beta carotene (347 of 1117 eyes). Comparing the lutein + zeaxanthin–containing AREDS supplements with the beta carotene–containing AREDS supplements resulted in HRs of 0.82 (95% CI, 0.69–0.96; $P = .02$) for progression to advanced AMD, 0.78 (95% CI, 0.64-0.94; $P = .01$) for neovascular AMD, and 0.94 (95% CI, 0.70-1.26; $P = .67$) for central geographic atrophy (Figure 6).

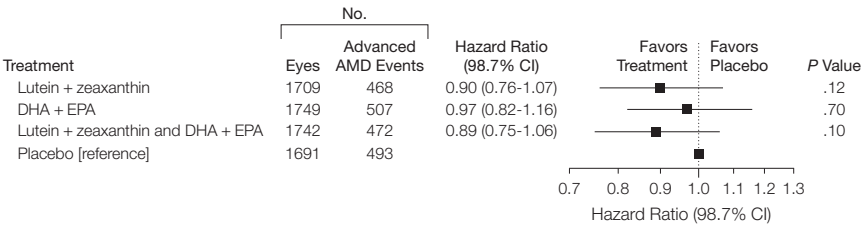
Visual Acuity

None of the nutrients affected development of moderate or worse vision loss, defined as a reduction of 15 or more letters from baseline or treatment for neovascular AMD. Compared with placebo, the HRs for development of moderate or worse vision loss were 0.95 (95% CI, 0.84-1.08; $P = .45$) for lutein + zeaxanthin, 0.96 (95% CI, 0.84-1.09; $P = .50$) for DHA + EPA, and 0.94 (95% CI, 0.83-1.07; $P = .34$) for lutein + zeaxanthin and DHA + EPA. No apparent effect on vision of eliminating beta carotene and reducing zinc dose was observed.

Safety Outcomes

Serious Adverse Events. No clinically or statistically significant differences in reported serious adverse events, including rates of development of neoplasms, were noted across the treatment groups in the primary randomization (TABLE 3). However, secondary randomization excluding participants who were smokers showed more lung cancers in the beta carotene group than in the no beta carotene group (23 [2.0%] vs 11 [0.9%]) (nominal $P = .04$). Thirty-one (91%) of participants who developed lung cancer were former smokers. In the original AREDS report, gastrointestinal conditions and hospitalizations for genitourinary diseases were significantly more common in participants randomized to receive zinc (80 mg) than to receive placebo. Rates of reported gastrointestinal disorders and hospitalizations for genitourinary diseases were similar in the 2 randomly assigned groups (high-dose zinc, low-dose zinc) in AREDS2. No clinically or statistically significant differences in reported serious adverse events were found in the analyses of the secondary randomization (TABLE 4). These analyses in-

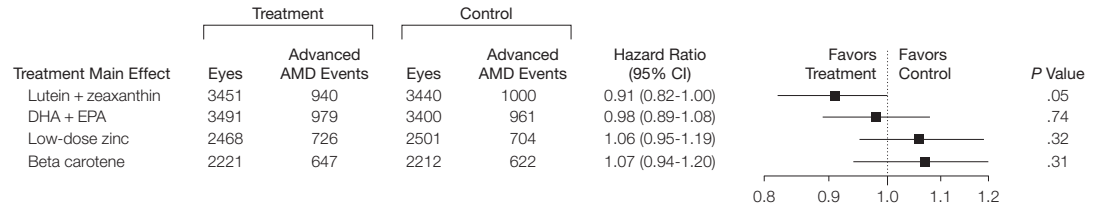
Figure 3. Primary Analyses of Lutein + Zeaxanthin + Omega-3 Long-Chain Polyunsaturated Fatty Acids vs Placebo for Treatment of Progression to Advanced Age-Related Macular Degeneration (AMD)



Treatment	No. Eyes	No. Advanced AMD Events	Hazard Ratio (98.7% CI)	P Value
Lutein + zeaxanthin	1709	468	0.90 (0.76-1.07)	.12
DHA + EPA	1749	507	0.97 (0.82-1.16)	.70
Lutein + zeaxanthin and DHA + EPA	1742	472	0.89 (0.75-1.06)	.10
Placebo [reference]	1691	493		

Participants assigned to the placebo group were also given Age-Related Eye Disease Study (AREDS) supplement either within or outside of the secondary randomization for the 4 variations of the AREDS supplements; thus, there is no true placebo group. DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid.

Figure 4. Main Effects of Lutein + Zeaxanthin, Omega-3 Long-Chain Polyunsaturated Fatty Acids, Zinc, and Beta Carotene on Progression to Advanced Age-Related Macular Degeneration (AMD)



Treatment Main Effect	Treatment Eyes	Treatment Advanced AMD Events	Control Eyes	Control Advanced AMD Events	Hazard Ratio (95% CI)	P Value
Lutein + zeaxanthin	3451	940	3440	1000	0.91 (0.82-1.00)	.05
DHA + EPA	3491	979	3400	961	0.98 (0.89-1.08)	.74
Low-dose zinc	2468	726	2501	704	1.06 (0.95-1.19)	.32
Beta carotene	2221	647	2212	622	1.07 (0.94-1.20)	.31

Participants assigned to the control group were also given Age-Related Eye Disease Study (AREDS) supplement either within or outside of the secondary randomization for the 4 variations of the AREDS supplements; thus, there is no true placebo group. DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid.

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cluded all the participants randomly assigned to the variations of the AREDS supplement, including smokers.

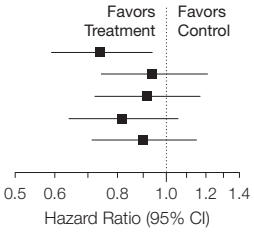
Mortality. Dietary supplementation with lutein + zeaxanthin, DHA + EPA, zinc, or beta carotene had no statistically significant effect on mortality (eFigure 2). The HR for mortality comparing lutein + zeaxanthin vs no lutein + zeaxanthin was 1.06 (95% CI, 0.87-1.31; $P = .56$) for lutein + zeaxanthin vs no lutein + zeaxanthin and 1.16 (95% CI, 0.94-1.42; $P = .16$) for DHA + EPA. There also were no differences for zinc main effect (low-dose zinc vs high-dose zinc) (HR, 1.02 [95% CI, 0.81-1.29]; $P = .87$) or for beta carotene main effect (beta carotene vs no beta carotene) (HR, 1.01 [95% CI, 0.78-1.31]; $P = .94$). Because of the potential for increased risk of mortality with beta carotene, analyses for competing risk were performed. These results showed little change from the original findings on mortality.

DISCUSSION

In this large, multicenter, placebo-controlled clinical trial in people at high risk for progression to advanced AMD, daily supplementation with lutein + zeaxanthin, DHA + EPA, or lutein + zeaxanthin and DHA + EPA in addition to the original AREDS formulation showed no statistically significant overall effect on progression to advanced AMD or changes in visual acuity. Primary, secondary, and subgroup analyses demonstrated no beneficial or harmful effects of DHA + EPA for treatment of AMD. These null results may be attributable to the true lack of efficacy. Other factors to consider include inadequate dose, inadequate duration of treatment, or both. The form of omega-3 long-chain polyunsatu-

Figure 5. Comparison of the Main Effects of Lutein + Zeaxanthin vs No Lutein + Zeaxanthin, Stratified by Quintiles of Dietary Intake of Lutein + Zeaxanthin, on Progression to Advanced Age-Related Macular Degeneration (AMD)

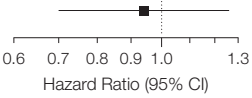
Lutein + Zeathanxin Dietary Intake, μg			Treatment		Control			
Quintile	Median[a]	Interquartile Range	Eyes	Advanced AMD Events	Eyes	Advanced AMD Events	Hazard Ratio (95% CI)	*P* Value
1	696	552-823	525	143	530	186	0.74 (0.59-0.94)	.01
2	1134	1030-1244	514	141	544	154	0.94 (0.74-1.21)	.65
3	1585	1465-1719	538	144	558	157	0.92 (0.72-1.17)	.49
4	2225	2036-2452	547	136	548	153	0.82 (0.64-1.06)	.72
5	3919	3201-5249	554	152	556	167	0.90 (0.71-1.15)	.41



Participants assigned to the control group were also given Age-Related Eye Disease Study (AREDS) supplement either within or outside of the secondary randomization for the 4 variations of the AREDS supplements; thus, there is no true placebo group. DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid.
[a] Median intake of dietary lutein + zeaxanthin (μg/1000 kcal per day).

Figure 6. Subgroup Analyses of Main Effects of Lutein + Zeaxanthin and the Comparison of Participants Randomized to Receive Lutein + Zeaxanthin and AREDS Supplements With Lutein + Zeaxanthin and Without Beta Carotene vs Those Randomized to Receive Original AREDS Supplements With Beta Carotene for Progression to Advanced Age-Related Macular Degeneration (AMD) and the 2 Forms of AMD, Neovascular AMD and Central Geographic Atrophy

	Treatment		Control			
Treatment	Eyes	Advanced AMD Events	Eyes	Advanced AMD Events	Hazard Ratio (95% CI)	*P* Value
Advanced AMD						
Lutein + zeaxanthin	3451	940	3440	1000	0.90 (0.82-0.99)	.04
AREDS supplement with lutein + zeaxanthin and no beta carotene vs original AREDS supplement with beta carotene	1114	310	1117	347	0.82 (0.69-0.96)	.02
Neovascular AMD						
Lutein + zeaxanthin	3451	607	3440	655	0.89 (0.79-1.00)	.05
AREDS supplement with lutein + zeaxanthin and no beta carotene vs original AREDS supplement with beta carotene	1114	209	1117	248	0.78 (0.64-0.94)	.01
Central geographic atrophy						
Lutein + zeaxanthin	3451	367	3440	398	0.92 (0.78-1.07)	.27
AREDS supplement with lutein + zeaxanthin and no beta carotene vs original AREDS supplement with beta carotene	1114	112	1117	117	0.94 (0.70-1.26)	.67



Participants assigned to the control group were also given Age-Related Eye Disease Study (AREDS) supplement either within or outside of the secondary randomization for the 4 variations of the AREDS supplements; thus, there is no true placebo group. DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid.

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rated fatty acids (ethyl ester) and the DHA:EPA ratio may be inappropriate, although trials of DHA and EPA for cardiovascular disease often tested doses and ratios similar to that used in AREDS2.[25,26]

The primary analyses demonstrated no beneficial or harmful effect of lutein + zeaxanthin for the treatment of advanced AMD. The exploratory analyses demonstrated results that suggest that the role of lutein + zeaxanthin needs to be examined further. Specifically, the nutritional status of the AREDS2 cohort and the competitive absorption of carotenoids require further assessments. AREDS2 participants' dietary intake of lutein + zeaxanthin is similar to that of other well-nourished populations. When exploratory subgroup analyses of the treatment effects were limited to those participants in the quintile with the lowest dietary intake of lutein + zeaxanthin, lutein + zeaxanthin demonstrated a protective effect for progression to advanced AMD (HR, 0.74 [95% CI, 0.59-0.94; $P = .01$), although there was no trend with increasing lutein + zeaxanthin intake. Similar findings were seen when the data were divided into tertiles, quartiles, and deciles.

We administered 2 carotenoids, beta carotene and lutein + zeaxanthin, either alone or in combination, through our secondary randomization. Achieved serum levels of lutein + zeaxanthin in AREDS2 participants randomly assigned to receive beta carotene were lower than levels in those not as-

Table 3. Serious Adverse Events by Treatment Group in the Primary Randomization to Lutein + Zeaxanthin and Omega-3 Long-Chain Polyunsaturated Fatty Acids

	No. (%)			
MedDRA System Organ Class	Placebo (n = 1012)	Lutein + Zeaxanthin (n = 1044)	DHA + EPA (n = 1068)	Lutein + Zeaxanthin and EPA + DHA (n = 1079)
Participants with ≥1 serious adverse event	479 (47.3)	484 (46.4)	505 (47.3)	519 (48.1)
Cardiac disorders	96 (9.5)	110 (10.5)	119 (11.1)	103 (9.5)
Gastrointestinal tract disorders	76 (7.5)	69 (6.6)	58 (5.4)	61 (5.7)
Infections	90 (8.9)	102 (9.8)	103 (9.6)	99 (9.2)
Neoplasms benign, malignant, and unspecified (including cysts and polyps)	80 (7.9)	88 (8.4)	83 (7.8)	92 (8.5)
Nervous system disorders	66 (6.5)	74 (7.1)	72 (6.7)	73 (6.8)
Respiratory tract, thoracic, and mediastinal disorders	44 (4.3)	43 (4.1)	37 (3.5)	46 (4.3)
Incident lung neoplasm	9 (0.9)	16 (1.5)	22 (2.1)	17 (1.6)

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

Table 4. Incidence of Serious Adverse Events in the Secondary Randomization to Variations of AREDS Supplements

	Treatment, No. (%)					
	Secondary Randomization (Variations of AREDS Supplement)[a]				No Secondary Randomization[b]	
Systems Organ Class	Original AREDS[c] (n = 659)	Without Beta Carotene (n = 863)	With Low-Dose Zinc[c] (n = 689)	With Low-Dose Zinc and No Beta Carotene (n = 825)	Original AREDS (n = 1148)	No AREDS (n = 19)
Participants with ≥1 serious adverse event	330 (50.1)	410 (47.5)	345 (50.1)	392 (47.5)	500 (43.6)	10 (52.6)
Cardiac disorders	69 (10.5)	90 (10.4)	66 (9.6)	107 (13)	93 (8.1)	3 (15.8)
Gastrointestinal tract disorders	39 (5.9)	55 (6.4)	37 (5.4)	57 (6.9)	75 (6.5)	1 (5.3)
Infections and infestations	56 (8.5)	78 (9)	65 (9.4)	84 (10.2)	110 (9.6)	1 (5.3)
Neoplasms benign, malignant, and unspecified (including cysts and polyps)	64 (9.7)	65 (7.5)	62 (9)	67 (8.1)	83 (7.2)	2 (10.5)
Nervous system disorders	48 (7.3)	58 (6.7)	55 (8)	54 (6.5)	65 (5.7)	5 (26.3)
Respiratory tract, thoracic, and mediastinal disorders	21 (3.2)	40 (4.6)	30 (4.4)	43 (5.2)	35 (3)	1 (5.3)
Incident lung neoplasm (MedDRA preferred term)	9 (1.4)	13 (1.5)	14 (2.0)	13 (1.6)	14 (1.2)	1 (5.3)

Abbreviations: AREDS, Age-Related Eye Disease Study; MedDRA, Medical Dictionary for Regulatory Activities.
[a] AREDS supplement: supplement consisting of vitamin C (500 mg), vitamin E (400 IU), beta carotene (15 mg), zinc (80 mg, as zinc oxide), and copper (2 mg, as cupric oxide).
[b] Participants declined to participate in secondary randomization.
[c] Smokers were not randomized to receive AREDS supplements containing beta carotene. These analyses of neoplasm and lung cancer include all participants, regardless of smoking status.

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signed to receive beta carotene (mean change, 175% vs 263%, respectively; $P = .02$). Animal[27] and human[28,29] studies suggest that the simultaneous administration of high doses of beta carotene and lutein + zeaxanthin may suppress serum and tissue levels of lutein + zeaxanthin because of competitive absorption of carotenoids. Post hoc analyses comparing AREDS2 participants randomized to receive lutein + zeaxanthin and AREDS formulation without beta carotene vs those randomized to receive no lutein + zeaxanthin and the original AREDS formulation suggest that lutein and zeaxanthin may play a role for reducing the risk of progression to advanced AMD when given without beta carotene. This hypothesis requires further study.

Two randomized controlled clinical trials of beta carotene reported an increase in lung cancer rates and associated mortality in cigarette smokers assigned to receive beta carotene.[21,22] AREDS2 participants who were not current smokers or who had stopped smoking more than 1 year prior to enrollment, and those assigned to one of the 2 groups receiving the AREDS formulation with beta carotene, showed an increased incidence of lung cancers (23 [2.0%] and 11 [0.9%], respectively; nominal $P = .04$). Thirty-one (91%) of those participants developing lung cancer were former smokers. AREDS2 found no increased risk of lung cancer with lutein + zeaxanthin supplementation.

Previous randomized controlled clinical trials of lutein + zeaxanthin, of short duration and with limited sample sizes, suggested improved visual function,[30-34] but AREDS2 showed no evidence that treatment affected visual acuity. One randomized trial of DHA and EPA found no effect on AMD progression.[35]

The limitations of this study include a complicated study design involving a secondary randomization, which may have affected our ability to evaluate the role of adding lutein + zeaxanthin and DHA + EPA to the AREDS formulation. Not all participants were taking the original AREDS formulation, with some taking only certain components of the AREDS formulation. This formulation was given as a mixture of antioxidant vitamins and minerals. It is not known whether a single specific ingredient is important or if the combination is essential for its therapeutic effect. In these analyses we assumed that there would be little interaction between the various nutrients tested. We found a potential interaction when administering 2 carotenoids (beta carotene; lutein + zeaxanthin) simultaneously; this may have influenced our primary analyses, which did not take this interaction into account. However, when we conducted the analyses with and without stratification for the secondary interventions, the results were almost identical. We also have not tested for equivalency between low-dose zinc and high-dose zinc and between no beta carotene and beta carotene.

Another limitation would be our inability to assess the effect of the potential increased risk of lung cancer associated with beta carotene on our analyses of mortality. The number of lung cancers was small, and the analyses of competing risk showed essentially no change in our mortality results.

These study results may not be generalizable, because the study population is a highly selected group of highly educated and well-nourished people. The strengths of this study include the low rates of loss to follow-up and consistently good adherence to the treatment regimen.

In summary, addition of lutein + zeaxanthin, DHA + EPA, or lutein + zeaxanthin and DHA + EPA to the AREDS formulation in primary analyses did not further reduce risk of progression to advanced AMD. Comparison of low-dose zinc vs high-dose zinc showed no evidence of a statistically significant effect, and there is insufficient evidence to provide a clinical recommendation. Based on apparent risks of beta carotene and possible benefits that are only evident within exploratory subgroup analyses, lutein + zeaxanthin requires further investigation for potential inclusion in the AREDS supplements.

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Author Contributions: Drs Chew and Clemons had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Acquisition of data: Danis, Elman, Antoszyk, Ruby, Orth, Bressler, Fish, Hubbard, Klein, Chandra, Blodi, Domalpally, Friberg, Wong, Rosenfeld, Toth, Bernstein.

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Critical revision of the manuscript for important intellectual content: Chew, Clemons, SanGiovanni, Danis, Ferris, Elman, Antoszyk, Ruby, Orth, Bressler, Fish, Hubbard, Klein, Chandra, Blodi, Domalpally, Friberg, Wong, Rosenfeld, Agron, Toth, Bernstein, Sperduto.

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Study supervision: Chew, Clemons, Danis, Ferris, Bressler, Domalpally, Friberg.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Ferris reported holding a patent for the Age-Related Eye Disease Study (AREDS) formulation with Bausch & Lomb. Dr Anto-

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szyk reported receiving grant and travel support from Southwest Clinical Research Associates LLC. Dr Ruby reported receiving payment for lectures from Genentech. Dr Bressler reported receiving grant and travel support from the Emmes Corporation; serving as a consultant for GlaxoSmithKline; receiving grants or grants pending from Allergan, Bayer Healthcare, Genentech, Lumenis Inc, Notal Vision Ltd, Novartis, Regeneron, Thrombogenics, and sanofi-aventis; receiving payment for lectures from providers of continuing medical education materials; and serving as an investigator on a grant to The Johns Hopkins University sponsored by Bausch & Lomb; this grant is negotiated and administered by the School of Medicine, which receives the grant through the Office of Research Administration (individual investigators who participate in such sponsored projects are not directly compensated by the sponsor but may receive salary or other support from the institution to support their effort on the projects). Dr Fish reported receiving grant and travel support from Texas Retina Associates. Dr Rosenfeld reported serving as a consultant for Oraya, Novartis, Chengdu Kanghong Biotech, Acucela, Thrombogenics, and Canon; receiving grants or grants pending from Carl Zeiss Meditec, Alexion, Potentia, and GlaxoSmithKline; and receiving payment for lectures from Carl Zeiss Meditec, Allergan, and Topcon. Dr Toth reported receiving grant and travel support from the Emmes Corporation and serving as a consultant to ALCON. Dr Bernstein reported serving as a consultant for Kemin Health, Kalsec, DSM, and Science Based Health. No other authors reported disclosures.

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Role of the Sponsor: The staff of the National Eye Institute and their contractors, led by Dr Chew, had complete control of the design and conduct of the study; the collection, management, analysis, and interpretation of the data; and the preparation, review, or approval of the manuscript. The contributing institutes from the NIH were consulted for various aspects of the study, including its design and conduct. The analyses were completed at the NEI/NIH. The sponsors who donated the study medications had no role in the design or conduct of the study and had no access to the data during the study.

Online-Only Material: The eAppendix, eTables 1-5, eFigures 1 and 2, and Author Video Interview are available at http://www.jama.com.

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