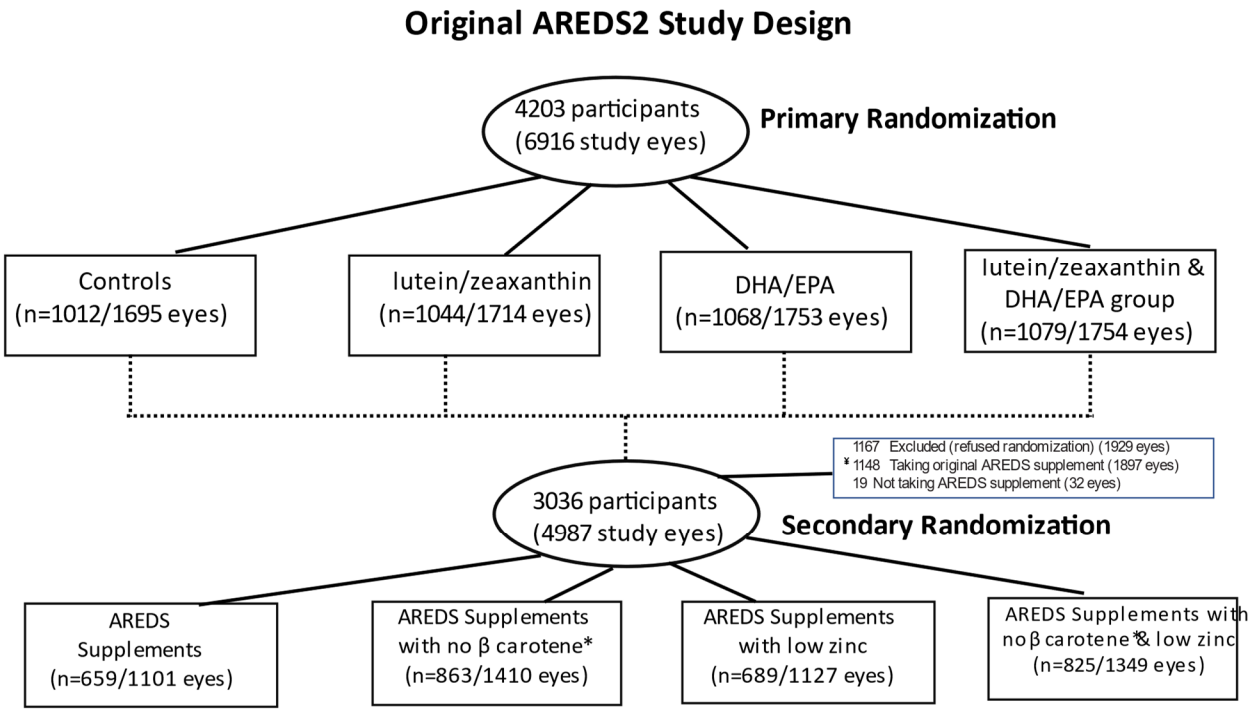
Supplementary Online Content

Chew EY, Clemons TE, Agrón E, et al; AREDS2 Research Group. Long-term outcomes of adding lutein/zeaxanthin and ω-3 fatty acids to the AREDS supplements on age-related macular degeneration progression: AREDS2 report 28. *JAMA Ophthalmol*. Published online June 2, 2022. doi:10.1001/jamaophthalmol.2022.1640

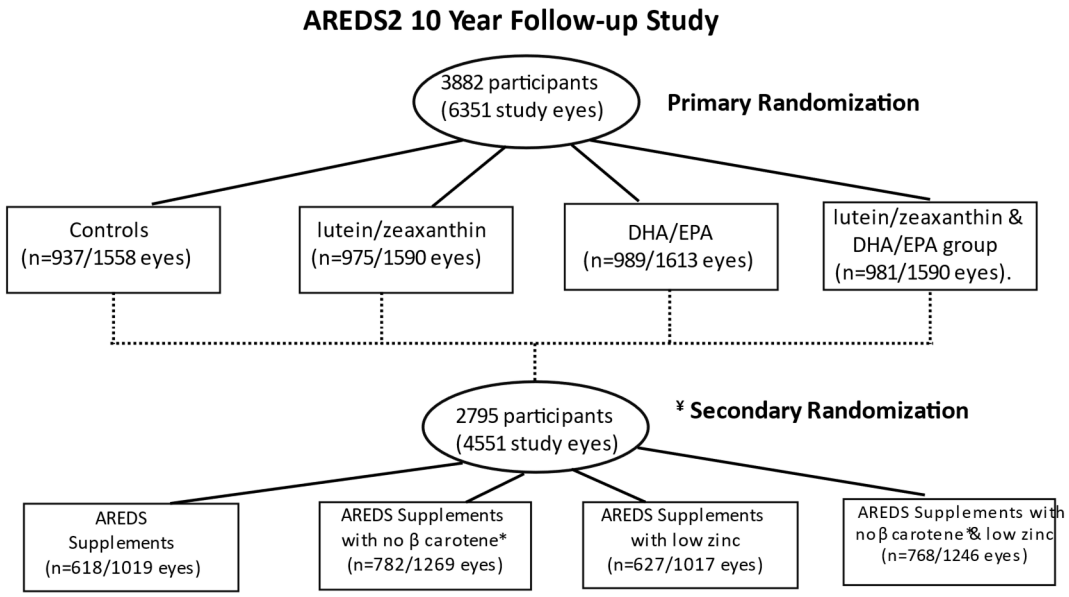
This supplementary material has been provided by the authors to give readers additional information about their work.

eFigure 1. Original AREDS2 Study Design[1]



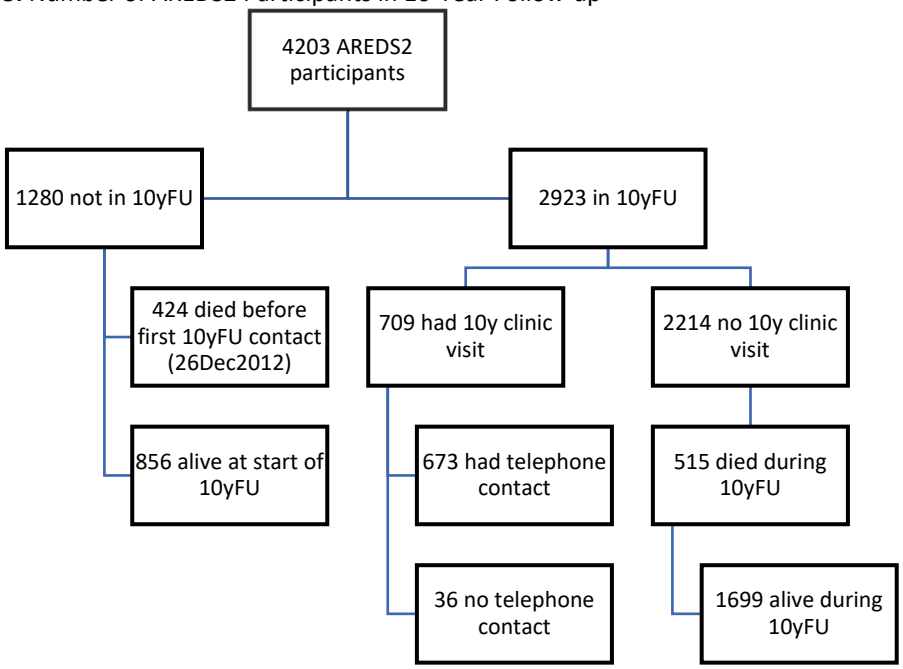
¥1148/4203 (original cohort) opted out of the secondary randomization. They chose to take the original AREDS supplements.
*Smokers were randomized only to these 2 groups (with no beta-carotene). Beta-carotene was given only to participants who were not current smokers or who stopped smoking for 1 or more year.

eFigure 2. AREDS2 10-Year Follow-up Study Design



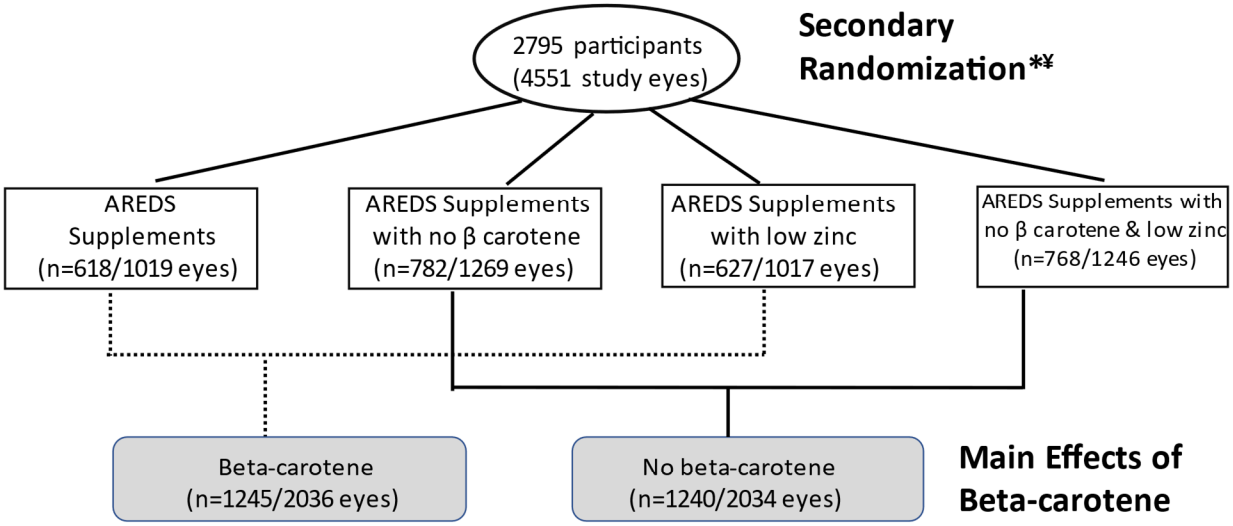
¥1087 (original cohort) opted out of the secondary randomization. They chose to take the original AREDS supplements.
*Smokers were randomized only to these 2 groups (with no beta-carotene). Beta-carotene was given only to participants who were not current smokers or who stopped smoking for 1 or more year.

eFigure 3. Number of AREDS2 Participants in 10-Year Follow-up



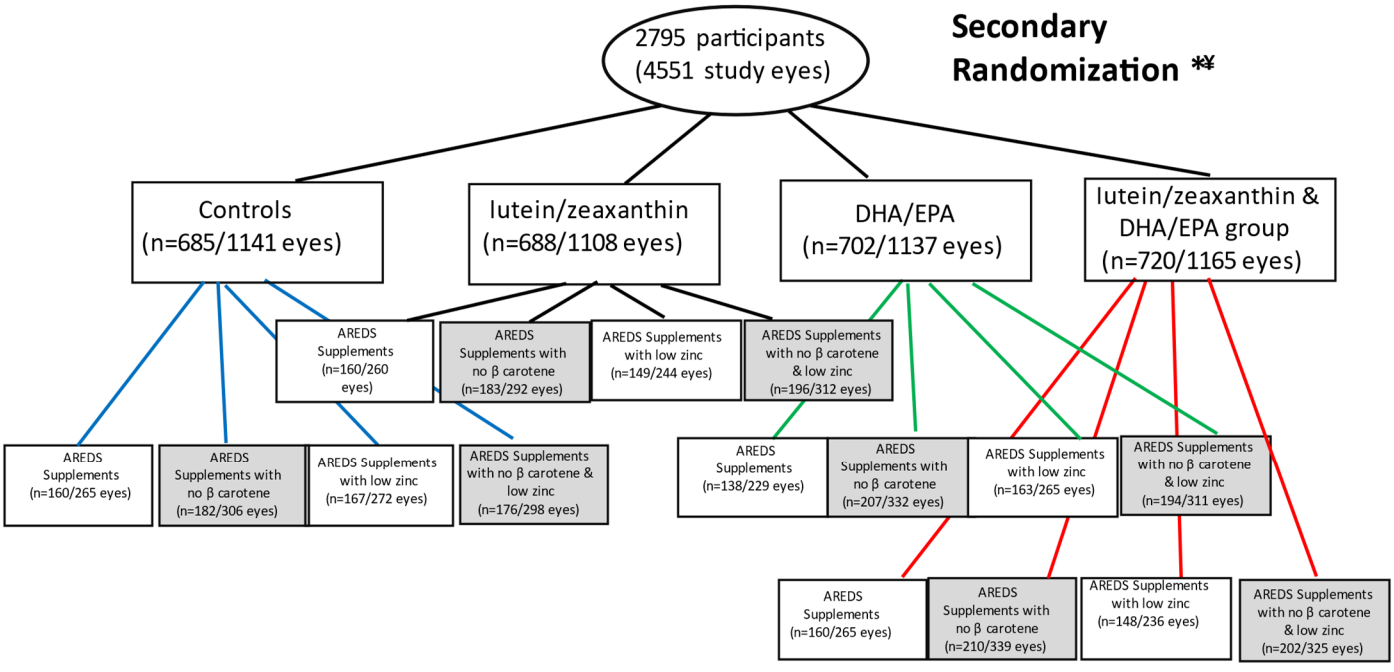
Footnotes:
Of the 424 that died before the first FO contact (26Dec2012), 407 died before the last 5-year close-out visit date (8Nov2012), and 17 died between the last close-out visit and the first 10yFO telephone call (26Dec2012)
*At baseline, 208 participants had bilateral late AMD, 111 had no follow-up color fundus photographs in either eye and 2 had unilateral late AMD at baseline but the non-AMD eye had no gradable follow-up color fundus photographs (leaving **3882** with intermediate AMD in at least one eye eligible for analyses of late AMD)

eFigure 4. Main Effects of Beta carotene Analysis



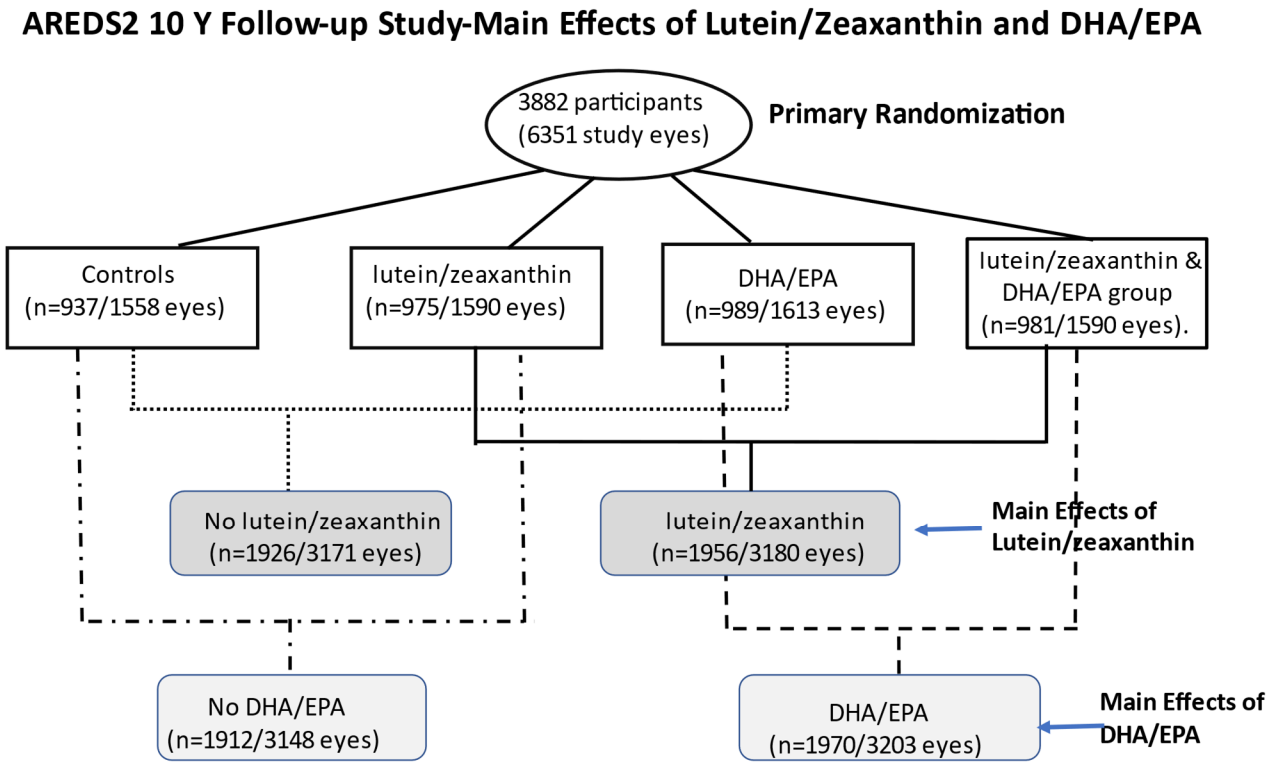
Smokers were randomized only to the groups with no beta-carotene. Beta-carotene was given only to participants who were not current smokers or who stopped smoking for 1 or more year.

eFigure 5. Detailed Evaluation of Beta carotene and Zinc in Secondary Randomization by the Primary Randomization Group

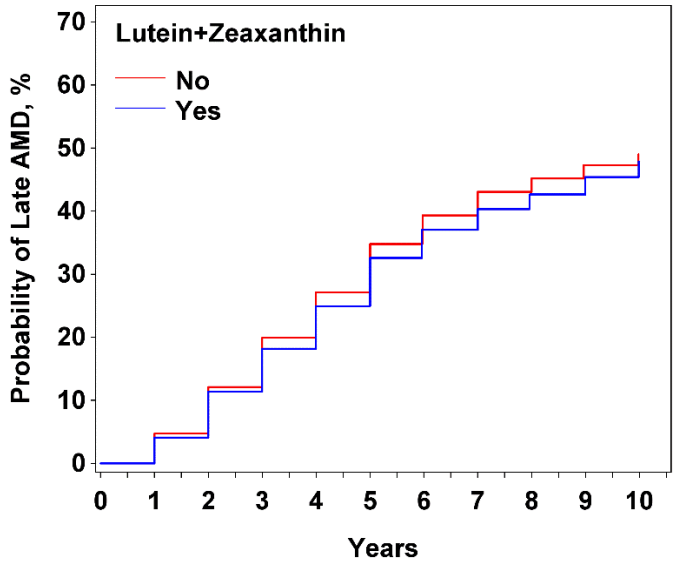


¥1087 (original cohort) opted out of the secondary randomization. They chose to take the original AREDS supplements.
*Smokers were randomized only to the groups with no beta-carotene. Beta-carotene was given only to participants who were not current smokers or who stopped smoking for 1 or more year.

eFigure 6. AREDS2 10-Year Follow-up Study—Main Effects of Lutein/Zeaxanthin and ω-3 Fatty Acids

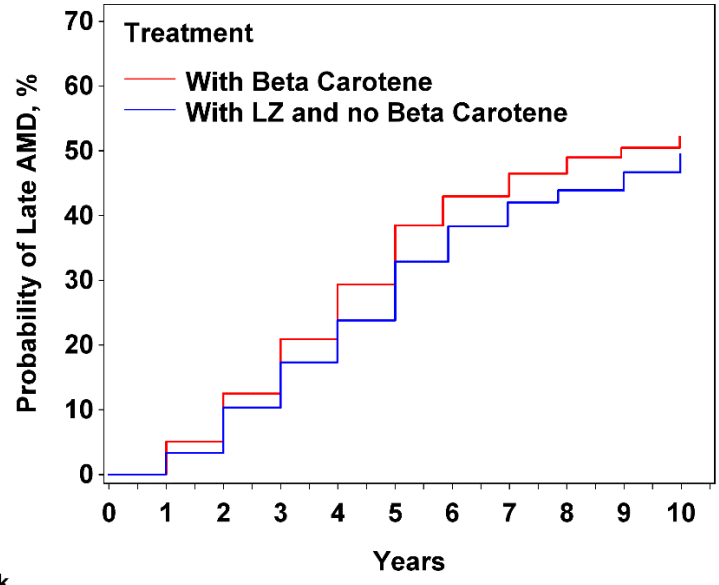


eFigure 7. Progression to Late AMD by Main Effects: Lutein/Zeaxanthin vs No Lutein/Zeaxanthin



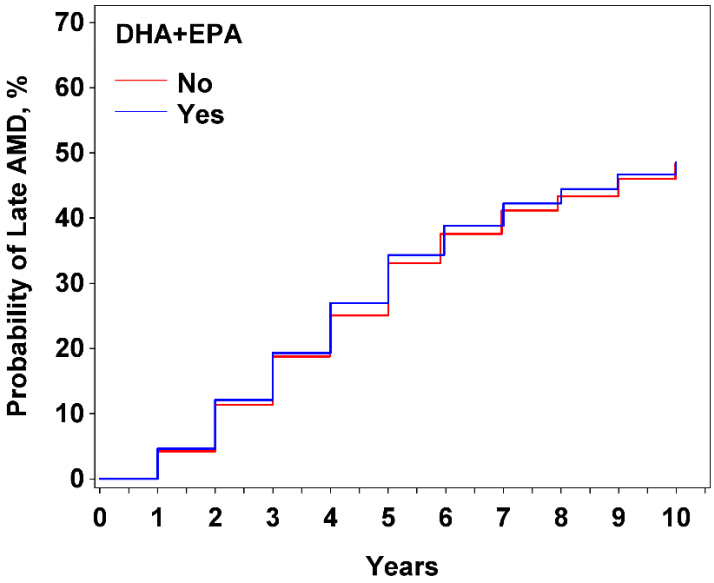
No. eyes at risk											
No Lutein+Zeaxanthin	3171	2990	2688	2385	2096	1724	1487	1361	1280	1192	1028
Lutein+Zeaxanthin	3180	3014	2705	2400	2126	1723	1524	1413	1315	1196	1051

eFigure 8. Progression to Late AMD by Comparing Directly Lutein/Zeaxanthin vs Beta carotene



No. eyes at risk											
With Beta Carotene	1031	965	856	756	649	517	444	409	382	356	311
With LZ and no BC	1026	979	887	786	701	550	473	435	414	375	329

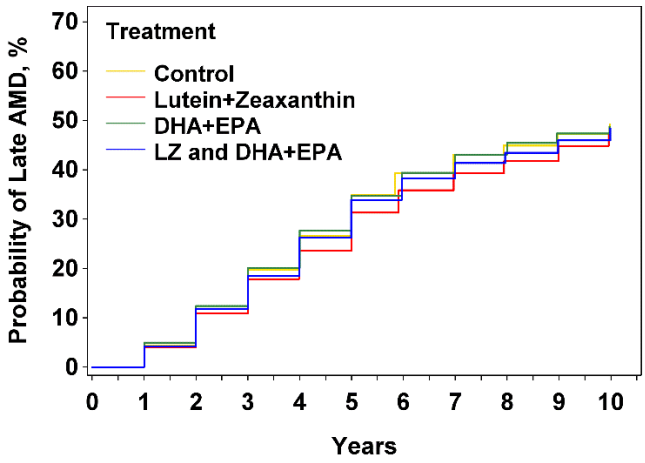
eFigure 9. Progression to Late AMD by Treatment With and Without ω-3 Fatty Acids



No. eyes at risk											
No DHA+EPA	3148	2984	2691	2397	2139	1741	1531	1412	1318	1207	1065
DHA+EPA	3203	3020	2702	2388	2083	1706	1480	1362	1277	1181	1014

DHA and EPA: docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]

eFigure 10. Progression to Late AMD by the 4 Primary Treatment Groups, Lutein/Zeaxanthin, DHA/EPA, Lutein/Zeaxanthin and DHA + EPA, and Controls

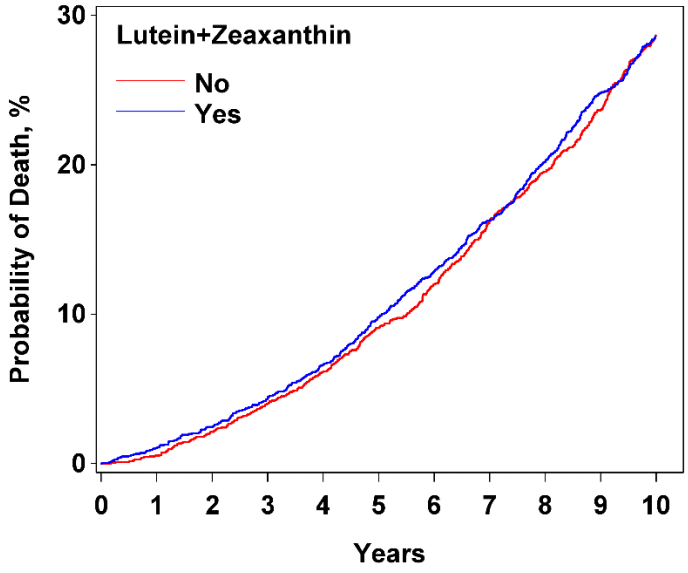


No. eyes at risk											
Control	1558	1475	1321	1176	1041	851	741	679	640	598	526
Lutein+Zeaxanthin	1590	1509	1370	1221	1098	890	790	733	678	609	539
DHA+EPA	1613	1515	1367	1209	1055	873	746	682	640	594	502
LZ and DHA+EPA	1590	1505	1335	1179	1028	833	734	680	637	587	512

DHA and EPA: docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]

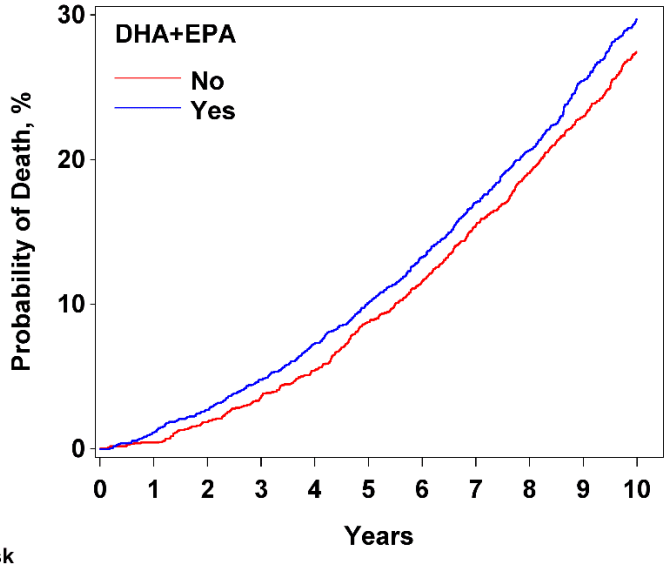
LZ: Lutein/zeaxanthin

eFigure 11. Mortality by Lutein/Zeaxanthin Assignment



No. at risk											
No Lutein+Zeaxanthin	2080	2064	2024	1976	1912	1757	1594	1510	1436	1350	1190
Lutein+Zeaxanthin	2123	2093	2060	2011	1937	1761	1593	1520	1434	1338	1218

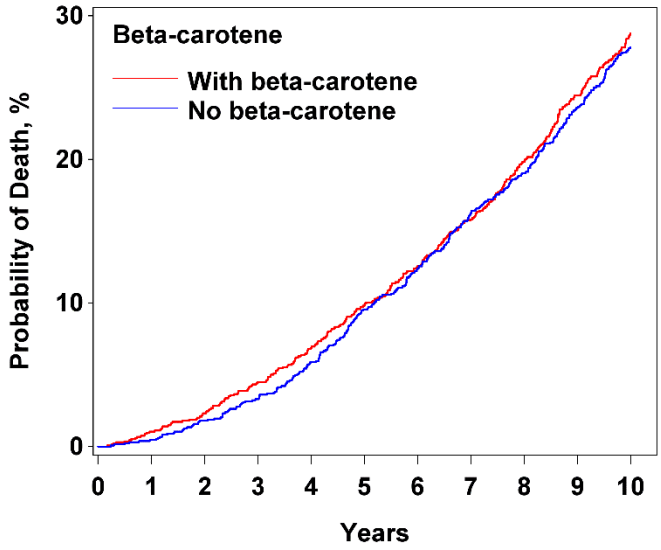
eFigure 12. Mortality by DHA/EPA Assignment



No. at risk											
No DHA+EPA	2056	2040	2007	1962	1898	1734	1562	1484	1403	1326	1191
DHA+EPA	2147	2117	2077	2025	1951	1784	1625	1546	1467	1362	1217

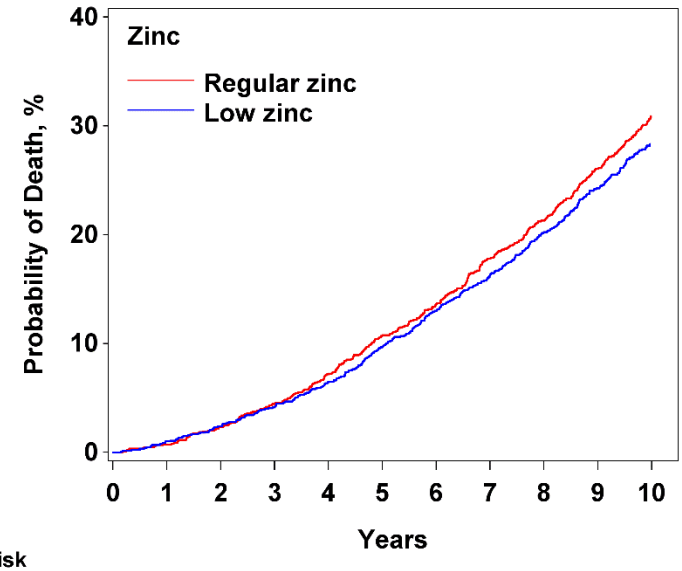
DHA and EPA: docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]

eFigure 13. Mortality by Beta carotene



No. at risk											
With beta-carotene	1348	1330	1310	1276	1229	1130	1021	977	919	861	783
No beta-carotene	1341	1329	1308	1283	1235	1117	1004	956	916	854	771

eFigure 14. Mortality by Zinc Level



No. at risk											
Regular zinc	1522	1507	1478	1439	1381	1251	1125	1063	1004	933	830
Low zinc	1514	1493	1568	1436	1385	1262	1138	1093	1033	973	887

Low zinc: 25 mg, Regular Zinc, 80 mg Zinc oxide

eTable. Characteristics of AREDS2 Participants by Participation Status in the 10-Year Follow-up				
	In 10-year FO	**Not in 10-year FO and alive at start of FO**	**Died prior to 10-year FO**	**P value**
Participants	2923	856	424	
Mean age (years)	72.0 (SD 7.7)	74.9 (SD 7.8)	77.4 (SD 5.9)	<.0001
Male				
Female	1688 (57.7)	507 (59.2)	192 (45.3)	<.0001
Male	1235 (42.3)	349 (40.8)	232 (54.7)	
Race				
Non-white	85 (2.9)	56 (6.5)	4 (0.9)	<.0001
White	2838 (97.1)	800 (93.5)	420 (99.1)	
Education				<.0001
Unknown	51 (1.7)	19 (2.2)	8 (1.9)	
High school or less	836 (28.6)	329 (38.4)	172 (40.6)	
At least some college	1376 (47.1)	370 (43.2)	178 (42)	
Post-graduate	660 (22.6)	138 (16.1)	66 (15.6)	
Smoking				.001
Never	1300 (44.5)	371 (43.3)	153 (36.1)	
Former	1449 (49.6)	421 (49.2)	227 (53.5)	
Current	174 (6.0)	64 (7.5)	44 (10.4)	
Aspirin use				.030
No	1497 (51.2)	457 (53.4)	192 (45.3)	
Yes, <2/day	1387 (47.5)	389 (45.4)	230 (54.2)	
Yes, 2+/day	39 (1.3)	10 (1.2)	2 (0.5)	
Acetaminophen use				.575
No	2658 (90.9)	769 (89.8)	387 (91.3)	
Yes	265 (9.1)	87 (10.2)	37 (8.7)	
NSAID use				.030
No	2596 (88.8)	757 (88.4)	394 (92.9)	
Yes	327 (11.2)	99 (11.6)	30 (7.1)	
Cholesterol drug use				.095
No	1655 (56.6)	463 (54.1)	219 (51.7)	
Yes	1268 (43.4)	393 (45.9)	205 (48.3)	
Diabetes				<.0001
No	2593 (88.7)	726 (84.8)	338 (79.7)	
Yes	330 (11.3)	130 (15.2)	86 (20.3)	
Hx hypertension				<.0001
Unknown	4 (0.1)	2 (0.2)	0 (0)	
No	1269 (43.4)	331 (38.7)	128 (30.2)	
Yes	1650 (56.4)	523 (61.1)	296 (69.8)	
Hx CHF				<.0001
Unknown	8 (0.3)	7 (0.8)	3 (0.7)	
No	2845 (97.3)	811 (94.7)	382 (90.1)	
Yes	70 (2.4)	38 (4.4)	39 (9.2)	
Hx CHD				<.0001
Unknown	21 (0.7)	13 (1.5)	3 (0.7)	
No	2659 (91)	752 (87.9)	350 (82.5)	
Yes	243 (8.3)	91 (10.6)	71 (16.7)	
Hx angina				0.0048
Unknown	11 (0.4)	4 (0.5)	2 (0.5)	
No	2789 (95.4)	810 (94.6)	389 (91.7)	
Yes	123 (4.2)	42 (4.9)	33 (7.8)	
Hx MI				<.0001
Unknown	11 (0.4)	2 (0.2)	0 (0)	
No	2752 (94.1)	788 (92.1)	357 (84.2)	
Yes	160 (5.5)	66 (7.7)	67 (15.8)	
Hx stroke				
Unknown	10 (0.3)	5 (0.6)	2 (0.5)	<.0001
No	2800 (95.8)	796 (93)	379 (89.4)	
Yes	113 (3.9)	55 (6.4)	43 (10.1)	
Hypercholesterolemia				.722
Unknown	7 (0.2)	3 (0.4)	3 (0.7)	
No	1244 (42.6)	355 (41.5)	185 (43.6)	
Yes	1672 (57.2)	498 (58.2)	236 (55.7)	
Baseline best AMD severity score				<.0001
Cannot determine	3 (0.1)	0 (0)	0 (0)	
1-6	1555 (53.2)	399 (46.6)	193 (45.5)	

7	1014 (34.7)	308 (36.0)	144 (34.0)	
8	232 (7.9)	98 (11.4)	52 (12.3)	
9	104 (3.6)	45 (5.3)	32 (7.5)	
10	5 (0.2)	2 (0.2)	0 (0)	
11	10 (0.3)	4 (0.5)	3 (0.7)	
Baseline worse AMD severity score				<.0001
Cannot determine	3 (0.1)	0 (0)	0 (0)	
1-6	708 (24.2)	170 (19.9)	63 (14.9)	
7	893 (30.6)	226 (26.4)	88 (20.8)	
8	266 (9.1)	88 (10.3)	48 (11.3)	
9	112 (3.8)	38 (4.4)	23 (5.4)	
10	94 (3.2)	41 (4.8)	28 (6.6)	
11	847 (29.0)	293 (34.2)	174 (41.0)	

eMethods

The AREDS2 was a double-masked placebo-controlled trial that used a 2x2 factorial design to evaluate the addition of lutein/zeaxanthin (10 mg/2 mg), omega-3 fatty acids (350 mg DHA and 650 mg EPA), or both, to the original AREDS formulation of 80 mg zinc oxide, 2 mg cupric oxide, 500 mg vitamin C, 400 international units vitamin E, and 15 mg beta-carotene.[1] Participants were offered an optional secondary randomization that tested modifications of the original AREDS supplement, specifically 25 mg vs. 80 mg of zinc oxide and 15 mg vs. no beta-carotene (eFigure 1). Only participants who never smoked or stopped smoking for more than 1 year were randomly assigned to the AREDS supplements that contained beta-carotene.

This phase 3 randomized controlled clinical trial enrolled, between 2006 and 2008, 4203 participants (aged 55 to 85 years) in 82 retinal specialty clinics in the United States. All participants had intermediate AMD (large drusen) in both eyes or in one eye while the fellow eye had late AMD. The research was conducted under the tenets of the Declaration of Helsinki and was compliant with the Health Insurance Portability and Accountability Act.

Rationale for an epidemiologic follow-up study. Long-term follow-up studies of completed randomized trials have often been used to assess the durability of the treatment effects, both risks and benefits, beyond the period of randomization. Examples include the long-term follow-up of AREDS to evaluate the effects of AREDS supplement on progression to late AMD;[2] the decades-long follow-up of patients enrolled in the Diabetes Control and Complications Trial (DCCT), named the Epidemiology of Diabetes Interventions and Complications (EDIC), designed to evaluate the effects of the treatment of tight glycemic control on progression of diabetic retinopathy;[3] and the long-term follow-up of participants enrolled in the Physicians' Health Study, assessing the post-trial effect of vitamins on cancer.[4] These all represent post-trial analyses from epidemiologic follow-up beyond the period of the randomized intervention while retaining the intent-to-treat principle analyses. They have the advantage of increasing study power, by longer follow-up time, while retaining the benefit of the original randomization (i.e., avoiding issues of confounding and reverse causation). They provide insights by relating health outcomes back to the original randomization, irrespective of subsequent exposures that might occur following the end of the clinical trial. Thus, the entire period during the clinical trial and the subsequent follow-up period were included in the analyses.

Progression to late AMD. In the AREDS2 follow-up study, participants who had bilateral late AMD at study enrollment at the beginning of the clinical trial were excluded. As previously reported, 11 patients who were enrolled into AREDS2 were subsequently determined to have had bilateral late AMD at baseline, and thus were excluded from those original analyses.[5] Our previous analyses defined late AMD as neovascular AMD or central geographic atrophy (GA) on color fundus photographs. Since the

publication of the results of the AREDS2 clinical trial, experts in the field of AMD research concluded that non-central GA should be included as part of this subtype of late AMD,[6] and the AREDS2 classification was updated to reflect the inclusion of non-central GA in late AMD. For this reason, we also excluded AREDS2 participants who had non-central GA at baseline of the clinical trial as they would have been considered to have late AMD. As was the case in the 5-year clinical trial, each participant included in the follow-up study had at least one eligible study eye that was free of late AMD.

eResults

The number of deaths is greater than the 368 deaths reported in the primary analysis[5], as the additional deaths were unknown and previously considered as losses to follow-up. Deaths in general were identified from reports from family and contacts of the participants and verified with death certificates. It should be noted that all participants who died during the study contributed to the analyses of this current study until the participant was censored at time of death, development of late AMD, study end or lost to follow-up.

As noted above, 11 participants were excluded from the original analyses because of the presence of bilateral late AMD at baseline. Following the change in the outcome measure of late AMD from central GA to include any GA, an additional 197 participants were found to have bilateral late AMD at baseline. After excluding them and the 113 participants with no follow-up fundus photographs, a total of 3882 participants (6351 study eyes) were eligible for the analyses of progression to late AMD. All participants (4203) enrolled in the original study were evaluated for mortality.

The baseline characteristics of both participants and non-participants of the follow-up study who were evaluated are presented in eTable 1. As expected, not all requests for medical records to validate the self-report diagnoses resulted in responses from the treating physicians. However, approximately 90% of all the self-reported cases were confirmed to be positive in the 10 year in-clinic visits while 35% of the self-reports of AMD progression were not fully validated. Approximately 50% of lung cancers were confirmed by medical records.

eDiscussion

Data from both human[7] and animal[8] studies demonstrate that the simultaneous consumption of high doses of lutein/zeaxanthin and beta-carotene suppresses both the serum and tissue levels of lutein/zeaxanthin, because of competitive absorption of carotenoids. Since both beta-carotene and lutein are transported in the blood by the same carrier, beta-carotene tends to suppress the absorption of lutein/zeaxanthin in both the blood and the tissues. This competitive absorption was well demonstrated in the AREDS serum data, the presence of beta-carotene suppressed the serum levels of lutein at years 1 and 5 by as much as 33% compared to baseline.[9] In the AREDS2 clinical trial, at year 5, the serum levels of lutein/zeaxanthin in those participants who were randomly assigned to receive lutein/zeaxanthin with the beta-carotene-containing AREDS supplement were lower (39.1 [SD, 18.7] μg/dL) than those participants who received the beta-carotene-free AREDS supplement (46.9 [SD, 20.3] μg/dL) (p=0.02).[5]

eReferences

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