Oral Supplementation of Lutein/Zeaxanthin and Omega-3 Long Chain Polyunsaturated Fatty Acids in Persons Aged 60 Years or Older, with or without AMD

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Purpose. Increased dietary intake of lutein/zeaxanthin and ω-long-chain polyunsaturated fatty acids (ω-3 LCPUFA) was found to be associated with reduced risk of advanced age-related macular degeneration (AMD). The purpose of the study was to examine the effect of oral supplementation of ω-3 LCPUFA on changes in serum levels of lutein/zeaxanthin during supplementation in persons 60 years of age and older, with or without AMD.

Methods. Forty participants with AMD of various degrees of severity received lutein (10 mg) and zeaxanthin (2 mg) daily and were equally randomized to receive ω-3 LCPUFA (350 mg docosahexaenoic acid [DHA] and 650 mg eicosapentaenoic acid [EPA]) or placebo for 6 months. Serum levels of lutein, zeaxanthin, and ω-3 LCPUFAs and macular pigment optical densities were measured at baseline, 1 week, and 1, 3, 6, and 9 months.

Results. By month 6, the median serum levels of lutein/zeaxanthin increased by two- to threefold compared with baseline. Increases in serum levels of lutein/zeaxanthin did not differ by ω-3 LCPUFA treatment ($P > 0.5$). After 1 month, in the ω-3 LCPUFA-treated group, the median levels of DHA and EPA increased and the placebo group had no changes. At month 6, participants with AMD had a lower increase in serum lutein concentration than did those without AMD ($P < 0.05$).

Conclusions. The addition of ω-3 LCPUFA to oral supplementation of lutein/zeaxanthin did not change the serum levels of lutein and zeaxanthin. A long-term large clinical trial is necessary to investigate the benefits and adverse effects of these factors for the treatment of AMD. (*Invest Ophthalmol Vis Sci.* 2008;49:3864–3869) DOI:10.1167/iovs.07-1420

Age-related macular degeneration (AMD) accounts for more than 50% of blindness in the United States.[1] The number of individuals affected will increase by 50% in the year 2020 with the increased longevity of the aging population.[2] Preventive therapies are important in reducing this increasing burden on society and on the affected individuals and their families. Epidemiologic data suggest that increased dietary intake of the macular xanthophylls lutein and zeaxanthin found in green leafy vegetables such as spinach, kale, and collard greens, is associated with a reduced risk of advanced AMD, with either neovascular[3–5] or geographic atrophy involving the center of fovea.[5] Similarly, epidemiologic data suggest that increased dietary intake of the ω-long-chain polyunsaturated fatty acids (ω-3 LCPUFA), found in fish products, is associated with a reduced risk of advanced AMD.[6,7] No randomized controlled clinical trials of long duration have been conducted to test the possible role of these nutrients in the treatment of AMD. In preparation for a large phase 3 study, the Age-Related Eye Disease Study 2 (AREDS2), which will evaluate these three nutritional supplements for AMD, we reported on a dose-ranging study of lutein.[8] The main purpose of this present study is to obtain data on the effects of ω-3 LCPUFA on the serum level of lutein as well as zeaxanthin. These data are important in the design of AREDS2. We now report on the effects of oral supplementation of ω-3 LCPUFAs on the serum levels of lutein and zeaxanthin of individuals, with or without AMD, who are receiving daily lutein and zeaxanthin.

Subjects and Methods

Study Objectives and Design

The primary objective of this pilot study was to assess whether additional daily oral supplementation of ω-3 LCPUFA (1 g/d, 350 mg docosahexaenoic acid [DHA] and 650 mg of [EPA]) to lutein (10 mg/d) and zeaxanthin (2 mg/d) would change the serum levels of lutein and zeaxanthin in participants over age 60, with or without AMD. The secondary objective was to study whether changes in serum levels of xanthophylls, lutein, and zeaxanthin after oral supplementation would result in changes in macular pigment density. The short-term adverse effects were also assessed for these nutrients.

The materials for the lutein, zeaxanthin, and ω-3 LCPUFAs and their matching placebos were donated by DSM Nutritional Products (Basel, Switzerland) and manufactured at Tishcon Laboratories (Salisbury, MD). Lutein was provided not as esters but as "lutein, 5% triglyceride (TG)" and "zeaxanthin, 5% TG", the beadlet form containing 5% free (unesterfied) lutein or zeaxanthin, respectively.

Forty participants who began taking lutein (10 mg/d) and zeaxanthin (2 mg/d) daily at baseline were randomized to receive ω-3 LCPUFAs (1 g/d) or a matching placebo (20 participants per treatment group) for 6 months. Follow-up continued for an additional 3 months without supplementation. The participants were instructed to take the study supplements together with breakfast, preferably with fat-containing foods. They had AMD of various degrees of severity, from mild to advanced stages including geographic atrophy involving the center of the macula or neovascular AMD in one eye; some had no AMD.

Fasting blood samples were drawn from participants, and serum levels of lutein, zeaxanthin, and other fat-soluble micronutrients were measured in a masked fashion at the Centers for Disease Control (CDC)

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and Prevention (Atlanta, GA). DHA, a major dietary ω-3 LCPUFA and a major structural lipid of retinal photoreceptor outer segment membranes was measured along with nine other ω-3 LCPUFAs, including EPA.[9] We report the serum levels of lutein, zeaxanthin, DHA, and EPA. Dietary sources of lutein from a food-frequency questionnaire (FFQ) that focused only on foods containing lutein (modified by the Minnesota Nutritional Coordinating Center, University of Minnesota, Minneapolis, MN), without adjustments for energy intakes, were collected at each visit. This limited FFQ served to provide documentation of any change in dietary intake of foods high in lutein during the duration of the study.

Macular pigment optical density (MPOD) was measured by heterochromatic flicker photometry (HFP) using a tabletop densitometer,[10] (Macular Metrics, Rehoboth, MA). Color vision testing (Panel D-15) was conducted before HFP. Using visual targets previously described,[11] MPOD was separately measured at 0.25°, 0.5°, 1°, and 1.75° eccentricity from the foveal center, using 468-nm/564-nm test lights that were presented on a 468-nm background (2.6 cd/m^2, 10° diameter); the reference target (1° diameter) was located at 7° eccentricity. A best-corrected visual acuity of 20/60 or better was necessary to visualize the fixation marks at the center of the foveal targets. Details of the results regarding the entire MPOD data set will not be reported here because of limited space, but a separate report will describe the results of the MPOD measurements.

Participants underwent a comprehensive ophthalmic examination at baseline and months 1, 3, 6, and 9, including best-corrected visual acuity, slit lamp biomicroscopy and dilated fundus examination. Fundus photography was conducted at baseline and month 9. Macular pigment measurements were conducted at all visits except for month 1. Fasting blood draws were obtained at each study visit. At the 1-week visit, blood draw, macular pigment measurements, and distribution of the study medications were performed.

The study protocol was approved by the Institutional Review Board of the National Eye Institute (National Institutes of Health, Bethesda, MD). The design and data were reviewed and monitored by the NEI Data and Safety Monitoring Committee. Informed consent was obtained from each study participant before enrollment, in compliance with the Declaration of Helsinki.

Fat-Soluble Micronutrients

Carotenoids, including total lutein and total zeaxanthin, were measured using a modification of a routine HPLC/UV-visible detection method with the major changes being utilization of a smaller volume of serum, a different internal standard, and gradient elution on a high-carbon-load C18 column to separate lutein from zeaxanthin.[12] Quantification was accomplished by comparing the peak height or area of the analyte in serum with the peak height or area of a known amount of standard in a calibrator solution. Calculations were corrected based on the peak height or peak area of the internal standard. Carotenoids were compared with apo-8′-carotenal at 450 nm. Values higher than NHANES III 99% reference ranges were repeated and confirmed. In many cases, samples were diluted by a factor of 2 or 3, and/or different volumes (5, 10, 15, and 30 μL) were injected to assess linearity, and/or samples were processed using multiple extractions to enhance recovery.

ω-3 Long Chain Polyunsaturated Fatty Acids

Total fatty acids were measured by using a modification of the method of Lagerstedt et al.[13] Briefly, esterified fatty acids were hydrolyzed from triglycerides, phospholipids, and cholesteryl esters, by using sequential treatment with mineral acid and base in the presence of heat, and hexane-extracted from the matrix along with an internal standard solution containing five stable isotopically labeled fatty acids to account for recovery. The extract was derivatized with pentaflurobenzyl bromide in the presence of triethylamine to form pentaflurobenzyl esters. The reaction mixture was injected onto a capillary gas chromatograph column to resolve individual fatty acids, which were detected using electron, capture negative-ion mass spectrometry on a single-quadrupole system (DSQ; Thermo Scientific, Waltham, MA). Four ω-3 and six ω-6 fatty acids were measured with selected ion monitoring. Quantitation was accomplished by comparing the peak area of the analyte in the unknown with the peak area of a known amount in a calibrator solution. Calculations were corrected based on the peak area of the internal standard in the unknown compared with the peak area of the internal standard in the calibrator solution.

Statistical Analysis Methods

The primary outcome measures in this study were the serum lutein, zeaxanthin, and fatty acid concentrations. To assess the reproducibility of the assay, serum concentrations were measured twice on a subset of samples with masked relabeling of vials. The results were compared with correlation coefficient analysis (test–retest correlations, $r > 0.94$). The short-term variability of the assay was assessed by a paired-*t*-test in comparing serum concentrations at baseline and week 1. Cross-sectional analyses of the primary and secondary outcomes for each study visit were examined with a two-sample mean test. When a moderately significant difference appeared between two groups at a scheduled visit, the generalized estimating equation method was applied to investigate the trends and variations of outcomes measured over the 6-month supplementation course of the study.[14]

Results

Forty participants were enrolled, ranging from 64 to 86 years (mean, 73 years) of age (Table 1). Thirty-six (90%) of the study participants were white; 23 (58%) were female. Fourteen of them had intermediate AMD (AREDS Category 3) with bilateral large drusen and hyper/hypopigmentary changes of the retinal pigment epithelium (RPE), whereas seven had advanced AMD, either neovascular or geographic atrophy involving the center of the fovea (AREDS category 4). All participants completed the 9-month final study visit.

Participant Adherence to Study Drugs

Study drug in tablet form was dispensed to participants at week 1. The bottles were weighed at each study visit and compared to the baseline weights for compliance. The bottle weights were similar in the two treatment groups and adherence rates of approximately 80% were estimated.

Table 1. Participants' Baseline Characteristics by Treatment Group

	Placebo	ω-3	Overall
Randomized participants	20 (100%)	20 (100%)	40 (100%)
Sex: female	11 (55%)	12 (65%)	23 (58%)
Race: white	17 (85%)	19 (95%)	36 (90%)
Age (Median)	73	72	72
Presence of AMD*	7 (35%)	14 (70%)	21 (53%)
Visual acuity (Better Snellen ≥ 20/20)	13 (65%)	15 (75%)	28 (70%)

* Advanced AMD, neovascular AMD or geographic atrophy involving the center of fovea.

Short-Term Variation

Serum concentrations of lutein, zeaxanthin, DHA and other micronutrients and ω-3 LCPUFAs were assessed at baseline and at the week 1 visit before oral supplementation. The two serum concentrations were found to be similar (mean difference in lutein, zeaxanthin, DHA, and EPA serum concentrations of −0.16 μg/dL, 0.12 μg/dL, 8.93 μmol/L, and 8.463 μmol/L and corresponding significance levels of paired *t*-test of 0.80, 0.15, 0.45, and 0.32, respectively). The average of the two baseline serum concentrations was used as the single baseline value in the repeated-measures analysis of serum concentration.

Changes in Lutein

Serum lutein concentrations significantly increased by the 1-month visit and then stabilized until the cessation of supplementation at month 6 (Fig. 1, left). After oral supplementation for 6 months, median lutein serum concentrations increased from 18.0 to 49.9 μg/dL (~2.8-fold) in the placebo group and from 21 to 37 μg/dL (~1.8-fold) in the ω-3 LCPUFA group (Table 2). Median changes from baseline in lutein serum concentrations are 27.1 and 19.3 μg/dL in the placebo group and ω-3 LCPUFA group, respectively. The magnitude of the increases at the month 6 visit did not differ between the two groups ($P = 0.68$ for *t*-test of mean change comparison and $P = 0.44$ for Wilcoxon rank sum test of median change comparison). Three months after cessation of the oral supplementations (at month 9), lutein serum concentrations returned to their baseline levels. Overall, increases in lutein serum concentration at any postrandomization visit did not differ between the two groups (with or without ω-3 LCPUFA). Therefore, the additional supplement of ω-3 LCPUFA (1 g/d) had no effect on the lutein serum concentration.

Changes in Zeaxanthin

Serum zeaxanthin concentrations significantly increased by the 1-month visit. Median zeaxanthin serum concentrations increased from 4.5 to 10.9 μg/dL (~2.4-fold) in the placebo group and from 4.5 to 8.6 μg/dL (1.9-fold) in the ω-3 LCPUFAs group (Fig. 1, right). Median changes from baseline in zeaxanthin serum concentrations are 5.3 μg/dL and 4.9 μg/dL in the placebo group and ω-3 LCPUFA group, respectively. These increases were not significantly different between the two treatment groups ($P > 0.80$).

Changes in Lutein and Zeaxanthin by AMD Status

Participants with AMD had an increase in serum lutein concentration of ~9.4 μg/dL lower than the participants without AMD during the 6-month supplementation period (95% CI, 0.05–18.78; $P = 0.049$) in repeated-measures analysis with adjustment for age, baseline serum level, and quadratic duration time effect (Fig. 2, left). Participants with AMD also had zeaxanthin levels of 0.60 μg/dL lower than normal participants, but the difference was not statistically significant ($P = 0.210$; Fig. 2, right).

Changes in DHA and EPA

Serum DHA concentrations in those supplemented with ω-3 LCPUFA increased at the month 1 visit and then stabilized until the cessation of supplementation at month 6 (Fig. 3, left). By month 6, the ω-3 LCPUFA-treated group had increase in DHA serum concentrations from 203 to 271 μmol/L (~1.3-fold). The placebo group without ω-3 LCPUFA had been stable but was ~25 μmol/L lower than baseline DHA serum concentrations. The magnitude of changes was significantly different between the two groups ($P < 0.0001$ for *t*-test of mean change comparison and Wilcoxon rank sum test of median change comparison). Three months after the oral supplementation (at month 9) was stopped, DHA serum concentrations returned to baseline levels.

Similar differences appeared in serum EPA concentrations (Fig. 3, right). By month 6, the ω-3 LCPUFA-treated group had an increase in EPA serum concentrations from 94 to 234 μmol/L (~2.5-fold). The placebo group without ω-3 LCPUFA had maintained stable levels but the concentration was approximately 20 μmol/L lower than baseline DHA serum levels.

Changes in FFQ

Dietary intake of lutein and zeaxanthin was estimated from the modified FFQ, which was designed to capture only lutein- and zeaxanthin-containing foods at baseline and at 1-, 3-, 6-, and 9-month visits. Average dietary levels in each dose group remained unchanged during the follow-up (data not shown).

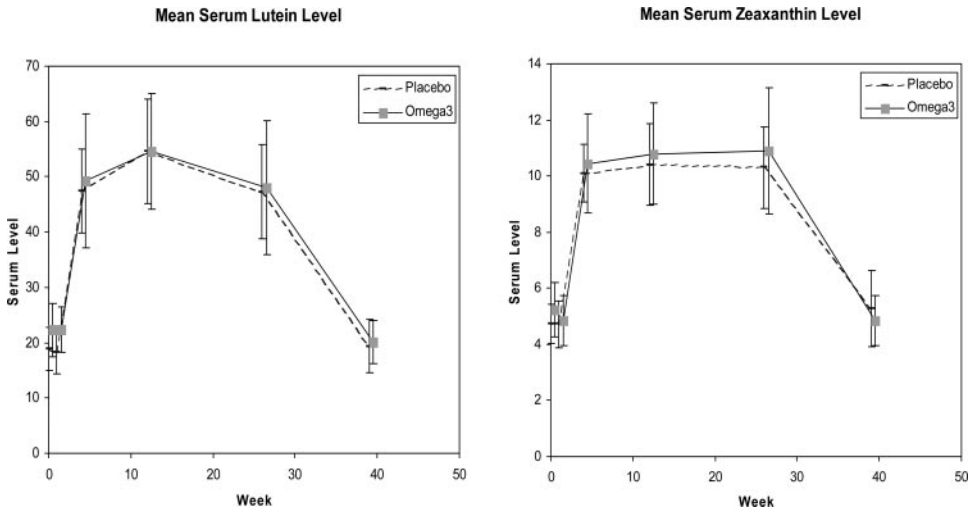

FIGURE 1. Mean serum concentrations of lutein and zeaxanthin by randomization to placebo versus ω-3 LCPUFA with 95% CI. No statistically significant difference was seen in the treatments groups. The concentrations of lutein and zeaxanthin were elevated while the subjects were receiving supplementation, with a return to pretreatment concentrations 3 months after the cessation of the supplements at 9 months.

TABLE 2. Lutein Serum Concentration by Treatment Group

	Mean		Change from Baseline		
Visit	**Placebo**	**ω-3**	**Placebo**	**ω-3**	***P*†/*P*‡**
Week 0					
n	20	20			
Median*	18.0	21.0			
Mean*	18.8	22.3			
(95% CI)	(14.7–23.0)	(17.2–27.4)			
Week 1					
n	19	20			
Median*	17.2	22.9			
Mean*	18.2	22.4			
(95% CI)	(14.0–22.3)	(18.0–26.7)			
Month 1					
n	20	20	20	20	0.80/0.34
Median*	51.2	42.0	32.4	25.2	
Mean*	47.5	49.3	28.5	27.0	
(95% CI)	(39.4–55.5)	(36.3–62.3)	(22.8–34.2)	(15.9–38.1)	
Month 3					
n	20	20	20	20	0.59/0.46
Median*	55.3	51.3	37.7	31.4	
Mean*	54.6	54.6	35.6	32.3	
(95% CI)	(44.4–64.7)	(43.4–65.8)	(26.5–44.7)	(23.3–41.2)	
Month 6					
n	20	20	20	20	0.68/0.44
Median*	49.9	37.0	27.1	19.3	
Mean*	47.3	48.0	28.3	25.7	
(95% CI)	(38.1–56.4)	(35.1–60.9)	(20.8–35.8)	(15.2–36.2)	
Month 9					0.22/0.19
n	20	20	20	20	
Median*	14.4	19.6	0.7	−1.6	
Mean*	19.4	20.2	0.4	−2.1	
(95% CI)	(14.2–24.6)	(16.0–24.3)	(−2.8–3.7)	(−5.0–0.7)	

* Data are in micrograms per deciliter.

P†/*P*‡, *P* of *t*-test/Wilcoxon rank sum test for comparing the mean/median of the two treatment groups.

Thus, the increases in serum lutein and zeaxanthin concentrations were attributed to the supplement.

Visual Acuity

Best-corrected visual acuity, using the ETDRS visual acuity chart, was obtained at each study visit, according to a standardized protocol. Proportions of participants whose visual acuity was at least 20/20 in the better-seeing eye were 65% and 75% for the placebo and the ω-3 LCPUFA-treated groups, respectively, and 74% (14/19) and 67% (14/21) for the participants without AMD and for those with AMD, respectively. No significant changes in visual acuity over the 9-month follow-up were found (data not shown).

Adverse Events

No serious adverse side effects resulting from study medications were reported during the 9-month follow-up. The number of subjects was small and the duration of follow-up was short. A larger sample and longer trial would be necessary to

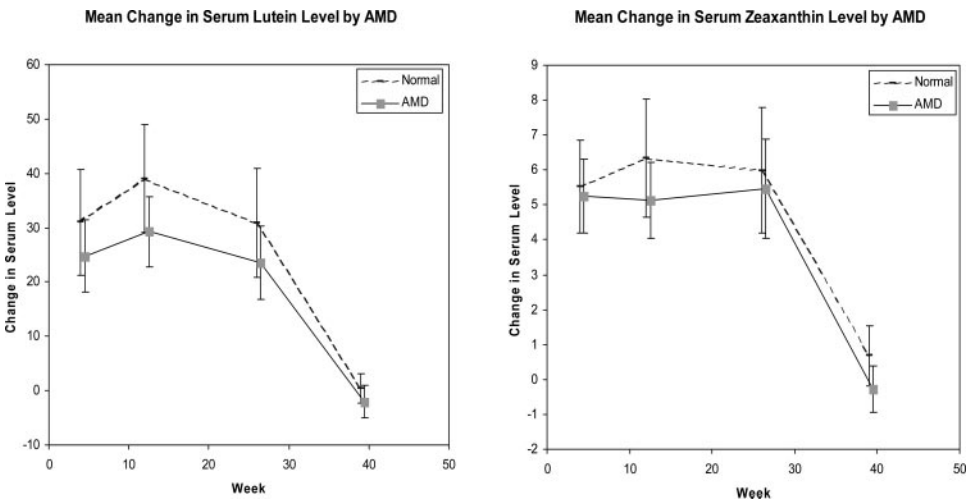

FIGURE 2. Mean serum concentrations of lutein and zeaxanthin by the presence of AMD versus no evidence of AMD, with 95% CIs. The serum concentrations of lutein were lower in persons with AMD ($P = 0.049$ in repeated-measures analyses). The serum concentrations of zeaxanthin were not different among those with or without AMD. Normal, 19 participants (13, placebo group; 6, ω-3 LCPUFA group) with no indication of AMD at baseline. AMD, 21 participants (7, placebo group; 14, ω-3 LCPUFA group) with the presence of any evidence of AMD at baseline.

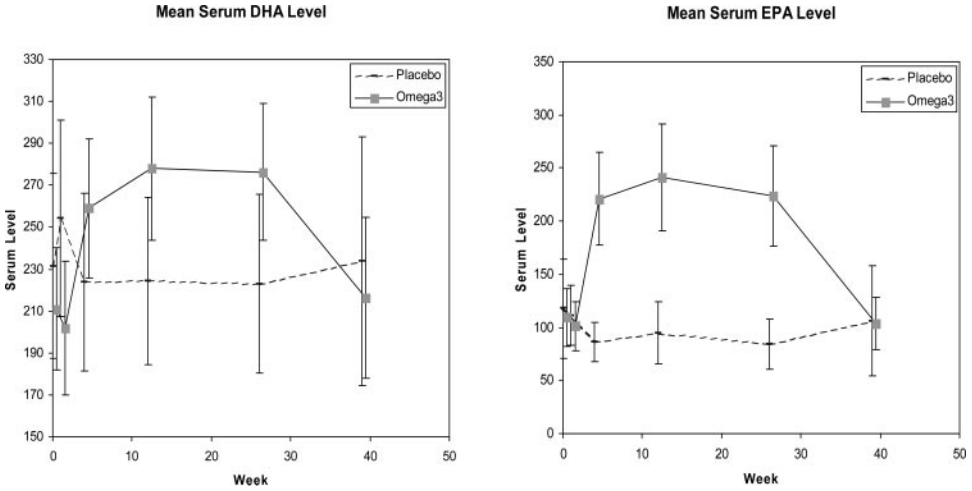

FIGURE 3. Mean serum concentrations of DHA and EPA by treatment group. There are significant differences between these two treatment groups for both DHA and EPA levels while the subjects were receiving supplements.

evaluate the potential adverse effects of these nutritional supplements.

DISCUSSION

In this small pilot study, we were not able to detect any effect of supplementation with ω-3 LCPUFA on the serum concentration of lutein and zeaxanthin. Previous studies showed that taking lutein ester supplements with a high-fat meal increased the plasma lutein substantially when compared with the levels achieved with a low-fat meal (207% vs. 88% increase).[15–17] The bioavailability of lutein also depends on the type of formulation. For example, crystalline preparation of lutein appeared to vary widely within and between subjects. The absorption may be facilitated with cosupplementation with vitamin C.[18]

Serum concentrations of lutein and zeaxanthin in the present study remained elevated during oral supplementation with these xanthophylls for up to 6 months. When the supplements were stopped, the levels returned to baseline within 3 months. It appeared that participants with AMD, while on lutein supplementation, had lower serum lutein levels than those without AMD, similar to the finding in another study of lutein supplements.[8] Subgroup analysis of the data from a previous lutein dose-range study of 5 participants without AMD and 10 participants with AMD taking lutein 10 mg also showed that serum lutein concentrations was approximately 17.2 μg/dL lower in the participants with AMD than those without AMD during the 6-month supplementation period ($P = 0.030$ in the same model). Although statistically significant, these moderately significant differences should be examined in a larger study population. It is well known that repeated serum measurements of many fat-soluble micronutrients in the same person on different occasions show substantial variation. Intraindividual coefficients of variation for carotenoids are typically between 18% and 26%.[19] The concentrations of lutein achieved using a similar supplement in our earlier study,[8] were 0.32-fold higher than in the present study. The differences in serum concentrations of lutein found in these two studies may be due to formulation differences in the supplement or differences in patient instructions or blood-sampling differences. The variation in the serum concentrations of lutein between studies is unlikely to be due to assay differences, because when a subset of 21 samples from the earlier study were retested 4 years later while testing the specimens from the present study, the repeat lutein results were approximately 5% higher (95% CI of the ratio of repeat to original result: 0.991–1.104) and zeaxanthin results were only approximately 7% lower (95% CI, 0.863–0.992). These differences are within the expected analytical variability of the assay and cannot explain the magnitude of the difference in serum concentration from one study to another.

The present study demonstrated that it is feasible to increase the serum concentration of these supplements in participants with and without AMD. In addition, no adverse side effects were detected in this study of relatively small sample size. Testing the efficacy and adverse effects of these nutritional supplements for the therapy of AMD would necessitate further study. Currently, using the results of AREDS and the present study, the National Eye Institute has started a large-scale randomized controlled trial of lutein/zeaxanthin and ω-3 LCPUFA, specifically DHA and EPA, to evaluate the effects on the incidence and progression of AMD in the AREDS 2 (http://www.areds2.org). Four thousand participants with either large drusen or advanced AMD in one eye will be recruited throughout the nation in at least 80 clinical sites. This study will also provide an opportunity to refine the AREDS formulation by assessing formulations without β-carotene and lower doses of zinc. The data from AREDS 2 will provide valuable information regarding the role of these additional nutritional supplements in the development and progression of AMD, a disease of significant public health importance.

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