Relation between dietary intake, serum concentrations, and retinal concentrations of lutein and zeaxanthin in adults in a Midwest population[1–3]

Joanne Curran-Celentano, Billy R Hammond Jr, Thomas A Ciulla, Dale A Cooper, Linda M Pratt, and Ronald B Danis

ABSTRACT

Background: Information on concentrations of retinal carotenoids (macular pigment, or MP) is of particular interest because MP protects against age-related macular degeneration, the leading cause of irreversible blindness in the United States.

Objective: This study was designed to evaluate the relation between dietary intake, blood concentrations, and retinal concentrations of carotenoids in a large group of volunteers.

Design: Two hundred eighty volunteers in the Indianapolis area completed health and diet questionnaires, donated a blood sample, and participated in MP density assessment to determine retinal carotenoid status. Dietary intake was assessed by food-frequency questionnaire. Serum concentrations of lutein, zeaxanthin, and β-carotene were measured by HPLC. MP optical density (MPOD) was determined psychophysically with a 460-nm, 1° test stimulus.

Results: Average MPOD was 0.21 ± 0.13. Average intakes of lutein + zeaxanthin and β-carotene were 1101 ± 838 and 2935 ± 2698 μg/d, respectively. Although several key dietary intake variables (eg, lutein + zeaxanthin and β-carotene) differed by sex, no significant sex differences were found in either serum concentrations of lutein and zeaxanthin or MPOD. Serum β-carotene concentrations were significantly higher in women than in men. Serum lutein + zeaxanthin and dietary intake of lutein + zeaxanthin were significantly correlated and significantly related to variations in MPOD ($r = 0.21$, $P < 0.001$, and $r = 0.25$, $P < 0.001$, respectively).

Conclusions: Retinal carotenoids can be measured in epidemiologic studies. In this study, MPOD was associated with lutein + zeaxanthin in the diet and the serum. Retinal concentrations, however, were influenced by other factors as well. To understand the effect of dietary lutein + zeaxanthin intake on the retina and risk of age-related eye disease, future studies should include measures of macular concentrations of these pigments. *Am J Clin Nutr* 2001;74:796–802.

KEY WORDS Retinal carotenoids, macular pigment, age-related macular degeneration, lutein, zeaxanthin, β-carotene, macular pigment optical density

INTRODUCTION

Evidence continues to accumulate suggesting that increased consumption of fruit and vegetables is associated with decreased risk of several chronic diseases (1–3). Understanding how the phytochemical content of dietary plants influences health risk is complex. The most compelling evidence comes from data that provide clear biological explanations for how specific phytochemicals prevent specific diseases at the site where damage leading to the disease occurs (4). For example, the prostate contains significant concentrations of lycopene, which may explain the reduced risk of prostate cancer associated with the consumption of lycopene-rich foods like tomatoes (5, 6).

Another example is the finding that lutein and zeaxanthin, as measured in the diet (2) and in serum (7, 8), are related to reduced risk of age-related macular degeneration. Lutein and zeaxanthin, to the exclusion of other carotenoids, are concentrated in the foveal pit of the human retina and when deposited in this region are referred to as macular pigment (MP) (9, 10). The concentration of the MP carotenoids in human retina varies widely (11, 12). Increased MP optical density (MPOD) has been linked directly to preserved foveal function in patients with annular maculopathy (13) and to preserved visual sensitivity in normal-aged subjects (14, 15). This protection may be because MP is localized within the inner foveal layers and absorbs short-wave light before it can damage vulnerable lipid-rich membranes in the outer segments of photoreceptors. The identification of lutein and zeaxanthin oxidation products within the human retina supports the possibility that the pigments may also serve to deactivate reactive oxygen species often generated within the retina (16).

MPOD can be modified by increasing intake of lutein-rich foods or purified lutein and zeaxanthin supplements (17, 18). Preliminary evidence suggests that lutein supplementation may improve symptoms of retinal degeneration in its earliest stages (19, 20). This confluence of data suggests that individual differences in MPOD are meaningful. Because past epidemiologic

[1] From the Department of Animal and Nutritional Sciences, University of New Hampshire, Durham; the Department of Psychology, University of Georgia, Athens; the Department of Ophthalmology, University of Indiana Medical School, Indianapolis; and The Food and Beverage Products Division, The Procter and Gamble Company, Cincinnati.

[2] Scientific contribution #2112 from the New Hampshire Agriculture Experiment Station.

[3] Address reprint requests to J Curran-Celentano, Department of Animal and Nutritional Sciences, Kendall Hall, Durham, NH 03824. E-mail: joannec@christa.unh.edu.

Received June 2, 2000.

Accepted for publication February 9, 2001.

work assessed the relation between risk of age-related macular degeneration and lutein and zeaxanthin measured in the diet and serum, the relation between MPOD and these variables is also of interest (21). Although normative values for serum concentrations and dietary intakes of carotenoids have been published, no large studies have measured normative values of MPOD and its relation to dietary intake and serum concentration of these pigments. Moreover, the question of whether MPOD can be measured effectively in a clinical research setting has not been addressed.

SUBJECTS AND METHODS

Subject recruitment and inclusion and exclusion criteria

Two hundred eighty healthy adult volunteers in Indianapolis and bordering counties were recruited to make a single clinic visit to Indiana University–Purdue University at Indianapolis (IUPUI). Students or employees of IUPUI or the IU Medical Center were excluded. Eligibility criteria included age between 18 and 50 y (to minimize the possibility of undiagnosed eye disease), residence in Indianapolis or bordering counties for the past year, and lack of known ocular disease. Other inclusion criteria included willingness to complete a comprehensive questionnaire on medical history and lifestyle, willingness to provide a fasting blood sample, and ability to give informed consent. The Institutional Review Board of the Indiana University School of Medicine and the University of New Hampshire approved the appropriate protocols.

Subject evaluation

Subjects were asked to fast (only water and non-energy-containing beverages were allowed) for ≥6 h before the planned blood draw. A core questionnaire that included questions on demographics, lifestyle, medical history, and health was completed by the subjects. The details of the questionnaire were reported previously (22); the information collected included data on medication use, nutritional supplementation, height and weight, smoking, and physical activity.

Assessment of macular pigment optical density

MPOD was measured with a psychophysical technique as described in Snodderly and Hammond (23). All MPOD measurements were made on the subjects' right eye. Subjects wore their own corrective lenses, or trial lenses, so that their near visual acuity was 20/25 or better during the test.

MPOD was measured with a 1° test stimulus. The 1° test stimulus was presented near the center of a 6°, 1.5 log trolands, 470-nm circular background. The test stimulus was alternately composed of a 460-nm measuring field (peak MP absorbance) and a 570-nm, 1.7 log trolands reference field (minimal MP absorbance). The troland values were calculated assuming a 3-mm pupil. The measuring and reference fields were superposed and presented out of phase at an alternation rate of 11–12 Hz in the foveal condition and 6–7 Hz in the parafoveal condition. Subjects adjusted the radiance of the 460-nm measuring field to achieve minimal flicker with the 570-nm reference field. This measurement was made in the fovea (where MP is the most dense) and 4° in the parafovea (where light absorption by MP is negligible). A tiny (5 min) opaque fixation point was located on the left edge of the background, and subjects fixated on this point when making the parafoveal measurement. Subtracting the foveal from the parafoveal sensitivity measurement yields a measure of MPOD. Subjects were given brief instructions on the method and a practice trial before 5 foveal and 5 parafoveal measurements were made. In a small portion of cases, either the foveal or parafoveal readings were repeated because the foveal readings had a range >100 units or the parafoveal readings had a range >75 units. The foveal and parafoveal values were calculated from the average of the final 5 readings, and these averages were then used to calculate the MPOD.

The apparatus used for the MP measurement delivered the stimulus in natural view, but used a stimulus configuration that was similar to configurations used in past studies in which the stimulus was presented in Maxwellian view (24–26). Recent evidence has shown, however, that MPOD measured in natural view, and with slight differences in stimulus configuration (eg, this study used a 4° rather than a 6° parafoveal reference), provides the same values as MPOD measured in Maxwellian view (27). Light for the 10° background was produced by 3 LEDs (packed tightly in a triangular array) with peak energy at 470 nm and half-widths of ≈20 nm. Light for the 570-nm reference field was produced by an LED with peak energy at 570 nm (half-width = 20 nm). Light for the 460-nm measuring field was produced by 2 LEDs with peak energy at 458 nm (half-width = 20 nm). Light from the LED sources was collimated with planoconvex lenses and was then passed through polycarbonate diffusers (high-efficiency, holographic type; Physical Optics Co, Torrance, CA), which served essentially as back projection screens.

The size of the background and test stimulus was defined by circular apertures (constructed by computer-generated images exposed on high-density, photographic, oriented polyester film) placed after the collimating lenses. The background and test stimulus were then combined and reflected to the subject by a 5-cm (2-in) beam splitter whose front surface was located ≈41 cm (16 in) from the subject's eye. The entire optical system was contained in a rectangular, black thermoplastic box. One side of the box contained a 2.5-cm (1-in) hole centered on the subject's optical axis through which the stimulus could be viewed. The subject's head was aligned by using an adjustable head and chin rest assembly; when the head was properly aligned, the subject viewed the hole in the box as slightly larger and concentric with the background field.

Stimuli were calibrated by using a photocell (PIN-10; UDT Sensors, Inc, Hawthorne, CA). The LEDs were driven by a constant-current power supply. Radiance variation was achieved by varying the frequency of a 1.5-ms pulse over a range of 300–300 000 Hz. Our calibration of the high-frequency pulse rate showed that the frequency delivery is nearly perfectly proportional to the radiance output. Thus, MPOD values could be derived by simply calculating the log ratio of the frequencies of the 460-nm measuring field at the foveal and parafoveal eccentricities, respectively.

Serum carotenoid assessment

Blood samples were collected into serum separator tubes and protected from direct light exposure during processing. Clotted blood was separated by centrifugation at 2000 × *g* at 4°C for 15 min. Serum was portioned into cryovials and shipped on dry ice to the University of New Hampshire, where it was stored at −80°C until analyzed.

Carotenoids were separated and quantified by reversed-phase HPLC. Serum was precipitated with ethanol containing the internal standard and was extracted into hexane. The extraction was repeated twice and the hexane layers from both extractions were removed to a common amber vial. The hexane was evaporated to

TABLE 1
Descriptive characteristics of the population[1]

Variable	Value
Age (y)	36.0 ± 7.9
BMI (kg/m^2)	26.4 ± 6.27
Serum	
β-Carotene (μmol/L)	0.28 ± 0.29
Lutein (μmol/L)	0.28 ± 0.13
Zeaxanthin (μmol/L)	0.091 ± 0.044
Lycopene (μmol/L)	0.601 ± 0.288
Diet	
α-Tocopherol (μg/d)	9.10 ± 6.4
β-Carotene (μg/d)	2935 ± 2698
Lutein + zeaxanthin (μg/d)	1101 ± 838
Lycopene (μg/d)	8366 ± 6106
Fruit (servings/d)	1.19 ± 1.09
Vegetables (servings/d)	1.39 ± 1.07
MPOD	0.21 ± 0.13

[1] $\bar{x}$ ± SD; n = 278. Two outliers were excluded because their dietary values were 10 and 20 SDs above the mean, respectively. MPOD, macular pigment optical density (460 nm, 1° test).

dryness under a stream of nitrogen, the sample was resuspended in 200 μL ethanol, and 20 μL was injected by loop overfill for analysis. The serum was protected from light from the time of collection through analysis.

The reversed-phase gradient HPLC system was equipped with an HP 1100 (Hewlett-Packard, Burlington, MA) photo diode-array detector set at 292, 325, and 452 nm for tocopherol, retinol, and carotenoids, respectively. The analytic column was a 4.6 × 250 mm Bakerbond C_{18} column (Mallinckrodt Baker, Phillipsburg, NJ) following a Vydac (Western Analytic Products, Murietta, CA) high-performance 5-μm C_{18} guard column. The mobile phase consisted of 100% methanol buffered with 1% ammonium acetate with a flow rate of 1.5 mL/min that transitioned to methanol:methylene chloride (80:20, by vol) over the first 10 min and remained set for the remainder of the 15-min run before switching back to 100% methanol. The method allows for separation of lutein and zeaxanthin at 452 nm while maintaining a run time of 15 min. The method and data were stored by CHEMSTATION software (Hewlett-Packard HP 3365 Series II). Samples were quantified by using peak area ratios to internal standards and by simultaneously running lab standards and external standards. Analytic accuracy was assessed with use of the National Institutes of Standards and Technology (NIST; Gaithersburg, MD) Standard Reference Material SRM 986 and by participation in the NIST Micronutrients Measurement Quality Assurance Program.

Dietary consumption estimation

A 1-y food-frequency questionnaire was used to estimate the dietary consumption of lutein + zeaxanthin and other nutrients (28). The food-frequency questionnaire included questions about the usual intakes over the past year of 122 foods and food groups, as well as adjustment and summary questions (29). Also included was an addendum with questions about intake of regular, reduced-fat, fat-free, and olestra-containing savory snacks. The food-frequency questionnaires were processed at the Fred Hutchinson Cancer Research Center (Seattle), and average daily intakes of nutrients were determined by use of the center's database for average nutrient content of food categories, which was derived from the University of Minnesota Nutrition Coordinating Center nutrient database (Nutrition Coordinating Center, Minneapolis).

Statistical analysis

This study used a cross-sectional design to estimate the sample mean value of MPOD in a group of healthy adult volunteers. Before the start of the study, we performed power calculations that predicted that the study had sufficient power to detect a sample mean value within 5%, assuming an MPOD SD of ≈0.15 and a total of 275 subjects. For the final analysis, only one subject did not have a complete set of foveal and parafoveal readings, yielding 279 valid MPOD values. In addition, dietary consumption data were excluded for subjects reporting energy intakes <2090 or >20090 kJ/d, because such chronic intakes are not physiologically likely and indicate that the subject did not correctly complete the food-frequency questionnaire. This is a common practice in epidemiologic studies. Consequently, valid dietary consumption data were available for 278 subjects.

Several variables were assessed in these subjects: tobacco use; iris color; sex; serum concentrations of lutein and zeaxanthin; dietary lutein + zeaxanthin intake; race; refractive error; family history of age-related macular degeneration; pregnancy or lactation; vitamin E intake; serum β-carotene; hours of sleep; age; dietary intake of fruit and vegetables, fat, iron, fiber, and olestra; use of supplements; visual acuity and ocular health; skin tone; use of prescription medications; body mass index; physical activity; and sun exposure. Values are reported as means ± SDs. For the present study, we limited our analysis of this data set to those variables that were relevant to the dietary habits of the participants. These variables, along with descriptive statistics, are listed in **Table 1**. One exception was the analysis of olestra use and its relation to MPOD and serum carotenoid concentrations. These data are presented in Cooper et al (30). The analysis of variables relating to personal characteristics such as race, iris color, and refractive error is provided in a separate article by Pratt et al (31). The primary statistical analyses conducted were correlational (Pearson's r) and inferential (Student's t tests) to analyze mean differences. Analyses were performed with use of

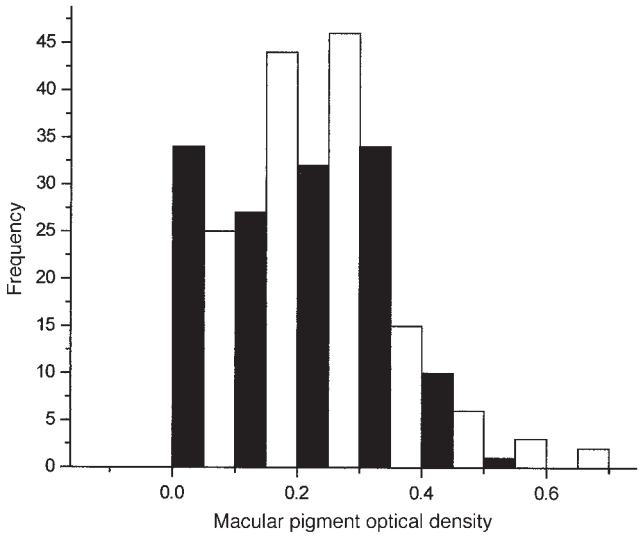

FIGURE 1. Frequency distribution of macular pigment optical density (460 nm, 1° test) in men (■; n = 138) and women (□; n = 142).

TABLE 2
Comparison of descriptive characteristics in smokers and nonsmokers (both past and never smokers)[1]

Variable	Current smokers (n = 70)	Never and past smokers (n = 208)
Age (y)	37 ± 8.7	35.70 ± 7.7
BMI (kg/m^2)	26.4 ± 6.1	26.40 ± 6.3
Serum		
β-Carotene (μmol/L)	0.325 ± 0.35	0.27 ± 0.27
Lutein (μmol/L)	0.26 ± 0.11	0.28 ± 0.13
Zeaxanthin (μmol/L)	0.09 ± 0.05	0.09 ± 0.04
Lycopene (μmol/L)	0.60 ± 0.27	0.60 ± 0.29
Diet		
α-Tocopherol (μg/d)	9.18 ± 5.08	9.08 ± 6.76
β-Carotene (μg/d)	3087 ± 2703	2814 ± 2301
Lutein + zeaxanthin (μg/d)	1183 ± 978	1078 ± 797
Lycopene (μg/d)	8747 ± 5893	8261 ± 6173
Fruit (servings/d)	1.3 ± 1.13	1.17 ± 1.09
Vegetables (servings/d)	1.59 ± 1.29	1.34 ± 1.00
MPOD	0.20 ± 0.13	0.21 ± 0.13

[1] $\bar{x}$ ± SD. MPOD, macular pigment optical density (460 nm, 1° test). There were no significant differences between groups.

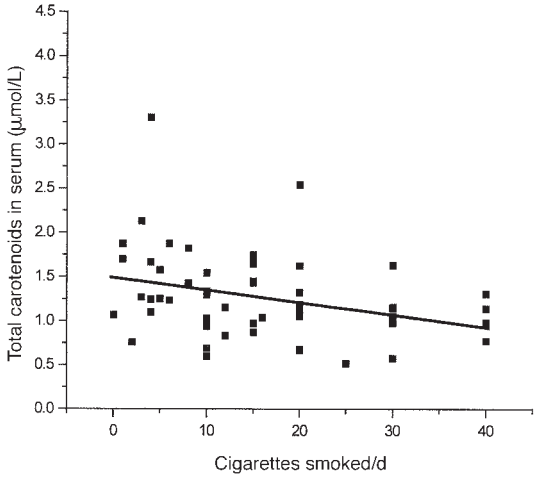

FIGURE 2. Relation between the number of cigarettes smoked per day for current smokers and total serum carotenoid content (r = –0.32, P < 0.005; n = 70).

MICROCAL ORIGIN software (version 5.0; Microcal Software, Northampton, MA).

RESULTS

Descriptive characteristics of the study population are provided in Table 1. Almost one-half (45.4%) of the subjects were between the ages of 31 and 40 y. There were 138 men and 142 women. The ethnic distribution matched closely with regional demographics; 239 (85%) of the subjects were white. There were 150 nonsmokers, 58 former smokers, and 70 current smokers. According to body mass index, 53 (18.9%) of the subjects were overweight and 38 (13.6%) were obese (32).

Dietary intake, serum concentrations of carotenoids, and MPOD measurements are also summarized in Table 1. The distribution of MPOD within the sample is shown in **Figure 1**. Average MPOD did not differ significantly between the men (0.215 ± 0.13) and women (0.207 ± 0.13). This similarity in MPOD is surprising given the large disparity in dietary carotenoid intake. Women had 19% higher lutein + zeaxanthin intake (P < 0.01) and 23% higher β-carotene intake (P < 0.05) than did the men, despite significantly (P < 0.0001) lower average fat (70.9 ± 38.9 compared with 103.4 ± 85.5 g/d) and energy (7654 ± 3296 compared with 10 254 ± 2475 kJ/d) intakes.

MPOD in the current smokers did not differ significantly from that in past and never smokers. Lutein and zeaxanthin concentrations were also not significantly different between current smokers and past and never smokers (**Table 2**). In fact, the current smokers had a slightly more carotenoid-dense diet overall than did the never and past smokers. To analyze whether smoking was directly related to serum carotenoid concentrations, the relation between smoking frequency (number of cigarettes smoked per day) and total carotenoid concentrations was analyzed. As shown in **Figure 2**, total carotenoids in serum were inversely related (r = –0.32, P < 0.005) to the number of cigarettes smoked per day by the current smokers in a dose-response manner. A significant dose-response relation was also found between serum lutein + zeaxanthin and smoking frequency (r = –0.26, P < 0.025). The relation between smoking frequency and carotenoid intake was not significant in current smokers.

TABLE 3
Pearson correlation matrix[1]

	Serum L	Serum Z	Serum BC	Total carotenoids	Dietary L + Z	Dietary BC	Dietary fat	Dietary energy	MPOD
Serum L	1.0	0.77[2]	0.31[2]	0.62[2]	0.19[2]	0.11	0.01	−0.01	0.26[2]
Serum Z	0.77[2]	1.0	0.20[2]	0.59[2]	0.03	0.02	0.08	0.04	0.20[2]
Serum BC	0.31[2]	0.20[2]	1.0	0.74	0.06	0.16[2]	−0.09	0.08	0.06
Total carotenoids	0.62[2]	0.59[2]	0.74[2]	1.0	0.05	0.12[3]	−0.06	−0.08	0.13[3]
Dietary L + Z	0.19[2]	0.03	0.06	0.05	1.0	0.73[2]	0.38[2]	0.46[2]	0.21[2]
Dietary BC	0.11	0.02	0.16[2]	0.12[3]	0.73[2]	1.0	0.49[2]	0.57[2]	0.20[2]
Dietary fat	0.01	0.08	−0.09	−0.06	0.38[2]	0.49[2]	1.0	0.96[2]	0.03
Dietary energy	−0.01	0.04	−0.07	−0.12[3]	0.22[2]	0.57[2]	0.96[2]	1.0	0.03
MPOD	0.26[2]	0.20[2]	0.06	0.13	0.21[2]	0.20[2]	0.03	0.03	1.0

[1] n = 278. Two outliers were excluded because their dietary values were 10 and 20 SDs above the mean, respectively. Total carotenoids is the sum of lutein + zeaxanthin + β-carotene. L, lutein; Z, zeaxanthin; BC, β-carotene; MPOD, macular pigment optical density (460 nm, 1° test).
[2] P < 0.001 (one-tailed).
[3] P < 0.01 (one-tailed).

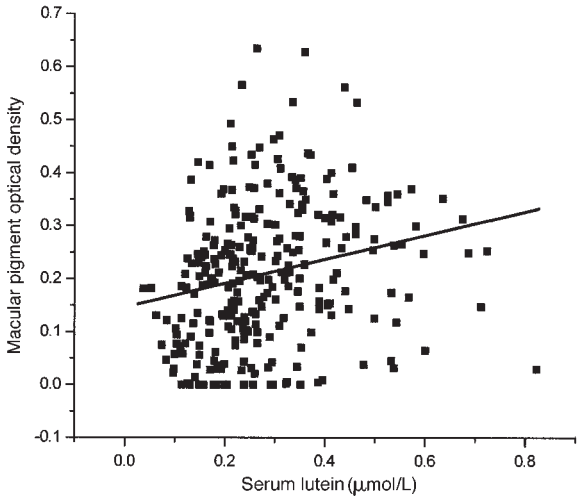

FIGURE 3. Relation between macular pigment optical density (460 nm, 1° test) and serum lutein ($r = 0.26$, $P < 0.0001$; $n = 278$).

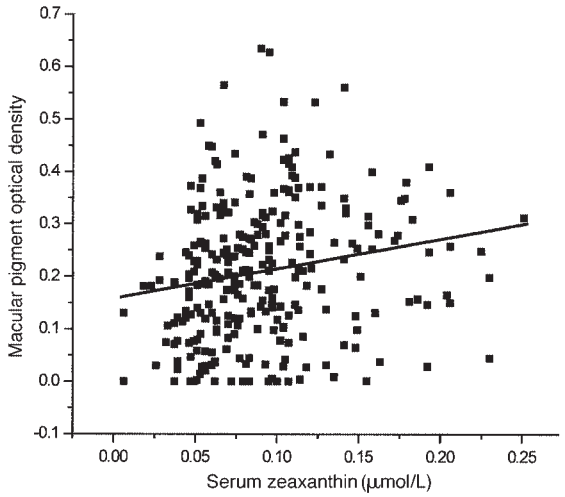

FIGURE 4. Relation between macular pigment optical density (460 nm, 1° test) and serum zeaxanthin ($r = 0.20$, $P < 0.0001$; $n = 278$).

The relation between MPOD, dietary carotenoid intake, and serum carotenoid concentrations was also analyzed (**Table 3**). MPOD was significantly related to both serum lutein and zeaxanthin and dietary intake of lutein + zeaxanthin. The relation between MPOD and serum lutein, serum zeaxanthin, and dietary lutein + zeaxanthin is illustrated in **Figures 3**, **4**, and **5**, respectively. As shown in the figures, each covariate had a wide range; thus, although some associations were significant, variation made it impossible to generalize to individual cases. For example, one 38-y-old male nonsmoker with dark brown irises had a baseline serum lutein + zeaxanthin concentration of 1.02 μmol/L but an MPOD value of 0.03. Another 29-y-old male nonsmoker with light blue irises had a serum lutein + zeaxanthin concentration of 0.763 μmol/L but an MPOD of 0.045.

DISCUSSION

Although optimal MP values have yet to be established, data showing a protective effect of MP on risk of age-related macular degeneration are consistent yet inconclusive. For example, females, smokers, persons with lighter-colored irises, and persons with carotenoid-deficient diets may be at greater risk of age-related macular degeneration (21). Past data have suggested that subjects with these characteristics also tend to have lower MPODs (33–35). Lower MP content could predispose subjects to greater retinal damage over time. For example, exposure to energetic short-wave light may increase the risk of age-related macular degeneration (36), and MP can attenuate the short-wave exposure of vulnerable foveal outer segments by as little as 0% or as much as 98% (37). This suggests that subjects with denser pigment will be most protected.

We report on the MPOD of 280 subjects in the Midwest. The recruitment criteria excluded subjects associated with the local university or medical school to avoid possible selection bias. In previous reports, the average MPOD value when testing subjects from a Northeast population was 0.35 (37). Thus, the average MPOD of 0.21 seen in the Midwest population is ≈40% less than that seen in the Northeast. The average MPOD in the Midwest population is similar to that found in a Southwest population (38) tested by similar procedures ($n = 217$; average MPOD = 0.22 ± 0.13). There was a significant relation between MPOD and dietary lutein + zeaxanthin and serum lutein in each of the studies. It is interesting to note the similarity between our average serum and dietary carotenoid values and those taken from large random samples (7, 8, 39; **Table 4**). It is too early to suggest that serum or dietary carotenoid concentration can predict MPOD. However, because methods are now available to measure retinal carotenoids without the use of extensive optical systems (27), future studies should make use of such measurements to better understand the relation of dietary carotenoids to MP and to visual health.

In contrast with the findings of past studies, in the present study, MPOD was not significantly correlated with differences in sex or smoking behavior. This may be related to the better average diet of the women than the men in this sample. Although the

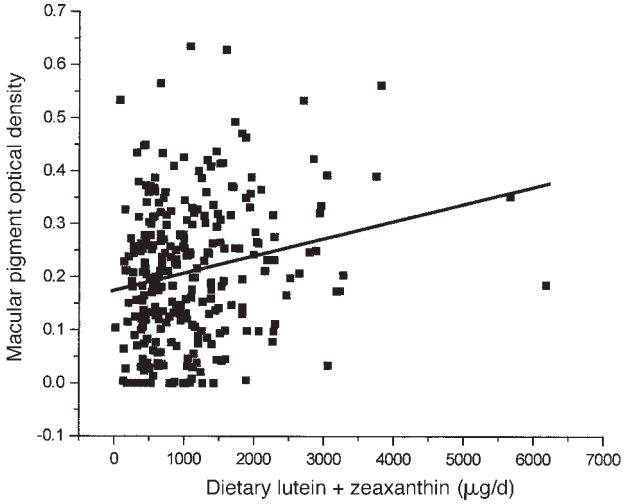

FIGURE 5. Relation between macular pigment optical density (460 nm, 1° test) and dietary intake of lutein + zeaxanthin ($r = 0.21$, $P < 0.0005$; $n = 278$).

TABLE 4
Distributions of lutein and zeaxanthin in the diet and serum of different study populations compared with the data from the present sample (Midwest population)[1]

Study population	Years of study	*n*	Intake percentile		
			10th	50th	90th
Diet (μg/d)					
Beaver Dam (baseline, women) (40)	1988–1990	1190	421	867	1773
Eye Disease Case Control Study (7, 8)	1986–1990	876	561	1708	5757
Midwest population	1998	278	343	892	2099
Serum (μmol/L)					
Beaver Dam (baseline, women) (40)	1988–1990	220	0.16	0.27	0.46
NHANES III (women aged ≥40 y) (41)	1988–1994	4989	0.19	0.39	0.72
Midwest population	1998	278	0.18	0.33	0.62

[1] NHANES III, third National Health and Nutrition Examination Survey.

men had significantly higher energy intakes, their carotenoid intakes were significantly lower. Because MP is derived from dietary carotenoids and responds to dietary supplementation, sex differences in diet would predict higher MP concentrations in the women. The present results are therefore consistent with past observations that MP accumulation by women may be influenced by their reproductive biology, such as cyclical variation in hormones or menopause. Because women may be more susceptible to several ocular diseases that are linked to carotenoid utilization, this issue deserves further study. In addition, although the fat content of the diet differed between the men (103 g/d) and the women (71 g/d), this is unlikely to have accounted for the lack of significant difference in MPOD despite increased intake of lutein + zeaxanthin in females. Fat in excess of 30 g/d is unlikely to influence carotenoid absorption (42).

We found no significant relation between smoking and MPOD. This result is consistent with the null effect of smoking on serum lutein and zeaxanthin concentrations in this sample (Table 2). This lack of effect may be due to the slightly more carotenoid-dense diet of the current smokers and the preponderance of light smokers in the study. Past studies have suggested that smoking <20 cigarettes/d does not have measurable effects on MPOD. This is consistent with the dose-response curve shown in Figure 2, in which heavier smoking had the greatest effect on serum carotenoid concentrations.

All the correlations must be viewed in light of the restricted range of MP values in this Midwest population. For example, the highest MP value in this sample was less than one-half of the highest value in Northeast samples that we have studied (37). The fact that significant correlations were found under these conditions suggests that the coefficients may underestimate the strength of the relation.

Whereas dietary lutein + zeaxanthin and serum lutein and zeaxanthin were significantly correlated with each other and with MPOD, the relations appeared to be less robust than predicted. Biological factors such as individual absorption profiles and day-to-day variation in blood concentrations contribute substantially to the attenuation of diet-serum correlations. Moreover, errors in measurement of dietary intake, serum carotenoid concentrations, and MPOD undoubtedly attenuate correlations. With the enhanced database information on dietary carotenoid values, improved methods for measuring the distribution of MP in a population-based study, and a better understanding of the transport and deposition of carotenoids in tissue, future studies should enhance our understanding of the links between diet, blood, and tissue concentrations and disease risk.

REFERENCES

1. CARIG (Carotenoid Research Interactive Group). Beta-carotene and the carotenoids: beyond the intervention trials. Nutr Rev 1996; 54:185–8.
2. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. JAMA 1994;272:1413–20.
3. Goldberg J, Flowerdew G, Smith E, Brody JA, Tso MOM. Factors associated with age-related macular degeneration: an analysis of data from the First National Health and Nutrition Examination Survey. Am J Epidemiol 1988;128:700–10.
4. Joseph JA, Shukitt-Hale B, Deisova NA, et al. Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. J Neurosci 1999;19:8114–21.
5. Clinton SK, Emenhiser C, Schwartz SJ, et al. *Cis-trans* lycopene isomers, carotenoids, and retinol in the human prostate. Cancer Epidemiol Biomarkers Prev 1996;5:823–33.
6. Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC. Intake of carotenoids and retinol in relation to risk of prostate cancer. J Natl Cancer Inst 1995;87:1767–76.
7. EDCCSG (Eye Disease Case Control Study Group). Antioxidant status and neovascular age-related macular degeneration. Arch Ophthalmol 1993;111:104–9.
8. EDCCSG (Eye Disease Case Control Study Group). Risk factors for neovascular age-related macular degeneration. Arch Ophthalmol 1992;110:1701–8.
9. Bone RA, Landrum JT, Tarsis SL. Preliminary identification of the human macular pigment. Vision Res 1985;25:1531–5.
10. Snodderly DM, Brown PK, Delori FC, Auran JD. The macular pigment. 1. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. Invest Ophthalmol Vis Sci 1984;25:660–73.
11. Snodderly DM, Handleman GJ, Adler AJ. Distribution of individual macular pigment carotenoids in the central retina of macaque and squirrel monkeys. Invest Ophthalmol Vis Sci 1991;32:268–79.
12. Bone RA, Landrum JT, Fernandez L, Tarsis SL. Analysis of the macular pigment by HPLC: retinal distribution and age study. Invest Ophthalmol Vis Sci 1988;29:843–9.
13. Weiter JJ, Delori F, Dorey CK. Central sparing in annular macular degeneration. Am J Ophthalmol 1988;106:286–92.
14. Haegerstrom-Portnoy G. Short-wavelength-sensitive-cone sensitivity loss with aging: a protective role for macular pigment? J Opt Soc Am 1988;5:2140–4.

15. Hammond BR Jr, Wooten BR, Snodderly DM. Preservation of visual sensitivity of older subjects: association with macular pigment density. Invest Ophthalmol Vis Sci 1998;39:397–406.
16. Khachik F, Bernstein PS, Garland D. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. Invest Ophthalmol Vis Sci 1997;38:1802–11.
17. Hammond BR, Johnson EJ, Russell RM, et al. Dietary modification of human macular pigment density. Invest Ophthalmol Vis Sci 1997;38: 1795–801.
18. Landrum JT, Bone RA, Joa H, Kilburn MD, Moore LL, Sprague KE. A one year study of the macular pigment: the effect of 140 days of a lutein supplement. Exp Eye Res 1997;65:57–62.
19. Richer S. Part II: ARMD—pilot (case series) environmental intervention data. J Am Optom Assoc 1999;70:24–36.
20. Zorge I, McDonald G, Dagnelie G. Lutein improves visual function in some patients with congenital retinal degeneration: a pilot study via the internet. Invest Ophthalmol Vis Sci 1999:40:S697 (abstr).
21. Snodderly DM. Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. Am J Clin Nutr 1995;62(suppl):1448S–61S.
22. Ciulla TA, Curran-Celentano J, Cooper D, et al. Determinants of macular pigment density in adult volunteers in the Indianapolis area. Invest Ophthalmol Vis Sci 2001;108:730–7.
23. Snodderly DM, Hammond BR. Chapter 13. In vivo psychophysical assessment of nutritional and environmental influences on human ocular tissue: lens and macular pigment. In: Taylor A, ed. Nutritional and environmental influences on the eye. New York: CRC Press, 1999: 251–73.
24. Hammond BR, Fuld K, Curran-Celentano J. Macular pigment density in monozygotic twins. Invest Ophthalmol Vis Sci 1995;36:2531–41.
25. Hammond BR, Fuld K. Interocular differences in macular pigment density. Invest Ophthalmol Vis Sci 1992;33:350–4.
26. Werner JS, Cicerone CM, Kliegl R, DellaRosa D. Spectral efficiency of blackness induction. J Opt Soc Am A 1984;11:981–5.
27. Wooten BR, Hammond BRJ, Land RI, Snodderly DM. A practical method for measuring macular pigment optical density. Invest Ophthalmol Vis Sci 1999;40:2481–9.
28. Patterson RE, Kristal AR, Coates RJ, et al. Low-fat diet practices of older women: prevalence and implications for dietary assessment. J Am Diet Assoc 1996;96:670–6.
29. Kristal AR, Patterson RE, Neuhouser ML, et al. Olestra Postmarketing Surveillance Study: design and baseline results from the sentinel site. J Am Diet Assoc 1998;98:1290–6.
30. Cooper DA, Curran-Celentano J, Ciulla TA, et al. Olestra consumption is not associated with macular pigment optical density in a cross-sectional volunteer sample in Indianapolis. J Nutr 2000;130: 642–7.
31. Pratt LM, Ciulla TA, Danis RP, Curran-Celentano J, Cooper DA, Hammond BR. Determinants of macular pigment density in adult volunteers in the Indianapolis area. Invest Ophthalmol Vis Sci 1999;40:S165 (abstr).
32. Sichieri R, Everhart JE, Hubbard VS. Relative weight classifications in the assessment of underweight and overweight in the United States. Int J Obes Relat Metab Disord 1992;16:303–12.
33. Hammond BR Jr, Curran-Celentano J, Judd S, et al. Sex differences in macular pigment optical density: relation to plasma carotenoid concentrations and dietary patterns. Vision Res 1996;36:2001–12.
34. Hammond BR, Fuld K, Snodderly DM. Iris color and macular pigment optical density. Exp Eye Res 1996;62:293–7.
35. Hammond BR, Wooten BR, Snodderly DM. Cigarette smoking and retinal carotenoids: implications for age-related macular degeneration. Vision Res 1996;36:3003–9.
36. McCarty C, Taylor H. Light and risk for age-related eye diseases. In: Taylor AJ, ed. Nutritional and environmental influences on vision. Boca Raton, FL: CRC Press, 1999:135–50.
37. Hammond BR Jr, Wooten BR, Snodderly DM. Individual variations in the spatial profile of the human macular pigment. J Opt Soc Am A 1997;14:1187–96.
38. Hammond BR Jr, Caruso-Avery M. Macular pigment optical density in a Southwestern sample. Invest Ophthalmol Vis Sci 2000;41: 1492–7.
39. Mares-Perlman JA, Klein R. Diet and age-related macular degeneration. In: Taylor AJ, ed. Nutritional and environmental influences on vision. Boca Raton, FL: CRC Press, 1999:251–73.
40. Mares-Perlman J, Brady W, Klein R, et al. Serum antioxidants and age-related macular degeneration in a population-based case-control study. Arch Ophthalmol 1195;113:1518–23.
41. Briefel R, Sowell A, Huff D, et al. The distribution of serum carotenoids in the US population (1988–94): results from the third National Health and Nutrition Examination Survey (NHANES III). FASEB J 1996;10:A4700 (abstr).
42. Roodenberg AJC, Leenen R, van het Hof KH, Weststrate JA, Tijburg LBM. Amount of fat in the diet affects bioavailability of lutein esters but not of α-carotene, β-carotene, and vitamin E in humans. Am J Clin Nutr 2000;71:1187–93.