Original Investigation | CLINICAL TRIAL

Secondary Analyses of the Effects of Lutein/Zeaxanthin on Age-Related Macular Degeneration Progression
AREDS2 Report No. 3

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

IMPORTANCE The Age-Related Eye Disease Study (AREDS) formulation for the treatment of age-related macular degeneration (AMD) contains vitamin C, vitamin E, beta carotene, and zinc with copper. The Age-Related Eye Disease Study 2 (AREDS2) assessed the value of substituting lutein/zeaxanthin in the AREDS formulation because of the demonstrated risk for lung cancer from beta carotene in smokers and former smokers and because lutein and zeaxanthin are important components in the retina.

OBJECTIVE To further examine the effect of lutein/zeaxanthin supplementation on progression to late AMD.

DESIGN, SETTING, PARTICIPANTS The Age-Related Eye Disease Study 2 is a multicenter, double-masked randomized trial of 4203 participants, aged 50 to 85 years, at risk for developing late AMD; 66% of patients had bilateral large drusen and 34% had large drusen and late AMD in 1 eye.

INTERVENTIONS In addition to taking the original or a variation of the AREDS supplement, participants were randomly assigned in a factorial design to 1 of the following 4 groups: placebo; lutein/zeaxanthin, 10 mg/2 mg; omega-3 long-chain polyunsaturated fatty 3 acids, 1.0 g; or the combination.

MAIN OUTCOMES AND MEASURES Documented development of late AMD by central, masked grading of annual retinal photographs or by treatment history.

RESULTS In exploratory analysis of lutein/zeaxanthin vs no lutein/zeaxanthin, the hazard ratio of the development of late AMD was 0.90 (95% CI, 0.82-0.99; $P = .04$). Exploratory analyses of direct comparison of lutein/zeaxanthin vs beta carotene showed hazard ratios of 0.82 (95% CI, 0.69-0.96; $P = .02$) for development of late AMD, 0.78 (95% CI, 0.64-0.94; $P = .01$) for development of neovascular AMD, and 0.94 (95% CI, 0.70-1.26; $P = .67$) for development of central geographic atrophy. In analyses restricted to eyes with bilateral large drusen at baseline, the direct comparison of lutein/zeaxanthin vs beta carotene showed hazard ratios of 0.76 (95% CI, 0.61-0.96; $P = .02$) for progression to late AMD, 0.65 (95% CI, 0.49-0.85; $P = .002$) for neovascular AMD, and 0.98 (95% CI, 0.69-1.39; $P = .91$) for central geographic atrophy.

CONCLUSION AND RELEVANCE The totality of evidence on beneficial and adverse effects from AREDS2 and other studies suggests that lutein/zeaxanthin could be more appropriate than beta carotene in the AREDS-type supplements.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT00345176

JAMA Ophthalmol. doi:10.1001/jamaophthalmol.2013.7376
Published online December 5, 2013.

Editorial

Supplemental content at jamaophthalmology.com

***AREDS2 Research Group Writing Team** The members of the writing team and their affiliations are found at the end of this article.

Group Information: The AREDS2 Research Group is found online in the Supplement (eAppendix 2).

Corresponding Author: Emily Y. Chew, MD, Division of Epidemiology and Clinical Research, National Institutes of Health, 10 Center Dr, MSC1204, Bldg 10-CRC, Room 3-2531, Bethesda, MD 20892-1204 (echew@nei.nih.gov).

Age-related macular degeneration (AMD) is the leading cause of blindness in the United States.[1] Despite widespread use of highly effective intravitreal injections of drugs that inhibit vascular endothelial growth factor for neovascular AMD,[2] there is still no effective therapy for the atrophic form of AMD. We have demonstrated that the original Age-Related Eye Disease Study (AREDS) formulation consisting of vitamin C, vitamin E, beta carotene, and zinc reduced the 5-year risk for developing late AMD in persons at risk by an estimated 25%.[3] This beneficial treatment effect, mostly for reducing the risk for progression to neovascular AMD, persisted for the 5 years following cessation of the controlled, randomized clinical trial.[4] Observational studies suggest that higher dietary intake of lutein/zeaxanthin and/or omega-3 long-chain polyunsaturated fatty acids (LCPUFAs) are associated with a decreased risk for developing late AMD.[5-20] Lutein had been considered for use in the original AREDS formulation because of this reported association. However, lutein was not commercially available at the start of AREDS. Lutein and zeaxanthin are of interest because they are the major components of the macular pigment and may serve a variety of functions including filtering of presumably damaging blue and ultraviolet light and providing antioxidant capability.[21]

The Age-Related Eye Diseases Study 2 (AREDS2) was designed to test whether adding the oral supplements of lutein/zeaxanthin and/or omega-3 LCPUFAs to the AREDS formulation might further reduce the risk for progression to late AMD. The primary analyses in AREDS2 compared each individual treatment group of approximately 1000 participants with the placebo group of approximately 1000 participants. These AREDS2 primary analyses demonstrated no beneficial or harmful effect of lutein/zeaxanthin, omega-3 LCPUFAs, or the combination on the progression to late AMD compared with placebo.[22] A prespecified analysis consisting of a comparison of lutein/zeaxanthin vs no lutein/zeaxanthin (main effect) using the entire study cohort of approximately 4000 participants demonstrated a beneficial effect of lutein/zeaxanthin (hazard ratio [HR], 0.90; 95% CI, 0.82-0.99; P = .04) for progression to late AMD. This beneficial effect was beyond the effects of the AREDS supplements, while we found no such indication of a beneficial effect of omega-3 LCPUFAs.

In addition, we reported prespecified analyses of the main effect of lutein/zeaxanthin that were stratified by quintiles of baseline dietary lutein/zeaxanthin intake. For persons in the lowest quintile (lowest dietary intake), comparison of lutein/zeaxanthin vs no lutein/zeaxanthin resulted in a HR of 0.74 (95% CI, 0.59-0.94; P = .01) for progression to late AMD.[22] Previous exploratory analyses were performed to evaluate the effects of lutein/zeaxanthin vs no lutein/zeaxanthin on the 2 forms of late AMD. The HRs were 0.89 (95% CI, 0.79-1.00; P = .05) for the development of neovascular AMD and 0.92 (95% CI, 0.78-1.07; P = .27) for development of central geographic atrophy (CGA) (**Figure 1**). In this article, we present detailed results from both prespecified and exploratory analyses examining the effect of lutein/zeaxanthin supplementation on progression to late AMD.

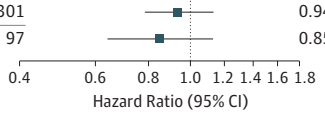
Methods

Study Population

Details of the study design have been published previously.[23] The Age-Related Eye Diseases Study 2 restricted enrollment to people at high risk for progressing to late AMD and those with either bilateral large drusen or large drusen in 1 eye and late AMD in the fellow eye. A total of 4203 participants, with a mean (SD) age of 73.1 (7.7) years, were enrolled between October 17, 2006, and September 28, 2008, at 82 clinical sites across the United States. Candidates were considered eligible only if they took at least 75% of the run-in medications (study's placebo and AREDS formulation) and if they agreed to take the AREDS2 supplements and stop the use of other study supple-

Figure 1. Comparison of Lutein/Zeaxanthin vs No Lutein/Zeaxanthin for the Development of Late Age-Related Macular Degeneration (AMD), Neovascular AMD, and Central Geographic Atrophy

	Lutein/Zeaxanthin		No Lutein/Zeaxanthin			
	No. of Eyes	No. of Events	No. of Eyes	No. of Events	Favors Lutein/Zeaxanthin / Favors No Lutein/Zeaxanthin	*P* Value
Late AMD						
All eyes	3451	940	3440	1000	0.90 (0.82-0.99)	.04
Large drusen OU	2688	576	2742	667	0.87 (0.77-0.95)	.04
Late AMD in 1 eye	763	364	698	333	0.96 (0.83-1.11)	.59
Neovascular AMD						
All eyes	3461	607	3440	655	0.89 (0.79-1.00)	.05
Large drusen OU	2688	322	2742	407	0.80 (0.68-0.95)	.01
Late AMD in 1 eye	763	285	698	248	1.02 (0.86-1.21)	.79
Central geographic atrophy						
All eyes	3451	367	3440	390	0.92 (0.78-1.07)	.27
Large drusen OU	2688	274	2742	301	0.94 (0.78-1.13)	.51
Late AMD in 1 eye	763	93	698	97	0.85 (0.64-1.13)	.27



Analyses were conducted for all Age-Related Eye Disease Study 2 participants and then subdivided by the baseline AMD status: bilateral (OU) large drusen and bilateral large drusen with late AMD in 1 eye.

ments. Of the 4203 participants, 3036 (72%) agreed to the secondary randomization evaluating the modifications to the AREDS supplements (eFigure 1 in Supplement). They had to satisfy the specified inclusion and exclusion criteria.[22] Institutional review boards from the clinical sites approved the AREDS2 research protocol and all participants provided written informed consent.

Interventions

The Age-Related Eye Diseases Study 2 is a randomized, double-masked, placebo-controlled, 2 × 2 factorial trial evaluating the risks and benefits of adding lutein/zeaxanthin, 10 mg/2 mg, and/or omega-3 LCPUFAs, specifically docosahexaenoic acid (DHA, 350 mg) and eicosapentaenoic acid (EPA, 650 mg), to the original AREDS formulation, or one of the variations of the AREDS formulation for the treatment of AMD. Study participants were randomized with equal probability to take 1 of the following study supplements daily: (1) placebo; (2) lutein/zeaxanthin; (3) DHA/EPA; or (4) lutein/zeaxanthin and DHA/EPA.

Because they are known to be at high risk for developing late AMD, all AREDS2 participants also were offered the original or a modified version of the AREDS formulation. A second randomization was conducted to evaluate the effect of eliminating beta carotene and/or lowering the zinc levels in the original AREDS formulation. Because beta carotene has been reported to increase the risk for lung cancer in cigarette smokers,[24,25] a version of the AREDS formulation without beta carotene was tested. A dose of 80 mg of zinc was used in the original AREDS formulation because this dose was used in an earlier trial suggesting efficacy.[26] A lower dose of zinc (25 mg) was tested in AREDS2 based on data suggesting this dose may be the maximal level that is absorbed.[27] Those who consented to the optional secondary randomization were randomly assigned to: (1) the AREDS formulation (vitamin C, 500 mg; vitamin E, 400 IU; beta carotene, 15 mg; zinc oxide, 80 mg; and cupric oxide, 2 mg), (2) the AREDS formulation minus beta carotene, (3) the AREDS formulation with low zinc (25 mg), or (4) the AREDS formulation minus beta carotene and low zinc. Current smokers and former smokers who had quit within 1 year before randomization and who agreed to this secondary randomization were randomized to 1 of the 2 arms without beta carotene. Participants who did not consent to this secondary randomization were provided with the original AREDS supplements, if they were not current smokers or had not smoked within the past year. Centrum Silver (Pfizer Inc) was offered to all study participants to standardize multivitamin intake. Participants and study personnel were masked to treatment assignment in each randomization.

Follow-up

Briefly, follow-up study visits were scheduled annually with telephone contacts at 6 months between visits and at 3 months postrandomization to collect information on AMD treatment and adverse events. Study visits included a comprehensive eye examination with best-corrected visual acuity (VA) using an electronic version of the Early Treatment Diabetic Retinopathy Study VA technique and standardized stereoscopic fundus photographs. Masked graders assessed the photographs at the reading center using a standardized protocol.

Pill counts at each annual visit and fasting blood samples at baseline and years 1, 3, and 5 were used to evaluate compliance with treatment assignments. Participants were followed up until October 2012, resulting in a median follow-up of 4.9 years (interquartile range, 4.3-5.1 years).

Outcome Measures

The primary outcome was the development of late AMD, defined as atrophy involving the center of the macula or neovascular changes of AMD that were detected on central grading of the stereoscopic fundus photographs for (1) definite central geographic atrophy, (2) retinal features of choroidal neovascularization, or (3) history of treatment for AMD. Prespecified secondary outcomes included progression along the detailed 11-step AREDS AMD scale[28] and VA losses of 10 or more letters or 15 or more letters from baseline. Eyes that received treatment for neovascular AMD were counted as events in both analyses. Such eyes may also have experienced decreased vision prior to onset of neovascular AMD. When this occurred, the decrease in vision was counted as the event.

Exploratory secondary analyses included progression to the 2 forms of late AMD, neovascular AMD, or CGA: (1) progression to late AMD stratified by baseline AMD status; (2) progression to more severe vision loss of worse than 20/100; (3) progression to loss of 30 or more letters from baseline; (4) analyses of a head-to-head comparison of the AREDS formulation minus beta carotene but with lutein/zeaxanthin added vs the original AREDS formulation including beta carotene but without lutein/zeaxanthin; and (5) analyses of the AREDS formulation with beta carotene and lutein/zeaxanthin vs the AREDS formulation with beta carotene (without lutein/zeaxanthin).

Statistical Analyses

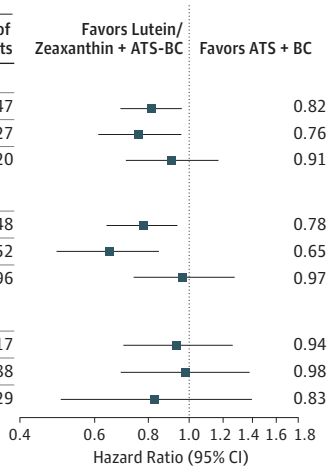
The unit of analysis for ophthalmic outcomes was by eye. The secondary and exploratory ophthalmic outcomes were assessed using Cox proportional hazards models with the Wei et al[29] method for obtaining robust variance estimates that adjusted for dependence among multiple event times (multiple study eyes) adjusted for baseline AMD status only. The assumptions for proportional hazards models were tested and met for all outcomes. Participants lost to follow-up or who died during the course of the study were censored at the time of the last contact. Hazard ratios and 95% CIs were computed. When analyses were restricted to participants taking beta carotene, these analyses were restricted to nonsmokers only because they were the only participants eligible for randomization to beta carotene vs no beta carotene. All analyses were conducted following the intention-to-treat principle and using SAS software version 9.2 (SAS Institute Inc).

Results

Baseline characteristics of the AREDS2 cohort were comparable across the 4 treatment groups in the primary randomization.[22] The baseline characteristics of the partici-

Figure 2. Comparison of Lutein/Zeaxanthin Plus Age-Related Eye Disease Study (AREDS) Supplements Without Beta Carotene (BC) vs AREDS Supplements With BC and No Lutein/Zeaxanthin

	Lutein/Zeaxanthin + ATS-BC		ATS + BC			
	No. of Eyes	No. of Events	No. of Eyes	No. of Events	Favors Lutein/ Zeaxanthin + ATS-BC / Favors ATS + BC	*P* Value
Late AMD						
All eyes	1114	310	1117	347	0.82 (0.69-0.96)	.02
Large drusen OU	862	176	886	227	0.76 (0.61-0.96)	.02
Late AMD in 1 eye	252	134	231	120	0.91 (0.71-1.17)	.47
Neovascular AMD						
All eyes	1114	209	1117	248	0.78 (0.64-0.94)	.01
Large drusen OU	862	99	886	152	0.65 (0.49-0.85)	.002
Late AMD in 1 eye	252	110	231	96	0.97 (0.74-1.28)	.84
Central geographic atrophy						
All eyes	1114	112	1117	117	0.94 (0.70-1.26)	.67
Large drusen OU	862	84	886	88	0.98 (0.69-1.39)	.91
Late AMD in 1 eye	252	28	231	29	0.83 (0.50-1.40)	.49



Head-to-head analysis of lutein/zeaxanthin vs BC for progression to late age-related macular degeneration (AMD) and the 2 forms of late AMD, neovascular AMD and central geographic atrophy. These were also subdivided by the baseline AMD status and bilateral (OU) large drusen or bilateral large drusen with late AMD in 1 eye. ATS indicates AREDS-type supplement.

pants assigned to lutein/zeaxanthin vs no lutein/zeaxanthin (eTable 1 in Supplement) as well as the cohort randomized to beta carotene vs no beta carotene (eTable 2 in Supplement) were comparable. The ocular and other characteristics regarding compliance and follow-up are found in eAppendix 1 (Supplement).

Dietary and Serum Levels of Lutein/Zeaxanthin

Compared with general-population participants sampled in the National Health and Nutrition Survey 2005-2006 of similar ages,[22] AREDS2 participants had a much higher dietary intake and mean serum levels of lutein/zeaxanthin. Baseline dietary intake of the study nutrients, including those of the AREDS supplements, was balanced across treatment groups.[22]

The serum levels of the study nutrients at baseline were balanced across the treatment groups.[22] The median baseline serum levels of lutein/zeaxanthin in participants randomized to lutein/zeaxanthin increased by 190% to 210% at years 1, 3, and 5, while those randomized to placebo showed essentially no change. Participants randomized to lutein/zeaxanthin and beta carotene had a similar increase in serum lutein/zeaxanthin as those randomized to lutein/zeaxanthin without beta carotene; however, at year 5, these levels were lower in the participants receiving lutein/zeaxanthin and beta carotene than observed in those randomized to lutein/zeaxanthin alone ($P = .05$) (eTable 3 in Supplement).

Lutein/Zeaxanthin vs Beta Carotene

In an exploratory subgroup analysis, participants assigned to lutein/zeaxanthin and the AREDS formulation minus beta carotene (n = 1114 eyes) were compared with those assigned to no lutein/zeaxanthin and the original AREDS formulation with beta carotene (n = 1117 eyes); HRs were 0.82 (95% CI, 0.69-0.96; $P = .02$) for progression to late 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 CGA (**Figure 2**).

Lutein/Zeaxanthin Plus Beta Carotene vs Beta Carotene

Further exploratory analyses compared participants assigned to lutein/zeaxanthin and AREDS supplements with beta carotene (n = 1104 eyes) vs no lutein/zeaxanthin and AREDS supplements with beta carotene (n = 1117 eyes), with HRs of 0.82 (95% CI, 0.69-0.97; $P = .02$) for development of late AMD, 0.72 (95% CI, 0.59-0.89; $P = .002$) for neovascular AMD, and 1.07 (95% CI, 0.81-1.42; $P = .62$) for CGA (eFigure 2 in Supplement).

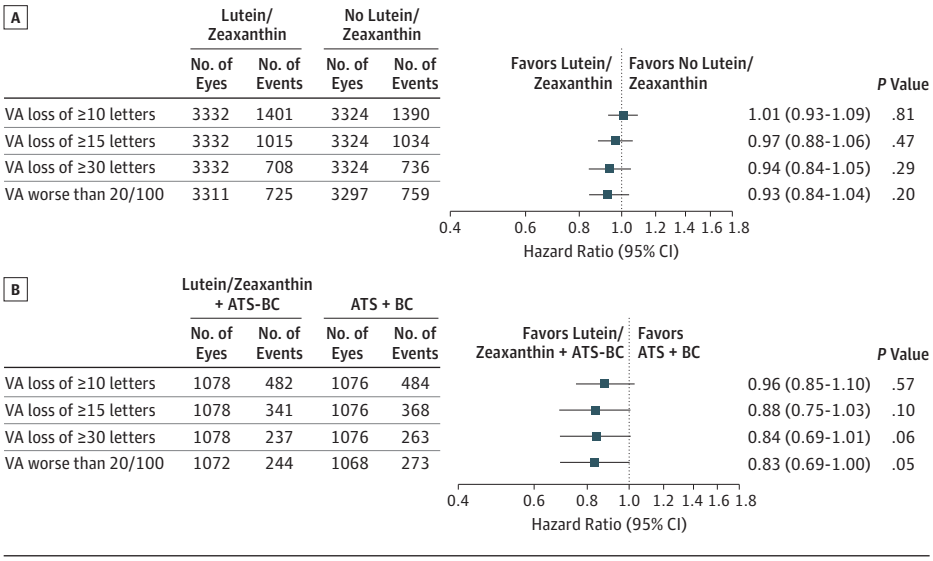
Progression to Late AMD Stratified by Baseline AMD Status

In exploratory analyses stratified by baseline AMD severity and bilateral large drusen or late AMD in 1 eye, the lutein/zeaxanthin vs no lutein/zeaxanthin comparison for progression to late AMD demonstrated HRs of 0.87 (95% CI, 0.77-0.95; $P = .04$) for those who had bilateral large drusen at baseline and 0.96 (95% CI, 0.83-1.11; $P = .59$) for those with late AMD in 1 eye (Figure 1). For the development of neovascular AMD in the comparison of lutein/zeaxanthin vs no lutein/zeaxanthin, the results included HRs of 0.80 (95% CI, 0.68-0.95; $P = .01$) and 1.02 (95% CI, 0.86-1.21; $P = .79$) for bilateral large drusen and late AMD in 1 eye at baseline, respectively (Figure 1). Again, when comparing lutein/zeaxanthin vs no lutein/zeaxanthin for the development of CGA, the results included HRs of 0.94 (95% CI, 0.78-1.13; $P = .51$) and 0.85 (95% CI, 0.64-1.13; $P = .27$) for bilateral large drusen and late AMD in 1 eye at baseline, respectively (Figure 2).

Progression Along the AREDS AMD Scale

A detailed severity scale for AMD progression was developed using the AREDS data.[28] A prespecified analysis compared lutein/zeaxanthin vs no lutein/zeaxanthin for progression along the scale or the development of late AMD. Eyes with the most severe stages of AMD at baseline (steps 10 and 11, which indicated CGA and neovascular AMD, respectively) were excluded from these analyses. Comparison of lutein/zeaxanthin vs no lutein/zeaxanthin for progression along the

Figure 3. Comparisons of Treatments



A, Comparison of lutein/zeaxanthin vs no lutein/zeaxanthin for effects on visual acuity (VA) loss (≥10 letters, ≥15 letters, and ≥30 letters lost compared with baseline) and the development of VA worse than 20/100. B, Comparison of lutein/zeaxanthin plus AREDS supplement without beta carotene (BC) vs AREDS formulation with beta carotene (without lutein/zeaxanthin) on VA outcomes. ATS indicates AREDS-type supplement.

AREDS AMD scale showed a HR of 0.96 (95% CI, 0.89-1.03; $P = .26$) for 2 or more step changes. Additionally, in similar analyses restricted to those randomly assigned to lutein/zeaxanthin and AREDS supplements minus beta carotene vs no lutein/zeaxanthin and AREDS supplements with beta carotene, a HR of 0.87 (95% CI, 0.77-0.98; $P = .03$) was found for 2 or more step progression. Similar analyses that evaluated various combinations of lutein/zeaxanthin plus beta carotene vs beta carotene alone were supportive of lutein/zeaxanthin (data not shown).

Visual Acuity Outcomes

The prespecified secondary analyses of lutein/zeaxanthin vs no lutein/zeaxanthin for outcomes were vision loss with a decrease in VA from baseline of 15 or more letters and vision loss of 10 or more letters from baseline and demonstrated no apparent treatment effect, with HRs of 1.01 (95% CI, 0.93-1.09; $P = .81$) and 0.97 (95% CI, 0.88-1.06; $P = .47$) for a loss of 10 or more letters and a loss of 15 or more letters, respectively (**Figure 3**A). Exploratory comparisons of lutein/zeaxanthin vs no lutein/zeaxanthin for vision loss of 30 or more letters from baseline or the need for AMD treatment and for VA worse than 20/100 or the need for treatment resulted in HRs of 0.94 (95% CI, 0.84-1.05; $P = .29$) and 0.93 (95% CI, 0.84-1.04; $P = .20$), respectively.

The exploratory comparison of lutein/zeaxanthin and the AREDS formulation without beta carotene vs AREDS formulation with beta carotene for the various VA outcomes are demonstrated in Figure 3B. Those comparisons of the head-to-head analyses of lutein/zeaxanthin vs beta carotene favored lutein/zeaxanthin for reducing the VA loss from baseline.

Discussion

In this large, multicentered, placebo-controlled randomized clinical trial of people at high risk for developing late AMD, daily additional supplementation with lutein/zeaxanthin and omega-3 LCPUFAs (DHA/EPA) combined with modified versions of the AREDS formulation showed no clinically or statistically significant overall effect on progression to late AMD in the primary analyses. Because DHA/EPA and the varying doses of zinc appeared to have no apparent effect on the outcome,[22] the prespecified comparison of those taking and not taking lutein/zeaxanthin (main-effects analysis) was appropriate.

This prespecified main-effects analysis demonstrated a favorable effect of lutein/zeaxanthin for progression to late AMD. Other prespecified analyses included the main effects of lutein/zeaxanthin on progression along the AMD scale and on VA outcomes of losses of 10 or more letters or 15 or more letters from baseline. Visual acuity outcomes showed no difference, while the remaining results generally favored lutein/zeaxanthin.

Exploratory analyses included stratified analyses by baseline AMD status and progression to the 2 forms of late AMD. Other exploratory analyses included the comparison of the secondary randomization of participants assigned to various combinations of the carotenoids (lutein/zeaxanthin plus the AREDS formulation with or without beta carotene vs AREDS with beta carotene) for progression to the 2 forms of late AMD, severe visual loss outcomes, and progression along the AMD scale. The pure head-to-head exploratory analyses of lutein/zeaxanthin alone vs beta carotene alone showed beneficial effects of lutein/zeaxanthin for reducing progression to late AMD particularly neovascular AMD. These data were further strengthened by the additional analyses of comparison of those assigned to lutein/zeaxanthin plus beta carotene vs beta carotene alone because lutein/zeaxanthin was again beneficial in reducing the risk for late AMD and neovascular AMD. These analyses suggest that beta carotene does not contribute to a synergistic effect to lutein/zeaxanthin because of similar point estimates in favor of lutein/zeaxanthin in these comparisons. Additional exploratory analyses of lutein/zeaxanthin plus the AREDS formulation with beta

carotene vs lutein/zeaxanthin plus AREDS without beta carotene revealed a HR of 1.00 (95% CI, 0.84-1.19). These analyses support the notion that lutein/zeaxanthin may be an important carotenoid to consider for the AREDS supplement.

When the analyses were conducted to evaluate the treatment effect on the 2 forms of late AMD, there was a trend toward a reduction particularly in the rates of development of neovascular AMD, although the lower rates of development of CGA in AREDS2 may have limited our power to evaluate the treatment effect on geographic atrophy. Similarly in AREDS, the long-term assessment of the beneficial effects of the AREDS formulation was most prominent in preventing the development of neovascular AMD.[4] It is plausible that the AREDS formulation and the addition of lutein/zeaxanthin did not have any effect on geographic atrophy. The AREDS long-term follow-up data[30] demonstrated that 30% of participants with geographic atrophy will develop neovascular AMD in 5 years, further providing evidence for participants with geographic atrophy to consider taking the AREDS formulation.

Additional exploratory analyses restricted to AREDS2 participants with bilateral large drusen at baseline also pointed toward a beneficial effect of lutein/zeaxanthin for progression to late AMD but not for participants with baseline late AMD in 1 eye. Inadequate sample size may be a reason for different results based on baseline AMD status or it may be owing to problems with subgroup analyses. In contrast, in the original AREDS, the beneficial effect of the AREDS formulation was demonstrated in the subgroup analyses of those participants with late AMD in 1 eye at baseline. Based on current clinical data, it would be difficult to speculate whether there is a different mechanism of action with progression to late disease in participants who had different baseline AMD severities.

In AREDS, there was also an accompanying statistically significant reduction in VA loss in those assigned to the AREDS formulation, while the beneficial effect of lutein/zeaxanthin was evident only in the AREDS2 exploratory analyses of the more severe VA loss. This may be partially explained by the introduction of anti-vascular endothelial growth factor therapy for neovascular AMD since the start of AREDS2 resulting in less vision loss. The comparison group of the AREDS cohort was a true placebo group, while the AREDS2 comparison group included participants taking the AREDS formulation. This may account for VA improvements evident in AREDS.

In analyses restricted to nonsmokers, incident lung cancers were more frequent in the AREDS participants assigned beta carotene (28 of 1348 [2.1%]) than those not assigned to beta carotene (11 of 1341 [0.9%]) ($P = .04$; χ^2 goodness-of-fit test for equal proportions).[22] Of those who developed lung cancer, 91% were former smokers. We specifically evaluated the rate of incident lung cancer in all participants including smokers. There were similar rates of lung cancer in the lutein/zeaxanthin and no-lutein/zeaxanthin groups (33 of 2123 [1.6%] vs 31 of 2080 [1.5%]; $P = .80$), with 62% occurring in former smokers in both treatment arms. These data, combined with results from previous studies, suggest that beta carotene supplements should not be recommended for current or former smokers, who comprise a large proportion of the population older than age 60 years. In AREDS and AREDS2, 50% were former smokers and 7% to 13% were current smokers. Estimates of the proportion of smokers and former smokers in population-based studies exceed 50% and the proportion of current smokers may be as high as 25%.[31-34] Providing an AREDS formulation without beta carotene would eliminate the risk for lung cancer that is associated with beta carotene supplementation.

The strengths of this study included the high statistical precision for our primary outcomes, low rates of losses to follow-up, and consistently good adherence to the treatment regimen. There were several limitations of this study. Generalizability of our results may be limited because the AREDS2 population appeared to be well nourished with above-average intake of dietary nutrients. Another major limitation of this report was that it was largely based on exploratory analyses in the face of negative primary study results. Multiple comparisons were conducted without adjustments. Whether a more stringent 99% confidence bounds should have been performed is balanced by the fact that an individual association cannot be more or less likely to be caused by chance based on how many other associations were assessed.[35,36] Ultimately, we reported both significant and nonsignificant findings along with corresponding confidence intervals and *P* values. The interpretations of the results were based not just on the *P* values but also on previous analyses of nutrition and AMD and biologic plausibility of the results. When all subgroup analyses that evaluated the effect of lutein/zeaxanthin supplementation on progression to late AMD were inspected, point estimates were uniformly in the direction of a protective effect. For safety reasons, especially for current and former smokers, it is important to have an AREDS-type formulation without beta carotene. The totality of evidence on the beneficial and adverse effects from AREDS2 and other studies suggest that lutein/zeaxanthin could be more appropriate than beta carotene for the new AREDS2 formulation.

ARTICLE INFORMATION

Submitted for Publication: June 18, 2013; final revision received October 23, 2013; accepted October 29, 2013.

Published Online: December 5, 2013. doi:10.1001/jamaophthalmol.2013.7376.

AREDS2 Research Group Authors/Writing Team: The writing team included the following investigators: Emily Y. Chew, MD; Traci E. Clemons, PhD; John Paul SanGiovanni, ScD; Ronald P. Danis, MD; Frederick L. Ferris III, MD; Michael J. Elman, MD; Andrew N. Antoszyk, MD; Alan J. Ruby, MD; David Orth, MD; Susan B. Bressler, MD; Gary E. Fish, MD; George Baker Hubbard, MD; Michael L. Klein, MD; Suresh R. Chandra, MD; Barbara A. Blodi, MD; Amitha Domalpally, MD; Thomas Friberg, MD; Wai T. Wong, MD, PhD; Philip J. Rosenfeld, MD; Elvira Agrón, MA; Cynthia A. Toth, MD; Paul S. Bernstein, MD, PhD; Robert D. Sperduto, MD.

AREDS2 Research Group Authors/Writing Team Affiliations: Division of Epidemiology and Clinical Research, National Institutes of Health, Bethesda, Maryland (Chew); EMMES Corp, Rockville, Maryland (Clemons, Sperduto); National Eye Institute, Clinical Trials Branch, Bethesda, Maryland (SanGiovanni); Department of Ophthalmology, University of Wisconsin–Madison (Danis); National Eye Institute, Bethesda, Maryland (Ferris, Agrón); Elman Retina Group, Baltimore, Maryland (Elman); Charlotte Eye Ear Nose and Throat, Charlotte, North Carolina (Antoszyk); Associated Retinal Consultants, William Beaumont Hospital, Royal Oak, Michigan (Ruby); Ingalls Memorial Hospital, Harvey, Illinois (Orth); Department of Ophthalmology, Wilmer Eye Institute, Johns Hopkins Hospital, Baltimore, Maryland (Bressler); Texas Retina Associates, Dallas, Texas (Fish); Emory

University, Atlanta, Georgia (Hubbard); Department of Ophthalmology, Casey Eye Institute, Oregon Health and Science University, Portland (Klein); Department of Ophthalmology and Visual Sciences, University of Wisconsin–Madison School of Medicine and Public Health (Chandra, Blodi, Domalpally); Fundus Photograph Reading Center, University of Wisconsin–Madison (Blodi); Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania (Friberg); Unit on Neuron-Glia Interactions in Retinal Disease, National Eye Institute, National Institutes of Health, Bethesda, Maryland (Wong); Department of Ophthalmology, Bascom Palmer Eye Institute, Miami, Florida (Rosenfeld); Duke University Medical Center, Durham, North Carolina (Toth); Department of Ophthalmology and Visual Sciences, Moran Eye Center, University of Utah Health Sciences Center, University of Utah, Salt Lake City (Bernstein).

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.
Study concept and design: Chew, SanGiovanni, Danis, Ferris, Elman, Chandra, Friberg, Sperduto.
Acquisition of data: Danis, Elman, Antoszyk, Ruby, Bressler, Fish, Hubbard, Klein, Chandra, Blodi, Domalpally, Friberg, Wong, Rosenfeld, Toth, Bernstein.
Analysis and interpretation of data: Chew, Clemons, Danis, Ferris, Elman, Antoszyk, Orth, Bressler, Blodi, Domalpally, Friberg, Wong, Rosenfeld, Agrón, Bernstein, Sperduto.
Drafting of the manuscript: Chew, Clemons, SanGiovanni, Bressler, Friberg, Rosenfeld, Agrón.
Critical revision of the manuscript for important intellectual content: Chew, Clemons, Danis, Ferris, Elman, Antoszyk, Ruby, Orth, Fish, Hubbard, Klein, Chandra, Blodi, Domalpally, Friberg, Wong, Rosenfeld, Agrón, Toth, Bernstein, Sperduto.
Statistical analysis: Clemons, Friberg, Agrón, Sperduto.
Obtained funding: Chew, Ferris, Blodi.
Administrative, technical, or material support: Chew, Danis, Ferris, Elman, Antoszyk, Ruby, Klein, Chandra, Domalpally, Sperduto.
Study supervision: Chew, Clemons, SanGiovanni, Danis, Ferris, Elman, Bressler, Friberg, Wong, Toth.

Conflict of Interest Disclosures: Dr Ferris holds a patent for the Age-Related Eye Disease Study (AREDS) formulation with Bausch and Lomb and also receives royalties. Dr Antoszyk receives grant and travel support from Southwest Clinical Research Associates LLC. Dr Ruby receives payment for lectures from Genentech. Dr Bressler receives grant and travel support from Emmes Corp and the Jaeb Center; received a physician-scientist grant from Research to Prevent Blindness; serves as a consultant for GlaxoSmithKline; receives grants or grants pending from Allergan, Bayer Healthcare, Genentech, Lumenis Inc, Notal Vision Ltd, Novartis, Regeneron, Thrombogenics, and sanofi-aventis; received payment for lectures from providers of continuing medical education materials; and served as an investigator on a grant to Johns Hopkins University sponsored by Bausch and Lomb (this grant was 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 has received grant and travel support from Texas Retina Associates. Dr Rosenfeld has served as a consultant for Oraya, Novartis, Chengdu Kanghong Biotech, Acucela, Thrombogenics, and Canon; has received grants or grants pending from Carl Zeiss Meditec, Alexion, Potentia, and GlaxoSmithKline; and has received payment for lectures from Carl Zeiss Meditec, Allergan, and Topcon. Dr Toth has received grant and travel support from Emmes Corp and served as a consultant to ALCON for surgical technologies and research support for Genentech. Dr Bernstein has served as a consultant for Kemin Health, Kalsec, DSM, and Science Based Health. No other disclosures were reported.

Funding/Support: This study was supported by the intramural program funds and contracts from the National Eye Institute/National Institutes of Health (NEI/NIH), Department of Health and Human Services, Bethesda, Maryland (contract No. HHS-N-260-2005-00007-C and ADB contract No. N01-EY-5-0007). Funds were contributed to these contracts by the following NIH institutes: 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. The study medications and raw materials were provided by Alcon, Bausch and Lomb, DSM, and Pfizer.

Role of the Sponsor: The funding agencies had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

REFERENCES

1. Congdon N, O'Colmain B, Klaver CC, et al; Eye Diseases Prevalence Research Group. Causes and prevalence of visual impairment among adults in the United States. *Arch Ophthalmol*. 2004;122(4):477-485.

2. Brown DM, Kaiser PK, Michels M, et al; ANCHOR Study Group. Ranibizumab versus verteporfin for neovascular age-related macular degeneration. *N Engl J Med*. 2006;355(14):1432-1444.

3. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol*. 2001;119(10):1417-1436.

4. Chew EY, Clemons TE, Agrón E, et al; Age-Related Eye Disease Study Research Group. Long-term effects of vitamins C and E, β-carotene, and zinc on age-related macular degeneration: AREDS report no. 35. *Ophthalmology*. 2013;120(8):1604-1611; e4. Epub 2013 Apr 10.

5. SanGiovanni JP, Chew EY, Clemons TE, et al; Age-Related Eye Disease Study Research Group. The relationship of dietary carotenoids, vitamin E, and vitamin C with age-related macular degeneration in a case-control study: AREDS report no. 22. *Arch Ophthalmol*. 2007;125(9):1225-1232.

6. Eye Disease Case-Control Study Group. Antioxidant status and neovascular age-related macular degeneration [published correction appears in *Arch Ophthalmol*. 1993;111(1):1499]. *Arch Ophthalmol*. 1993;111(1):104-109.

7. Seddon JM, Ajani UA, Sperduto RD, et al; Eye Disease Case-Control Study Group. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *JAMA*. 1994;272(18):1413-1420.

8. Mares-Perlman JA, Fisher AI, Klein R, et al. Lutein and zeaxanthin in the diet and serum and their relation to age-related maculopathy in the third national health and nutrition examination survey. *Am J Epidemiol*. 2001;153(5):424-432.

9. Snellen EL, Verbeek AL, Van Den Hoogen GW, Cruysberg JR, Hoyng CB. Neovascular age-related macular degeneration and its relationship to antioxidant intake. *Acta Ophthalmol Scand*. 2002;80(4):368-371.

10. Moeller SM, Parekh N, Tinker L, et al; CAREDS Research Study Group. Associations between intermediate age-related macular degeneration and lutein and zeaxanthin in the Carotenoids in Age-related Eye Disease Study (CAREDS): ancillary study of the Women's Health Initiative. *Arch Ophthalmol*. 2006;124(8):1151-1162.

11. Tan JS, Wang JJ, Flood V, Rochtchina E, Smith W, Mitchell P. Dietary antioxidants and the longer-term incidence of age-related macular degeneration: the Blue-Mountains Eye Study. *Ophthalmology*. 2008;115(2):334-341.

12. Seddon JM, Rosner B, Sperduto RD, et al. Dietary fat and risk for advanced age-related macular degeneration. *Arch Ophthalmol*. 2001;119(8):1191-1199.

13. Seddon JM, Cote J, Rosner B. Progression of age-related macular degeneration: association with dietary fat, transunsaturated fat, nuts, and fish intake. *Arch Ophthalmol*. 2003;121(12):1728-1737.

14. Seddon JM, George S, Rosner B. Cigarette smoking, fish consumption, omega-3 fatty acid intake, and associations with age-related macular degeneration: the US Twin Study of Age-Related Macular Degeneration. *Arch Ophthalmol*. 2006;124(7):995-1001.

15. Chua B, Flood V, Rochtchina E, Wang JJ, Smith W, Mitchell P. Dietary fatty acids and the 5-year incidence of age-related maculopathy. *Arch Ophthalmol*. 2006;124(7):981-986.

16. Augood C, Chakravarthy U, Young I, et al. Oily fish consumption, dietary docosahexaenoic acid and eicosapentaenoic acid intakes, and associations with neovascular age-related macular degeneration. *Am J Clin Nutr*. 2008;88(2):398-406.

17. Swenor BK, Bressler S, Caulfield L, West SK. The impact of fish and shellfish consumption on age-related macular degeneration. *Ophthalmology*. 2010;117(12):2395-2401.

18. SanGiovanni JP, Chew EY, Agrón E, et al; Age-Related Eye Disease Study Research Group. The relationship of dietary lipid intake with incident age-related macular degeneration: AREDS report no. 23. *Arch Ophthalmol*. 2008;126(9):1274-1279.

19. SanGiovanni JP, Agrón E, Clemons TE, Chew EY. Omega-3 long-chain polyunsaturated fatty acid intake inversely associated with 12-year progression to advanced age-related macular degeneration. *Arch Ophthalmol*. 2009;127(1):110-112.

20. SanGiovanni JP, Agrón E, Meleth AD, et al; Age-Related Eye Disease Study Research Group. {omega}-3 Long-chain polyunsaturated fatty acid intake and 12-year incidence of neovascular

age-related macular degeneration and central geographic atrophy: a prospective cohort study from the Age-Related Eye Disease Study. *Am J Clin Nutr*. 2009;90(6):1601-1607.

21. Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. *Mol Aspects Med*. 2005;26(6):459-516.

22. Age-Related Eye Disease Study 2 Research Group. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA*. 2013;309(19):2005-2015.

23. Chew EY, Clemons T, SanGiovanni JP, et al; AREDS2 Research Group. The Age-Related Eye Disease Study 2 (AREDS2): study design and baseline characteristics (AREDS2 report number 1). *Ophthalmology*. 2012;119(11):2282-2289.

24. Albanes D, Heinonen OP, Huttunen JK, et al. Effects of alpha-tocopherol and beta-carotene supplements on cancer incidence in the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study. *Am J Clin Nutr*. 1995;62(6)(suppl):1427S-1430S.

25. Omenn GS, Goodman GE, Thornquist MD, et al. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. *J Natl Cancer Inst*. 1996;88(21):1550-1559.

26. Newsome DA, Swartz M, Leone NC, Elston RC, Miller E. Oral zinc in macular degeneration. *Arch Ophthalmol*. 1988;106(2):192-198.

27. Hambidge M. Underwood Memorial Lecture: human zinc homeostasis: good but not perfect. *J Nutr*. 2003;113(5)(5, suppl 1):1438S-1442S.

28. Davis MD, Gangnon RE, Lee LY, et al; Age-Related Eye Disease Study Group. The Age-Related Eye Disease Study severity scale for age-related macular degeneration: AREDS report no. 17. *Arch Ophthalmol*. 2005;123(11):1484-1498.

29. Wei LJ, Jin DY, Weissfeld L. Regression analysis of multivariate incomplete failure time data by modeling marginal distributions. *J Am Stat Assoc*. 1989;84(408):1065-1073. doi:10.1080/01621459.1989.10478873.

30. Chew EY, Clemons TE, Agrón E, et al; Age-Related Eye Disease Study Research Group. Ten-year follow-up of age-related macular degeneration in the Age-Related Eye Disease Study: AREDS report no. 36. *JAMA Ophthalmol*. In press.

31. Tan JSL, Mitchell P, Kifley A, Flood V, Smith W, Wang JJ. Smoking and the long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study. *Arch Ophthalmol*. 2007;125(8):1089-1095.

32. Tomany SC, Wang JJ, Van Leeuwen R, et al. Risk factors for incident age-related macular degeneration: pooled findings from 3 continents. *Ophthalmology*. 2004;111(7):1280-1287.

33. Tseng TS, Lin HY, Martin MY, Chen T, Partridge EE. Disparities in smoking and cessation status among cancer survivors and non-cancer individuals: a population-based study from National Health and Nutrition Examination Survey. *J Cancer Surviv*. 2010;4(4):313-321.

34. Garrett BE, Dube SR, Trosclair A, Caraballo RS, Pechacek TF; Centers for Disease Control and Prevention (CDC). Cigarette smoking: United States, 1965-2008. *MMWR Surveill Summ*. 2011;60(suppl):109-113.

35. Katz MH. *Multivariable Analysis: A Practical Guide For Clinicians*. 2nd ed. Cambridge, England: Cambridge University Press; 2006:203.

36. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology*. 1990;1(1):43-46.