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Research article

Serum and retinal responses to three different doses of macular carotenoids over 12 weeks of supplementation

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ABSTRACT

The macular carotenoids lutein (L), zeaxanthin (Z), and mesozeaxanthin (MZ) have been shown to have neuroprotective and visual performance benefits once deposited in retinal tissues. The purpose of this 12-week trial was to determine biweekly the absorption kinetics, efficiency of retinal deposition, and effects on the spatial profile of macular pigment for three levels of L + Z + MZ supplement.

This study was a double-blind, placebo-controlled 12-week trial. Twenty-eight healthy subjects, aged 18–25 yrs participated. Subjects were randomly assigned to one of four daily supplementation groups: placebo (safflower oil; n = 5), 7.44 mg total macular carotenoid (n = 7), 13.13 mg total macular carotenoid (n = 8), and 27.03 (n = 8) mg total macular carotenoid. Ratios of the three carotenoids were virtually identical for the three levels of supplement (83% L, 10% Z, 7% MZ). At baseline and every two weeks thereafter over the 12-week study period, a fasting blood draw was conducted and, via heterochromatic flicker photometry, spatial profiles of macular pigment optical density (MPOD) were determined.

Compared to placebo, serum concentrations of both L and total Z, for each of the supplement levels, were found to increase significantly from baseline after two weeks of daily ingestion ($p < 0.001$). Likewise, MPOD increased significantly in all treatment groups ($p < 0.001$) compared to placebo. Serum responses (L, Z, and L + Z) were linearly related to dose ($p < 0.001$ for all), but not to retinal response. L: Z serum response ratios decreased exponentially with increases in dose ($p = 0.008$). The ratio of MPOD change: total serum response was found to be highest for the 13.13 mg level of supplement ($p = 0.021$), followed by 27.03- and 7.44-mg doses. The very center of the spatial profile of MPOD increased in a fashion commensurate with dose level.

Although L serum responses increased with dose, the slope of increase was shallower than for Z. Given the higher levels of L in the supplements, this is suggestive of a compressed response with relatively high doses of L. Although all three doses significantly augmented MPOD, the 13.13 mg/day L + Z supplement level was the most efficient in doing so. The data regarding efficiency may inform recommendations regarding macular carotenoid supplementation for age-related macular degeneration. Lastly (although not statistically significant), the shift toward a more pronounced central peak in the spatial profile of MPOD in all treatment groups suggests that central retinal deposition of Z and MZ was efficient and can be seen after a short period of supplementation, especially with higher (e.g. 27.03 mg) daily doses of macular carotenoids.

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1. Introduction

Lutein (L) and zeaxanthin (Z) are diet-derived, yellow-orange colored carotenoids obtained primarily from leafy-green vegetables (Sommerburg et al., 1998). L and Z are not synthesized by the body, and therefore must be obtained via dietary means; those who have diets rich in leafy greens, or supplement with sufficient L and Z tend to maintain and accumulate higher blood and tissue concentrations (Ciulla et al., 2001; Bone et al., 2003). One of the conspicuous features of L and Z is their specific accumulation in the macular retina (Snodderly et al., 1984b), where they can reach extremely high

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concentrations (e.g. Hammond et al., 1997); it is not uncommon to see concentrations in the fovea that exceed 10,000 times that seen in the blood (Bone et al., 1993). Once deposited in the retina, some of the L is converted to a stereoisomeric form of zeaxanthin, called mesozeaxanthin (MZ; Neuringer et al., 2004). Although rare, MZ has been shown to exist in nature, and indeed in the human food chain – its presence has been recently verified in salmon, trout, and sardine skin, and also trout flesh (Nolan et al., 2014). Importantly, MZ has been shown to be readily deposited in the retina when taken in supplement form (Loughman et al., 2012). The accumulation of these three carotenoids in the macula yields a yellowish coloration, classically known to ophthalmologists as the "macula lutea" ("yellow spot"; first noted by Buzzi, 1782). Today, this collective pigmentation is commonly referred to as macular pigment (MP; Wald, 1945), with concentrations typically expressed in terms of optical density (MPOD).

Xanthophyll carotenoids such as L, Z, and MZ are especially potent antioxidants (Krinsky et al., 2003). Via a process called triplet excitation transfer (Ruban et al., 2002), L, Z, and MZ can regenerate to repeatedly "quench" the energy of singlet oxygen. This makes them capable of long-term accumulation in target tissues such as the retina, where they can provide protection against oxidative stress. Another critical function of the macular carotenoids involves their optical properties within the eye. As noted above, one of the primary tissue targets for these carotenoids is the retina, where they accumulate (as MP) in very high densities in the fovea (Snodderly et al., 1984b). Specifically, L, Z, and MZ are deposited in retinal layers anterior to the lipid-rich photoreceptor outer segments, which are vulnerable to oxidation by radiant energy (Wiegand et al., 1983). The central, pre-receptoral location of macular pigment is therefore advantageous in at least three ways: 1) It enables the yellow-orange MP to filter high-energy short-wavelength (blue) light (Snodderly et al., 1984a) before it can cause damage via lipid peroxidation of the photoreceptor outer segments, 2) Its central location in the fovea preferentially protects the cones serving high-performance central vision (whose densities in humans can reach 400,000/mm^2 and perhaps even higher (Curcio et al., 1990), and 3) The filtration of short-wave (blue) light can yield visual performance benefits, such as improvements in contrast sensitivity (Loughman et al., 2012; Kvansakul et al., 2006; Stringham et al., 2011; Yao et al., 2013; Sasamoto et al., 2011), parameters of visual performance in glare (Stringham et al., 2011; Hammond et al., 2013), chromatic contrast (Hammond et al., 2013) and outdoor vision through atmospheric haze (Fletcher et al., 2014). Importantly, the long-term protection conferred to the retina by the impressive antioxidant and filtering capability of MP translates to a significantly reduced risk of developing diseases that are brought on by cumulative tissue damage, including age-related macular degeneration (AMD; e.g. Seddon et al., 1994), the leading cause of blindness in the Western world (Klaver et al., 1998).

Given the many benefits of a diet rich in L and Z, and relatively high tissue densities of L, Z, and MZ, a pressing question going forward involves the response kinetics of people to different levels of carotenoid ingestion. The development of reliable dose/response curves for these carotenoids would enable us to better understand dietary need and its relationship to health and performance benefits. Additionally, the fact that carotenoids are often affected by competitive absorption with each other (Wang et al., 2010) suggests that the relationship between dietary intake of a mixture of carotenoids and absorption profile could be vastly different. Previous studies have determined that supplementation with the macular carotenoids generally yields significant increases in serum concentrations and MPOD in healthy subjects over study periods ranging from 8 weeks (Connolly et al., 2010) to 1 year (Nolan et al., 2011). The purpose of this study was to determine, with relatively fine resolution, relationships among dose, relative and temporal kinetics of serum absorption for L vs. the collective zeaxanthin isomers (Z + MZ), and subsequent MPOD response. To this end, we conducted a fasting blood draw and assessed spatial profiles of MPOD in subjects consuming three different levels of a macular carotenoid supplement vs. placebo, at baseline and every two weeks thereafter for 12 weeks.

2. Materials and methods

This study was reviewed and approved by the University of Georgia Institutional Review Board. Informed consent was obtained for each subject, and the study adhered to the tenets of the Declaration of Helsinki. Thirty-two University of Georgia students, aged 18–25 yrs, enrolled in the study. Twenty-eight completed the entire 12-week trial. Subjects were randomly assigned to one of four daily supplement groups: placebo (n = 5), 7.44 mg L + total Z (n = 7), 13.13 mg L + total Z (n = 8), or 27.03 L + total Z (n = 8). Pills were provided by Omniactive Health Technologies, Inc., and were brown-colored, soft gelatin capsules, with L and Z suspended in safflower oil. Independent analysis of 100 pills in each dose category indicated that the 7.44 mg group supplement contained 6.18 mg L/0.73 mg Z/0.53 mg MZ, the 13.13 mg group supplement contained 10.86 mg L/1.33 mg Z/0.94 mg MZ, and the 27.03 mg group supplement contained 22.33 mg L/2.70 mg Z/2 mg MZ. Placebos contained no L or Z, but only safflower oil. All reported values were within ±5% variability. Subjects were instructed to ingest one pill with a meal (preferably lunch or dinner) every day. Compliance was ensured with weekly phone calls and pill counts.

To ensure subjects met inclusion criteria for participation, biometric data (e.g. height, weight, body fat percentage), as well as health habits data (e.g. whether or not a smoker) were obtained at the screening/intake visit. Subjects were excluded from participation in the study if they were determined to have a BMI higher than 27, if they currently smoked, or currently took supplements containing any of the carotenoids involved in the study. Subjects were instructed to maintain their current diet; those that were planning on changing their diet (for whatever reason) were excluded from consideration for the trial. In consideration of MPOD testing, all subjects had uncorrected or contact lens-corrected visual acuity of 20/20 or better in the test (right) eye, and had no current or previous history of ocular pathology. After being familiarized with the study, subjects were instructed to visit the laboratory every 2 weeks, in order to participate in vision testing and phlebotomy. Phlebotomy was conducted after fasting for at least 10 h, and subjects were given some food (e.g. a bagel or a breakfast bar) and water immediately after the blood draw. Macular pigment measurement occurred shortly thereafter.

2.1. Measurement of macular pigment optical density (MPOD)

The spatial profile of MPOD was assessed with a non-invasive, perceptual task called customized heterochromatic flicker photometry (cHFP; Stringham et al., 2008). A densitometer (Macular Metrics Corp., Rehoboth, MA) described by Wooten et al. (1999) was used for this purpose. The densitometer, detailed measurement procedures, and the principle of HFP have been fully described in earlier publications (Wooten et al., 2005). Briefly, subjects are presented with two superimposed lights that are temporally alternated in square-wave counterphase. This gives the subject an impression of a flickering disc of light. The peak (550 nm) of the spectral composition of one of the lights is chosen to bypass the absorption of MP, and the other (460 nm) is strongly absorbed by MP. The subject's task is to adjust the relative radiance of the two

lights until a percept of no flicker is achieved. All other factors being equal, a subject that requires more short-wave (i.e., 460 nm) relative to middle-wave (i.e., 550 nm) light to achieve null flicker has higher MPOD. This task is performed for the locations of interest within the fovea, which presumably contain MP, and for a reference location in the parafovea that does not (about 7° eccentricity). To obtain a measure of MPOD at a given test locus, the logarithmic ratio of short-to middle-wave radiance (for null flicker) at the reference location is subtracted from the corresponding logarithmic ratio found at the test locus. Measurements were taken at baseline and every 2 weeks over the 12-week study period. We obtained spatial profiles of MPOD at each visit, with measures at 10′, 20′, 30′, 1.75°, and 2.75° of retinal eccentricity. MPOD for centrally-viewed, relatively small (<= 1.5°) circular targets has been shown to correspond to the edge of the disc (Smollon et al., 2015). Therefore, our stimuli of 20′, 40′, and 1° of visual angle corresponded to 10′, 20′, and 30′ retinal eccentricities. For measures corresponding to 1.75° and 2.75° retinal eccentricity, subjects viewed a small fixation dot in the center of annuli with diameters of 3.5° and 5.5°, respectively.

2.2. Blood collection

Fasting blood was collected between 9 a.m. and 11 a.m., by a licensed phlebotomist. Subjects′ whole blood was collected into a serum separator vacutainer tube (SST) via venipuncture. Blood was allowed to clot for 30 min at room temperature before centrifugation for 15 min at 1000*g*. Serum was then removed and stored in microvials at −20 C until analysis. Samples were taken at baseline and every 2 weeks over the 12-week study period.

2.3. High-performance liquid chromatography (HPLC)

Sample extractions and analyses were completed under yellow light. Serum proteins were precipitated with an equal volume of ethanol (1% BHT) containing the internal standard, trans-β-apo-8'-carotenal. After centrifugation, samples were extracted three times with n-hexane, by mixing and centrifugation. Organic layers were pooled and evaporated to dryness with nitrogen and re-suspended in the mobile phase. An Agilent 1200 series HPLC system consisting of a quaternary pump with degasser, autosampler, thermostated column compartment, UV–Vis diode array detection (DAD) with standard flow cell, and 3D ChemStation software (Agilent Technologies, Santa Clara, CA, USA) was employed for the chromatography. A reversed-phase YMC C30 column (4.6 × 250 mm, 5-μm particle size) was utilized. A stepwise elution consisting of mobile phase A (95% methanol) and mobile phase B (methyl tert-butyl ether) from 15 to 85% B over a 27-min period at a flow rate of 1 mL/min was employed. A volume of 100 μL was injected for each of the serum samples. Detection wavelengths were $\lambda = 447$ nm (lutein) and 450 nm (total sum zeaxanthin).

2.4. Dietary characterization

Subjects were instructed to maintain their current diet over the 12-week study period to the best of their ability. To ensure the consistency of dietary intake with regard to foods that contain L and Z, a short food frequency questionnaire was used. This questionnaire contained 12 items, which assessed subjects′ dietary intake of different leafy-green vegetables, colored fruits, and eggs. The questionnaire required roughly 1 min to complete.

2.5. Statistical analysis

One-way and repeated-measures analysis of variance, curve fitting, and correlational analyses were conducted with the statistical software package Origin Pro 9.3 (OriginLab; Northampton, MA). Tukey's posthoc tests were used to determine differences between groups for the various time points. Statistical significance was determined at the $p \leq 0.05$ level.

3. Results

3.1. Serum response to different carotenoid supplement levels

Table 1 presents descriptive data relevant to dose, serum response, and retinal response.

Based on baseline data from Figs. 1 and 2, it is clear that our subjects' dietary intake of L, Z, and MZ was quite low. This was corroborated with self-reported dietary intake data, which indicated that, on average, subjects consumed roughly 1–3 mg of L and Z (data not shown). As can be seen in Figs. 1 and 2, serum concentration of even the relatively low 7.44 mg group increased at least 5 fold over baseline levels of both L and Z. Fig. 1 shows subjects' serum L response to the different doses of macular carotenoids over the 12-week study period. A repeated-measures ANOVA with Tukey's posthoc analysis revealed significant differences from baseline in serum L at the first time point (2 weeks) and at each time point thereafter for all levels of the carotenoid supplement ($p < 0.05$ for all). Additionally, all carotenoid groups were found to be significantly different from placebo at all of the time points, save baseline ($p < 0.05$ for all). Serum concentrations of L did not change in the placebo group over the 12-week period. Serum L concentration increase for the 27.03-mg total L + Z supplement group was found to be significantly different from the 7.44 mg supplement group for all post-baseline measures, and significantly different from the 13.13-mg supplement group at all post-baseline time points except 2 and 8 weeks ($p < 0.05$ for 4, 6, 10, and 12 week time points). For all doses, response appeared to plateau somewhere near 6–8 weeks after the onset of supplementation. With regard to the three supplement groups, serum L concentration increase at response plateau was linear, but not proportional to dose. The slope of the line describing this relationship was 0.068 (see Fig. 3).

Fig. 2 shows subjects′ serum Z response to the different levels of supplement. In many respects, the Z data are similar to the L data. For example, significant differences from baseline were found in all supplement groups for all measures beyond baseline ($p < 0.05$ for all), and each supplement group was found to be significantly different ($p < 0.05$ for all) from placebo at all of the time points (with the exception of baseline). There are a couple of notable differences, however, between the L and Z serum response plots. The slope describing the linear function between Z dose and serum Z increase at response plateau was higher (0.077; see Fig. 4) than for L (0.068), although this difference was not statistically significant ($p = 0.14$). Also, the response plateau appears to occur earlier for Z (roughly 4–6 weeks compared to 6–8 weeks for L). As indicated by the differential slopes for the serum concentration increases of L vs. Z, the absorption of Z increased relative to L as a function of dose. This can be seen graphically in Fig. 5. This occurred despite the virtually equivalent L: Z ratios found in each supplement level (see Table 1). In terms of efficiency of serum response, based on the plateau-generated average serum concentration increase, a one-way ANOVA revealed that the 7.44 mg dose produced a significantly higher serum increase/dose ratio (0.247) than either the 13.13 mg dose (0.168) or the 27.03 mg dose (0.122).

3.2. Macular pigment optical density

MPOD responses across the study at the standard, 30′ retinal locus are shown in Fig. 6. As can be seen in the Figure, response in

Table 1
Macular carotenoid summary data for dose, serum response, and retinal response. Means ± 1 SD. Serum increases defined as the increase in concentration (average of the 8-, 10-, and 12-week measures) over baseline for the designated group. Total serum increase/total dose is the combined total of L + Z serum concentration increases, divided by the total L + Z dose (mg). MPOD response is the optical density difference between final and baseline visits. a = significantly different from 7.44 mg group; b = significantly different from 13.13 mg group; c = significantly different from 27.03 mg group (determined with Tukey's HSD posthoc test), $p < 0.05$. * = significantly different from baseline at the $p = 0.05$ level. ¥ = significantly different from 7.44 mg dose at both 10 and 12 week measures.

Total Dose (mg)	L dose (mg)	Z dose (mg)	L/Z Dose ratio	L serum increase (μg/mL)	Z serum increase (μg/mL)	Total serum increase /total dose	L/Z Serum ratio	MPOD response	MPOD change /total serum increase
7.44	6.18	1.29	4.77	1.64 ± 0.21[c]	0.19 ± 0.07[b,c]	0.247[b,c]	8.20[c]	0.111 ± 0.03*	0.0603[b]
13.13	10.86	2.27	4.78	1.94 ± 0.33[c]	0.26 ± 0.078[a,c]	0.168[a]	7.5	0.169 ± 0.042*a¥	0.0765 [a,c]
27.03	22.33	4.70	4.75	2.75 ± 0.51[a,b]	0.45 ± 0.091[a,b]	0.122[a]	7.25[a]	0.196 ± 0.074*a¥	0.0594[b]

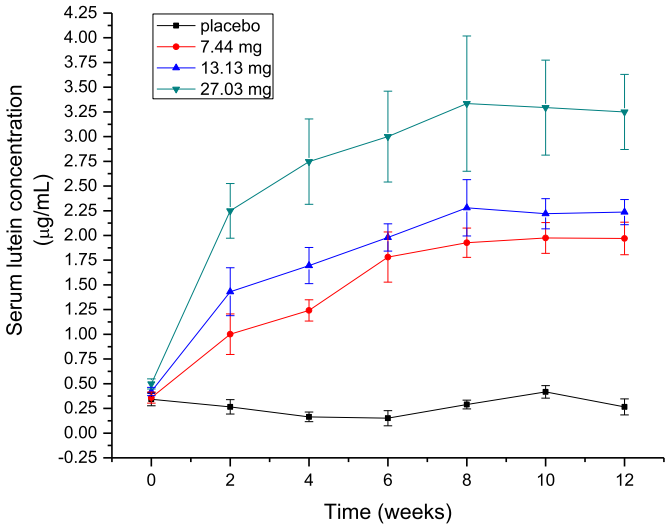

Fig. 1. Subjects' serum lutein concentration as a function of time. Carotenoid supplement groups noted in legend. Symbols indicate means, error bars are±1 SD.

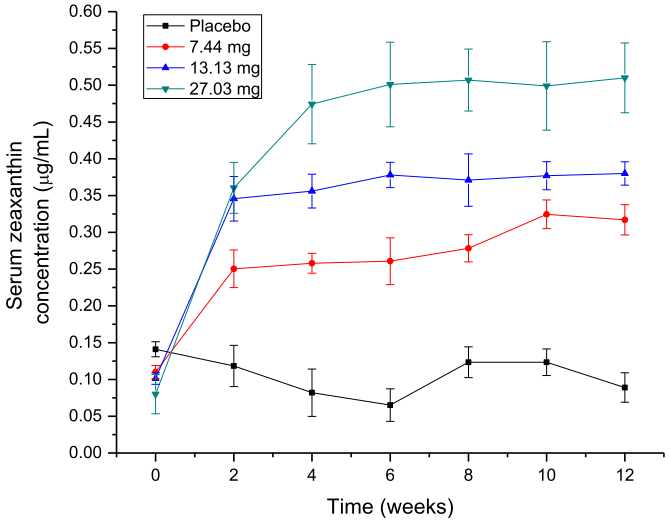

Fig. 2. Subjects' serum zeaxanthin concentration as a function of time in the study. Carotenoid supplement groups noted in legend. Symbols indicate means, error bars are±1 SD.

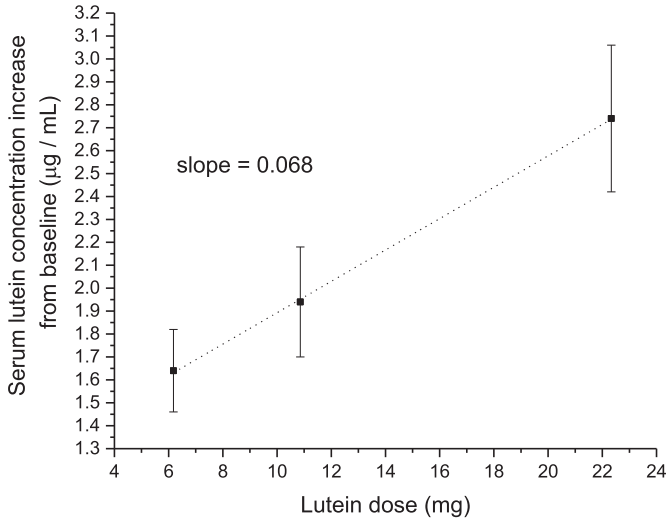

Fig. 3. Serum lutein concentration increase from baseline, as a function of lutein dose for the three dose levels used in the study. The slope of the best-fit line (0.068) is noted. Square symbols are means, ± 1 SD.

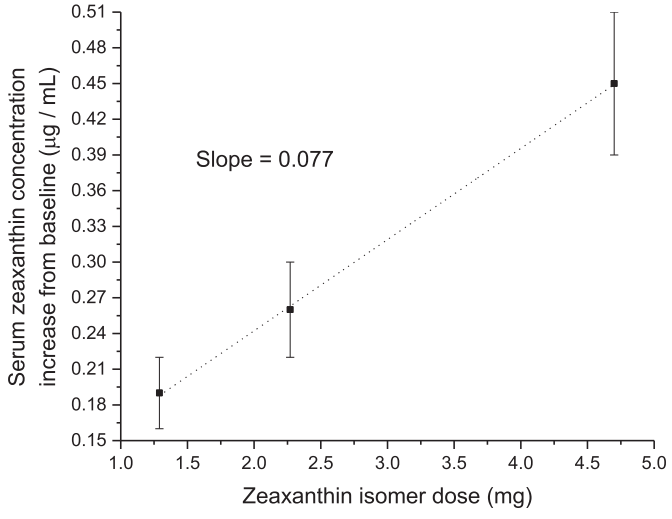

Fig. 4. Serum zeaxanthin concentration increase from baseline, as a function of zeaxanthin dose, for the three dose levels used in the study. The slope of the best-fit line (0.077) is noted. Squares are means, ± 1 SD.

the retina for all supplement groups was not detectable until the third study visit (week 6). A repeated-measures ANOVA revealed significant increases from baseline for the 7.44 mg group at the 12-week visit ($p = 0.046$); for the 13.13 mg group at visits 8, 10, and 12 ($p < 0.001$), and for the 27.03 mg group at weeks 8, 10, and 12 ($p < 0.001$). From Fig. 6 it can be seen that the response to each level

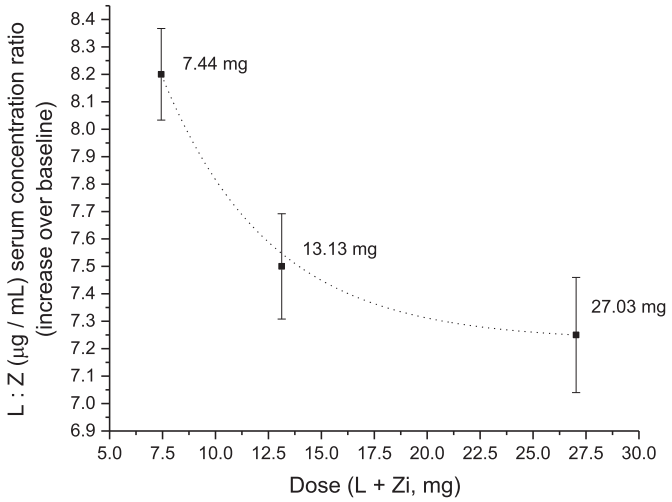

Fig. 5. L: Z serum concentration increase from baseline ratio (at plateau), as a function of dose. Means±SEM. Data fit with first-order decreasing exponential function: $Y = 5.18^{(-x/3.97)} + 5.27$.

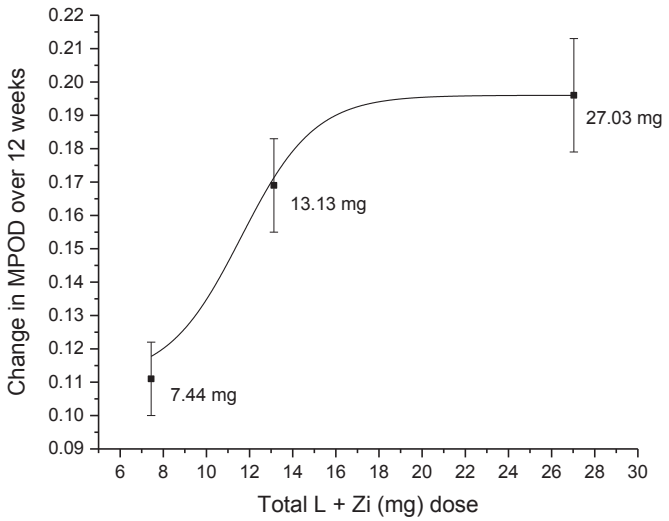

Fig. 7. Change in MPOD after 12 weeks of macular carotenoid supplementation, as a function of total dose. Means±SEM. Data fit with a dose-response function: $Y = 0.111 + (0.196-0.111)/(1 + 10^{((11.61 - x) * 0.26)})$.

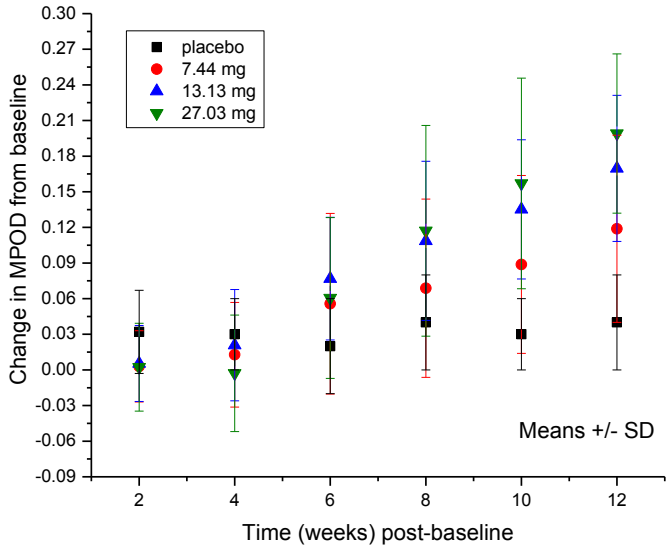

Fig. 6. Macular pigment optical density change from baseline as a function of time in the study, for different levels of daily L, Z, and MZ supplementation, versus placebo. Error bars = ± 1 SD. Dose levels noted in legend.

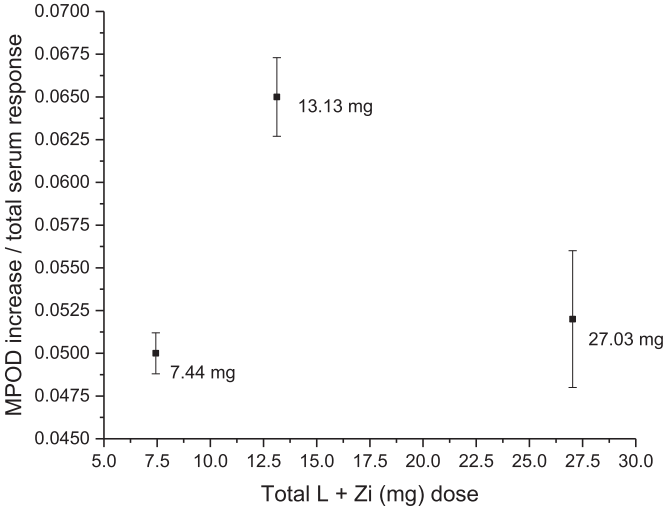

Fig. 8. Ratio of MPOD increase: total L + Z serum concentration increase, as a function of supplement dose, for the three doses tested in the study. The 13.13 mg dose differs significantly from both 7.44 and 27.03 mg doses ($p < 0.05$ for both). Square symbols indicate means, ± 1 SD.

of supplement was roughly linear as a function of time, and response was shown to be greater with time as a function of the level of supplement. The increase in MPOD for both 13.13 and 27.03 mg groups was determined to be significantly different from the 7.44 mg group at both 10 and 12 week measures ($p < 0.05$ for both doses at both time points). In terms of efficiency of retinal uptake, however, the relationship between dose level and resultant change in MPOD (presented in Fig. 7) is suggestive of a compression in response with increases in dose. Additionally, based on the ratio of MPOD change: total serum concentration change, the 13.13 mg dose was the most efficient for augmenting MPOD (one-way ANOVA; $p = 0.021$; see Fig. 8). This was true despite the fact that the most efficient dose in terms of serum response was 7.44 mg (see Table 1, "Total serum increase/total dose ratio" column).

Because it is considered to be the "standard" measure of MPOD, the 30′ retinal locus was used for the analysis in Fig. 6. Similar results, however, were found for those retinal loci near the center of the spatial distribution (i.e. 10′ and 20′′ loci – see Fig. 9). It can be seen in Fig. 9 that the average baseline spatial distribution of MPOD appears to exhibit an appreciable "shoulder," notable here at 1.75°. On this point, and in addition to the general increase in MPOD at all eccentricities tested, the shape of MPOD spatial profile appears to become more exponential in nature, with greater increases in optical density at central loci as a function of dose (see Fig. 9).

4. Discussion

The results of this 12-week macular carotenoid intervention are supportive of many conclusions. First, serum responses for L and Z, respectively, were linear as a function of dose. The slopes of these functions, however, were not equivalent – the slope for Z (0.077; see Fig. 4) was steeper than that for L (0.068; see Fig. 3). This could be taken to mean that the isomers of zeaxanthin are more efficiently absorbed than L. There are other plausible explanations,

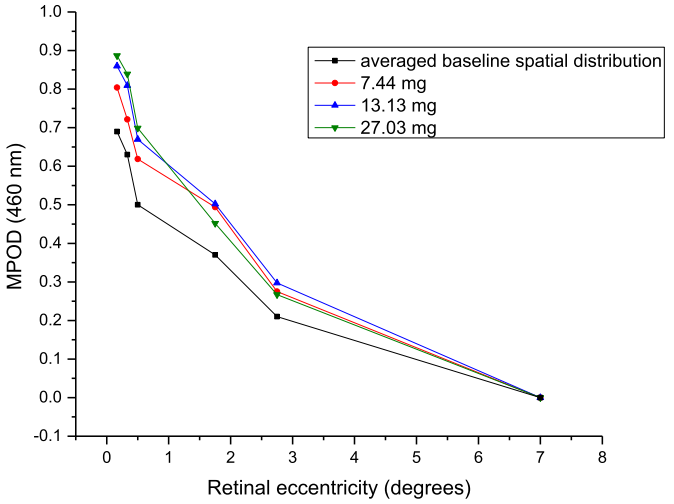

Fig. 9. Average spatial profile of MPOD in each supplement group, after the 12-week study period, compared to averaged baseline MPOD spatial profile. Carotenoid supplement groups noted in legend. For clarity, error bars are not shown.

however. For example, the apparently reduced efficiency of absorption for L could be an effect of abundance – the amount of L was roughly 5 times that of the Z in the supplements, and the absolute level of L was roughly 15 times that of the average adult American diet (Johnson et al., 2010) for the highest dose of L (22.33 mg). It is possibly the case that efficiency of L absorption simply decreases as a function of dose. It could also be the case that, as Z doses increased, competitive absorption reduced the relative efficiency of the absorption of L. Lastly, in a general sense, it could be that absorption of L (and Z) is proportionally reduced as doses reach some critical level. This can be described by a classic dose-response curve, and the general idea can be seen in Fig. 7 for retinal response where, when fit with a dose-response function, there is a clear tapering of MPOD with increased dose. This kind of dose vs. serum response relationship has been found for other nutrients, including vitamin D (Gallagher et al., 2012). This finding is also related to the overall efficiency of carotenoid absorption (total serum response/total dose – see Table 1) – it was found that the low (7.44 mg) dose produced the most efficient serum response; efficiency appeared to decrease with increasing dose. Again, it is perhaps the case that efficiency of absorption falls off with increases in dose. Nevertheless, as noted above, higher doses produced higher serum concentration increases, and these increases were related directly to changes in MPOD.

Another point of interest is the significant difference in efficiency for the three levels of supplement in terms of augmenting MPOD. Fig. 8 illustrates this graphically. Although both total serum L + Z and MPOD increased with increasing dose, the "middle," 13.13 mg total dose yielded the greatest increase in MPOD per unit serum response. This result, coupled with the aforementioned dose-response relationship presented in Fig. 7, suggests that (in terms of efficiency of MPOD response) the optimal daily dose of macular carotenoids is perhaps near 13.13 mg, and probably lies somewhere between 7.44 mg (the lowest dose) and 27.03 mg (the highest dose). Because L and Z are known to be deposited in many locations throughout the body (e.g. skin, adipose tissue, or the brain), another possible explanation for the non-linear retinal response with increasing dose could be preferential deposition of the carotenoids in locations other than the retina with higher doses/serum concentrations. We did not obtain data from these other tissues, and so are unable to comment on "prioritization" of carotenoid deposition; we are also unaware of any data on the prioritization of specific tissue targets for L or Z.

Although the general increase in MPOD over a relatively short time period was encouraging, perhaps more interesting was the change in MPOD spatial profile for the different supplement levels. As can be seen at baseline in Fig. 9, subjects' averaged MPOD spatial profile exhibited a "shoulder," extending from 30′ to roughly 1.75 degrees of retinal eccentricity. Despite a general MPOD response, supplementation with 7.44 mg of L, Z, and MZ did not appear to modify the shoulder. The 13.13-mg group, however, exhibited a slight change in shoulder slope over the study, with the overall spatial distribution trending toward a more peaked shape. Finally, the 27.03-mg group's average spatial distribution after 12 weeks exhibits much less of a clearly defined shoulder. Indeed, the 27.03-mg group's MPOD spatial profile became a smoother, more peaked distribution, consistent with a decreasing exponential function (Snodderly et al., 1984b). Although this effect was not statistically significant, the rate at which the trend occurred (12 weeks), coupled with the relation to dose level, makes it notable. This smoothing effect as a function of supplement level could potentially be explained by an "availability effect," whereby (should sufficient L, Z, and MZ be present), deposition proceeds in a fashion consistent with what Wenzel et al. (2007) found: Increasingly greater accumulation found at eccentricities closer to the center of the fovea. Wenzel's subjects consumed 30 mg L + 2.7 mg Z daily – a relatively high level, somewhat comparable with our 27.03 mg total carotenoid dose. Another potential explanation for this finding involves the formulation of the supplements, namely the inclusion of MZ. Nolan et al. (2012) convincingly showed central MPOD increases in subjects with atypical spatial profiles upon supplementation with relatively high doses of MZ. Their subjects exhibited so-called "ring-like" (Berendschot and van Norren, 2006) distributions of MPOD, where instead of a peak in the very center of the fovea, there is a dip. The central depressions in subjects' MPOD were corrected upon daily ingestion of supplements containing MZ, but not exclusively L and Z, after a supplementation period of only 8 weeks. This effect is most certainly due to the specificity of location in the fovea: In adults, MZ has been found to be nearly exclusively deposited in the very center of the fovea, with Z and L filling out the distribution with increasing eccentricity (Landrum and Bone, 2001). Nolan et al.'s supplements contained significantly more MZ than our 27.03-mg total supplement, but it could nevertheless be the case that even small amounts (e.g. 1–3 mg) of daily MZ can appreciably modify the central distribution of MPOD after 12 weeks. The apparent change in the shape of the spatial profile with increased dose in the present study should be qualified by the fact that no formal statistical analysis was conducted. The variability in MPOD response data (see Fig. 6) prevented this kind of analysis; we are therefore limited to comment on the visual appearance of the curves.

In terms of direct comparison to previous studies, the most relevant is Bone and Landrum's (2010) 140 day investigation of serum and MPOD response. They studied 100 subjects during 140 days of L supplementation at three levels: 5, 10, and 20 mg daily. They too found a linear response to dose in serum L concentration, and (considering our slightly higher doses), the magnitude of their L responses (2.57, 3.35, and 8.15-fold for their respective doses), lines up reasonably well with ours (5.75, 6.85, and 8.75-fold). With regard to MPOD, their data indicated a linear dose-response relationship. This relationship was somewhat complicated by the fact that their 5-mg group appeared to decrease in MPOD compared to placebo, and therefore we cannot compare or comment on our apparent diminishing returns finding. Overall, the MPOD response rates in the present study were higher. This discrepancy could simply be a result of aforementioned response variability, our relatively small sample sizes, or perhaps the fact that roughly 17% of

the formulation in the present study was composed of Z (compared to 5% for Bone and Landrum's study).

Other studies of effects of macular carotenoid supplementation on MPOD have generally painted a picture of linear response kinetics over time, although there is variability. For example, for 6-month, 12-mg L + Z/day doses (10 mg L/2 mg Z), Trieschmann et al. (2007) and Stringham and Hammond (2008) determined quite different MPOD response rates of 0.548 vs. 0.877 milliabsorbance units/day. In the present study, the value for the 13.13 mg dose (most comparable to Trieschmann et al. (2007) and Stringham and Hammond (2008)) was 2.01 milliabsorbance units/day. These differences are probably indicative of real variability in response to supplementation with L and Z, most notably age. The average age of Treischmann et al.'s sample, for example, was 71.5 yrs, compared to 23.9 yrs for Stringham and Hammond (2008). Moreover, augmentation of MPOD was found to be 5-fold lower in subjects over 50 yrs of age compared to those under 30 yrs in the Bone and Landrum (2010) study. The question, of course, is what specifically accounts for the variability in response to macular carotenoid supplementation? Bone and Landrum (2010) found that roughly 29% of the rate of change in their subjects' MPOD could be accounted for by the fractional change in serum L (plateau − baseline/baseline). For our (decidedly smaller) sample in the present study, we calculate a value of 39%. What this indicates is that, contrary to what might seem to be reasonable logic, roughly 34% of retinal response can be explained by response in the serum (based on an average of Bone and Landrum's (2010) and our findings). Much of the variability may therefore be explained by mechanisms involved in transport (Sato et al., 2013), binding (Vachali et al., 2012) and/or perhaps demand for these carotenoids for more immediate uses, such as the reduction of systemic inflammation or oxidation (Tian et al., 2013).

Based on the results of our study, it appears that even a relatively low (7.44 mg) daily dose of retinal carotenoids can produce meaningful responses in the serum and retina over 12 weeks. At the other end of the scale, our serum and retinal response results indicate that the body is capable of processing and using relatively high amounts (27.03 mg) of retinal carotenoids. The data from our study indicate that somewhere between these two poles lies a point of efficient uptake, transport, and deposition in the retina. The point of most efficient retinal deposition is very important when considering macular carotenoid dose recommendations for AMD patients or those seeking to take preventative measures against AMD. Our data suggest that a relatively modest daily dose of macular carotenoids produces the most efficient (and significant) retinal response. This lines up well with the AREDS 2 supplement formulation (AREDS 2 Research Group, 2013), which contains (based on capsule information) 12 mg of L + Z (similar to our 13.13 mg dose). The primary, and perhaps very meaningful, difference is the inclusion of MZ in the supplements used in the present study. Given the healthcare cost and personal burden of AMD, the finding of macular deposition efficiency at a modest dose is encouraging.

There is clearly much potential for the macular carotenoids to positively impact health and performance. Whether or not they are transported to the eye and brain is a question not only of transport and deposition mechanisms, but also perhaps of systemic health status, or other yet-to-be discovered factors. This provides the impetus for much future study.

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References

Age-Related Eye Disease Study 2 Research Group, 2013. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. JAMA 15 (309(19)), 2005–2015.
Berendschot, T.T., van Norren, D., 2006. Macular pigment shows ringlike structures. Invest Ophthalmol. Vis. Sci. 47 (2), 709–714.
Bone, R.A., Landrum, J.T., Hime, G.W., Cains, A., Zamor, J., 1993. Stereochemistry of the human macular carotenoids. Invest Ophthalmol. Vis. Sci. 34, 2033–2040.
Bone, R.A., Landrum, J.T., Guerra, L.H., Ruiz, C.A., 2003. Lutein and zeaxanthin dietary supplements raise macular pigment density and serum concentrations of these carotenoids in humans. J. Nutr. 133 (4), 992–998.
Bone, R.A., Landrum, J.T., 2010. Dose-dependent response of serum lutein and macular pigment optical density to supplementation with lutein esters. Arch. Biochem. Biophys. 504 (1), 50–55.
Buzzi, F., 1782. Nuove sperienze fatte sulle' occhio umano. Opuscoli Scetti Sulle Sci. Sulle 5, 87.
Ciulla, T.A., Curran-Celantano, J., Cooper, D.A., Hammond Jr., B.R., Danis, R.P., Pratt, L.M., Riccardi, K.A., Filloon, T.G., 2001. Macular pigment optical density in a midwestern sample. Ophthalmology 108 (4), 730–737.
Connolly, E.E., Beatty, S., Thurnham, D.I., Loughman, J., Howard, A.N., Stack, J., Nolan, J.M., 2010. Augmentation of macular pigment following supplementation with all three macular carotenoids: an exploratory study. Curr. Eye Res. 35 (4), 335–351.
Curcio, C.A., Sloan, K.R., Kalina, R.E., Hendrickson, A.E., 1990. Human photoreceptor topography. J. Comp. Neurol. 292 (4), 497–523.
Fletcher, L.M., Engles, M., Hammond Jr., B.R., 2014. Visibility through atmospheric haze and its relation to macular pigment. Optom. Vis. Sci. 91 (9), 1089–1096.
Gallagher, J.C., Sai, A., Templin 2nd, T., Smith, L., 2012. Dose response to vitamin D supplementation in postmenopausal women: a randomized trial. Ann. Intern Med. 20 (156(6)), 425–437.
Hammond Jr., B.R., Fletcher, L.M., Elliott, J.G., 2013. Glare disability, photostress recovery, and chromatic contrast: relation to macular pigment and serum lutein and zeaxanthin. Invest Ophthalmol. Vis. Sci. 54 (1), 476–481.
Hammond Jr., B.R., Johnson, E.J., Russell, R.M., Krinsky, N.I., Yeum, K.J., Edwards, R.B., Snodderly, D.M., 1997. Dietary modification of human macular pigment density. Invest Ophthalmol. Vis. Sci. 38 (9), 1795–1801.
Johnson, E.J., Maras, J.E., Rasmussen, H.M., Tucker, K.L., 2010. Intake of lutein and zeaxanthin differ with age, sex, and ethnicity. J. Am. Diet. Assoc. 110, 1357–1362.
Klaver, C.C.W., Wolfs, R.C.W., Vingerling, J.R., Hofman, A., De Jong, P.T.V.M., 1998. Age-specific prevalence and causes of blindness and visual impairment in an older population: the Rotterdam Study. Arch. Ophthalmol. 116, 653–658.
Krinsky, N.I., Landrum, J.T., Bone, R.A., 2003. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. Ann. Rev. Nutr. 23, 171–201.
Kvansakul, J., Rodriguez-Carmona, M., Edgar, D.F., Barker, F.M., Kopcke, W., Schalch, W., Barbur, J.L., 2006. Supplementation with the carotenoids lutein or zeaxanthin improves human visual performance. Ophthalmic Physiol. Opt. 26 (4), 362–371.
Landrum, J.T., Bone, R.A., 2001. Lutein, zeaxanthin, and the macular pigment. Arch. Biochem. Biophys. 385 (1), 28–40.
Loughman, J., Nolan, J.M., Howard, A.N., Connolly, E., Meagher, K., Beatty, S., 2012. The impact of macular pigment augmentation on visual performance using different carotenoid formulations. Invest Ophthalmol. Vis. Sci. 53 (12), 7871–7880.
Neuringer, M., Sandstrom, M.M., Johnson, E.J., Snodderly, D.M., 2004. Nutritional manipulation of primate retinas, I: effects of lutein or zeaxanthin supplements on serum and macular pigment in xanthophyll-free rhesus monkeys. Invest Ophthalmol. Vis. Sci. 45 (9), 3234–3243.
Nolan, J.M., Akkali, M.C., Loughman, J., Howard, A.N., Beatty, S., 2012. Macular carotenoid supplementation in subjects with atypical spatial profiles of macular pigment. Exp. Eye Res. 101, 9–15.
Nolan, J.M., Beatty, S., Meagher, K.A., Howard, A.N., Kelly, D., Thurnham, D.I., 2014. Verification of Meso-zeaxanthin in fish. J. Food Process Technol. 5 (6), 335.
Nolan, J.M., Loughman, J., Akkali, M.C., Stack, J., Scanlon, G., Davison, P., Beatty, S., 2011. The impact of macular pigment augmentation on visual performance in normal subjects: COMPASS. Vis. Res. 51 (5), 459–469.
Ruban, A.V., Pascal, A., Lee, P.J., Robert, B., Horton, P., 2002. Molecular configuration of xanthophyll cycle carotenoids in photosystem II antenna complexes. J. Biol. Chem. 277 (45), 42937–42942.
Sato, Y., Kondo, Y., Sumi, M., Takekuma, Y., Sugawara, M., 2013. Intracellular uptake mechanism of lutein in retinal pigment epithelial cells. J. Pharm. Pharm. Sci. 16 (3), 494–501.
Sasamoto, Y., Gomi, F., Sawa, M., Tsujikawa, M., Nishida, K., 2011. Effect of 1-year lutein supplementation on macular pigment optical density and visual function. Graefes Arch. Clin. Exp. Ophthalmol. 249 (12), 1847–1854.
Seddon, J.M., Ajani, U.A., Sperduto, R.D., Hiller, R., Blair, N., Burton, T.C., Farber, M.D., Gragoudas, E.S., Haller, J., Miller, D.T., et al., 1994. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. JAMA 18, 1413–1420. Erratum in: JAMA. 1995;8:622.
Smollon Jr., W.E., Wooten, B.R., Hammond, B.R., 2015. Stimulus edge effects in the

measurement of macular pigment using heterochromatic flicker photometry. J. Biomed. Opt. 20 (11), 115004.
Snodderly, D.M., Brown, P.K., Delori, F.C., Auran, J.D., 1984a. The macular pigment. I. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. Invest Ophthalmol. Vis. Sci. 25 (6), 660–673.
Snodderly, D.M., Auran, J.D., Delori, F.C., 1984b. The macular pigment. II. Spatial distribution in primate retinas. Invest Ophthalmol. Vis. Sci. 25 (6), 674–685.
Sommerburg, O., Keunen, J.E., Bird, A.C., van Kuijk, F.J., 1998. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. Br. J. Ophthalmol. 82 (8), 907–910.
Stringham, J.M., Garcia, P.V., Smith, P.A., McLin, L.N., Foutch, B.K., 2011. Macular pigment and visual performance in glare: benefits for photostress recovery, disability glare, and visual discomfort. Invest Ophthalmol. Vis. Sci. 52, 7406–7415.
Stringham, J.M., Hammond, B.R., Nolan, J.M., Wooten, B.R., Mammen, A., Smollon, W., Snodderly, D.M., 2008. The utility of using customized heterochromatic flicker photometry (cHFP) to measure macular pigment in patients with age-related macular degeneration. Exp. Eye Res. 87 (5), 445–453.
Stringham, J.M., Hammond, B.R., 2008. Macular pigment and visual performance under glare conditions. Optom. Vis. Sci. 85 (2), 82–88.
Tian, Y., Kijlstra, A., van der Veen, R.L., Makridaki, M., Murray, I.J., Berendschot, T.T., 2013. The effect of lutein supplementation on blood plasma levels of complement factor D, C5a, and C3d. Plos One 8 (8), e73387.
Trieschmann, M., Beatty, S., Nolan, J.M., Hense, H.W., Heimes, B., Austermann, U., Fobker, M., Pauleikhoff, D., 2007. Changes in macular pigment optical density and serum concentrations of its constituent carotenoids following supplemental lutein and zeaxanthin: the LUNA study. Exp. Eye Res. 84 (4), 718–728.
Vachali, P., Li, B., Nelson, K., Bernstein, P.S., 2012. Surface plasmon resonance (SPR) studies on the interactions of carotenoids and their binding proteins. Arch. Biochem. Biophys. 1 (519(1)), 32–37.
Wald, G., 1945. Human vision and the spectrum. Science 101 (2635), 653–658.
Wang, Y., Roger Illingworth, D., Connor, S.L., Barton Duell, P., Connor, W.E., 2010. Competitive inhibition of carotenoid transport and tissue concentrations by high dose supplements of lutein, zeaxanthin and beta-carotene. Eur. J. Nutr. 49 (6), 327–336.
Wenzel, A.J., Sheehan, J.P., Gerweck, C., Stringham, J.M., Fuld, K., Curran-Celentano, J., 2007. Macular pigment optical density at four retinal loci during 120 days of lutein supplementation. Ophthalmic Physiol. Opt. 27 (4), 329–335.
Wiegand, R.D., Giusto, N.M., Rapp, L.M., Anderson, R.E., 1983. Evidence for rod outer segment lipid peroxidation following constant illumination of the rat retina. Invest Ophthalmol. Vis. Sci. 24 (10), 1433–1435.
Wooten, B.R., Hammond, B.R., Smollon, B., 2005. Assessment of the validity of heterochromatic flicker photometry for measuring macular pigment optical density in normal subjects. Optom. Vis. Sci. 82, 378–386.
Wooten, B.R., Hammond, B.R., Land, R., Snodderly, D.M., 1999. A practical method of measuring macular pigment optical density. Invest Ophthalmol. Vis. Sci. 40, 2481–2489.
Yao, Y., Qiu, Q.H., Wu, X.W., Cai, Z.Y., Xu, S., Liang, X.Q., 2013. Lutein supplementation improves visual performance in Chinese drivers: 1-year randomized, double-blind, placebo-controlled study. Nutrition 29 (7–8), 958–964.

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