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Randomized, double-blind, placebo-controlled study of zeaxanthin and visual function in patients with atrophic age-related macular degeneration

The Zeaxanthin and Visual Function Study (ZVF) FDA IND #78, 973

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KEYWORDS
Zeaxanthin;
Lutein;
Carotenoids;
Macular pigment;
Atrophic age-related macular degeneration

Abstract

BACKGROUND: The purpose of this study is to evaluate whether dietary supplementation with the carotenoid zeaxanthin (Zx) raises macula pigment optical density (MPOD) and has unique visual benefits for patients with early atrophic macular degeneration having visual symptoms but lower-risk National Institute of Health/National Eye Institute/Age-Related Eye Disease Study characteristics.

METHODS: This was a 1-year, n = 60 (57 men, 3 women), 4-visit, intention-to-treat, prospective, randomized controlled clinical trial of patients (74.9 years, standard deviation [SD] 10) with mild-to-moderate age-related macular degeneration (AMD) randomly assigned to 1 of 2 dietary supplement carotenoid pigment intervention groups: 8 mg Zx (n = 25) and 8 mg Zx plus 9 mg lutein (L) (n = 25) or 9 mg L ("Faux Placebo," control group, n = 10). Analysis was by Bartlett's test for equal variance, 3-way repeated factors analysis of variance, independent *t* test ($P < 0.05$) for variance and between/within group differences, and post-hoc Scheffé's tests. Estimated foveal heterochromic flicker photometry, 1° macular pigment optical density (MPOD QuantifEye®), low- and high-contrast visual acuity, foveal shape discrimination (Retina Foundation of the Southwest), 10° yellow kinetic visual fields (KVF), glare recovery, contrast sensitivity function (CSF), and 6° blue cone ChromaTest® color

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thresholds were obtained serially at 4, 8, and 12 months.

RESULTS: Ninety percent of subjects completed ≥ 2 visits with an initial Age-Related Eye Disease Study report #18 retinopathy score of 1.4 (1.0 SD)/4.0 and pill intake compliance of 96% with no adverse effects. There were no intergroup differences in 3 major AMD risk factors: age, smoking, and body mass index as well as disease duration and Visual Function Questionnaire 25 composite score differences. Randomization resulted in equal MPOD variance and MPOD increasing in each of the 3 groups from 0.33 density units (du) (0.17 SD) baseline to 0.51 du (0.18 SD) at 12 m, ($P = 0.03$), but no between-group differences (Analysis of Variance; $P = 0.47$). In the Zx group, detailed high-contrast visual acuity improved by 1.5 lines, Retina Foundation of the Southwest shape discrimination sharpened from 0.97 to 0.57 ($P = 0.06$, 1-tail), and a larger percentage of Zx patients experienced clearing of their KVF central scotomas ($P = 0.057$). The "Faux Placebo" L group was superior in terms of low-contrast visual acuity, CSF, and glare recovery, whereas Zx showed a trend toward significance.

CONCLUSION: In older male patients with AMD, Zx-induced foveal MPOD elevation mirrored that of L and provided complementary distinct visual benefits by improving foveal cone-based visual parameters, whereas L enhanced those parameters associated with gross detailed rod-based vision, with considerable overlap between the 2 carotenoids. The equally dosed (atypical dietary ratio) Zx plus L group fared worse in terms of raising MPOD, presumably because of duodenal, hepatic-lipoprotein or retinal carotenoid competition. These results make biological sense based on retinal distribution and Zx foveal predominance.

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Age-related macular degeneration (AMD) is the leading cause of vision loss in aging western populations, particularly among aging U.S World War II veterans, with dry AMD constituting 90% of all cases. While some 5.5 million Americans are projected to require anti-vascular endothelial growth factor and other medical treatments to avoid catastrophic vision loss from neovascular (wet) AMD by the year 2050, 10 times this number, or some 55 million Americans, will have less-severe, but nonetheless visually disabling, retinal pigment epithelium (RPE)/photoreceptor atrophy. From 1991 through 1995, we hypothesized and published a pre-Age-Related Eye Disease Study (AREDS) multicenter, randomized control trial (RCT) showing that AMD is a nutrition-responsive disease.[1-4] Atrophic AMD patients taking broad-spectrum multivitamin mineral capsules maintained or stabilized declining visual function.[2,3] The National Institutes of Health (NIH)/AREDS in 2001 substantiated that AMD is nutrition responsive.[5] From 1996 through 1999, we published a metric for evaluating atrophic AMD with visual function tests beyond high-contrast visual acuity, determining, through open case series experiments, that 10 mg of the carotenoid lutein (L) from spinach or supplements, improved multiple low-contrast and glare function parameters in atrophic AMD.[5-7] The 10-mg dose of L was later adopted as a treatment arm by NIH/National Eye Institutes (NEI)/AREDS II. In 2000 through 2004, we published a pre-AREDS II RCT showing improvement, and not mere stabilization, in an array of visual function parameters adversely affected in early and moderate atrophic AMD.[8] The psychophysical tests of vision, which depend on the functional status of the photoreceptor-RPE complexes, may detect subtle alterations in the macula before morphologic fundus changes are apparent by a fundus examination and before traditional measures of visual acuity exhibit deterioration. These tests are useful tools for assessing and monitoring patients with AMD.[9]

The possibility for AMD vision improvement by macular pigment enhancement with carotenoids or nutritional cofactors has been confirmed in the Phototrop study,[10] the Lutein Xanthophyll Eye Accumulation (LUXEA) Study,[11] the LUtein Nutrition effects measured by Autofluorescence study (LUNA),[12] the Taurine, Omega-3 Fatty Acids, Zinc, Antioxidant, Lutein study,[13] the Carotenoids and Antioxidants in Age-Related Maculopathy Italian Study,[14] the Carotenoids in Age-Related Maculopathy Study,[15] and a recent macular pigment consensus paper.[16] We report, in this article, on zeaxanthin (Zx), the primary human foveal carotenoid, at a 4 times larger dose than under evaluation in AREDS II,[17] for protection against catastrophic high-risk AMD.

Macular pigment is composed principally of 2 isomeric carotenoids, L, Zx, and the L metabolite meso-Zx (meso-Zx is also found in minimal amounts in seafood). In the central 3 mm of the macula, L, Zx, and MZ are present in approximately equal amounts.[18] The macula selectively concentrates L and Zx at levels up to 1,000 times greater than found in any other body tissues.[19,20] Furthermore, the macula selectively places Zx in its foveal center where the greatest protection is needed, which is last to degenerate. Zx has a chemical structure with an extra conjugated double bond (11 conjugated double bonds vs. 10 for L) and may make this a superior antioxidant. Zx is a minor component of the diet of the population of the United States (chiefly corn and yellow peppers), being no greater than one fourth as prevalent as L. By contrast, a cup of Chinese goji (Wolfberry, *Fructus barbarum*) berries is the highest known Zx food source, having some 200 times the amount in a cup of corn and 65 times the Zx dose found in a cup of yellow peppers.[21] Despite the fact that L is at least 4 times as prevalent as Zx in human serum, there is a greater concentration of Zx in the central portion of the macula by 2 times. This fact has been shown in quail, monkeys, and humans.[22-24] Also, relative intake of Zx to L decreases with age.[25]

Two animal experiments found the protective effect of Zx. Quail were raised for 6 months on carotenoid-deficient, normal, or Zx-supplemented diets before exposure to brighter light. Quail on the carotenoid-deficient diet showed extensive retinal damage. The group with normal dietary levels of Zx showed significantly less retinal damage than did the Zx-deprived group, whereas the quail group receiving normal dietary levels of Zx had fewer signs of photoreceptor death.[26] Zx and L protect cytochrome oxidase against the permanent damage caused by phototoxic fluorophores, such as A2E combining with light.[27] After long-term xanthophyll deficiency, L or Zx supplementation has also been shown to protect the primate fovea from blue light damage.[23]

It is known that dietary L and Zx increases human MPOD.[19] As Carpentier et al. suggest in their recent review, few studies have focused on the impact of dietary L and Zx on retinal function and the potential to preserve vision and prevent further degeneration, although we know visual function is reduced in AMD.[28-31]

The 2 Zx and Visual Function study (ZVF) objectives are 1) evaluate whether dietary supplementation with 8 mg Zx alone increases MPOD (primary outcome) and has visual benefit (secondary outcome) for patients with AMD and visual symptoms but lower-risk NEI/AREDS characteristics, and 2) evaluate whether supplemental 8-mg Zx has additional visual benefit when added to L, which was previously found beneficial in early and moderate AMD.

Methods

The study design was a 1-year, staggered recruitment, prospective, double-blind, intention-to-treat RCT of patients with early and moderate AMD (ICD9 362.51), but not advanced disease. The sample included 60 patients (119 eyes), predominantly male (57 male and 3 female), 74.9 years of age, with an SD of 10 years. Subjects were allocated randomly to 1 of 2 dietary supplement treatment carotenoid pigment arms: 8 mg Zx (n = 25 subjects), a higher-dose 8-mg Zx/9-mg L combination (n = 25), or "Faux Placebo" 9-mg L supplement control group (n = 10). The U.S. Food and Drug Administration (FDA), in the absence of a large-scale randomized, controlled study, had no issue with L being considered a "Faux Placebo" in approving our application to study Zx, hence the designation "Faux Placebo."

Randomization, allocation, concealment, and implementation

Capsules were formulated, chemically verified for carotenoid content, and packaged by Chrysantis, Inc. (West Chicago, Illinois) into identical 120-capsule, 4-month bottles. Owing to unequal group sizes (10, 25, and 25), the manufacturer assigned a 4-digit randomly generated number to each of the 60 subjects, which in turn was simultaneously linked (internal to Chrysantis, Inc.) to 1 of 3 randomly assigned interventions. Capsule bottles were identified only by the first randomly generated numeric code and randomly dispensed by the Pharmacy Service of the Department of Veterans Affairs (DVA) Medical Center directly to the subject who was unaware of the specific intervention group. No individual at DVA Medical Center (including the principal investigator) knew the identity of the contents within the bottles with respect to intervention group.

The ZVF study was approved and monitored by the DVA office of Research and Development and Human Subjects protection (FDA IND #78, 973) and registered with *www.clinicaltrials.gov*. ZVF utilized staggered recruitment and commenced on November 26, 2007. The last subject completed his 12-month visit on May 19, 2009, at which time the data were presented to the statistician and the intervention group-link disclosed.

Inclusion criteria

We recruited patients from the DVA Medical Center Eye Clinic, with early and moderate AMD retinopathy. These patients had symptoms and measurable deficits on the contrast sensitivity chart or demonstrated glare disturbances, Amsler grid abnormalities, or subjective functional night driving or reading disturbances that they wished to improve. The inclusion criteria used in this ZVF study are identical to those used in the Lutein Antioxidant Supplementation Trial RCT.[8]

Exclusion criteria

Subjects were excluded if they had either high-risk retinal characteristics for advanced AMD or if they manifested advanced AMD for which existing medical or surgical options were available. Such patients typically required an AREDS I NEI PreserVision® (B + L Global Pharmaceuticals, Madison, New Jersey) type formula to protect the uninvolved or less-involved retina in combination with anti–vascular endothelial growth factor agents. Disqualifying retinal characteristics included presence of significant active exudative AMD pathology by fluorescein angiography or optical coherence tomography (OCT), but also a single large drusen, > 15 multiple intermediate drusen, parafoveal geographic atrophy, or loss of vision in 1 eye because of advanced AMD.[5] Additional exclusion criteria included consumption of L (or Zx) beyond the minimal 250 μg/d commonly found in pabulum-type daily multivitamins within 6 months. Patients known to suffer from active comorbidities, such as uncontrolled and severe diabetes, glaucoma, uveitis, or optic neuritis, or subjects with Alzheimer's disease or non-Alzheimer's dementia or schizophrenia were excluded. Subjects using retinotoxic medications were also excluded (*see* the ZVF Participation Flow Chart [*see* Appendix 1] and the 3 high-performance liquid chromatography certificates of assays for the "Faux Placebo" and 2 intervention groups [*see* Appendices 2A-C]).

Basic ophthalmologic examinations were completed and eligibility was determined at the prestudy visit. Subjects were debriefed, questions were answered, and verbal and written informed consent was obtained. The methodology described below includes in detail the demographic parameters, MPOD, baseline macular visual function, and baseline ocular health, i.e., lens/macular retinal assessments. Initial procedures involved 4 hours of subject-examiner testing. All examinations were accomplished by a trained single full-time technician. After baseline examinations, subjects were instructed to take 1 capsule of the randomly assigned carotenoid pigment(s) per day with a meal. Subjects returned at 4 months, 8 months, and 12 months for serial evaluation. They received $25 at each visit for transportation expenses, for a total of $100 for their full participation. In addition to monetary compensation and provision of nutritional products, serial telephone query, unused capsule counting, and skin carotenoid scores were all used to assure and gauge compliance in the absence of direct serum carotenoid assays.

Demographic, dietary, skin carotenoids, confounding lens opacification and NEI Visual Function Questionnaire 25 AMD symptom assessment

Demographic parameters included a query of age, gender, months since AMD diagnosis, smoking in pack years, alcohol consumption in ounces per day, physical activity, and diabetes. Iris color was noted as blue, green, or brown. Physical assessment included body mass index (BMI), hand grip, and body fat percentage measured by bioelectric impedance (Omron Corp, Japan) at the baseline and final 12-month visit, as adipose tissue is a known reservoir for carotenoids.[32,33]

Diet was assessed using the Harvard School of Public Health Food Frequency Intake Questionnaire, version GP88, for the presence of AREDS and AREDS II nutrients, dietary omega n3 fatty acids, and carotenoids, i.e., L, Zx, and miscellaneous nutrients within the diet at the beginning and end of the study.[34] Specifically, higher intake of vegetable and transunsaturated fats, and, to a lesser extent, animal fat increases the rates of progression; therefore, food groups with higher levels of these fats, particularly baked goods, are associated with a higher progression of AMD, except for nuts and fish, which are protective. We evaluated the omega n3 content of the diet (AREDS Report #20), and the carotenoid content (AREDS Report #22)—both found to be protective against the development of advanced AMD in AREDS post-hoc data analysis.[35,36] Subjects were urged not to alter their diets.

Skin carotenoids, a surrogate nonserum measure of fruit and vegetable and carotenoid intake/tissue absorption, were measured at baseline and each visit with the Biophotonic® Scanner (Pharmanex, Inc, Provo, Utah). This test has been performed on 8 million Americans (score range, 10,000 to 50,000; average, 21,000) and is a validated surrogate measure of both total carotenoid skin content and overall systemic antioxidant protection status.[37]

The degree of cataract lens opacification was determined with a Lens Opacification Cataract Scale (LOCS III) image at baseline and final dilated examination, with analogous methodology described in the LAST RCT.[8] The dimensions evaluated for each lens included nuclear color (NC), nuclear opacification (N), cortical opacification (C), and post-subcapsular opacification (P) on a 7-interval scale.[8,38]

The self-administered version of the NEI VFQ 25 (Vision Function Questionnaire) was utilized to subjectively evaluate baseline and incremental changes in visual function impairment and health-related quality-of-life parameters on a range of activities of daily living, such as driving, reading, and watching television; this test has demonstrated good reliability and construct validity as a measure of vision-related functioning outcomes in patients with AMD.[39]

Primary outcome measure—estimated central foveal 1° MPOD and 3-dimensional autofluorescence MPOD distribution

Replicate measures of foveal 1° estimated central MPOD were evaluated with the QuantifEye® MPS 9000 macular pigment screener (ZeaVision, Inc., Chesterfield, Missouri), a modified heterochromic flicker photometer (HFP). (In HFP, the peripheral test may not be reliable, depending on cataract stage and cognitive ability.) It uses alternating blue and green flickering light-emitting diodes and fixation on a 1° target.[40] The method has good repeatability (r = 0.97), and the data are comparable with an objective optical method based on retinal reflectometry (r = 0.78).[41] Center-only QuantifEye estimates compared with central-peripheral measurements in 5,616 eyes show a 95% limit of agreement for the 2 estimates of 0.13 du.[42] Estimated foveal MPOD was chosen for the ZVF because the majority of older subjects had concurrent cataracts (more so than in the LAST study), introducing additional variability, in addition to fatigue and uncertainty, should peripheral measurements be attempted. There are 2 other reasons for selecting a center estimate. The presence of residual Zx carotenoids at eccentric retinal positions, presumed "null" reference points, has been proven in the LUXEA study, adding to the uncertain of traditional HFP [11] and the presence of topographic complexity with a central dip that increases with aging and in smokers.[32,43]

Objective MPOD techniques overcome HFP difficulties. Yet cataracts, especially their nuclear components, affect MPOD levels measured by autofluorescence spectrometry as well.[44] A 7°, 3-dimensional MPOD spatial distribution using an autofluorescence technique was used to determine the distribution of MPOD in patients without significant lenticular confounding, i.e., pseudophakes.[45]

Retinal lipofuscin autofluorescence imaging

Excessive accumulation of lipofuscin granules in the lysosomal compartment of retinal pigment epithelium cells

represents a common downstream pathogenetic pathway in various hereditary and complex retinal diseases including AMD.[46] Retinal autofluorescence is a noninvasive method of imaging the fundus to observe the presence of lipofuscin.[47] Baseline and final visit 50° digital retinal and autofluorescent lipofuscin images were taken with a modified Kowa Digital VK2® system, (KOWA Optimed, Tokyo, Japan) using 481 nm excitation/660 nm barrier filters. Pairs of fundus digital images (baseline/final n = 119 images) were masked and AREDS graded by a retinal specialist. Fundus images were graded for the presence of retinal autofluorescence. These patterns were measured at the beginning and at the conclusion of the study for each participant. Intake and exit images were paired, placed on a computer viewer, and graded simultaneously. Pairs of acceptable-quality images (baseline/final, n = 103 images, irrespective of degree of retinopathy) were masked and graded by a trained clinical optometrist as to whether there appeared to be less, same, or more autofluorescence. On some occasions, there were grading compensations. These compensations included variations to image contrast between image pairs using the optic nerve head as a reference tissue, discrepancies caused by sharpness of focus between image pairs using the retinal blood vessels as a reference tissue, and fluctuation of the shadowing between image pairs caused by differences in the illumination of the fundus.

Macular visual function test battery

Foveal testing

Testing was exclusively monocular, with the best refraction by a single examiner used for subsequent testing at baseline and serial visits. Conventional high-contrast Early Treatment of Diabetic Retinopathy Study (ETDRS) distance visual acuity was assessed to a fractional line (single letter), displayed randomly on a video projection system at 10 feet (M&S Technologies, Smart Systems II, Park Ridge, Illinois). Measurements were converted to LogMAR visual acuity. One-degree foveal function was also assessed with the 4-circle shape-discrimination test. In each group, only 1 of the circles is distorted while the others are perfectly symmetrical. The distorted circle becomes more difficult for the subject to identify as the test proceeds. The test continues until the subject is unable to tell the difference between the 4 circles. Studies have found that it is difficult for patients with AMD to detect subtle distortions of these circular shapes even when visual acuity is good.[48,49]

Parafoveal testing

Parafoveal function was assessed with a number of tests. The commercial ChromaTest System® (CH Electronics, Bromley, United Kingdom) was used to measure the loss of large receptive field blue-cones via a staircase threshold measurement of large briefly flashed optotypes on a computer screen, determining the length of the B-Y MacAdam ellipse (just noticeable differences of chromaticity) according to published protocols.[50-52] By the time the patient with AMD is in danger of an acute degenerative change, a flashed optotype of a size that more than covers the fovea is indistinguishable no matter how intense the color.

Low-contrast near visual acuity, an important measure of AMD parafoveal rod function impairment, was assessed with a 10% Weber fraction Colenbrander Mixed Contrast Reading Card® (#4031, Precision Vision, LaSalle, Illinois) at 40 cm to a fractional line (single letter) with a LogMAR conversion.[53] Distance photopic contrast sensitivity function (CSF) at 5 spatial frequencies (1.5, 3, 6, 12, and 20 cycles per degree) was determined with the Functional Vision Analyzer® (Stereo Optical Co., Inc., Chicago, Illinois).[8,54]

The photostress glare recovery test involves exposing an individual eye to intense light or retinal bleach for a set duration and measuring the time taken for visual acuity to recover to a predetermined level.[55] Glare photo-stress recovery (in seconds) following 30 seconds of continuous retinal bleach was assessed using a 2-line, suprathreshold, low-contrast, randomly presented Landolt C using the KOWA AS14B Night Vision Tester (KOWA Optimed, Tokyo, Japan).

Scotomas within the central 20° visual field were assessed at 5 contrast levels (20, 40, 60, 80, and full contrast) with a SimulEyes® Kinetic Visual field test (Rush Ophthalmics, Gold Beach, Oregon). The color yellow was further used to avoid confounding by the lens (i.e., nuclear yellowing). Subjects outlined the boundaries of their scotoma(s) on an area-integrating and recording touch screen monitor displaying a central fixation point and movable horizontal/vertical raster lines.[56]

Statistical analysis and blinding (masking)

The primary outcome measure was a change in the MPOD by the intervention group using a 3-factor repeated measures analysis of variance (ANOVA). Post-hoc tests were conducted using Scheffe's test, and the equal variances assumption was ascertained using Bartlett's test. When Bartlett's test violated ANOVA's equal variances assumption, a Welch's *t* test for unequal variances was used. Between-group differences were ascertained using a 2-sample *t* test with equal variances assumption. Based on prior higher trending MPOD and visual function data with carotenoid L and Zx supplementation, 1-tailed *t* tests were used.[8,11] The participants and those administering and assessing the outcomes were blinded to group assignment, which was held offsite by the grant administrator. Average-eye data from a single subject, and significance, is typically shown for simplicity, in cases in which the analysis did not differ appreciably from evaluating right eyes and left eyes separately.

Results

In ZVF, 90% of subjects completed at least 2 study visits with 96% pill intake compliance gauged by unused pill count,

periodic (daily, weekly, then monthly) telephone queries, and measuring the near universal increase in skin carotenoids (*see* ZVF non-responder section below). One patient in the "Faux Placebo" L group (subject Z46, age 77) died before the 4-month visit, and 1 subject in the Zx group (subject Z31, age 60) died before the 8-month visit. The cause of these deaths were reviewed by the Human Subjects Institutional Review Board and deemed unrelated to the study interventions. One subject in the Zx group (subject Z44) was disqualified because of a herpes zoster corneal infection after his 8-month visit. One subject in the Z plus L group (subject Z20) became ill with pneumonia week 1 and decided to drop out. Two subjects dropped out for no apparent reason, and 3 additional subjects dropped out because of the "tedious nature" of the ZVF testing protocol or to take care of an ill spouse. No other significant adverse events were encountered or reported by telephone query or physician report.

Baseline demographic and clinical characteristics

Table 1 presents population and group baseline demographic data averages, SDs and significance. On average, 60 older near obese (BMI 29.1, SD 5) AMD subjects, 74.9 (SD 10) years of age, (57 men and 3 women), with brief average AMD duration of 3.5 years from diagnosis, participated in the RCT. There was a minimal current average population smoking history of only 0.2 (SD 0.5) packs per day and alcohol consumption of only 0.8 (SD 1) ounces of alcohol per day. Population diabetes duration was only 0.2 (SD 0.4) years. Skin carotenoid scores of 18,467 (SD 8,829) were below average compared with an average of 21,000 in the 8 million U.S. subject database.[37] Finally, ZVF subjects manifested an AREDS Report #18 retinopathy grade of 1.33 (SD 0.9) of a possible bilateral score of 4, reflecting minimal bilateral AMD retinopathy consistent with the duration of their disease. There were no significant differences by group at baseline, with the notable exception of subjects in the Zx group having significantly greater retinopathy than the other 2 subgroups (1-way ANOVA, $P < 0.007$) at baseline.

Estimated foveal 1° central MPOD for the ZVF population was "low normal" at 0.32 du, standard error (SE) 0.024. There were no a priori statistically significant subgroup differences (Zx, 0.36 du, SE 0.05; Zx plus L, 0.27 du, SE 0.03; and "Faux Placebo" control L, 0.37 du, SE 0.05). Owing to the advanced age of the subjects and presence of cataracts in most patients (6.2 SD, 4.1 NC/N/C/P composite score right eyes; 6.4 SD, 3.8 NC/N/C/P composite score left eyes), the usable (4 visit) 3-dimensional autofluorescence MPOD data were available for only 16% of subjects. The images were nonetheless useful in the ZVF post-hoc context evaluating individual Zx supplemented subjects who manifested resolution of their central foveal scotomas. Two Zx subject examples along with their corresponding 3-dimensional autofluorescence MPOD distributions appear in Appendix 3.

Table 1 ZVF baseline demographic statistics

Parameter (AVG/SD)	Population, n = 60	L ("Faux Placebo"), n = 10	Zx, n = 25	Zx + L, n = 25	Statistic	
					Bartlett	1-way ANOVA
Age (y)	74.9, SD 10	73.9, SD 9	74.4, SD 11	75.8, SD 9	NS	NS
Smoking (pack/d/5 y)	0.2, SD 0.5	0.3, SD 0.5	0.7, SD 0.2	0.2, SD 0.7	NS	NS
Body mass index	29.1, SD 5	29.8, SD 5*	28.6, SD 5*	29.4, SD 5*	NS	
Diabetes mellitus II	0.2, SD 0.4	0.3, SD 0.5	0.2, SD 0.4	0.2, SD 0.4	NS	NS
AMD duration (mo)	41.4, SD 41	28.0, SD 26	42.8, SD 47	45.5, SD 41	NS	NS
ETOH (oz)	0.8, SD 1	1.0, SD 1	0.9, SD 1	0.6, SD 1	NS	NS
Pharmanex	18,467, SD 8,829	18,400, SD 7,168	18,000, SD 8,010	18,960, SD 10,378	NS	NS
AREDS report #18 retinal grade	1.33, SD 0.9	0.9, SD 0.7	*1.78, SD 1.0	1.1, SD 0.8	NS	0.007

ZVF = Zeaxanthin and Visual Function study; AVG/SD = average/standard deviation; L = lutein; Zx = zeaxanthin; ANOVA = Analysis of Variance; NS = not significant; AMD = age-related macular degeneration; ETOH = ethyl alcohol; AREDS = Age-related Eye Disease Study.

Note. The 3 groups were well matched after randomization except for greater AMD retinopathy in the Zx group.

* Clinical obesity in all 3 groups.

Table 2 Baseline ZVF vision parameters are mostly matched after randomization

					Statistic	
	Population, n = 60	L ("Faux Placebo"), n = 10	Zx, n = 25	Zx + L, n = 25	Bartlett	1-way ANOVA
VFQ	85, SD 10	89.7, SD 8	87.0, SD 10	86.0, SD 13	NS	NS
ETDRS distance visual acuity	Right 95.2, SD 8 Left 91.0, SD 16	98.5, SD 5	95.4, SD 7	93.7, SD 9	0.09	NS
100% and 10% Colenbrander near visual acuity	88.7, SD 13 76.2, SD16	93.3, SD 8 81, SD 10	88.3, SD 10 77.2, SD 12	86.8, SD 12 72.7, SD 16		NS
Smith Kettlewell Institute Low Luminance	Right 57.7, SD 17 Left 61.6, SD 15	64.5, SD 10 66.3, SD 12	60.6, SD 14 63.6, SD 13	52.2,* SD 20 57.8, SD 17		0.04*
CSF photopic distance (mean)	204 SD 125	212, SE 34	201, SE 22	204, SE 30	NS	NS
Glare recovery	34.1, SD 30	52.9, SE 16	26.7, SE 5	35.6, SE 6	NS	NS
Shape discrimination	0.8, SD 0.8	0.7, SE 0.2	1.0, SE 0.2	0.7, SD 0.1	NS	NS
6.5° Tritan threshold	right 6.9, SD 10 Left 8.2, SD 11	Right 4.9, SD 4 Left 4.2, SD 4	Right 6.0, SD 9 Left 7.7, SD 10	Right 8.6, SD 12 Left 10.6, SD 14	NS	0.001
100% kinetic field	2,738, SD 4471	5514 SE 2074	2,649, SE 750	1,717, SE 765	0.06	0.07
Est MP		0.37 du, SE 0.05	0.36 du, SE 0.05	0.27 du, SE 0.03	NS	NS

ZVF = Zeaxanthin and Visual Function study; L = lutein; Zx = zeaxanthin; ANOVA = Analysis of Variance; VFQ = Visual Function Questionnaire; SD = standard deviation; ETDRS = Early Treatment of Diabetic Retinopathy Study; SE = standard error; CSF = contrast sensitivity function; Est MP = estimated macula pigment; NS = not significant; du = density units.

* $P < 0.05$.

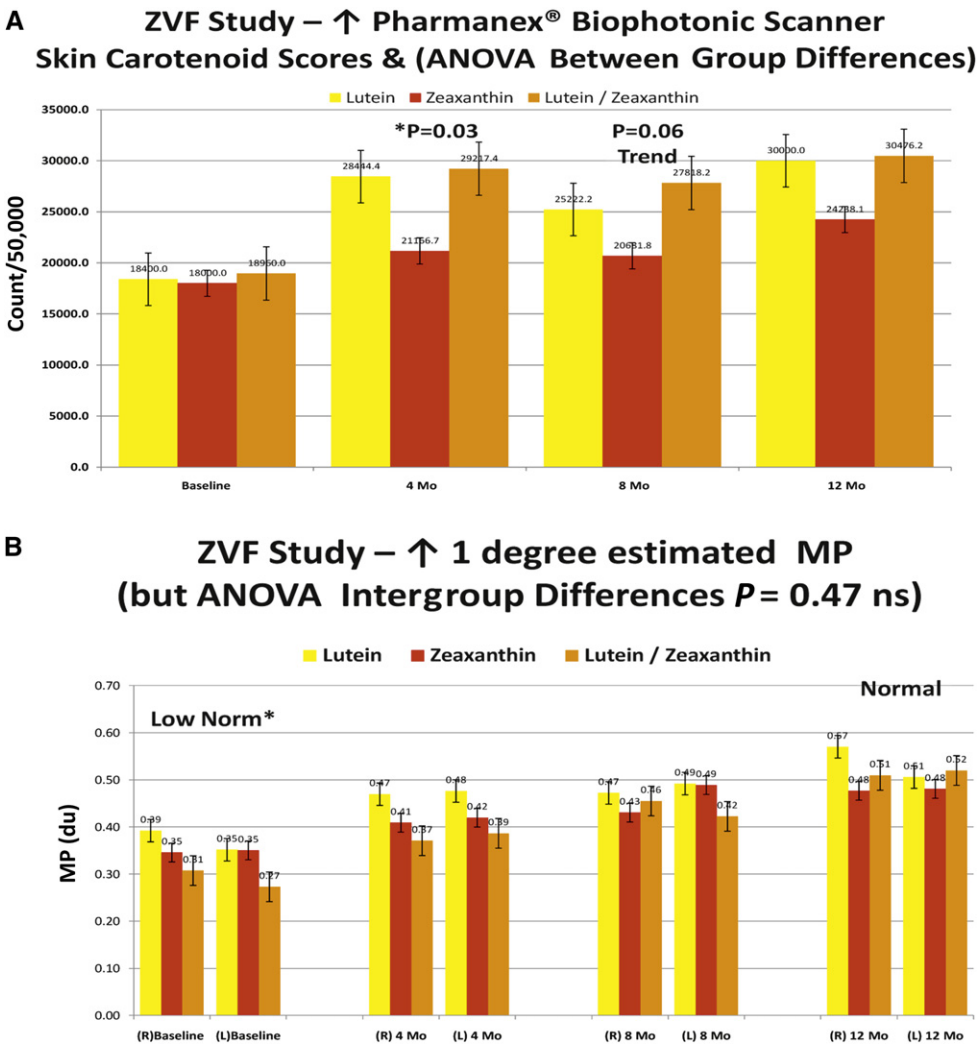

Figure 1 **A**, Pharmanex Biophotonic Skin carotenoid scores, initially equal at baseline, differentially increased during ZVF with less increase in the Zx group and greater effect in the higher-dosed combined carotenoid group (2-sample *t* test, equal variance, baseline to 12 months. L, $P = 0.02$; Zx, $P = 0.04$; and L plus Zx, $P = 0.0008$ and ANOVA, $P = 0.03$ at 4 months and $P = 0.06$ for trend at 8 months, but indistinguishable between-group differences by 12 months). **B**, Foveal (1°) estimated macular pigment using the clinical QuantifEye modified heterochromic flicker photometer. There were no intergroup differences at baseline among the 3 groups at baseline or during the study (ANOVA, $P = 0.47$, not significant). By 12 months, foveal MP increased in all 3 groups from low-normal to normal density (2-sample *t* test, equal variance, single-sided *t*, baseline to 12 months, L, $P = 0.03$; Zx, $P = 0.03$; and L plus Zx, $P = 0.06$ for trend).

Visual function reduction in AMD patients at baseline

Table 2 displays baseline ZVF visual function data. The population composite NEI VFQ25 score was 85 of 100 with no subgroup differences. The ETDRS (high-contrast) visual acuity score was 95.2 (SD 8) or 20/25+1 right eyes, 91.0 (SD 16) or 20/30 left eyes, with no subgroup differences. However, the low-contrast Colenbrander near visual acuity score was reduced, more for left eyes 88.7 (SD 13) R eyes, 76.2 (SD 16) L eyes with no subgroup differences. The Smith Kettlewell Institute Low Luminance low-contrast near test was similarly reduced at 57.7 (SD 17) for the right eyes, 61.6 (SD 15) for the left eyes, with right eyes in the Zx plus L subgroup having significantly poorer function (1-way ANOVA, $P < 0.04$) consistent with their greater retinopathy (*see* later discussion). The area under the curve (AUC) composite CSF score was low-normal at 204 (SD 125), average eye glare recovery was reduced by 34.1 (SD 30) seconds, and 1° foveal shape discrimination at 0.8 (SD 0.8) was as well reduced, consistent with atrophic AMD, with no subgroup differences in any of these visual parameters. Parafoveal blue cone tritan thresholds were reduced to 6.9 decibels (db) (SD 10) in the right eyes, 8.2 db (SD 11) in the left eyes, with the Zx plus L subgroup displaying an even higher threshold than the other 2 groups (1-way ANOVA, $P = 0.001$). Finally, a number of subjects had scotomas (L, 55% [11/20 eyes]; Zx, 73% [36/49 eyes], and Zx plus L, 58% [29/50 eyes]). The average eye 100% composite threshold scotoma count was 2,738 (SD 4,471) with no statistically significant subgroup differences.

On the whole, the baseline visual function of the population was well matched among subgroups after randomization. Although visual acuity was largely preserved

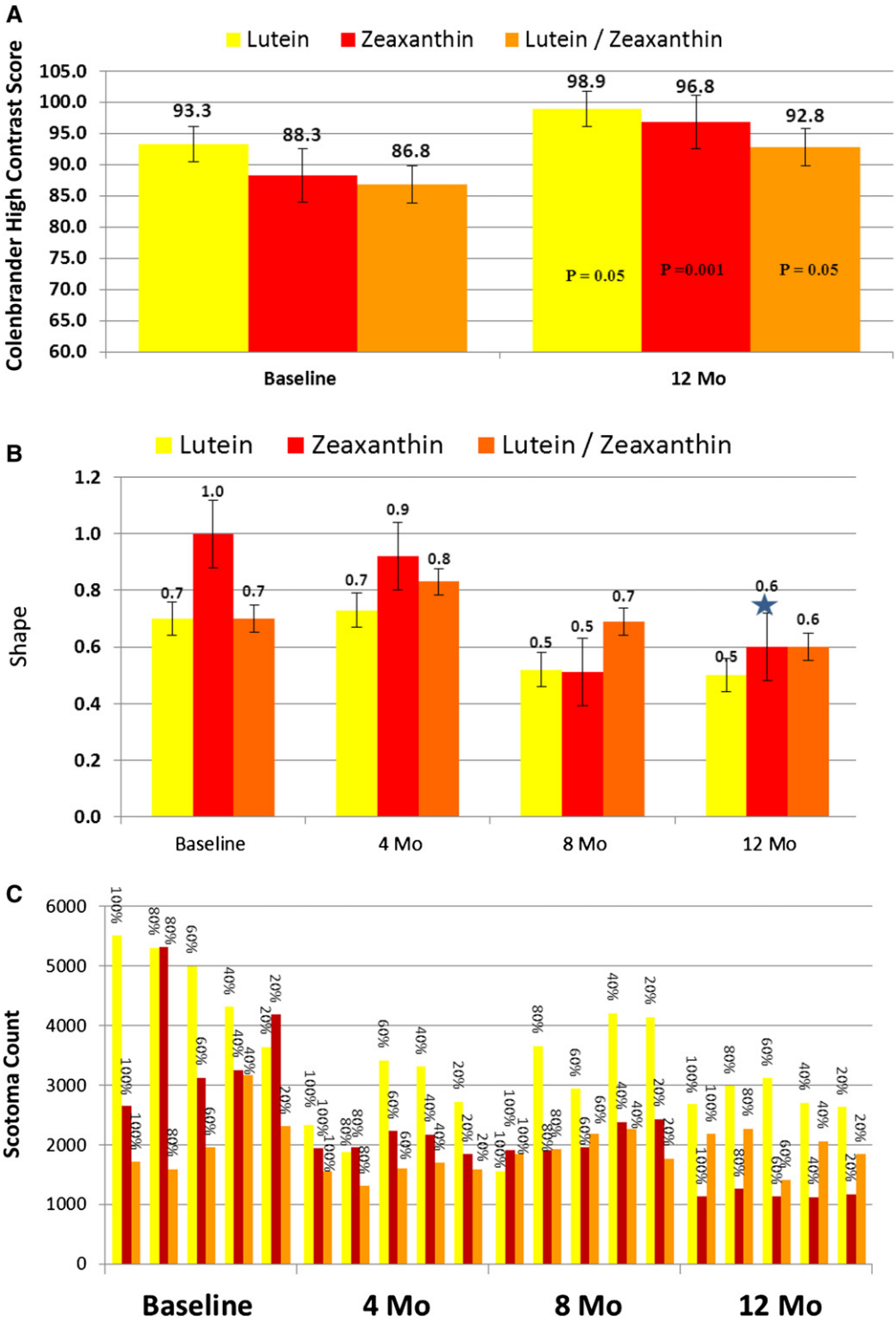

Figure 2 **A**, Colenbrander average eye near high-contrast visual acuity improved at least 1 line in all 3 intervention groups (L, 5.6 letters; Z plus L, 6.0 letters; $P = 0.05$), but the greatest (1.5 lines/8.5 letters) increase was with Zx alone, $P = 0.001$, a carotenoid that anatomically predominates in the central fovea. **B**, Shape discrimination, average eye, showed a near statistically significant increase with Zx by 12 m (but ANOVA intergroup differences $P = 0.74$) Improved Average Foveal Shape Discrimination Baseline and Final 2-Sample pair ($T > t$) Alternate hypothesis: Difference > 0. L (not significant); Z ($P = 0.06$); L plus Z (not significant). **C**, Twenty-degree Kinetic Field Analyzer, average eye, scotoma count at 5 contrast levels: 20%, 40%, 60%, 80%, and 100% for each intervention group. Zx proved most efficient at improvement of scotomas count at 20% contrast ($P = 0.03$, paired T baseline to 12 m), 40% contrast ($P = 0.03$), and 60% contrast targets ($P = 0.06$ for trend).

and retinas manifested minimal (visible) AMD retinopathy, depressed VFQ25 scores, abnormal CSFs, glare recovery, measures of low-contrast VA impairment, elevated parafoveal blue-yellow increment thresholds, and presence of scotomas reflected significant visual impairment. CSF and blue cone parafoveal loss were correlated with accelerated objective SD OCT parafoveal thinning compared with aging "normal" retinas, the subject of a separate article.[57]

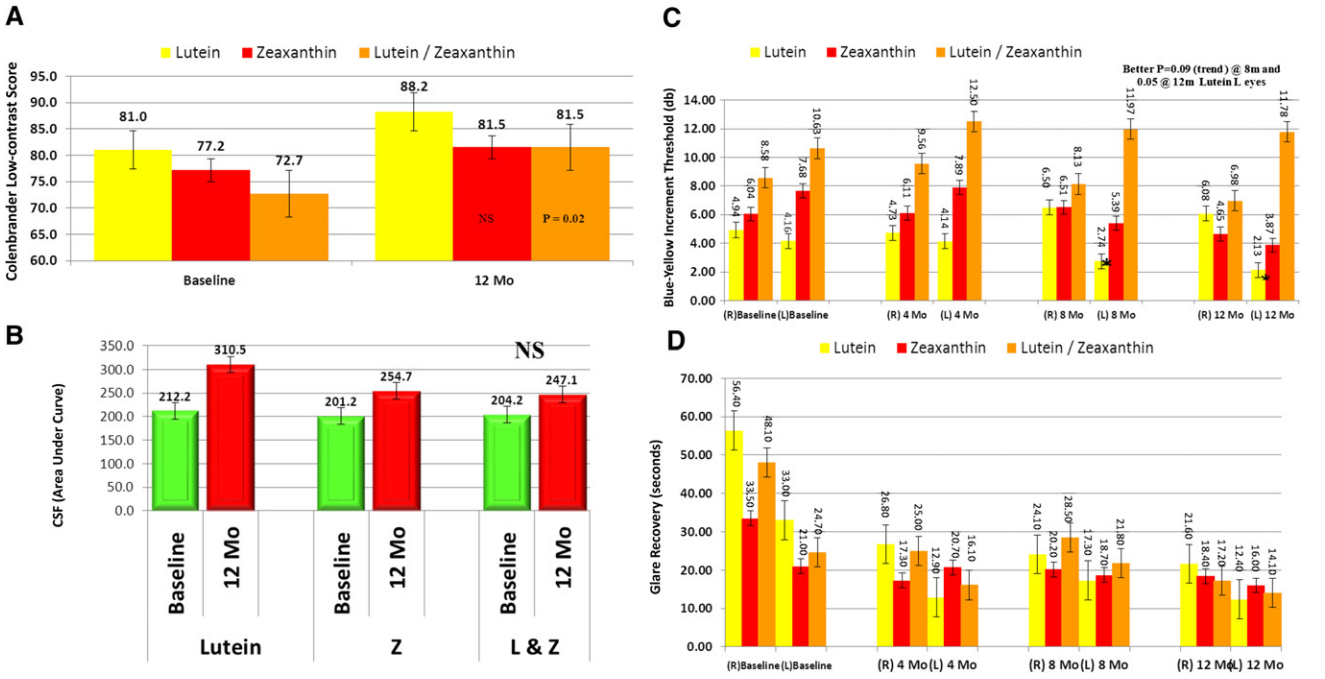

Figure 3 **A**, Baseline, average eye, low-contrast near visual acuity was inferior to that of high-contrast letters by at least 2 lines. By 12 months, the Colenbrander low-contrast letters were better visualized with either L (+7.2 letters, $P = 0.04$) or L plus Zx (+8.8 letters, $P = 0.02$) but nonsignificantly improved with Zx alone (+4.3 letters, P value not significant). **B**, Baseline, average eye, and final AUC CSF (at 5 spatial frequencies) for L (difference improvement, +48%; $P = 0.05$) and Zx (+24%; $P = 0.09$ for trend) but surprisingly not for and L plus Zx (+20%; not significant). **C**, Blue cones, equally spaced within the parafovea provide an independent measure of parafoveal function. Of all 3 intervention groups, L proved best ($P = 0.09$ for trend ANOVA at 8 m and $P = 0.05$ ANOVA at 12 m). **D**, Glare recovery (seconds) improvement was significant for L ($P = 0.02$) and particularly the combined L plus Zx group ($P = 0.002$) with only a trend for improvement with the Zx subgroup ($P = 0.09$), paired t test (baseline to 12 m).

Primary outcome and significance

Figure 1A depicts universal increased palm skin carotenoid scores with carotenoid supplementation. The scores were equal at baseline, but differentially increased during the ZVF study, with a lower increase seen in the Zx group (*see* nonresponder section below) and with a greater effect in the higher-dosed combined carotenoid group (2-sample t test, equal variance, baseline to 12 months; L, $P = 0.02$; Zx, $P = 0.04$, and L plus Zx, $P = 0.0008$; ANOVA, $P = 0.03$ at 4 months; $P = 0.06$ for trend at 8 months, but indistinguishable subgroup differences by 12 months).

Foveal estimated 1° MPOD is the major ZVF independent variable, as all subsequent visual function and retinal parameters are dependent on the intervention group. Figure 1B depicts a universal increase in MPOD over time with no intergroup differences at baseline between the 3 groups or during ZVF (ANOVA, $P = 0.47$, not significant). By 12 months, foveal MPOD increased in all 3 groups from low-normal to normal density (2-sample t test, equal variance, single-sided t, baseline to 12 months; L, $P = 0.03$; Zx, $P = 0.03$; and L plus Zx, $P = 0.06$ for trend).

Secondary outcomes and significance

Foveal vision

Figures 2A-C depict the effects of carotenoid supplementation on conventional average eye high-contrast visual acuity, shape discrimination, and kinetic visual fields, which measure central high-resolution cone-predominating foveal function where Zx outperformed L. In Figure 2A, average eye Colenbrander near visual acuity improved at least 1 line in all 3 intervention groups (L, 5.6 letters; Zx plus L, 6.0 letters; $P = 0.05$), with the greatest 1.5-line/8.5-letter increase with Zx alone, $P = 0.001$ (a carotenoid that anatomically is predominate in the fovea). Similarly in Figure 2B, average eye foveal 1° shape discrimination showed a near statistically significant increase with Zx by 12 m Zx ($P = 0.06$; intergroup differences, $P = 0.74$). As with visual acuity, combining both carotenoids at near equivalent dosages (L plus Zx group) proved less effective. The increase in visual acuity at 1 line in the "Faux Placebo" L group is identical to that reported in our LAST study with a similar dose of L in a near-obese group of slightly younger men.[8] Figure 2C depicts average eye 20° kinetic field analyzer scotoma count at 5 contrast levels: 20%, 40%, 60%, 80%, and 100% for each intervention group. The intergroup difference was not significant. However, Zx proved most efficient at improvement of the scotoma count for 20% contrast targets ($P = 0.03$), 40% contrast targets ($P = 0.03$), and 60% contrast targets ($P = 0.06$ for trend) by paired t test baseline to 12 months. The non-statistically significant, but improved, scotoma resolution phenomenon for L was also noted in our LAST study.[8] However, in ZVF, the L group had an increase in the number of eyes with new scotomas (13 of 18 eyes or 72% at final visit worsening from 55% at baseline). This was different than that in the 2 Zx groups. The Zx group had 24 of 41 eyes or 59%

Table 3 ZVF lens data and significance

	LOCS III (sum NC/N/C/P) mean (SE) right lens			LOCS III (sum NC/N/C/P) mean (SE) left lens		
	L	Zx	L + Zx	L	Zx	L + Zx
Baseline	6.5 (1.2)	5.6 (0.8)	6.7 (0.9)	6.4 (1.2)	6.4 (0.8)	6.3 (0.8)
12 months	6.6 (1.5)	6.0 (0.9)	6.0 (0.9)	6.1 (1.4)	6.8 (0.8)	6.3 (1.0)
P value 2-sample *t*	NS	NS	NS	NS	NS	NS

ZVF = Zeaxanthin and Visual Function study; LOCS III = Lens Opacification Cataract Scale; NC/N/C/P = nuclear color/nuclear opacification/cortical opacification/post-subcapsular opacification; SE = standard error; L = lutein; Zx = zeaxanthin; NS = not significant.

with complete scotoma resolution compared with 73% of eyes at baseline. The Zx plus L group had only 23 of 42 eyes, or 55% complete scotoma resolution compared with 58% of eyes at baseline.

Parafoveal vision. Figure 3A-D analogously depicts measures of average eye low-contrast vision consisting of greater contributions of rod-based parafoveal vision in which L outperformed Zx. Average eye baseline low-contrast near VA was inferior to that of high-contrast letters by at least 2 lines (compare with Figure 2A). In Figure 3A, by 12 months, the Colenbrander low-contrast letters were better visualized with either L (+7.2 letters, $P = 0.04$) or L plus Zx (+8.8 letters, $P = 0.02$) but nonsignificantly improved with Zx alone (+4.3 letters, *P* value not significant). Figure 3B shows an overall increase in average eye AUC CSF, with the L group most effective. Baseline and final AUC CSF (AUC CSF at 5 spatial frequencies) for L (difference improvement, +48%; $P = 0.05$) and Zx (+24%; $P = 0.09$ for trend) but surprisingly not for equally weighted L plus Zx supplementation (+20%; *P* value not significant). Blue cones, equally spaced within the parafovea, provide an independent measure of parafoveal function. In Figure 3C, tritan parafoveal threshold, L proved best ($P = 0.09$ for trend ANOVA at 8 months and $P = 0.05$ ANOVA at 12 months). Because the ANOVA equal variances assumption was not met via Bartlett's test, we also performed *t* tests using Welch's formula accounting for unequal variances. There were between-group differences for L versus L plus Zx at 8 months ($P = 0.03$), and 12 months ($P = 0.02$). In Figure 3D, glare recovery, in seconds, improvement was significant for L ($P = 0.02$) and particularly for the combined L plus Zx group ($P = 0.002$) with only a trend for improvement with the Zx subgroup ($P = 0.09$), paired *t* test (baseline to 12 months).

Subjective vision, retinal lipofuscin, and cataract

Composite summed subjective VFQ25 questionnaire answers improved slightly (+2%) over 12 months, but were not statistically significant, with no summed category intergroup differences by ANOVA, except for the driving subscale that showed a near improvement in the Z group ($P < 0.057$ for trend) in a linear regression model.[58] The AREDS report #18 bilateral simplified AREDS score showed a nonsignificant improvement for Zx subjects who had higher (worse) starting baseline AMD retinopathy ($P < 0.007$, ANOVA). The improvement, however, was not statistically significant, with no intergroup ANOVA changes over time (L, 0.90/4.0 baseline; 1.56/4.0 final; Zx, 1.78 baseline, 1.68 final; L plus Zx, 1.08 baseline, 1.14 final). The autofluorescence lipofuscin data for right eyes (n = 51) showed that 88% of retinas (n = 45) remained the same, 2 retinas worsened, and 4 retinas improved with no intergroup differences by Scheffe's Interval test statistic ($P = 0.05$). However, for left eyes (n = 52), 44 remained the same (88% of retinas), 6 worsened, and 2 improved with a beneficial Scheffe's Interval test statistic ($P = 0.04$) for Zx (but more stringent [$P = 0.07$] for trend using the Welch Test ANOVA violation of equal variances test). The LOCS III subjective lens opacity scores decreased slightly from 6.2 (SD 4.1) composite NC/N/C/P score to 6.1 (SD 4.3) and was statistically insignificant. Subgroup cataract data are presented in Table 3. Neuropsychological data were obtained at baseline and 12 months by an American Psychological Association–certified neuropsychologist and are presented separately.

ZVF nonresponders and AREDS/AREDS II nutrient intake changes

Sixteen of 60 ZVF subjects (27%) did not show a response in terms of > 5,000 unit increase in their skin carotenoid scores, and 75% of these were in the Zx group. The biophotonic skin carotenoid skin scanner is not efficiently tuned to Zx compared with L.[59] Five individuals (8.3%) failed to experience greater than a 10% MPOD increase. These individuals included 2 smokers who were of similar age, BMI, percentage of body fat, AREDS retinopathy score, clinical evidence of gallbladder disease (none), liver enzyme abnormalities, nephropathy, presence of diabetes, and similar vitamin A but lower omega n3 dietary intake. The MP nonresponders did, however, manifest higher serum triglycerides (> 150 mg %) and lower high-density lipoprotein (< 40 mg %), and 4 of 5 used cholesterol-lowering medications, compared with roughly 50% for the entire study population. Loane et al.[60] recently noted

these same trends in a younger aged cohort, finding a statistically significant and positive association between MPOD and both serum cholesterol and HDL concentration, but an inverse association with serum triglycerides, suggesting HDL to be particularly important for the transport of carotenoids in serum. Finally, 4 individuals had both low skin and low retinal pigment.

The estimated dietary intake of L was between 2.5 mg and 4 mg throughout the ZVF study, a low value similar to that encountered in the LAST Study.[8] The Harvard Food Frequency Intake Evaluation did not allow for specific determination of estimated Zx dietary intake. With the exception of a trend in vitamin C intake intragroup differences ($P < 0.08$), there were no other statistically significant intragroup and baseline/final differences in combined dietary/supplemental intake of the AREDS nutrients: B carotene, vitamin C, vitamin E , zinc, and copper or the n3 fatty acids and total dietary carotenoids (*see* Appendix 4).

Discussion

Preliminary results of a single-center (n = 53) patient substudy, within AREDS II, suggests that serum and skin carotenoid measurements are not reliable biomarkers for MPOD.[61] There are also formidable technical difficulties in reliably measuring MPOD in elderly patients with cataracts; better objective imaging techniques are desperately needed. Nonetheless, the "low-normal" macular pigment values encountered in ZVF are similar to those encountered in our LAST Study, when adjusted for the QuantifEye 0.1-du center estimate over estimation, and are comparable with low macular pigment values found in most all AMD studies to date.[8,16,42] In ZVF, near equally dosed Zx or L significantly elevated low-normal MPOD to normal ranges in our predominantly older male AMD population who were supplemented for 1 year, and ZVF subjects benefited visually from this intervention. However, our results may not be generalized to women, who typically have a higher percentage of adipose fat and different lipoprotein profiles.

Our first objective was to evaluate properties of Zx independent of L. Our data suggest that Zx has unique visual cone-enhancing attributes consistent with its foveal position, a crucial retinal location particularly deserving of clinical attention and protection.[62] Indeed, Zx improved high-contrast visual acuity by 1.5 lines and sharpened 1° Foveal Shape Discrimination, a test of foveal cone alignment. In some cases, the macula kinetic visual field data and correlative 3-dimensional MPOD plots demonstrated complete resolution of central scotomas, even in retinas that started out with a comparatively greater degree of AMD retinopathy. The autofluorescence lipofuscin data suggested amelioration, and this pilot data should be repeated. In contradistinction, the rod-dominant parafovea, disproportionately affected in geographic atrophy, allows us to see gross details and shadows.[63] Here L, ostensibly owing to its more parafoveal retinal distribution, proved superior, with statistically significant improvement in contrast sensitivity, glare recovery, and enhanced blue-yellow increment db thresholds from parafoveal blue cones, which are equally spaced throughout the parafovea. These visual effects were not mutually exclusive, as there were weak trends toward significance with Zx for rod-dominant visual parameters, while the L group also manifested overlapping properties with respect to cone function. (L is also present in the fovea, thus, these 2 carotenoids are complementary.) That there were no changes in lens opacification in ZVF could reflect the short duration of this study as Delcourt et al.[64] found that patients with the highest levels of Zx had a 77% reduction in nuclear cataracts in a 3-year population-based prospective cohort of the French population.

The second objective of ZVF was to assess whether there was added benefit of Zx to traditional L supplementation. In this case, higher dose 1:1 ratio of Zx plus L barely increased MPOD ($P = 0.057$ for trend) and inconsistently enhanced visual function, suggesting carotenoid duodenal, hepatic-lipoprotein, or retinal competition when introduced at equal supplement doses. This is not surprising, as dietary L predominates 5:1 and foveal Zx predominates 2:1 over L, so equal doses of each carotenoid are unusual from a dietary or tissue standpoint. The choice of a 5:1 dose, based on the U.S. diet, for AREDS II, appears judicious.[17] Finally in ZVF, not everyone who was supplemented experienced elevated MPOD, as reported by others.[59,64]

Although the focus of ZVF was evaluation of vision function and not prevention of advanced AMD, "macular pigment" is associated with all 3 primary AMD risk factors—age, smoking, and obesity.[65-68] The 2001 AREDS I results demonstrated that dietary antioxidants can intervene in the late stages of AMD, significantly increasing the credibility of one of the theorized protective mechanisms of Zx. Evidence from epidemiologic studies consistently shows high dietary intake of U.S. fruits and vegetables rich in L and Zx reduces the risk of AMD as well as lens opacification, cataract onset, and cataract extraction risk. Both AREDS I and the Blue Mountain study demonstrated a protective carotenoid effect.[36,69] More specifically, a 2003 report by Gale et al.[70] found that those whose plasma concentration was in the lowest third for Zx had an odds ratio for AMD risk of 2.0 (95% confidence interval, 1 to 4.1) after adjustment for age and other risk factors, a relationship that did not exist for L, and a 2006 study suggests serum Zx is strongly associated with reduced risk of advanced AMD. The POLA study evaluated a French/Mediterranean cross-sectional population with 899 subjects, showing that patients in the highest quintile of serum Zx were associated with a 93% risk reduction of AMD, $P = 0.005$, whereas patients with the highest plasma L had a 79% reduction of AMD.[70] Again, we can only conjecture whether raising foveal Zx in the diet beyond the 2-mg dose in AREDS II might offer better clinical protection. Subretinal choroidal neovascular occult membranes, for example, manifest 80% foveal predilection. The concept of "prescriptive carotenoids" based on drusen location, pathological SD OCT appearance, and macular pigment distribution also warrants further study.

ZVF is consistent with other studies, showing that Zx raises MPOD.[11,66] Regardless of whether the 2-mg dose of Zx within AREDS II contributes to prevention of catastrophic end-stage vision loss in patients with high-risk retinopathy, there is little doubt that raising macular pigment via Zx supplementation alone results in salutary visual benefits to AMD patients with lesser disease, through enhancement of visual acuity, shape discrimination, scotoma resolution, as well as weaker negative effects on CSF, glare recovery, and blue-yellow color thresholds.

We believe patients with AMD, particularly those short of catastrophic end-stage disease, might want to enhance their declining vision with carotenoids apart from the pending results of AREDS II. Prescriptive MPOD enhancement via dietary manipulation is scientifically possible. Visual function can further be monitored via utilization of clinical MPOD instruments and visual metric(s), such as glare recovery, contrast sensitivity, central visual field scotoma count, and foveal shape discrimination. Clinical foveal MPOD estimation has recently become widely available; there now exists both a clinical central field instrument (Ellex MAIA Macular Integrity Assessment; *www.ellex.com*) and dark adaptometer (Apeliotus AdaptRx; *www.hersheyresearch.com*). All 3 instruments are devoted to early AMD preophthalmoscope surrogate disease markers.

MPOD measurement and assessment of vision function, beyond using an 1862 Snellen visual acuity chart, are rarely used together in the typical adult 50+ eye examination. This results in failure to capture the subtleties of disease progression that we can correlate to patient complaints. It is even more important to ascertain this information before recommending dietary carotenoids or supplements and represents a new "metric" in optometry. The aging of the U.S. population and attendant increase in AMD has implications for night driving and public safety well beyond its potential role in the AREDS II study that focuses on the crucial but narrow hypothesis of prevention of catastrophic retinal disease.[71] In ZVF, increasing MPOD was related to NEI VFQ subscale driving performance in a multivariate regression model, and Zx benefited patients self-described driving performance ($P = 0.057$ for trend). This important data, along with ZVF neuropsychological findings, are the subjects of separate articles (in process). The remarkable increased visual acuity and heightened foveal shape discrimination with Zx potentially applies to sports vision (i.e., baseball players) and has military application (i.e., sharpshooters) for the young as well as old.

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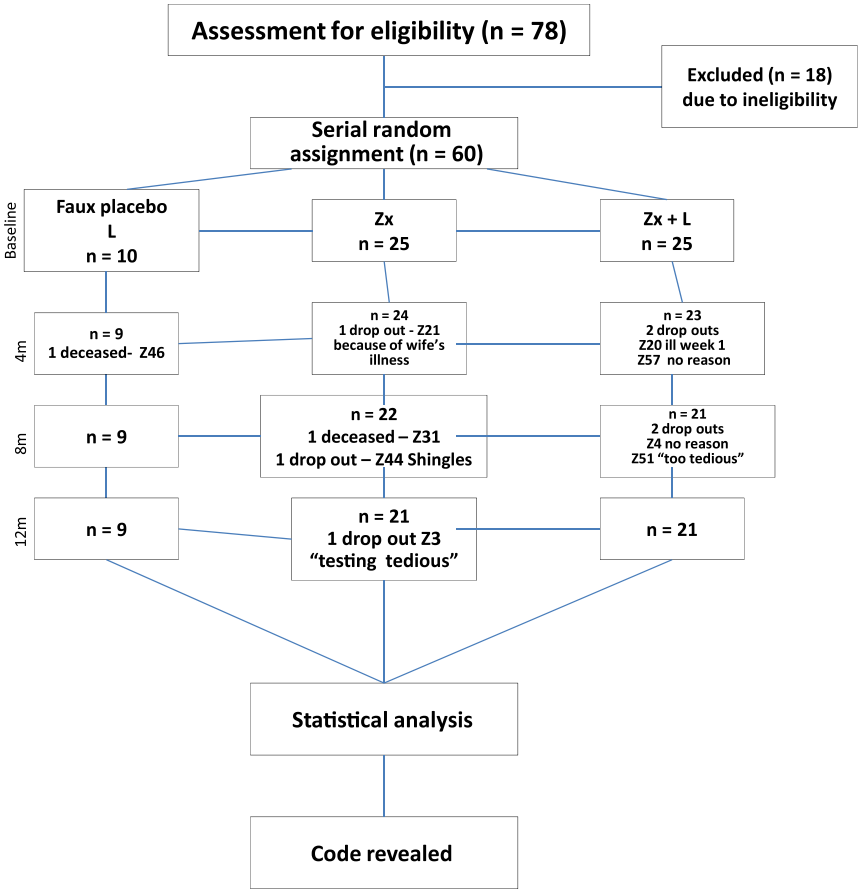
Appendix 1 ZVF Participation Flow Chart



Appendix 2A

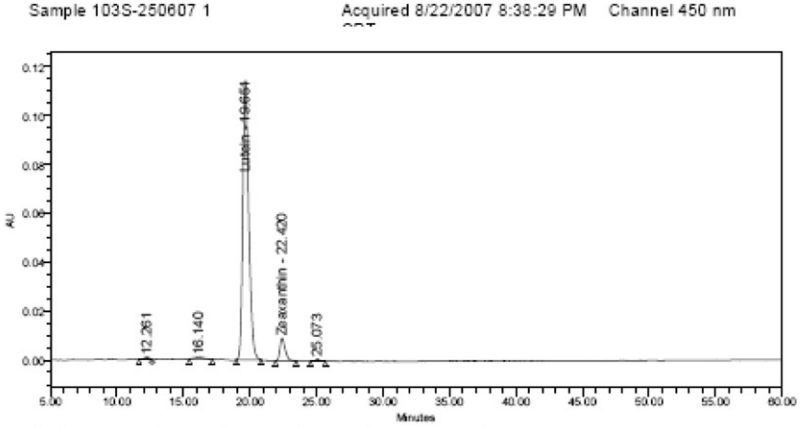
Lutein Softgel Lot 103S-250607

Carotenoid Profile

Zeaxanthin	6.2 %
Lutein	91.3 %

Carotenoid Content *

Total Carotenoids	9.7 mg/softgel
Zeaxanthin	0.6 mg/softgel
Lutein	8.9 mg/softgel



	Peak Name	RT	Area	% Area	Peak Lambda Max.
1		12.261	28577	0.7	431.8
2		16.140	36806	1.0	445.0
3	Lutein	19.651	3530157	91.3	445.0
4	Zeaxanthin	22.420	240693	6.2	452.3
5		25.073	19724	0.5	446.2

Microbiological Assay

Test	Specifications	Results
Standard Plate count	5000 CFU/g max	<10 CFU/g
Yeast/Mold	50/g max	<10 CFU/g
E. Coli	Negative/g	Negative
Salmonella	Negative/g	Negative

Analyst September 04, 2007 *Determined by spectroscopy, ± 5%Certificate of Analysis

Appendix 2B

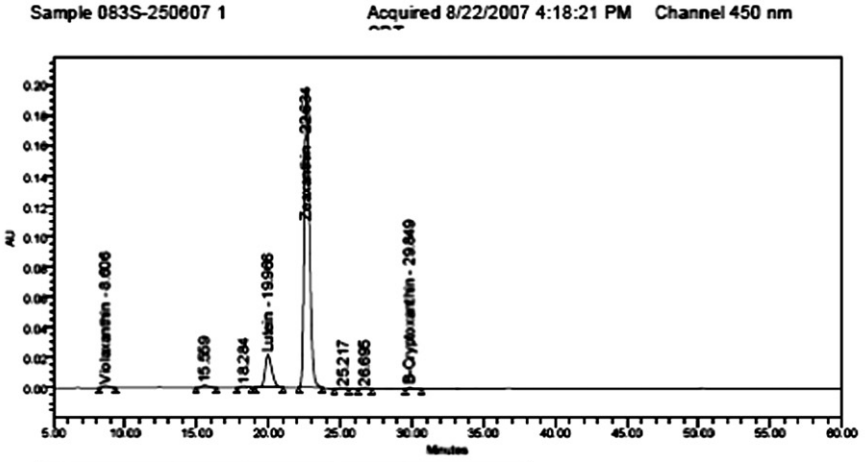
Zeaxanthin Softgel Lot 083S-250607

Carotenoid Profile

Zeaxanthin	85.1 %
Lutein	11.9 %

Carotenoid Content *

Total Carotenoids	9.5 mg/softgel
Zeaxanthin	8.1 mg/softgel
Lutein	1.1 mg/softgel



	Peak Name	RT	Area	% Area	Peak Lambda Max.
1	Violaxanthin	8.606	42098	0.7	445.0
2		15.559	59100	1.0	437.8
3		18.284	27677	0.5	428.1
4	Lutein	19.966	711935	11.9	445.0
5	Zeaxanthin	22.634	5094548	85.1	451.1

Microbiological Assay

Test	Specifications	Results
Standard Plate count	5000 CFU/g max	<10 CFU/g
Yeast/Mold	50/g max	<10 CFU/g
E. Coli	Negative/g	Negative
Salmonella	Negative/g	Negative

Analyst September 04, 2007 *Determined by spectroscopy, ± 5%

Appendix 2C

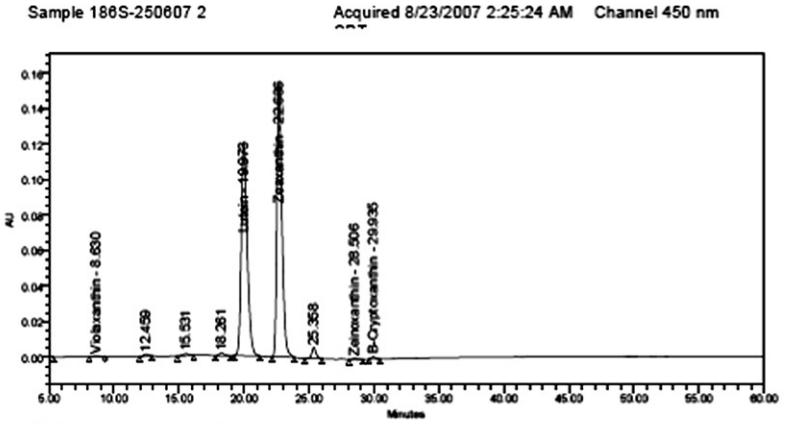
Lutein/Zeaxanthin Softgel Lot 186S-250607

Carotenoid Profile

Zeaxanthin	49.9 %
Lutein	46.2 %

Carotenoid Content *

Total Carotenoids	16.8	mg/softgel
Zeaxanthin	8.4	mg/softgel
Lutein	7.8	mg/softgel



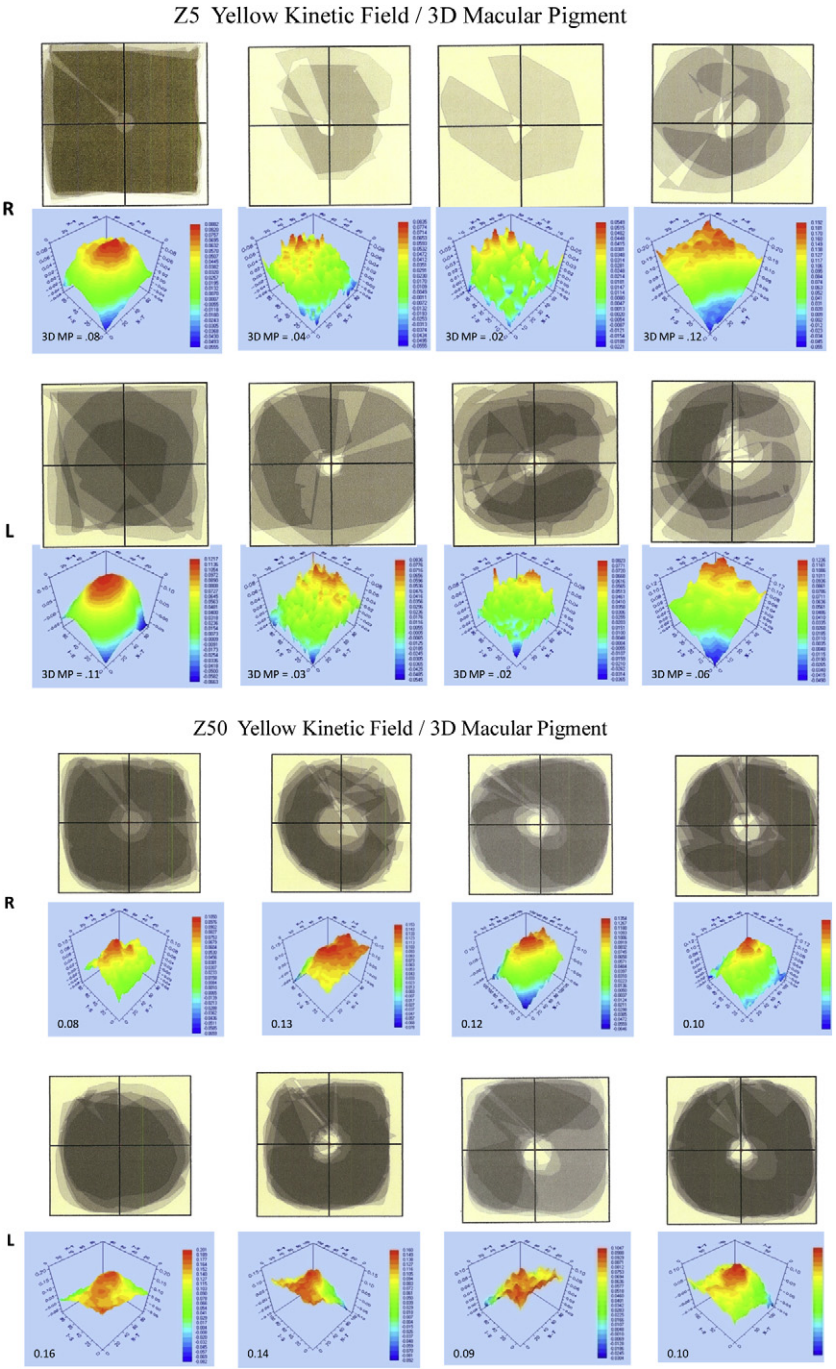
	Peak Name	RT	Area	% Area	Peak Lambda Max.
1		15.531	37605	0.5	437.8
2		18.261	45750	0.6	428.1
3	Lutein	19.973	3741429	46.2	445.0
4	Zeaxanthin	22.686	4035552	49.9	451.1
5		25.358	140152	1.7	443.8

Microbiological Assay

Test	Specifications	Results
Standard Plate count	5000 CFU/g max	<10 CFU/g
Yeast/Mold	50/g max	<10 CFU/g
E. Coli	Negative/g	Negative
Salmonella	Negative/g	Negative

Analyst September 04, 2007 *Determined by spectroscopy, ± 5%

Appendix 3 Examples of improvement in macular pigment and visual fields beginning at the 4-month visit, following zeaxanthin supplementation (patients Z5 and Z50)



Appendix 4 Selected AREDS/AREDS II nutrition per subgroup at baseline, final visit, and statistical significance

AREDS Nutrients (mean)

	Baseline			Significance	Final			Significance
	L	Z	L+Z	*P* values	L	Z	L+Z	*P* values
B carotene (IU)	4,758	5,167	4,488	Not significant	4,846	3,583	3,801	Not significant
Vitamin C (mg)	232	410	234	Not significant	468	302	186	(<0.08)
Vitamin E (IU)	29	72	29	Not significant	24	44	15	Not significant
Zinc (mg)	17	24	15	Not significant	21	25	17	Not significant
Copper (mg)	2	2	2	Not significant	2	3	2	Not significant

AREDS II Nutrients (mean)

	Baseline			Significance	Final			Significance
	L	Z	L+Z	*P* values	L	Z	L+Z	*P* values
N3 (g)	0.49	0.38	0.31	Not significant	0.29	0.34	0.29	Not significant
Carotenoids (µg)	8845	9387	8319	Not significant	9087	6586	7149	Not significant
Lutein (µg)	4025	4028	2702	Not significant	2826	2635	2556	Not significant