

## ***Erratum***

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On page 993, the first sentence under Experiment 3 should read: The next supplement to be tested was a commercially produced gel capsule containing 1.2 mg of L, unesterified, from marigolds.

## Lutein and Zeaxanthin Dietary Supplements Raise Macular Pigment Density and Serum Concentrations of these Carotenoids in Humans<sup>1,2</sup>

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**ABSTRACT** Age-related macular degeneration (AMD) is thought to be the result of a lifetime of oxidative insult that results in photoreceptor death within the macula. Increased risk of AMD may result from low levels of lutein and zeaxanthin (macular pigment) in the diet, serum or retina, and excessive exposure to blue light. Through its light-screening capacity and antioxidant activity, macular pigment may reduce photooxidation in the central retina. Lutein supplements, at 30 mg/d, were shown previously to increase serum lutein and macular pigment density in two subjects. In this study, we compared the effects of a range of lutein doses (2.4–30 mg/d), as well as a high zeaxanthin dose (30 mg/d), on the serum and macular pigment in a series of experiments. Serum carotenoids were quantified by HPLC. Macular pigment densities were determined psychophysically. Serum lutein concentrations in each subject reached a plateau that was correlated with the dose ( $r = 0.82$ ,  $P < 0.001$ ). Plateau concentrations ranged from  $2.8 \times 10^{-7}$  to  $2.7 \times 10^{-6}$  mol/L. Zeaxanthin was less well absorbed than an equal lutein dose, resulting in plateaus of  $\sim 5 \times 10^{-7}$  mol/L. The rate of increase in macular pigment optical density was correlated with the plateau concentration of carotenoids in the serum ( $r = 0.58$ ,  $P < 0.001$ ), but not with the presupplementation optical density ( $r = 0.13$ ,  $P = 0.21$ ). The mean rate of increase was  $(3.42 \pm 0.80) \times 10^5$  mAU/d per unit concentration (mol/L) of carotenoids in the serum. It remains to be demonstrated whether lutein or zeaxanthin dietary supplements reduce the incidence of AMD. *J. Nutr.* 133: 992–998, 2003.

**KEY WORDS:** • macular pigment • lutein • zeaxanthin • carotenoids • age-related macular degeneration

Current interest in the human macular pigment (MP),<sup>4</sup> consisting of lutein (L) and zeaxanthin (Z), is driven largely by its possible association with a reduced risk for age-related macular degeneration (AMD). Many dietary supplements consisting of, or containing, these carotenoids are now commercially available, promoted for their supposed benefits to the health of the eye. Over the course of several years, during which preparations of L and Z have become available for human consumption, we investigated their effects on MP density and on their concentration in the blood serum. The purpose of the present study was to consolidate our findings and determine whether any unifying conclusions could be drawn from them.

The *macula lutea*, or yellow spot, which characterizes the primate retina, is named for the region in and around the fovea

where L and Z are concentrated (1). These carotenoids appear mainly in the photoreceptor axon layer with an L/Z ratio of about 0.5 (2,3). At eccentricities beyond 1–2 mm from the fovea, the pigments are still present, though no longer visible, with an L/Z ratio of about 2.0 (3). In recent studies, L and Z have been found in the rod outer segments and retinal pigment epithelium of both the perifoveal and peripheral retina (4,5).

The central region of the retina is prone to the destructive effects of AMD, a leading cause of vision loss in the United States (6). The photooxidative processes by which blue light in particular may contribute to the etiology of AMD have been described by Schalch et al. (7). Remarkably, the wavelengths of light that damage the retina are essentially limited to the same range (400–500 nm) where MP absorbs most strongly (8). MP peak optical density (at 460 nm) in the central 1–2° lies in the range of 0.1 to 0.9 for most people (9,10). Thus, for a person having an optical density at the low end of this range, structures posterior to the MP will be exposed to approximately six times the blue light flux compared with those of a person at the other extreme. As a result, we might expect a greater incidence of AMD among people having a low MP density. Interestingly, in the advanced form of AMD known as geographic atrophy, the foveal center, which contains the highest concentrations of L and Z, tends to be spared until late in the course of the disease (11,12).

In addition to an ability to attenuate blue light and thereby reduce photooxidation, L and Z are effective quenchers of

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<sup>2</sup> Presented in part in abstract form at the annual meeting of the Association for Research in Vision and Ophthalmology, April 29–May 4, 2001, Fort Lauderdale, FL. [Bone, R. A., Landrum, J. T., Llerena, C. M., Ruiz, C. A. & Tibor, S. (2001) Dependence of macular pigment density increases on elevated serum levels of lutein and zeaxanthin resulting from supplementation. *Invest. Ophthalmol. Vis. Sci.* 42: S233.]

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<sup>4</sup> Abbreviations used: AMD, age-related macular degeneration; EDCCSG, Eye Disease Case-Control Study Group; L, lutein; MP, macular pigment; OD, optical density; Z, zeaxanthin.

singlet oxygen and reactive radicals (13). In this capacity, too, they can limit photooxidative processes, but only if they are in close proximity to the sites where oxidation may be occurring. The discovery of L and Z in the rod outer segments is of particular relevance in this regard (4,5).

There is evidence to support the hypothesis that MP provides protection against AMD. The Eye Disease Case-Control Study Group (EDCCSG) reported significant associations between high levels of L and Z in both the diet and serum of their subjects and a reduced risk of advanced, neovascular AMD (14,15). However, the Beaver Dam Study reported only slightly, and nonsignificantly, lower concentrations of serum L and Z in AMD subjects in comparison with controls (16). This may have been attributable to the relatively low intake of L and Z by these subjects compared with those in the EDCCSG study. In another study, L and Z concentrations in autopsy retinas from donors with and without AMD were compared. A significant trend for reduction in risk for AMD with increasing amounts of L and Z was noted (17). In a representative sample of the U.S. population, levels of lutein in the diet, but not the serum, were related to later, but not early, stages of AMD (18). However, these relationships were limited to subjects who were in the younger age categories at risk for these conditions. Unfortunately, the question of causality between L and Z levels and risk of AMD cannot be answered by these studies.

To investigate directly the efficacy of L and Z as prophylactics against AMD, intervention trials will be required. Because AMD is a disease that may be the final manifestation of a lifetime of contributory factors, such trials would necessarily be long term. A practical consideration, before the implementation of such a study, is the extent to which MP density can be increased through dietary modification that increases the intake of L and/or Z. Previous studies have focused primarily on the effects of single fixed dosages of L (19–24). In this study we compared the effects of L and Z dietary supplements, the former in a range of dosages, on the serum and the MP. We hypothesized that the blood serum level of L or Z would be correlated with the dose, and the increase in MP density would be correlated with the blood serum level of L and Z resulting from the supplement. We also hypothesized that the increase in MP density would be correlated with the presupplementation MP density.

## SUBJECTS AND METHODS

### Subjects

Subjects over the age of 18 y, and of either sex, were recruited from the University community. Having been informed of the nature of the study, the subjects signed an informed consent form approved by the Institutional Review Board. Subjects were given individual training in flicker photometry, the technique used to measure MP optical density. (See below.) Only if they demonstrated proficiency in the task and were able to give reproducible results were they accepted into the study. Approximately 90% of the screened subjects met the proficiency criterion. Those who failed tended to be older subjects with little or no scientific training. Each subject accepted into the study was given a full eye exam by an ophthalmologist to ensure that no pathologies were present. The only other eligibility requirement was (self-identified) good health.

### Supplementation

**Experiment 1.** The first available L supplement was made using an oleoresin containing natural L esters extracted from marigolds. The response to this supplement was reported previously (19), and the main outcome is included here for comparison purposes only. In brief, 30 mg doses of L equivalent were prepared, each being sus-

pending in 2 mL of canola oil. Two subjects, A and B, took the 30 mg daily dose for 140 d. The normal daily intake of L and Z in the United States is reported to be about 1 to 1.5 mg/d, and the L/Z ratio in the diet is about 4 or 5. Consequently, the 30 mg dose represents ~20 to 30 times the normal daily intake. It should also be noted that marigold extracts contain small amounts of Z, ~5% of the amount of L.

**Experiment 2.** Sufficient Z for two subjects was subsequently made available, crystalline, unesterified and encapsulated in gelatin/starch beadlets. The source of the Z was a commercial culture of *Flavobacteria*. Subject A and a new subject C took the supplement for 120 and 60 d, respectively. The daily dose was again 30 mg, suspended in 2 mL of canola oil, and represents about 100 times the average daily intake of Z. The last day of L supplementation for subject A preceded the 1st d of Z supplementation by 21 mo.

**Experiment 3.** The next supplement to be tested was a commercially produced gel capsule containing 1.2 mg of L, as esters, from marigolds. In addition, each capsule contained a number of herbal extracts including docosahexanoic acid, bilberry extract, lemon flavonoid concentrate and acerola concentrate. Twenty-one subjects took two capsules/d (2.4 mg of L) for 6 mo. The purpose of this experiment was to investigate the effects of a dose that provided a similar amount of L to that found in the average U.S. diet.

**Experiment 4.** The final supplements that we investigated consisted of gel capsules containing either 5 or 20 mg of L (as esters from marigolds), together with a small quantity of vegetable oil. Twelve subjects took 20 mg/d, and two subjects took 5 mg/d, for 120 d.

During all four studies, subjects were instructed to follow their normal, self-selected diet and to take the L or Z supplement with a meal, or shortly thereafter.

### Macular pigment measurements

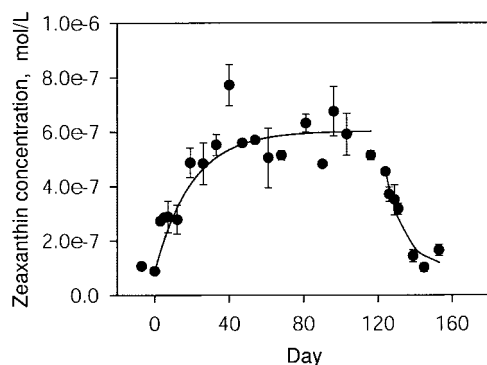
MP optical densities were determined psychophysically using the well-established technique of heterochromatic flicker photometry (19). The subject viewed a 1.5° circular stimulus that alternated at about 30 Hz between 460 nm, the wavelength of maximum MP absorbance, and 540 nm, where the absorbance is essentially zero. While fixating on the center of the stimulus, the subject adjusted the 460-nm intensity to produce a flicker null. A subject with a denser MP would need a higher intensity to compensate for the attenuation that the pigment produces. To account for differences among subjects in relative cone populations and/or lens absorption, both of which will affect the intensity setting, a second setting was made at about 15 Hz with the stimulus imaged 8° above the fovea. At this location, MP absorbance is essentially zero. The log ratio of intensity settings is equal to the MP optical density at 460 nm.

The subjects made 5–10 sequential settings each for the central and peripheral locations of the stimulus. Measurements were made in both eyes before, during and after the supplementation period, and were repeated on separate days at least twice/wk.

### Serum analysis

Blood samples were obtained from the subjects during the study period to monitor changes in carotenoid concentration. Generally, at least two samples were obtained in the 2 wk preceding supplementation, and at least one sample was obtained every 2 wk during supplementation. Finally, three samples were obtained in the 2 wk after supplementation. Subjects were not required to follow a more rigid schedule than this, nor were the blood sampling days related to flicker photometry sessions. For expts. 1 and 2, blood sampling was more frequent and flicker photometry was conducted 3–5 times/wk. Subject C (expt. 2), unfortunately, elected to leave the study after 60 d of Z supplementation, so no postsupplementation data are available. The subject became increasingly uncomfortable with venipuncture.

Blood samples were collected in Vacutainer™ serum separator tubes with no anticoagulant, and allowed to stand for 30 min to allow for coagulation. The samples were centrifuged for 10 min and the serum removed by pipette. Carotenoids were extracted from the serum by established techniques (25). For quantification purposes, 20



**FIGURE 1** Concentration of zeaxanthin (Z) in the serum of subject A. The Z supplementation period was from d 0 to d 120. Values are means  $\pm$  SD,  $n = 3$  replicates.

$\mu\text{L}$  of an ethanol solution containing 90 ng of monohexyl lutein ether were added to each 200  $\mu\text{L}$  of serum as an internal standard. To precipitate proteins, 2 mL of ethanol/water (50:50) were also added. To extract the carotenoids, 2 mL of hexane were added and the mixture vortexed for 1 min followed by centrifuging for 5 min. The hexane layer was removed and the extraction step repeated twice more. The pooled hexane was evaporated to dryness under a stream of nitrogen before analysis of the extract by HPLC.

HPLC was carried out on a reversed-phase system using a 250  $\times$  4.6 mm Ultracarb ODS 3- $\mu\text{m}$  column (Phenomenex, Torrance, CA). The mobile phase was acetonitrile/methanol (85:15) with 0.1% triethylamine added to inhibit degradation of carotenoids. The flow rate was 1 mL/min and detection was at 451 nm. The amounts of L and Z were determined by comparing their chromatogram peak areas with that of the internal standard.

### Statistical analyses

Results are expressed as means  $\pm$  SD. Increases in serum concentrations of L and Z, and increases in MP density resulting from supplementation were tested for significance using an independent-samples  $t$  test ( $\alpha = 2$ ). Values of  $P < 0.05$  were considered significant. Whether rates of increase in MP density resulting from supplementation were significantly different from zero was determined by use of a one-sample  $t$  test ( $\alpha = 2$ ). Again, values of  $P < 0.05$  were considered significant. Adjustments for other potentially influencing factors, such as body mass index, were not included in the analyses. We also applied a multiple linear regression model to the rate of increase in MP optical density using the elevated serum concentration of L and Z and the presupplementation optical density as independent variables.

## RESULTS

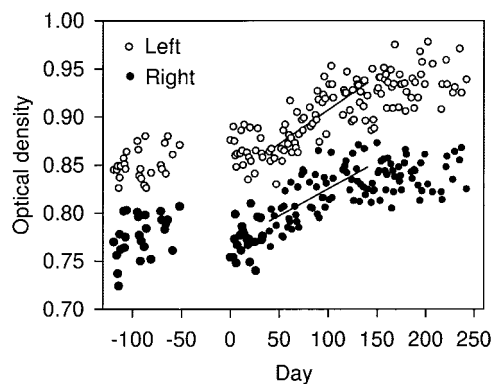
**Experiment 2.** Because expt. 1 (30 mg/d of L) was previously reported elsewhere (19), the similar results of expt. 2 (30 mg/d of Z) are described first. Subject A was a 53-y-old male and subject C was a 21-y-old male. Both subjects responded to the Z supplement, with 5- to 6-fold increases in serum Z concentration. For subject A, Z concentration increased from  $\sim 0.097 \times 10^{-6}$  mol/L before supplementation to a plateau at  $\sim 0.56 \times 10^{-6}$  mol/L over  $\sim 30$  d (Fig. 1). For subject C, the increase from  $\sim 0.086 \times 10^{-6}$  mol/L to a plateau at  $\sim 0.48 \times 10^{-6}$  mol/L was similar though much more rapid, occurring in  $\sim 10$  d. Upon discontinuing the Z supplement, the serum concentration of Z in subject A dropped roughly exponentially to near presupplementation levels over  $\sim 20$  d (Fig. 1). As stated earlier, no postsupplementation serum data are available for subject C.

The results of Z supplementation on MP optical density for

both eyes of subject A are shown in Figure 2. For approximately the first 40 d of supplementation, no effect was discernible, and the optical densities were essentially the same as those observed during an earlier  $\sim 70$  d period before supplementation. Thereafter, the optical densities increased in a more or less linear fashion at mean rates of  $0.71 \pm 0.08$  and  $0.56 \pm 0.09$  mAU/d in the left and right eyes, respectively (AU = absorbance unit). This trend continued throughout the 120-d supplementation period, and during the 20 d following, before leveling off. Measurements made during the succeeding  $\sim 100$  d showed no tendency to decline. For this subject, significant differences in optical densities between left and right eyes were maintained throughout the study period. The eyes of subject C were more closely matched. Presupplementation MP optical densities were 0.567 and 0.538 AU in the left and right eyes, respectively. After  $\sim 25$  d of supplementation, rates of increase in optical density of about 0.35 and 0.31 mAU/d were observed in the left and right eyes, respectively.

**Experiment 1.** Subject B was a 42-y-old male. Subjects A and B responded more robustly in both serum and retina to the 30 mg/d L supplement than did subjects A and C to the 30 mg/d Z supplement. Because the supplements were equal in amount, the pertinent features of the serum and retina responses are compared in Table 1 and Table 2, respectively.

**Experiment 3.** The results of this experiment will be reported fully elsewhere. The study group consisted of 17 females and 4 males ranging in age from 19 to 59 y with a mean of  $28 \pm 10$  y. In summary, for all 21 subjects taking 2.4 mg of L/d, a significant increase in serum L was observed ( $P < 0.05$ ), as determined by an independent-samples  $t$  test. The  $t$  test compared the mean serum L concentration for each subject before supplementation with the mean plateau concentration during supplementation. The serum L concentration for the group before supplementation was  $(0.245 \pm 0.120) \times 10^{-6}$  mol/L, and the plateau concentration was  $(0.484 \pm 0.176) \times 10^{-6}$  mol/L. The increases in serum L concentration determined in this way ranged from  $\sim 20$  to 300%, with a mean for the group of  $\sim 100\%$ . Complete MP optical density measurements were obtained from 20 of the 21 subjects. Over the 6-mo supplementation period, significant increases in MP optical density, as determined by an independent-samples  $t$  test, occurred in 12 subjects ( $P < 0.0005$  to  $P < 0.05$ ). Marginally significant increases occurred in three subjects ( $P < 0.1$ ), and no significant changes occurred in the remaining five



**FIGURE 2** Macular pigment (MP) optical density as a function of time of supplementation with zeaxanthin (Z) for subject A. The Z supplementation period was from d 0 to d 120. The rates of increase in MP optical density, represented by the regression lines, are  $0.71 \pm 0.08$  and  $0.56 \pm 0.09$  mAU/d for the left and right eyes, respectively.

TABLE 1

Characteristics of the serum response to lutein (L) or zeaxanthin (Z) supplements at 30 mg/d in three human subjects<sup>1</sup>

Supplement	Subject	Serum carotenoid concentration		Time to reach plateau	Time to return to baseline
		Presupplementation <sup>2</sup>	Postsupplementation <sup>3</sup>		
		$\times 10^{-6}$ mol/L		d	
L	A	0.150 $\pm$ 0.079	1.74 $\pm$ 0.12	~30	~40
L	B	0.165 $\pm$ 0.030	2.38 $\pm$ 0.28	~20	~25
Z	A	0.097 $\pm$ 0.007	0.56 $\pm$ 0.07	~30	~20
Z	C	0.086 $\pm$ 0.009	0.48 $\pm$ 0.05	~10	—

<sup>1</sup> Data are for three subjects, A, B and C.

<sup>2</sup> Data in the third column represent the mean  $\pm$  SD of the blood serum concentration of L or Z before supplementation with that carotenoid.

<sup>3</sup> Column 4 represents the mean  $\pm$  SD of the plateau level of blood serum concentration of the carotenoid during supplementation.

subjects ( $P \geq 0.18$ ). The mean increase for all 20 subjects was ~10%, representing an increase from  $0.443 \pm 0.173$  AU before supplementation to a postsupplementation value of  $0.489 \pm 0.174$  AU.

**Experiment 4.** The two subjects taking 5 mg of L/d were females ages 26 and 27 y. Their plateau concentrations of serum L were  $0.74 \times 10^{-6}$  and  $1.14 \times 10^{-6}$  mol/L, respectively. The mean rates of increase in MP optical density, averaged for both eyes, were 0.303 and 0.219 mAU/d, respectively. In one eye of the former subject, the rate of increase tended to be significantly different from zero ( $P < 0.1$ ), but in the other eye, it did not ( $P = 0.42$ ), as determined by one-sample *t* tests. Significant rates of increase were observed in both eyes of the other subject ( $P < 0.005$  and  $P < 0.02$ ). Of the 12 subjects taking 20 mg/d of L, eight were female and four were male. Their ages ranged from 19 to 60 y, with a mean of  $37 \pm 15$  y. The serum concentrations that were achieved ranged from  $0.77 \times 10^{-6}$  to  $2.45 \times 10^{-6}$  mol/L, with a mean of  $(1.30 \pm 0.44) \times 10^{-6}$  mol/L. The mean rates of increase in MP optical density, averaged for both eyes, ranged from 0.055 to 1.56 mAU/d. The mean for the group was  $0.53 \pm 0.41$  mAU/d. An example of a responding subject is shown in Figure 3. In three subjects, the rates of increase in MP optical density for both eyes were not different from zero, as deter-

mined by a one-sample *t* test ( $P \geq 0.18$ ). An example of one of these three nonresponding subjects is shown in Figure 4.

To determine the effect of supplement dosage, we examined the plateau L + Z concentration in the serum as a linear function of dosage (Fig. 5). The linear correlation coefficient  $r$  was 0.82 ( $P < 0.001$ ). We also examined the hypothesis that the concentration of L and Z achieved in the serum would be a major factor determining the response of the MP. In Figure 6, which includes all 38 subjects and a range of supplement dosages, the rate of increase in MP optical density is plotted as a function of the plateau concentration of combined L and Z in the serum. Linear regression analysis gave a correlation coefficient of 0.58 that was significant ( $P < 0.001$ ) but, as with the serum data (Fig. 5), there was considerable scatter. We also considered the possibility that the rate of increase in MP optical density would depend on the presupplement MP optical density but the correlation was not significant ( $r = 0.13$ ,  $P = 0.21$ ). The situation did not change as a result of fitting a multiple linear regression model to the rate of increase in MP optical density using the elevated serum concentration of L and Z and the presupplementation optical density as independent variables. The coefficient for serum was significant ( $B = 0.59$ ,  $P < 0.001$ ) but the coefficient for optical density was not ( $B = 0.14$ ,  $P = 0.64$ ).

TABLE 2

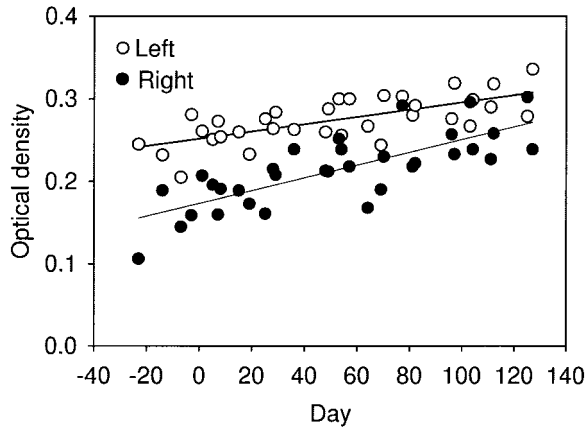
Characteristics of the macular pigment optical density (OD) response to lutein (L) or zeaxanthin (Z) supplements at 30 mg/d in three human subjects<sup>1</sup>

Supplement (duration)	Subject (eye)	Presupplementation OD	Postsupplementation OD	Increase in OD	Rate of increase in OD
				AU	%
L (140 d)	A (L)	0.756 $\pm$ 0.028	0.930 $\pm$ 0.020	23	1.21 $\pm$ 0.04
	A (R)	0.650 $\pm$ 0.020	0.852 $\pm$ 0.021	31	1.20 $\pm$ 0.05
L (140 d)	B (L)	0.576 $\pm$ 0.029	0.809 $\pm$ 0.019	40	1.14 $\pm$ 0.07
	B (R)	0.571 $\pm$ 0.024	0.793 $\pm$ 0.023	39	0.96 $\pm$ 0.06
Z (120 d)	A (L)	0.850 $\pm$ 0.016	0.933 $\pm$ 0.021	10	0.71 $\pm$ 0.08
	A (R)	0.775 $\pm$ 0.022	0.839 $\pm$ 0.017	8	0.56 $\pm$ 0.09
Z (60 d)	C (L)	0.567 $\pm$ 0.017	—	—	0.35 $\pm$ 0.08
	C (R)	0.538 $\pm$ 0.019	—	—	0.31 $\pm$ 0.08

<sup>1</sup> Data are for the left (L) and right (R) eyes of three subjects, A, B and C.

<sup>2</sup> The presupplementation macular pigment OD is the mean  $\pm$  SD of seven consecutive measurements made by the subject before supplementation, in absorbance units (AU).

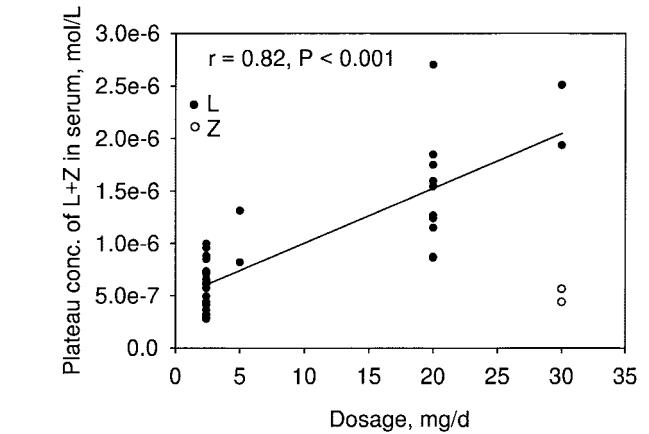
<sup>3</sup> The postsupplementation OD is the mean  $\pm$  SD of all measurements made on the subject after the OD stopped increasing (e.g., d 140 onward in Fig. 2).



**FIGURE 3** Macular pigment (MP) optical density as a function of time for a subject who responded to lutein (L) supplementation at 20 mg/d. The rates of increase in MP optical density are  $0.44 \pm 0.08$  and  $0.77 \pm 0.12$  mAU/d for the left and right eyes, respectively.

## DISCUSSION

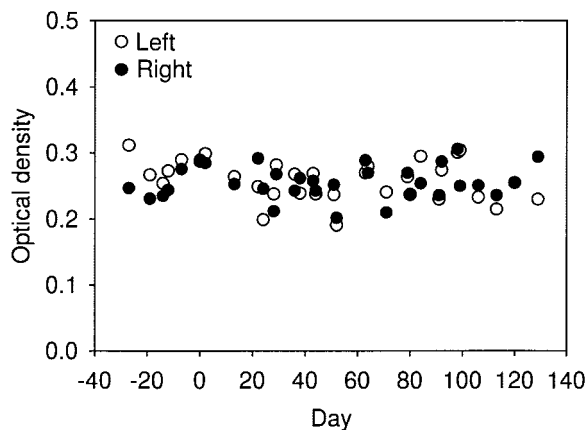
The primary aim of this study was to compare the effects of different L dosages as well as a high Z dosage on blood serum concentrations of these carotenoids and on MP density. The general trend observed in all 38 subjects was an increase in serum L or Z concentration to a plateau followed by an approximately exponential decline once supplementation ceased, as exemplified in Figure 1. In general, the higher L doses resulted in higher plateau concentrations than did the low L doses. Approximately 67% of the variance in serum L concentration could be attributed to a linear dependency on dose (Fig. 5). This is a similar degree of correlation to that found earlier when we examined serum carotenoid concentrations as a function of dietary intake of L and Z in unsupplemented individuals (26). Other researchers have found such correlations to be low (27). However, for each L dose in the present study, there was quite a wide spread in plateau concentrations, with the highest concentration occurring in a subject taking the 20 mg dose. A factor that has the potential to influence carotenoid uptake into the serum is co-consump-



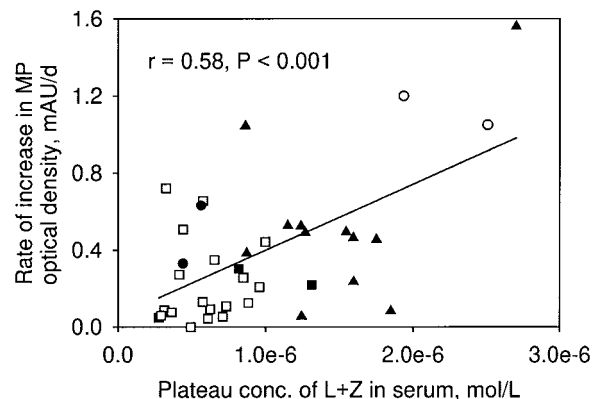
**FIGURE 5** Scatterplot showing the dependency on dose of the elevated plateau concentration of lutein (L) + zeaxanthin (Z) in the serum resulting from carotenoid supplementation. The regression line has a slope of  $(5.3 \pm 0.7) \times 10^{-8}$  (mol/L)/(mg/d).

tion of fat. Because subjects' diets in the present study were self-selected and not analyzed, we were unable to determine whether dietary fat was a significant factor for absorption of L into the serum.

The regression line in Figure 5 does not include the two data points (open circles) for the (30 mg/d) Z-supplemented subjects. The Z concentrations at the plateau were anomalously low compared with those achieved with the 30 mg/d L supplements. For subject A, who participated in both experiments, the serum concentration of Z achieved in expt. 2 was only about one third of the serum concentration of L achieved in expt. 1. It is unlikely that this reflects an efficiency of uptake of Z into the serum that was lower than that for L. The L/Z ratio in serum generally reflects the ratio found in the average diet ( $\sim 4:1$ ). In addition, Neuringer et al. (28) studied monkeys that were supplemented with either pure L or pure Z. They found that, although serum levels of L rose faster than those of Z, by  $\sim 16$  wk, both had stabilized at comparable concentrations of  $\sim 0.8 \times 10^{-6}$  mol/L. An alternative explanation is that the lower bioavailability that we found for Z may



**FIGURE 4** Macular pigment (MP) optical density as a function of time for a subject who did not respond to lutein (L) supplementation at 20 mg/d. The rates of change in MP optical density are  $-0.18 \pm 0.12$  and  $0.06 \pm 0.10$  mAU/d for the left and right eyes, respectively, and are not different from zero.



**FIGURE 6** Scatterplot showing the dependency of the rate of increase in macular pigment (MP) optical density on the elevated plateau concentration of lutein (L) + zeaxanthin (Z) in the serum resulting from carotenoid supplementation. Open squares, 2.4 mg L; filled squares, 5 mg L; filled triangles, 20 mg L; open circles, 30 mg L; filled circles, 30 mg Z. The regression line has a slope of  $(3.42 \pm 0.80) \times 10^5$  (mAU/d)/(mol/L).

be the result of the formulation of the product. The L was esterified and prepared as an oleoresin that was easily solubilized in vegetable oil before consumption. The Z was crystalline, unesterified and incorporated in gelatin/starch beadlets, and exhibited little or no tendency to dissolve in vegetable oil.

The factors that influence the transport of L and Z into the serum are presumed to be different from those that influence their subsequent transfer from the serum into the retinal tissues. Our hypothesis that the concentration of L and Z in the serum would be a major factor for this latter transfer was supported by the data. Because of the different periods of supplementation (60 d to 6 mo), rates of increase in MP optical density, rather than absolute increases, were plotted as a function of the plateau concentration of combined L and Z in the serum (Fig. 6). Approximately one third of the variance in the rate of increase in MP optical density could be attributed to a linear dependency on the plateau concentration (Fig. 6). Another factor that might be expected to affect the rate of increase in MP optical density is the presupplementation MP optical density. If the accumulation of L or Z in the macula eventually reaches a saturation level, we might expect a slower rate of increase of optical density in subjects whose density is already high. Conversely, a high density may be an indication that the subject readily absorbs L and Z into the retina. If that were the case, such subjects might be expected to respond with the higher rates of increase in MP optical density. However, analysis of the data indicated that presupplementation MP optical density was not a significant factor in determining the rate of increase in MP optical density ( $r = 0.13$ ,  $P = 0.21$ ).

The high rate of nonresponse of MP optical density among subjects who consumed the low L dosage (2.4 mg/d) is not altogether unexpected. The dose is somewhat higher than the average daily intake for the U.S. population, but still less than half of the daily intake of those in the upper quintile reported by the EDCCSG (14). No subject characteristic (age or gender) distinguished the responders from the nonresponders, but this is not surprising, given that most of the subjects in this group were young (under 30 y) and female. For this group, considered separately, the correlation between the rate of increase in MP optical density and the serum plateau concentration of L and Z was not significant.

Subject A's MP optical density began to level off at the same time that the serum concentration of Z returned to baseline, as indicated in Figure 2. The tendency for the post-supplementation optical density to remain elevated was previously observed for both subjects A and B in the earlier L study (expt. 1) (19), and was also reported by others (23). It reinforces the suggestion, made at the time, that there may be a very slow turnover of carotenoids in the retina. Carotenoids are generally unstable in the presence of heat, light and oxygen (29), but it is possible that they achieve a high level of stability when incorporated into the retinal tissues. If this is the case, continued high dosing with L or Z may not be necessary to maintain a high density of MP. Rather, a high dose could be employed initially to produce a timely increase in MP density; thereafter, a lower maintenance dose may be adequate to prevent any decrease from occurring. This statement is made with the realization that the beneficial effects of high MP density are tentative. Although there is compelling evidence for an association between the presence of L and Z in the diet, serum and eyes of individuals, and reduced risk of AMD, the association has not been demonstrated to be causal (14,15,17). The concept of L and Z acting as markers for some underlying factor, which is itself responsible for the disease, remains a possibility.

In summary, all subjects receiving L doses in the range 2.4–

30 mg/d, and both subjects receiving 30 mg/d of Z, responded to the supplement with significant, but varied, increases in the serum concentration of that carotenoid. Many subjects also responded with increases in the density of their macular pigment. Subjects who did not respond tended to be those who consumed the lower dosages, which generally produced lower serum concentrations of carotenoids.

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