Enrichment of Macular Pigment Enhances Contrast Sensitivity in Subjects Free of Retinal Disease: Central Retinal Enrichment Supplementation Trials – Report 1

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Purpose. The high-performance visual function associated with central vision is mediated by the macula (the central retina), which accumulates three diet-derived pigments (the carotenoids lutein [L], zeaxanthin [Z], and meso-zeaxanthin [MZ]). Our study sought to investigate the impact on visual function, including contrast sensitivity (CS), of supplementation with these naturally occurring carotenoids, in individuals with low retinal concentrations.

Methods. Subjects consumed daily a formulation containing 10 mg L, 2 mg Z, and 10 mg MZ (active group; $n = 53$) or placebo ($n = 52$) for a period of 12 months. Study visits were at baseline, 3, 6, and 12 months. Contrast sensitivity at 6 cycles per degree (cpd) was the primary outcome measure (POM). Secondary outcome measures included CS at other spatial frequencies, best-corrected visual acuity (BCVA), glare disability, photostress recovery, and light scatter. Macular pigment optical density (MPOD) was measured using dual-wavelength autofluorescence, and serum carotenoid concentrations were analyzed using high performance liquid chromatography (HPLC).

Results. Compared to placebo, statistically significant improvements from baseline CS were detected at 6 ($P = 0.002$) and 1.2 ($P = 0.004$) cpd in the active group. Additionally, improvements in CS were commensurate with the observed increases in retinal concentrations of these carotenoids ($r = 0.342$, $P = 0.002$ at 6 cpd).

Conclusions. These results indicate that dietary fortification with the macular carotenoids can have meaningful effects on visual function.

Keywords: macular pigment, contrast sensitivity, meso-zeaxanthin, lutein, visual function, visual acuity, glare disability, randomized clinical trial

Macular pigment (MP), a yellow pigment concentrated at the macula, is composed of the xanthophyll carotenoids lutein (L), zeaxanthin (Z), and meso-zeaxanthin (MZ; Fig. 1).[1–3] Studies on this pigment, and its constituent carotenoids, have intensified over the last two decades, with researchers hypothesizing, investigating, and reporting on its origins and functions.[4] Specifically, research has been conducted on the role of supplementation with MP's constituent carotenoids (L, Z, and MZ) on clinical course[5,6] and vision[7] in patients with established nonadvanced age-related macular degeneration (AMD). These studies were prompted by the observations that MP is a powerful antioxidant[8,9] and also acts as a filter of short-wavelength visible (blue) light[10] (given that AMD is attributable, at least in part, to oxidative stress and that irradiation with blue light induces oxidative stress in the retina).[11]

In 2013, the AREDS2 study concluded that supplementation with at least two of MP's constituent carotenoids (L and Z, along with coantioxidants, vitamin C, vitamin E, zinc, copper) is beneficial in terms of reducing disease progression and in terms of visual outcomes in patients with nonadvanced AMD.[6] However, from an evolutionary perspective, it is unlikely that humans have evolved to selectively accumulate three carotenoids (L, Z, and MZ) in the central retina to retard the natural course of an age-related disease.[12] In other words, it seems intuitive that the primary role of MP is other than protection against age-related macular disorders.

Accordingly, many have postulated that MP is important for vision in a nondiseased eye, and this view was first proposed by Schultze et al. in 1866.[13] In brief, it is proposed that MP's prereceptorial filtration of short-wavelength visible (blue) light optimizes and/or enhances visual function by its attenuation of chromatic aberration and by its attenuation of the visual impact of light scatter, phenomena that are largely restricted to short wavelengths of visible light (i.e., blue light).[12,14–17] However, there are optical effects of the eye that reduce overall chromatic aberration.[18] Moreover, visual acuity is largely driven by middle- and long-wavelength sensitive cones.[19] Both of these effects serve to reduce the capacity of short-wavelength light (and thereby limit MP's ability) to influence visual acuity.

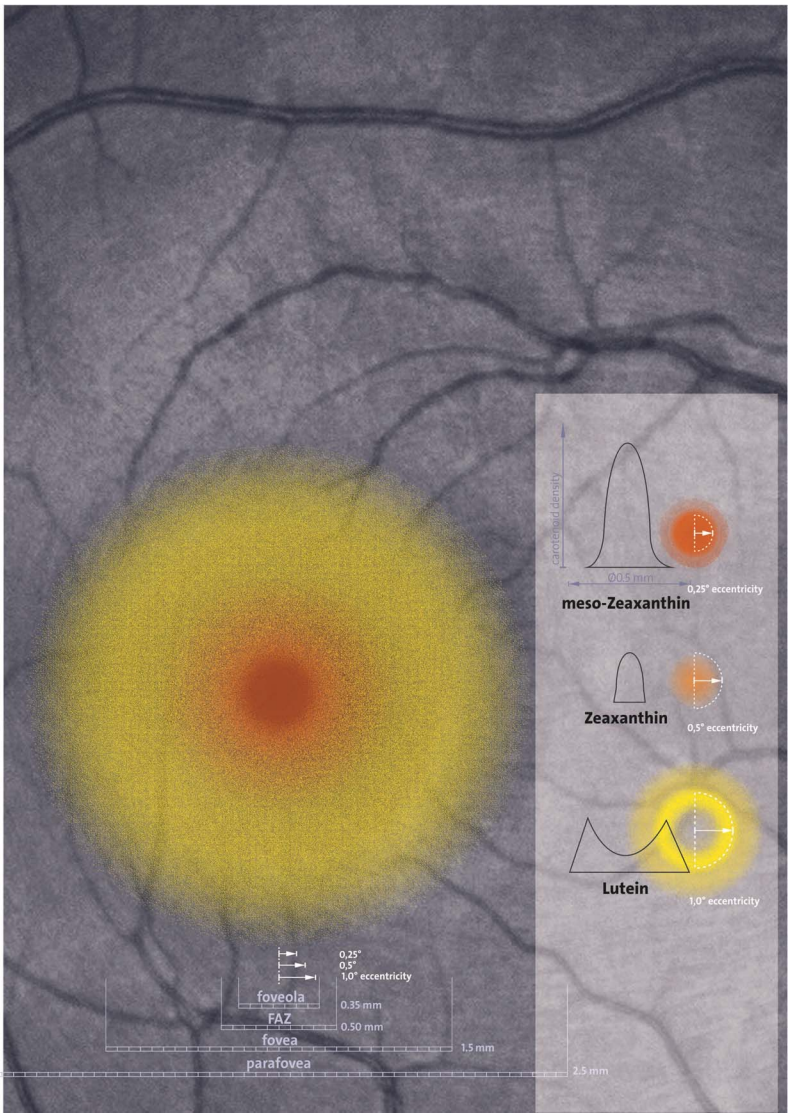

Figure 1. Distribution of MP's constituent carotenoids presented in scale onto a photograph of a healthy human retina. Figure courtesy of John Nolan, Robert Kuchling, and Kristiane Nöbel.

However, studies performed to date to test the above hypotheses have been limited in terms of their design (e.g., single-blind),[20] methodology (e.g., measurement devices not optimal or validated),[21,22] outcome measures (e.g., assessing visual function using, for instance, best corrected visual acuity [BCVA] only),[21] and interventions used (e.g., trials using supplements containing either low amounts of carotenoids [thereby limiting bioavailability][23] and, in most cases, the supplement formulations used only one or two of the three macular carotenoids [typically L and/or Z],[24,25] thereby precluding comment on the impact of supplementation with all three macular carotenoids, a desirable endeavor given that L, Z, and MZ are found in equal amounts at the macula[26]).

In July 2011, the European Research Council (ERC) awarded funding of €1,493,342 to support and conduct the Central Retinal Enrichment Supplementation Trials (CREST).[27] The CREST project was funded under the ERC "Ideas" Framework 7 program. The objective of CREST was to use a gold standard clinical trial design to study the "protective" and "visual function" hypotheses of MP. In brief, two clinical trials were established to investigate the impact of supplementation with a combined carotenoid formulation of MZ, L, and Z on visual function in normal subjects with low MP at baseline (Trial 1, the focus of the current report) and in subjects with early AMD (Trial 2, report to follow).

A novel and important feature of the CREST trials was the inclusion of MZ in the study intervention. Indeed, recent published data from our laboratory have shown that the addition of MZ to the carotenoid formulation, resulting in a MZ:L:Z (mg) ratio of 10:10:2, on a daily basis, results in optimal response in terms of: (1) total circulating serum carotenoid concentrations,[28] (2) enrichment of MP centrally and across its spatial profile,[28,29] (3) enhancements in visual function in subjects free of retinal disease,[30] and (4) enhancement of visual function in subjects with retinal disease (i.e., subjects with established nonadvanced AMD).[7,31] However, while these earlier and exploratory studies have added greatly to knowledge in the field regarding the importance (or not) of including all three of the macular carotenoids in a formulation, we felt that a gold-standard clinical trial, with optimal study design and appropriately informed outcome measures, was merited. With this objective in mind, the CREST study was designed and the findings of the CREST Normal trial (CREST Trial 1) are presented and discussed here.

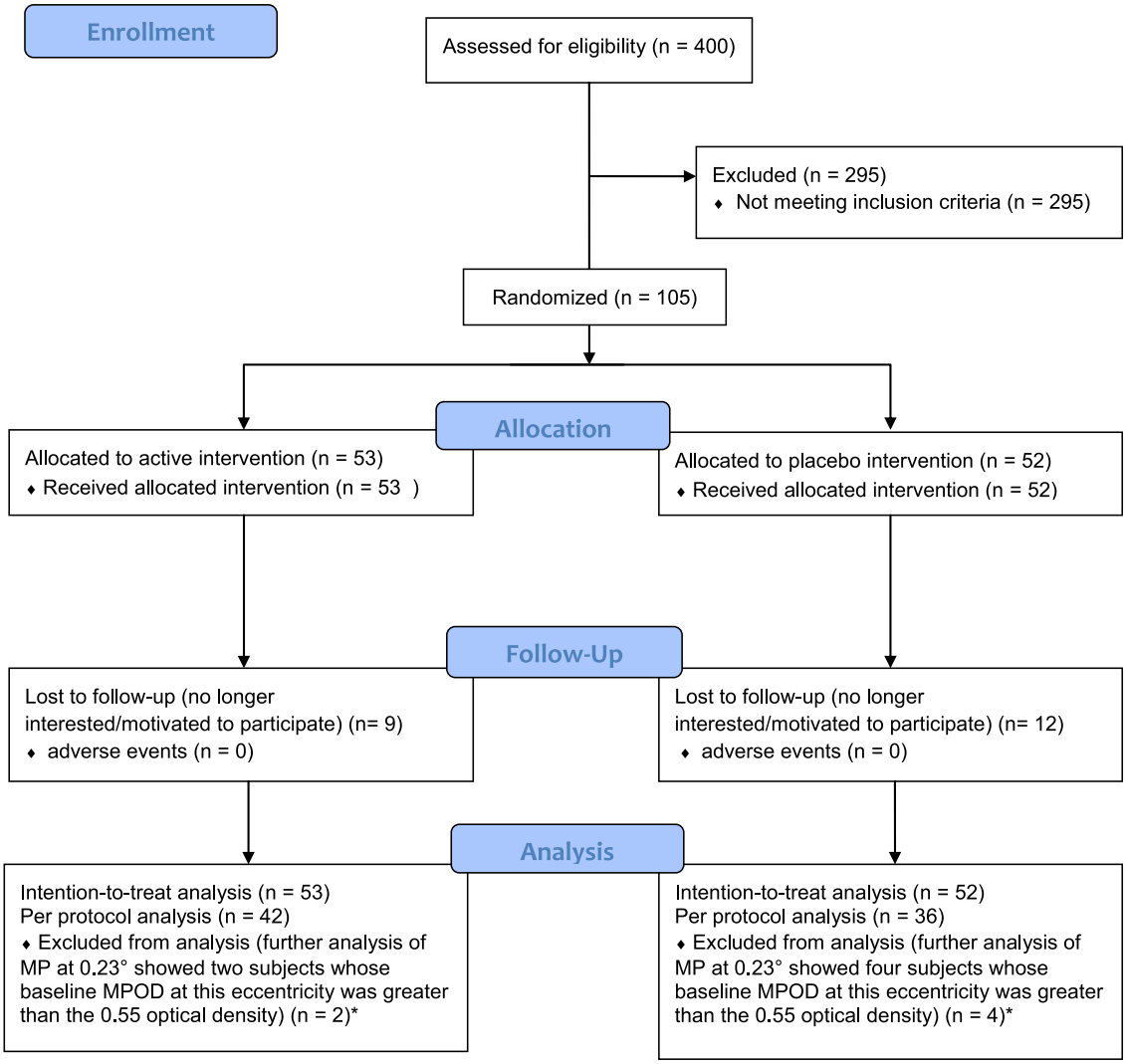

Figure 2. Consolidated Standards of Reporting Trials (CONSORT) Flow Diagram for CREST Trial 1.

Materials and Methods

The design and methodology of this study have been described in detail previously.[27] A summary of the methodology used in the CREST Trial 1 is presented below. In brief, CREST Trial 1 is a parallel group, double-blind, placebo-controlled, block-randomized trial investigating the impact of macular carotenoid supplementation on visual function in normal subjects with low MP at baseline (Trial registration No. ISRCTN68270512). The trial commenced in October 2012 (i.e., the first subject visit) and concluded in June 2015 (i.e., last subject 12-month visit).

Of 105 subjects (52 male, 53 female) originally recruited into the study, 10 were excluded before statistical analysis, as the threshold for defining "low" MP was set at 0.55 optical density units (for MP measured at 0.23° eccentricity, measured on the Heidelberg Spectralis [Heidelberg Engineering GmbH, Heidelberg, Germany], see Fig. 2). Before enrollment, all subjects provided written informed consent. Ethical approval was granted by the Research Ethics Committee of the Waterford Institute of Technology, Waterford, Ireland, and the Ethics Committee of the ERC. The CREST study adhered to the tenets of the Declaration of Helsinki, and followed the full code of ethics with respect to subject recruitment, subject testing, and data protection.

Inclusion criteria for participation in this study were as follows: age 18 years or older, monocular BCVA of 6/6 or

better, no more than ±5 diopters (D) spherical equivalence of refraction, no previous consumption of supplements containing the macular carotenoids (L, Z, and/or MZ), no ocular pathology, and MP at 0.23° of eccentricity ≤ 0.55 optical density units. A subject was defined as "normal" when he/she exhibited no vision-related abnormalities, which was assessed as follows: clinical examination, which consisted of ocular and medical history and general health questionnaire, BCVA measurement, MP measurement, optical coherence tomography (OCT), fundus photography, and completion of a general health questionnaire. This assessment battery was performed as part of a screening visit, which took place on a separate day, before a subject's baseline study visit (visit 1).

Subjects who passed the eligibility assessment were assigned to intervention groups in a ratio of 1:1 with no stratification using block randomization.[32] We randomly assigned 53 subjects to the active intervention, which contained 10 mg L, 10 mg MZ, and 2 mg Z in a sunflower oil suspension. There were 52 subjects randomly assigned to the placebo intervention, which contained just sunflower oil. Subjects were instructed to take one capsule daily with a meal. The intervention and placebo supplements were identical in external appearance and, therefore, the two treatments were indistinguishable from each other. Frequent phone calls and reminder text messages were sent to subjects to ensure compliance with consumption, and capsule counting was implemented at follow-up visits. Of note, capsule count was comparable between the active and placebo groups for each time point in the study.

Study visits occurred at baseline, and at 3-, 6-, and 12-month intervals. Study visits were conducted by one of two researchers (RP or JD). Statistical analysis by the research group statistician (JS) found no evidence of systematic difference in measurements, for any study outcome measure, between the two researchers. This was a single-site study, which presents advantages and limitations. The advantages of a study which involves only a single site include governance and validity/reproducibility of measurements, each being important in terms of standardization of methodology, quality control, and compliance with study visits/interventions. However, the principal disadvantage rests on the fact that, typically, a single site attracts only subjects from a given geographic area, and, therefore, is not necessarily generalizable to the overall population.

Demographic, Lifestyle, Medical, and Ophthalmic Assessment

Questionnaires were used to obtain demographic and lifestyle information at baseline. Medical and ocular histories also were documented. Body mass index (BMI) was calculated (kg/m^2) from height (m) and weight (kg) measurements recorded using the Leicester Height Measure and SECA weighing scales (SECA, Birmingham, UK), respectively. Weekly intake of carotenoid-rich foods (eggs, broccoli, corn, dark green leafy vegetables) was recorded using a dietary LZ screener previously used by our group and developed by Elizabeth Johnson.[33]

Assessing Visual Function

The eye with the best visual acuity was selected as the study eye for assessment. Where both eyes had the same BCVA, the right eye was chosen. Best corrected visual acuity was measured with a computerized LogMAR Early Treatment Diabetic Retinopathy Study (ETDRS) test chart (Test Chart 2000 Xpert; Thomson Software Solutions, Hatfield, UK). Letter contrast sensitivity (CS) was assessed using the computerized ETDRS test chart (Test Chart 2000 PRO) at five different spatial frequencies (1.2, 2.4, 6.0, 9.6, 15.15 cycles per degree [cpd]). Both visual performance tests used the Sloan optotypes and were viewed at a distance of 4 m. Contrast sensitivity also was assessed using the Optec Functional Vision Analyzer[34] (Stereo Optical Co., Inc., Chicago, IL, USA), which uses the functional acuity contrast test to assess CS at five different spatial frequencies (1.5, 3, 6, 12, 18 cpd). These methods have been described in more detail previously.[30]

The amount of intraocular straylight on the retina was measured using the C-Quant Straylight Meter (Oculus GmbH, Wetzler, Germany). Photostress recovery time was measured by assessing CS and investigating the impact of a light stress using a 300-watt tungsten spotlight (ARRI 300 Plus lamp; ARRI Lighting Solutions GmbH, Berlin, Germany) with a low-pass glass dichroic filter. A CS value of 0.30 log units (i.e., two lines on Letter CS) above the individual's contrast threshold was used. The time taken for the subject's study eye to recover (nonstudy eye was covered with an eye patch) and see all five letters on the chart after the 10-second exposure was taken as the photostress recovery time (seconds). Visual function was also assessed subjectively (at baseline and 12 months only) via questionnaire.[35]

Fundus Photography and Grading

All photography was performed by trained and certified photographers. Standard color fundus photographs centered on the macula were taken using the Zeiss Visucam 200 (Carl Zeiss Meditec AG, Jena, Germany) at a 45° magnification setting, following pupil dilation. These fundus photographs were reviewed by an ophthalmologist (SB) to exclude any ocular pathology.

Macular Pigment Measurement

Macular pigment was measured using the Heidelberg Spectralis HRA+OCT MultiColor (Heidelberg Engineering GmbH). Pupillary dilation was performed before measurement. This technology uses confocal scanning laser ophthalmoscopy (cSLO) imaging with diode lasers and uses dual-wavelength autofluorescence (AF) for measuring MP.[36] Dual-wavelength AF in this device uses two excitation wavelengths, one that is well-absorbed by MP (486 nm, blue), and one that is not (518 nm, green). A 30-second video was taken in simultaneous blue AF and green AF imaging mode for MP measurement acquisition. The video images were aligned and averaged using the Heidelberg Eye Explorer software (HEYEX, version 1.7.1.0), from which a MP density map was created. Central MP at 0.23° eccentricity and MP volume (calculated as MP average times the area under the curve out to 7° eccentricity) are reported here.

Serum Carotenoid Assessment

Nonfasting blood samples were collected at each study visit by standard venipuncture techniques in 9 mL vacuette tubes (BD Vacutainer SST Serum Separation Tubes; Becton, Dickinson and Company, Plymouth, UK) containing a "Z Serum Sep Clot Activator." All collection tubes were inverted a minimum of five times to ensure appropriate mixing of the clot activator. The blood samples were allowed to clot at room temperature for 30 minutes, after which they were centrifuged for 10 minutes at 725*g* in a Gruppe GC 12 centrifuge (Desaga Sarstedt, Hampshire, UK) to separate the serum from the whole blood. After centrifugation, serum was transferred to light-resistant microtubes and stored at circa –80°C until the time of batch analysis. Serum carotenoid analysis was done by

high performance liquid chromatography (HPLC) as described previously.[27,37]

Statistical Analysis

The statistical package IBM SPSS version 22 was used for all analyses. Contrast sensitivity at 6 cpd was the primary outcome measure (POM) of this study. Secondary outcome measures included CS at other spatial frequencies, visual acuity, glare disability, photostress recovery, light scatter, MP, serum carotenoid concentrations, and subjective visual function.

This was a double-blind, placebo-controlled, block-randomized clinical trial. Sample size was estimated as 45 in each of the active and placebo groups, based on an effect size of 0.15 log CS units in the POM (equivalent to one line on Letter CS [Thomson Test Chart 2000 PRO]), 80% statistical power, and a 1-tailed test at the 5% level of statistical significance. Estimates of standard deviations and pre–post correlation, needed for the sample size calculation, had been obtained from an earlier pilot study. The decision to use a 1-tailed test, in sample size calculation, also was based on the results of this pilot test, where we had found clear evidence that the POM improved significantly in the active supplement group relative to the placebo group. We had targeted to recruit 120 subjects into this trial (30 more than indicated by the sample power calculations), and we screened a total of 400 subjects to achieve this target of 120. In the event, only 95 subjects (24% of those screened [see Fig. 2]) eventually were deemed eligible (met all inclusion criteria, including MP at 0.23° of eccentricity $\leq$ 0.55 optical density units); 10 more subjects participated in the study but were excluded from statistical analysis due to exceeding the MP threshold.

No adjustment was made for multiple comparisons. Standard statistical tests, such as the independent samples *t*-test for quantitative variables, and the contingency table χ^2 test for categorical variables, were used to compare active and placebo groups at baseline. Repeated measures ANOVA was used for the between-group comparisons of change in outcome variables over time. As specified in the CREST methodology study,[27] subjects who failed to complete the full 12 months of trial were not included in final between-group analysis; however, we performed additional intention to treat analysis using Last Observation Carried Forward (LOCF), for purposes of comparison whenever the main analysis, excluding missing values, produced statistically significant results. Statistical significance was set at the standard $P < 0.05$ for all analyses.

Results

Baseline

Table 1 presents baseline summary statistics for demographic, health, lifestyle, and vision study variables, in the active and placebo intervention groups. There were no statistically significant differences between treatment groups for any of these variables at baseline.

Change in Outcome Variables Over Time

Change in Contrast Sensitivity. Table 2 shows (for active and placebo study groups) changes, over the 12-month study period, in mean CS at five different frequencies, as well as changes in mean BCVA. The final column of Table 2 displays the *P* values for the time-group interaction effects; that is, it identifies those outcome variables for which the mean change, after 12 months, was significantly different between active and placebo groups. Figure 3 displays graphically the mean CS curves for the active and placebo groups at baseline and 12 months. As seen in Figure 3 and Table 2, mean changes in two CS outcome measures (CS at 1.2 and 6 cpd [POM]) were statistically significantly different between the active and placebo intervention groups by 12 months. These statistically significant differences constituted an improvement in CS in the active treatment group. Of note, intention to treat analysis also gave statistically significant results for both of these CS outcome measures.

Change in Serum Carotenoids and MP. Figure 4 shows (for the active and placebo groups) mean change in serum L, MZ, and Z, over the 12-month study period. Of note, for each carotenoid analyzed, a drop in serum concentration was seen at V4, which may reflect the influence of a regulatory process governing uptake of circulating carotenoids.

Figure 5 shows the corresponding results for MP at 0.23° and MP volume. The error bars do not overlap, indicating statistically significant increases in the active intervention group compared to the placebo group, with the exception of serum Z at 12 months.

Change in Other Outcome Variables: Visual Acuity, CS at Other Eccentricities, Glare Disability, Photostress Recovery, Light Scatter and Subjective Visual Function. Repeated measures ANOVA of all other study variables (Table 1) did not reveal any statistically significant differences, over the 12-month study period, between active and placebo intervention groups.

When Did Significant Change Occur?

In this study, the statistically significant differential in CS in the active treatment group versus the placebo group was not observed until 12 months (i.e., with no significant change in CS by 3 or 6 months). However, the statistically significant increases in serum L, Z, and MZ, and in MP (at 0.23° and MP volume) all occurred by 3 months ($P < 0.0005$ for all, repeated measures ANOVA).

Relationship Between Change in Serum Concentrations of L, Z, and MZ, Change in MP, Versus Change in CS

We also investigated the relationship of change in CS (at 6 and 1.2 cpd) versus change in MP, measured over the 12-month study period. We did this for placebo and active intervention groups combined, using Pearson correlation analysis, and found positive and statistically significant relationships between change in MP and change in CS.

The following relationships were positive and statistically significant: change in central MP and change in CS at 6 cpd (POM, $r = 0.342$, $P = 0.002$), change in MP volume and change in CS at 6 cpd (POM, $r = 0.255$, $P = 0.024$), change in central MP and change in CS at 1.2 cpd ($r = 0.249$, $P = 0.028$), and change in MP volume and change in CS at 1.2 cpd ($r = 0.293$, $P = 0.009$). Thus, in general, greater changes in subjects' MP were associated with greater changes in CS.

Of note, the relationship between change in each of the serum carotenoids, and change in MP, over the 12-month study period, was positive and statistically significant ($P < 0.01$ for all). Thus, in general, greater changes in subjects' serum carotenoid concentrations were associated, in this study, with greater changes in MP. However, no statistically significant relationships were observed between change in serum concentrations of L (or MZ) and change in CS at any spatial frequency ($P > 0.05$, for all).

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Table 1. Demographic, Health and Lifestyle, Vision, and MP Data of the Active and Placebo Intervention Groups

Variables	Active Intervention, $n = 48$	Placebo Intervention, $n = 47$	Sig.
Demographic and health			
Age, y	44.83 ± 11.46	46.49 ± 13.07	0.513
BMI, kg/m^2	27.32 ± 4.69	26.32 ± 4.58	0.319
Exercise, min/wk	288.72 ± 306.51	286.63 ± 296.98	0.973
Diet, estimated intake of L and Z	24.13 ± 14.69	21.5 ± 12.8	0.357
Sex, % male	47.9	53.2	0.607
Education, highest level %			0.903
Primary	2.1	2.1	
Secondary	22.9	19.1	
Higher, third level	75	78.7	
Smoking, %			0.720
Never smoked	45.8	46.8	
Past smoker	35.4	31.9	
Current smoker	16.7	21.3	
Alcohol frequency, %			0.103
Never	6.4	2.1	
Special occasions	6.4	19.1	
1–2 times/mo	23.4	21.3	
1–2 times/wk	63.8	51.1	
Everyday	0	6.4	
AMD family history, % yes	10.4	17	0.370
Vision			
BCVA	105.67 ± 3.79	106.41 ± 4.26	0.373
CS 1.2 cpd	1.96 ± 0.10	1.98 ± 0.12	0.398
CS 2.4 cpd	1.94 ± 0.12	1.95 ± 0.17	0.919
CS 6 cpd	1.68 ± 0.15	1.69 ± 0.21	0.841
CS 9.6 cpd	1.47 ± 0.14	1.51 ± 0.21	0.254
CS 15.15 cpd	1.17 ± 0.19	1.19 ± 0.25	0.512
Light scatter	1.17 ± 0.17	1.22 ± 0.22	0.285
PRT, seconds	22.88 ± 17.04	23.96 ± 16.40	0.753
MPOD 0.23°	0.38 ± 0.08	0.38 ± 0.10	0.925
MPOD volume	3992.98 ± 1288.81	3792.94 ± 1597.32	0.504

BCVA was reported in visual acuity rating, CS was reported using the Thomson Test Chart 2000PRO, and PRT was reported in seconds. Data displayed are mean ± SD for numerical data and percentages for categorical data. Variables, variables analyzed in the study; Active Intervention, group supplemented with 10 mg L, 10 mg MZ, and 2 mg Z in a sunflower oil suspension; Placebo Intervention, group supplemented with sunflower oil; Sig., the statistical difference (*P* value) between the groups; BMI, the body mass divided by the square of the body height, expressed in units of kg/m^2; Exercise, total exercise measured as minutes per week engaged in physical or sporting activity; Diet score, estimated dietary intake of lutein and zeaxanthin; Education (highest level %), highest level to which subject was educated; Smoking (%), current smoker (smoked ≥100 cigarettes in lifetime and at least one in the last year), past smoker (smoked ≥100 cigarettes in lifetime and none in past year), or nonsmoker (smoked < 100 cigarettes in lifetime); Alcohol frequency, frequency of consumption of Alcohol; AMD family history (% yes), the percent of subjects with a confirmed family history of AMD for a first degree relative; MPOD 0.23°, MPOD at 0.23° of retinal eccentricity; MPOD volume, the volume of macular pigment out to 7° of retinal eccentricity.

Discussion

The CREST Trial 1 is a double-blind, placebo-controlled, block-randomized clinical trial, designed to investigate the impact of supplementation with all three macular carotenoids (in a MZ:L:Z [mg] ratio of 10:10:2) on visual function in individuals free of retinal disease, but with low MP at study baseline. The design and methodology of this study have been informed by the published literature, and in consultation with the world's leading macular carotenoid vision scientists.[27] Of note, to our knowledge this is the first study of rigid and gold standard design and with published a priori outcome measures (POM, CS at 6 cpd) designed to investigate the impact, if any, of supplemental macular carotenoids on visual function in nondiseased eyes. The principal finding was that, following supplementation with the macular carotenoids in a MZ:L:Z (mg) ratio of 10:10:2 for 12 months, the POM (CS at 6 cpd) exhibited significant improvement.

Visual performance, in spite of the multifaceted composite that it represents, is typically oversimplified to measures of visual acuity, even by eye health professionals. Although visual acuity is, indeed, an important aspect of visual performance, it is by no means a surrogate for an individual's visual performance and experience, and other variables relating to visual function should be investigated when conducting a scientific study of this nature.

Vision is a composite of optical, physiologic, and neural processes. One could argue that visual acuity is largely determined by the optics of a healthy eye, reflected in the observation that optical resolving power is the principal determinant of acuity.[38] Some parameters of visual performance, however, such as dark adaptation,[39] are mediated primarily by physiologic processes, whereas others, such as color constancy,[40] are the result of visual processing at higher levels (i.e., cortex). Interestingly, the results of our study suggest an outcome governed by some or all of the determinants of visual performance: for example, visual acuity did not change over the study period for subjects in the active supplement group, whereas CS did (Fig. 3B; Table 2), and this

Table 2. Repeated Measures Analysis of Visual Function Variables From Baseline to 12 Months Showing the Time Group Interaction

	Active Intervention				Placebo Intervention				Time X Group Interaction
	Baseline		12 Mo		Baseline		12 Mo		
Variables	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*
BCVA	105.67	3.79	105.7	4.25	106.41	4.26	106.12	3.56	0.398
CS 1.2 cpd	1.96	0.1	2.08	0.12	1.98	0.12	1.98	0.12	0.004*
CS 2.4 cpd	1.94	0.12	2.00	0.15	1.95	0.17	1.98	0.12	0.13
CS 6 cpd	1.68	0.15	1.76	0.16	1.69	0.2	1.68	0.2	0.002*
CS 9.6 cpd	1.47	0.14	1.46	0.18	1.51	0.21	1.51	0.21	0.761
CS 15.5 cpd	1.17	0.19	1.16	0.23	1.2	0.25	1.22	0.22	0.967

Contrast sensitivity was assessed using the Thomson Test Chart 2000PRO.

* Significant difference between groups at the 0.05 level; only subjects with MP at 0.23° eccentricity ≤ 0.55 optical density units were included. Per protocol analysis $n = 42$ in the active arm and $n = 36$ in the placebo arm (see Fig. 2 for full breakdown).

observation indicates that CS (although a correlate of visual acuity)[41] is influenced by factors other than the determinants of acuity, including retinal and/or cortical factors, thereby explaining the disparity of the results in terms of visual acuity versus CS.

In this study, we reported statistically significant improvements in CS at 1.2 and 6 cpd, associated with changes in MP, but a discussion on the clinical significance of this finding is merited. Clinical meaningfulness is difficult to define. However, having based our study sample size calculations on an effect size of 0.15 log CS units—one line on a contrast sensitivity chart—it seems reasonable also to define clinical significance in these terms. For CS at 6 cpd (POM), 28.5% of subjects in the active supplement group, and just 3.1% of subjects in the placebo group, improved CS by at least one line on a chart. The corresponding percentages for CS at 1.2 cpd were 37.5% in the active supplement group and 6.2% in the placebo group, who also improved CS by at least one line on a chart. Given that the carotenoid status of our subjects' retinas was significantly augmented over the study period, it is safe to assume that the observed impact on CS is attributable to the observed augmentation of MP.

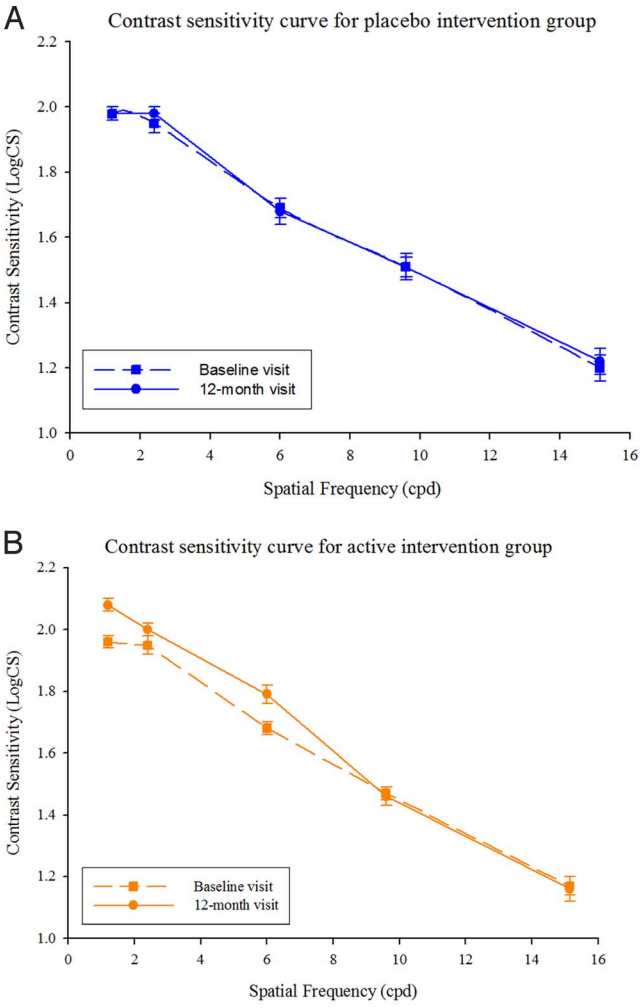

Figure 3. (**A**) Letter CS function for placebo intervention group. (**B**) Letter CS function for active intervention group.

All subjects in the active intervention arm of the study exhibited augmentation of MP, reflected in a mean (± SD) increase in MP volume of 2436 (± 1451), and a range of observed increases in MP volume of 738 to 6464. In percentage terms, MP volume increased by a mean (± SD) of 73% (± 62%), with a range of increases of 16% to 337%. This is an important observation, given that circa 20% of supplemented subjects do not normally exhibit any rise in MP in studies that did not include MZ in the formulation,[35,42] consistent with the view that some individuals lack the capacity to bioconvert retinal L to retinal MZ.[43,44]

We believe that the visual improvements observed herein are the result of at least one of two mechanisms. First, the prereceptoral filtration of blue light could reduce chromatic aberration and also reduce the impact of any (albeit mild) light scatter. These effects could plausibly improve CS, but arguments against the observed improvement being attributable to prereceptoral filtration of visible blue light include the fact that light and dark bars would be equally affected, thereby negating any perceived differences in luminance that would serve to enhance CS. Further, given the moderate light levels during testing in the current study, scattered light would not be expected to appreciably affect visual performance in an adverse way. Lastly, if prereceptoral absorption of blue light was driving the observed visual benefits reported herein, the effect would be at higher spatial frequencies than those that we observed because MP optical density (MPOD) peaks centrally, where the density of photoreceptors averages 200,000/mm^2 and where it can be much higher[45] (an observation that is responsible for very fine visual resolution [including CS for high spatial frequencies] at this locus). However, because CS improved only for frequencies near the peak of the contrast sensitivity function, and not for high spatial frequencies, it is likely that the observed visual benefits are primarily physiologic/retinal/cellular in origin, rather than

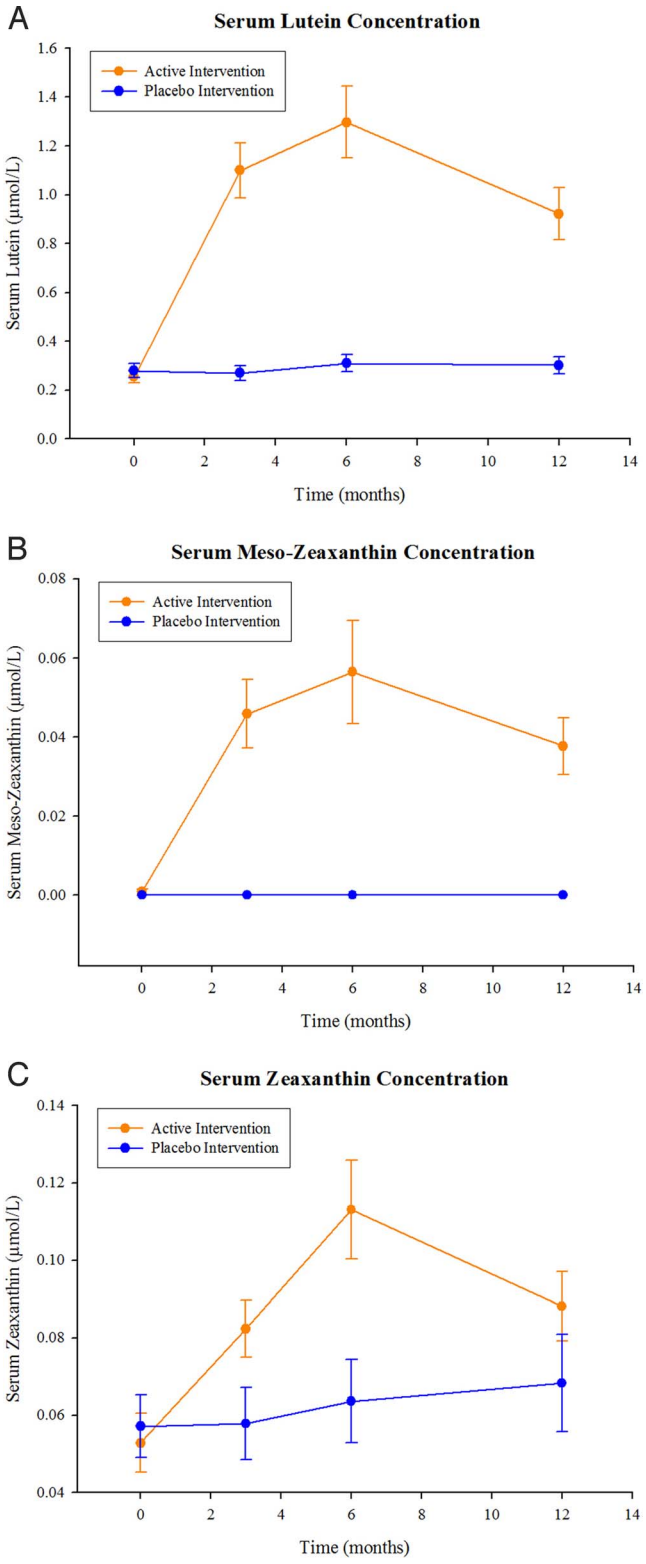

FIGURE 4. (A) Serum L response for the active and placebo groups over the study period. (B) Serum MZ response for the active and placebo groups over the study period. (C) Serum Z response for the active and placebo groups over the study period.

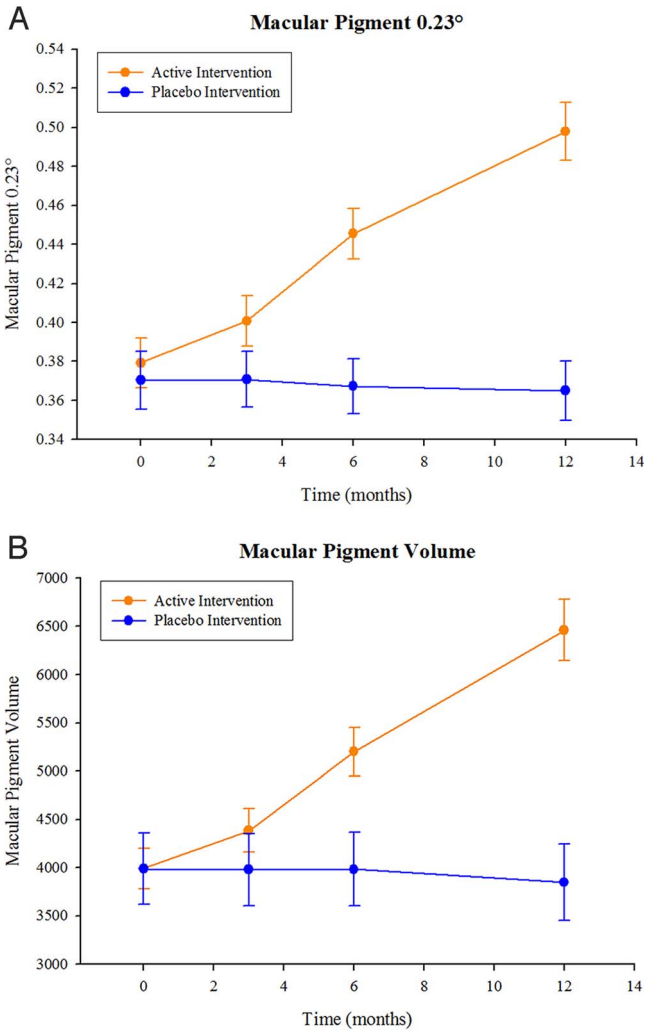

FIGURE 5. (A) Macular pigment response for the active and placebo groups at 0.23° of eccentricity over the study period. (B) Macular pigment volume response for the active and placebo groups over the study period.

being solely attributable to the optical impact of augmented MP.

This brings us to the second, and seemingly more plausible mechanism for our observations. The seminal work of Kuffler,[46] and several subsequent investigations,[47] have characterized the anatomic and neurophysiologic basis for CS lateral inhibition. In short, lateral inhibition is the result of retinal circuitry that is wired in such a way as to produce many thousands of overlapping, roughly concentric, subtractive regions called receptive fields.[48] Light differentially affects the center versus surround regions of the receptive field and, ultimately, the perceived difference between the two yields the visual system's ability to detect edges (i.e., contrast). The arrangement of the receptive fields is such that a difference in CS is a function of spatial frequency, and this phenomenon is known as the contrast sensitivity function (CSF), and, when tested with sinusoidal gratings, its peak generally is found to be approximately 4 cycles/deg (although the function is fairly broadly tuned).[49] Based on our results, in some manner, increased macular carotenoid concentration probably enhances lateral inhibitory processes that yield performance increases near the peak of the CSF. There is a plausible mechanism for this effect. For example, it could be that increased MP simply

leads to increased efficiency in the visual cycle. This idea is consistent with our findings, and, given the macular carotenoids' exceptional antioxidant properties,[9] is also consistent with the effect of visual cycle inhibition/disruption by oxidative stress.[50] It has been shown that the retinal carotenoids serve to strongly inhibit the activity of A2E, itself the product of oxidative stress and a potent visual cycle inhibitor.[51] At the level of perception, a more efficient visual cycle is likely to manifest as increased CS, especially for those neural networks that are under the greatest metabolic stress (i.e., near the peak of the CSF). In consideration of our finding of enhanced CS following enrichment of MP in the active group, this idea was first introduced by Stringham et al.,[52] who found a relationship between MPOD and CS for a slightly higher spatial frequency (10 cycles/deg). The idea was subsequently expanded upon,[53] and further supported by the suggestion of a plausible molecular mechanism involving the interplay of retinal carotenoids and nitric oxide,[54] whereby increased macular carotenoids facilitate the ability of nitric oxide to increase the signal-to-noise ratio of horizontal cells that serve center-surround receptive fields.[55]

An important distinction between the findings of our study and those of previous investigators is that we observed improvements in CS that were commensurate with MP augmentation, which suggests that the observed benefits are, indeed, attributable to observed increases in MP over the study period (and not attributable to interindividual variability in factor[s] related to MP). In other words, our findings are not simply associative, and inference of causality is justified.

In terms of everyday meaningfulness of improved CS following supplementation with the macular carotenoids, several practical and clinical benefits can be expected by the individual. Most obvious would be a general improvement in visual discrimination for objects in real-world scenes, such as resolving individual leaves on a tree, whereas perhaps before improvement, leaves would tend to blend together. Indeed, it has been found that the human CSF very closely follows the image characteristics of natural scenes, reflective of the evolution of spatial vision.[56] In an automobile driving situation, increased CS would allow for earlier and more accurate detection of objects.[57,58] Given that automobile safety often is the result of a split-second reaction to rapidly changing environmental conditions, this kind of improvement, no matter how small, would improve outcomes.[59] Indeed, some countries in Europe recently have added measures of CS (rather than performing measures of visual acuity alone) for assessing eligibility criteria to drive. In the United Kingdom, for example, the visual standard to hold a driver's license requires that the applicant achieves a visual acuity of 6/12 (20/40) or better (measured indoors) and demonstrates the ability to read a car number plate (measured [outdoors] at a specified distance),[60] in keeping with a European Union directive on driving licensure.[61] However, and given that subjects with reduced CS have greater difficulty outdoors,[62,63] it is unsurprising that visual acuity is not predictive of the ability to read a number plate in those with poor CS.[57] In other words, poor CS creates a disconnect between the ability to read a car number plate and visual acuity, thereby negating the value of acuity readings for the purpose of assessing a subject's eligibility to drive. Of note, CS also is important for train drivers (in Europe), as European Union (EU) legislation now specifies the need for good CS for those seeking certification to operate locomotives and trains on the EU railway system.[64]

Lastly, general quality of life would likely be improved by enhancements in CS (e.g., enjoying a scenic view, and so forth), and even small improvements in CS for those spatial frequencies near the peak of the CSF could have meaningful effects, for example, making printed text easier to process; thus, easing eye strain and fatigue over the course of a day. Moreover, those engaged in vision-dependent activities for the military (e.g., sniper units, aviators, and so forth) and sports (e.g., baseball players, tennis players, and so forth) could expect improvements in performance.

In conclusion, we found that in subjects free of retinal disease and with low MP, supplementation with a formulation containing all three macular carotenoids resulted in measurable improvements in vision, reflected in enhanced CS at 6 and 1.2 cpd. These findings may have important implications for those endeavoring to maximize their visual performance and experience, whether for professional or leisure activities.

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