

Macular Pigment and Lutein Supplementation in Retinitis Pigmentosa and Usher Syndrome

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PURPOSE. To determine macular pigment (MP) in patients with inherited retinal degeneration and the response of MP and vision to supplementation of lutein.

METHODS. Patients with retinitis pigmentosa (RP) or Usher syndrome and normal subjects had MP optical density profiles measured with heterochromatic flicker photometry. Serum carotenoids, visual acuity, foveal sensitivity, and retinal thickness (by optical coherence tomography [OCT]) were quantified. The effects on MP and central vision of 6 months of lutein supplementation at 20 mg/d were determined.

RESULTS. MP density in the patients as a group did not differ from normal. Among patients with lower MP, there was a higher percentage of females, smokers, and light-colored irides. Disease expression tended to be more severe in patients with lower MP. Inner retinal thickness by OCT correlated positively with MP density in the patients. After supplementation, all participants showed an increase in serum lutein. Only approximately half the patients showed a statistically significant increase in MP. Retinal nonresponders had slightly greater disease severity but were otherwise not distinguishable from responders. Central vision was unchanged after supplementation.

CONCLUSIONS. Factors previously associated with lower or higher MP density in normal subjects showed similar associations in RP and Usher syndrome. In addition, MP in patients may be affected by stage of retinal disease, especially that leading to abnormal foveal architecture. MP could be augmented by supplemental lutein in many but not all patients. There was no change in central vision after 6 months of lutein supplementation, but long-term influences on the natural history of these retinal degenerations require further study. (*Invest Ophthalmol Vis Sci.* 2001;42:1873-1881)

Retinitis pigmentosa (RP) is a genetically and clinically heterogeneous group of incurable retinal degenerative diseases. The association of RP and sensorineural hearing loss is termed Usher syndrome.¹ Despite this heterogeneity, most patients with RP (or Usher syndrome) tend to share the experience of diminishing peripheral vision at early disease stages and dependence at later stages on a residual central island of useful perception. Central or macular vision thus becomes of increasing importance to those with RP as the disease progresses, and attempts to preserve this vision are a worthy goal for intervention.

Macular pigment (MP) has been suggested to have a protective role for central vision from oxidative damage and such damage may be at least partly involved in loss of vision in degenerative retinal disease. The main focus for such consideration has been age-related macular degeneration.²⁻¹⁰ Lutein and zeaxanthin are the principal components of MP, a yellowish carotenoid complex most notably located within photoreceptor axons and the inner plexiform layer of the central retina.¹¹⁻¹⁶ Evidence for localization in photoreceptors has also been provided.^{17,18} For normal human subjects and non-human primates, MP is most dense in the central 1° to 2°, declining in exponential fashion to negligible levels by 5° to 10° radial eccentricity.^{10,19-21}

The present work attempts to set the foundation for testing the hypothesis that central retinal function in retinal degenerations may be stabilized with the use of the supplemental non-vitamin A carotenoid, lutein.²² First, we asked whether MP density was normal in patients with RP or Usher syndrome. The advent of a clinically feasible method of measuring MP density facilitated these investigations.^{23,24} Then, we studied a subset of these patients over a 6-month period of lutein supplementation to determine whether baseline serum and MP density could be modified. Considering recent reports of increased vision after lutein intake in retinal degenerations,^{25,26} we also measured central vision in the patients to determine whether there was any visual benefit of relatively short-term lutein supplementation.

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METHODS

Subjects

Patients with the diagnosis of RP ($n = 47$) or Usher syndrome ($n = 11$) and normal subjects ($n = 29$) participated in this study. Table 1 briefly describes the patient population as two groups: the entire study group of patients with retinal degeneration ($n = 58$) and a subset of this group who underwent a pilot trial of supplementation with lutein ($n = 23$). All subjects had a routine ocular examination and best corrected visual acuity determined with the Early-Treatment Diabetic Retinopathy Study (ETDRS) chart. Normal subjects had visual acuity of 20/20 or better. All patients with RP or Usher syndrome were examined and the diagnosis made by one of the authors (SGJ). These subjects were included because they had adequate visual acuity (20/63 or better in the test eye) and sufficient visual field (minimum kinetic visual field extent to the 10° isopter with a Goldmann V-4e target) to perform the MP density measurement. In the entire patient group, 39 patients were evaluated bilaterally and 19 patients unilaterally. Of the 23 patients of the subgroup who were taking the lutein supplement, 22 were tested bilaterally. Unilateral testing occurred when the other eye did not meet criteria (visual acuity or field) for performing the tests reliably. Ten

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TABLE 1. Patients with Retinal Degeneration

Patients*	Gender* (F/M)	RP*	Usher Syndrome* I/II	Age Range (y) (mean)	Iris Color* (%)		Smokers* (%)
					Light	Dark	
Entire patient population 58	31/27	47	5/6	11-59 (31)	26 (45)	32 (55)	10 (17)
Patient subgroup with supplemented lutein 23	12/11	21	0/2	12-59 (35)	10 (43)	13 (57)	3 (13)

* Number of patients.

patients representing 14 eyes with cystoid macular edema (CME) that met the visual criteria were included, but their results were analyzed separately. There were six patients with bilateral CME (one eye from each of two of these patients did not meet visual criteria) and four patients with unilateral CME. Informed consent was given by all subjects, institutional approval was obtained, and the tenets of the Declaration of Helsinki were followed.

Measurement of MP Optical Density

Heterochromatic flicker photometry (HFP) was used to estimate MP optical densities.^{10,20,27-29} This psychophysical technique compares flicker photometric sensitivity measured in the fovea, where MP is most dense, to that obtained at an eccentric retinal location (~5-8° parafovea) where the density of the MP is negligible. Sensitivity is determined by alternating a short wavelength test light (460 nm, peak absorption of MP) in counterphase with a longer wavelength reference light (560 nm, for example) that is not absorbed by the MP. The intensity of the 460-nm light is adjusted until the perception of flicker is minimized or eliminated, at which point the two lights are equated in intensity. The parafoveal/foveal sensitivity ratio is used to determine the peak density of the MP.

An LED-based MP densitometer (Macular Metrics Corp., Rehoboth, MA) was used to measure MP density in this study. Details of this instrumentation and methodology and the relationship of results to those from Maxwellian-view systems are published.^{23,24,30} In brief, flickering stimuli (460 nm, test; 570 nm, reference, 1.7 log trolands [td]) were centered on a 6° diameter background field (1.5 log td, 470 nm). The four stimuli used consisted of two discs (0.34° and 1° diameter) and two annuli (2° and 4° diameter, 0.4° wide). Under the assumption that flicker perception is dominated by the edges,^{20,27} these stimuli represent eccentricities of 0.17°, 0.5°, 1°, and 2° and their results are plotted as such. Fixation was to a central 5' (min) spot. Parafoveal sensitivities were determined with a 2° diameter disc centered on the background. Subjects fixated to a small red LED situated to the left or right of the background field at 5° to 7° eccentricity (the 5° locus had to be used in many patients because of the limited extent of their central island of function). The flicker frequency was optimized for each stimulus to achieve a clear flicker null over a small range (10-15 Hz for the centrally viewed stimuli and 7-12 Hz for the peripherally viewed stimulus).

MP density measurements were made using a psychophysical method that differs from previous work with this instrumentation.^{23,24,30} The experimenter rather than the subject made the intensity adjustment. Subjects were asked to press a button to indicate when the perception of flicker was eliminated or minimized. The procedure began with the intensity of the test light much brighter than the reference, so that perception of flicker was obvious. Test light intensity was gradually reduced until the subject indicated that flicker had stopped or was minimal. The intensity of the test light was then offset in the other direction (dimmer than the standard) until flicker was obvious. The experimenter then increased the intensity until flicker was nulled again. Flicker frequency was adjusted to minimize the null range. The mean of this range was taken as the point of minimal flicker. The subject was given several practice trials until comfortable with the

task. This procedure was repeated, and a total of three to eight measurements were collected for each stimulus, depending on the consistency of the observer.

Supplementation with Lutein

A subset of 23 patients with retinal degeneration (Table 1) and 8 normal subjects participated in a 6-month pilot trial of lutein supplementation. In this pilot investigation, there was no placebo control group and no attempt to mask the patient as to the content of the supplement. After two baseline visits (separated by no more than one month), subjects supplemented their diets with a commercially available form of lutein at 20 mg per day (Twin Laboratories, Inc., Ronkonkoma, NY).²² Subjects were instructed to take the lutein supplement with dinner and not in combination with other medications or nutrients they had been taking.²² Subjects who were taking other nutrient supplements were encouraged to continue them as before. A subgroup of patients (13/58) was taking vitamin A orally at 15,000 IU/d before and throughout this study. Subsequent visits after beginning to take the supplement included a fasting (overnight) blood sample for serum carotenoids and measurements of ETDRS visual acuity, MP optical density, and absolute sensitivity at the fovea.

Other Methodology

Serum carotenoids, specifically lutein and zeaxanthin, were measured in all patients and in normal subjects ($n = 24$) using high-performance liquid chromatography³¹ by an analytical laboratory (Craft Technologies, Inc., Wilson, NC). Foveal sensitivity was measured in the dark-adapted state with a 650-nm target (1.7°; 200 msec duration) using a modified automated perimeter.³² The participants also provided dietary information through the Health Habits and History Questionnaire (HHHQ) developed by the National Cancer Institute (Bethesda, MD).³³ Data were analyzed using the revised HHHQ Diet System Analysis.³⁴

Optical coherence tomography (OCT) was performed with a commercial instrument (Humphrey Instruments, San Leandro, CA). The principles of the instrument and our technique have been published.³⁵⁻³⁸ Horizontally and vertically oriented scans (15° in extent) crossing fixation were obtained in patients and compared with scans from normal subjects. In 49 patients ($n = 75$ eyes) and 19 normal subjects ($n = 27$ eyes), scan quality permitted measurement of inner retinal thickness. Using the pseudocolor images, the central 1° of inner retinal thickness (defined from vitreoretinal interface to the onset of the outer retinal-choroidal complex³⁸⁻⁴⁰) was outlined manually, and the number of pixels within these boundaries was quantified by computer. Patients with CME, defined clinically and/or by OCT, were not included in this analysis.

Data Analyses

Data analyses were performed by computer with statistical software (SAS, ver. 8.00; SAS, Cary, NC). Mean values from the two baseline visits were used in describing the study groups and in calculating change after supplementation with lutein. In addition, the average of the measurements from each eye was used to establish person-specific characteristics. Signed and absolute differences of measurements be-

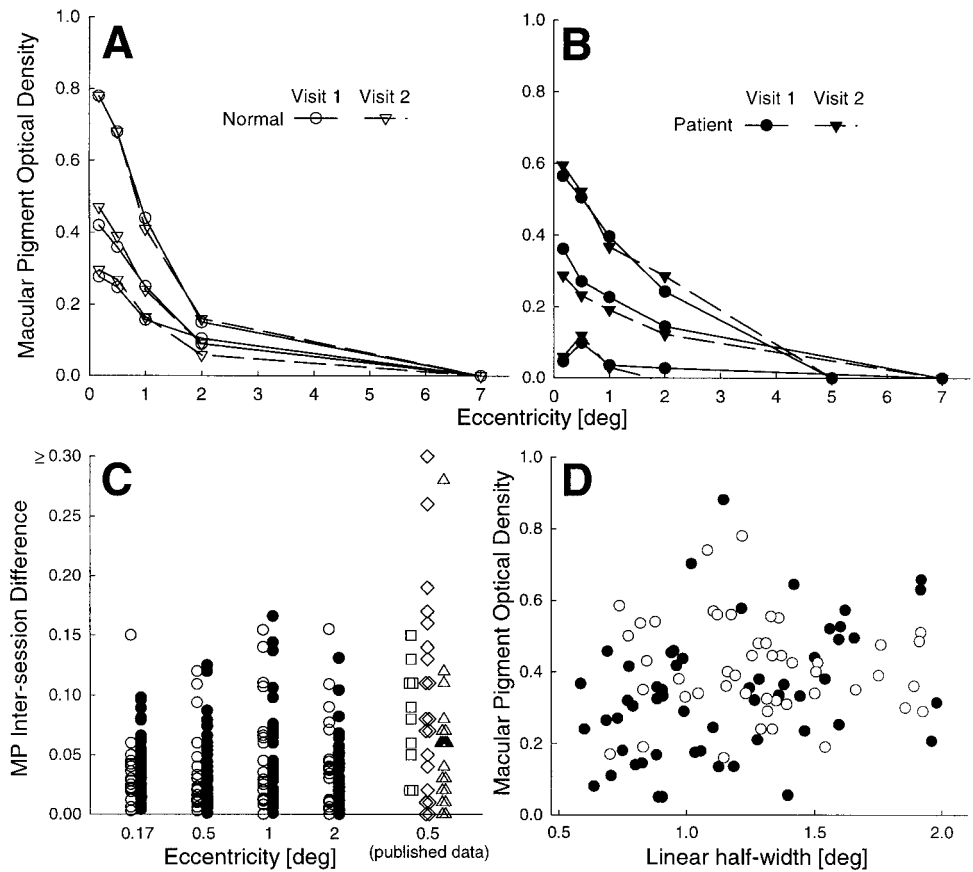


FIGURE 1. Spatial MP profiles and their variation in normal subjects and patients with inherited retinal degeneration. (A) Three normal subjects tested at two different visits; (B) three patients tested at two different visits; (C) intersession absolute differences in MP in normal subjects (*open symbols*) and patients (*closed symbols*), using stimuli at four different eccentricities. *Open symbols at right* are published data from three studies using a 0.5° eccentricity (1° diameter) stimulus and an HFP technique (\square^{20} , \diamond^{43} , Δ^{42}); (D) variation in spatial profiles shown as a plot of MP density (at 0.17° eccentricity) versus half-width at half-height of this MP density. *Open symbols:* normal subjects; *solid symbols:* patients.

tween the first and second baseline visits were used to assess intersession variability. Intersession differences were examined for the correlation between eyes of the same patient. No pattern of consistent (direct or inverse correlation) or significant correlation was observed between eyes. Therefore, analyses of intersession variability treat the data from each eye as an independent observation. Means of intersession differences and person-specific variables were compared with independent *t*-tests. Proportions were compared using χ^2 tests with exact computation of the *P* values. The *t*-tests comparing means and significance levels for correlation coefficients involving data from two eyes of the same person were performed using a robust variance estimator to accommodate the correlation between eyes.⁴¹

RESULTS

MP in Patients with Retinal Degeneration

MP density peaked within the central 1° and declined with increasing retinal eccentricity in the normal subjects and most of the patients in this study. Representative spatial profiles of MP density are shown for two visits separated by less than 1 month in one eye of three normal subjects (Fig. 1A) and of three patients with retinal degeneration (Fig. 1B). The examples indicate there was a range of measurable MP density levels in both normal subjects and patients and that some patients could display relatively flat spatial profiles with little or no measurable MP.

How variable are MP density measurements in the same individual on two visits (Fig. 1C) and is there any predictability to the shape of MP spatial profiles in patients or normal subjects (Fig. 1D)? Absolute and signed differences of MP density between the first and second baseline visits were used to assess intersession variability. In Figure 1C, we display the absolute intersession differences in 38 eyes of patients and make com-

parisons with results from 20 eyes of our normal subjects and with other groups of normal subjects tested similarly.^{20,42,43} The mean absolute difference between values was similar for patient and normal eyes for each of the four eccentricities tested. The mean values were 0.044 and 0.038 at 0.17°, 0.040 and 0.035 at 0.5°, 0.050 and 0.052 at 1.0°, and 0.037 and 0.043 at 2.0°, for patient and normal eyes, respectively. The SDs of the distributions for each subject group and each stimulus were approximately 0.04. Previous studies using HFP methods of assessing MP have mainly examined MP density using a 1.0° stimulus (0.5° eccentricity). The reported data from five earlier studies give the following mean (\pm SD) intersession absolute differences: 0.06 \pm 0.06, $n = 20^{42}$; 0.10 \pm 0.10, $n = 20^{43}$; 0.06 \pm 0.04, $n = 10^{20}$; 0.08 \pm 0.02, $n = 13^{44}$; and 0.08 \pm 0.09, $n = 37^7$.

Taking our normal data together with those reported in the literature, it can be concluded that the intersession variation in MP density levels in the patients with retinal degeneration is within the expected range of normal. Signed differences between visits (i.e., baseline session two minus session one) were then explored to determine whether there was any increase in measured MP density that would suggest a systematic learning effect in subjects. There was no substantial increase between sessions for any of the four stimuli either in patient eyes or in normal eyes. The mean differences were -0.01 and 0.02 at 0.17°, -0.00 and 0.02 at 0.5°, -0.01 and 0.03 at 1.0°, and 0.00 and 0.02 at 2.0°, for patient and normal eyes, respectively. None of the mean signed differences reached statistical significance.

Individual variation in the shape of MP density profiles has been noted previously in normal subjects^{20,44} and was evident in both our normal subjects and patients. A trend of higher peak MP density with wider half-width at half-height has been

reported in normal subjects.²⁰ The attraction of characterizing the entire MP profile from a single peak value led us to ask whether this trend was also evident in our data. Figure 1D plots MP density (for the smallest stimulus) versus half-width at half-peak MP level in all eyes of patients and normal subjects. The width of the MP distribution was not related to MP peak density in normal subjects ($r = -0.132$) or patients ($r = 0.209$).

Is MP density in patients with retinal degeneration as a group different from normal? A frequency histogram is shown of MP densities from the patients, measured with the conventional 1° stimulus (Fig. 2A). Each individual in this analysis is represented as a single MP density value (derived from results of one eye on one visit or, when available, from an average of results of both eyes on one or two visits). Above the patient data are displayed, for comparison, box plots of these data (d) and of normal values from this study (a) and two recent studies (b, c) that used the same instrumentation and target.^{23,24} The patients had an average MP density (\pm SD) of 0.29 ± 0.18 . The MP density of normal subjects in our study was 0.33 ± 0.11 . Normal data from two other studies showed mean MP densities of 0.26 ± 0.16 ²³ and 0.24 ± 0.13 ²⁴. A comparison of these groups of normal subjects with the patient data for MP density showed no statistically significant differences between patients and any of the normal groups.

The basis for the wide range of MP density levels observed in normal subjects has been explored in previous studies, and there are "lifestyle variables"²⁰ and personal characteristics that are associated with lower versus higher MP levels.^{4,8,24,45-47} A single measure of MP density from one eye usually has been used to relate to variables such as diet, serum levels of carotenoids, gender, smoking, and iris color (e.g., Ref. 24), on the assumption that normal MP interocular variability is no greater than intersession variability.⁴² Interocular variability of MP (mean absolute difference, 0.03) and intersession variability (mean absolute difference, 0.04) were also similar in our normal subjects. Among patients, the interocular variability (mean absolute difference, 0.05) in MP density was slightly greater than the intersession variability (mean absolute difference, 0.04) within eyes, but not to a statistically significant degree ($P = 0.08$; Fig. 2B). Single MP densities with the 1° stimulus (as in Fig. 2A) were thus used in examining associations among MP, dietary intake, serum levels of lutein, and personal characteristics among our patient and normal groups.

Dietary intake of lutein showed a modest relationship to serum lutein in the patients ($r = 0.32$; $P = 0.05$) but not in normal subjects ($r = 0.22$; $P = 0.31$). MP density was not related to dietary intake of lutein ($r = -0.05$; $P = 0.71$) or serum lutein ($r = 0.14$; $P = 0.44$) in patients. In normal subjects, there was no significant correlation of MP with dietary intake ($r = 0.04$; $P = 0.84$) but a significant correlation with serum lutein ($r = 0.50$; $P = 0.01$). MP was not correlated with serum zeaxanthin in either the patient ($r = 0.04$; $P = 0.81$) or the normal group ($r = 0.21$; $P = 0.34$). To further examine the associations of MP density in the patients, they were arbitrarily divided into low (≤ 0.2) and high (> 0.4) groups (Fig. 2C). Consistent with the correlation analysis above, the mean serum lutein (\pm SD) was slightly higher in the high-MP group (mean, 0.16 ± 0.07 μ g/ml) than in the low-MP group (mean, 0.14 ± 0.05 μ g/ml), but not to a statistically significant degree ($P = 0.38$). Gender (female), smoking, and light-colored irides have been associated with lower MP in normal subjects.⁴⁵⁻⁴⁷ Among the patients with retinal degeneration with lower MP, there was a higher percentage of females (63% vs. 50%), smokers (26% vs. 14%), and individuals with light-colored irides (58% vs. 29%; Fig. 2C). The results are thus consistent with published work.

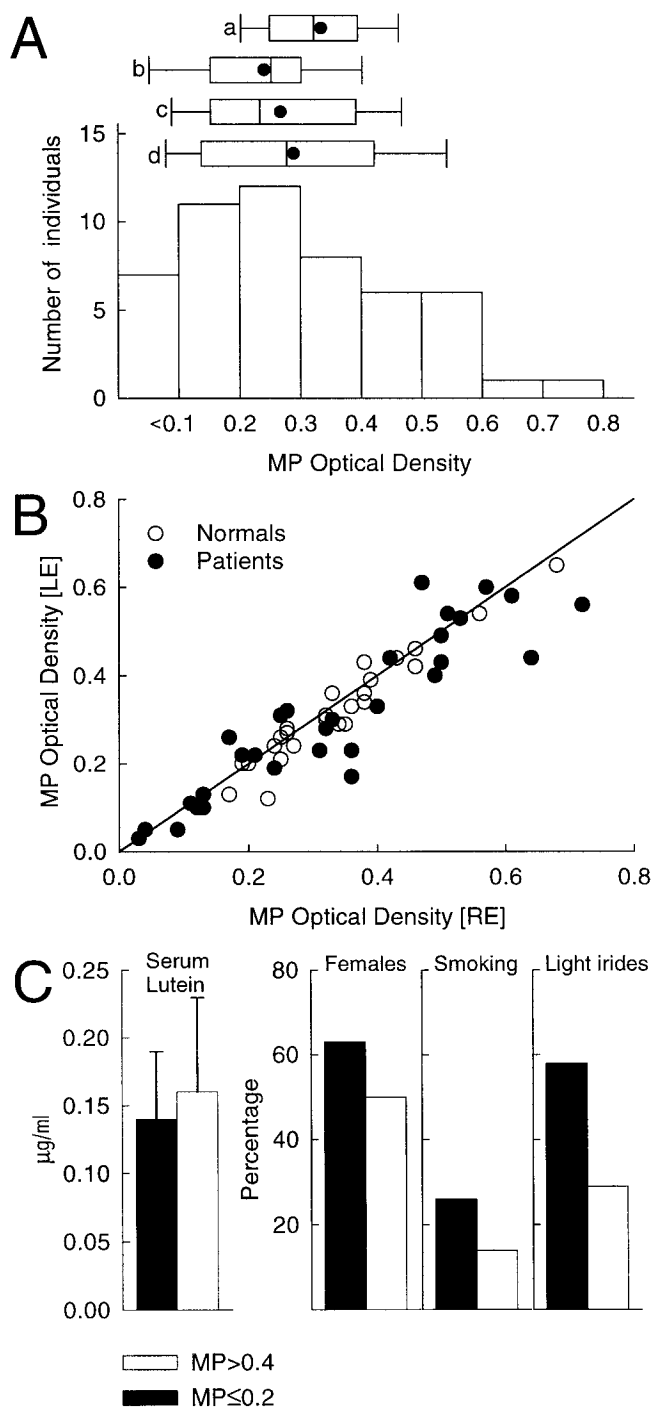


FIGURE 2. Group statistics for MP, interocular differences in MP, and relation of MP to systemic and ocular factors in the patients with retinal degeneration. (A) Frequency distribution of MP optical densities for the 0.5° eccentricity target in the patients. Above are box plots of MP density for this target in the same patients (d) compared with normal subjects from this study (a) and two other studies (b²⁴, c²³). Each box shows the median (solid line) and mean (filled symbol). The boxed region represents the interquartile data (25th–75th percentiles); whiskers, the 10th and 90th percentiles. (B) Interocular differences in MP for the 0.5° eccentricity target in normal subjects and patients. (C) Relationships of serum lutein, gender, smoking, and light irides in patients with low (≤ 0.2) and high (> 0.4) MP levels.

Is there an association between severity of retinal disease and MP density in patients? We considered MP results in relation to those of both retina-wide measures of function

(kinetic perimetry, full-field electroretinography) and central retinal function (dark-adapted foveal sensitivity, visual acuity). Using presence or absence of a detectable electroretinogram (ERG) to a standard maximal white stimulus in the dark-adapted state⁴⁸ as an estimate of retina-wide function, there was a higher percentage of patients with no detectable ERG in the low-MP group (52%) than in the high-MP group (31%). There were modest correlations between MP density and kinetic visual field extent to the V-4e target ($r = 0.30$; $P = 0.008$), log minimum angle of resolution (MAR) visual acuity ($r = -0.22$; $P = 0.04$), and foveal sensitivity ($r = 0.36$; $P = 0.002$). The results suggest a tendency for greater severity of disease expression to be associated with lower MP.

Foveal architecture has been postulated to be one of the factors that may contribute to differences in MP levels in humans.²⁰ Experimental studies in monkey retinas suggest individual variations in central retinal structure and MP.¹² We tested the hypothesis that inner retinal thickness in the central 1° of retina, as measured with the *in vivo* microscopy technique of OCT, was related to MP density. Figure 3 illustrates OCT scans through the fovea in two normal subjects showing variation in thickness (Figs. 3A, 3B) and in four patients (Figs. 3C-F). When inner retinal thickness was plotted versus MP density in normal subjects (Fig. 3G), there was modest correlation ($r = 0.39$; $P = 0.12$); in the patients (Fig. 3H), there was greater correlation ($r = 0.57$; $P < 0.001$).

The abnormalities in foveal architecture caused by CME led us to exclude from the analyses the results from eyes with this central retinal complication of RP and Usher syndrome.⁴⁹ Were there any detectable differences between MP in eyes with or without CME? In 10 patients with CME, MP density was measured in at least one eye. A total of 14 eyes were studied: both eyes of four patients with bilateral CME, one eye of two other patients with bilateral CME, and the affected eye of four patients with unilateral CME. Comparison of mean MP densities (1° target) showed that eyes with CME had lower MP (mean, 0.19 ± 0.19) than eyes without CME (mean, 0.29 ± 0.18). However, the difference did not reach statistical significance ($P = 0.12$). Among eyes with CME, mean logMAR visual acuity (mean, 0.24 ± 0.13) was approximately 0.4 line lower than in eyes without CME (mean, 0.20 ± 0.27 ; $P = 0.30$). There were no significant differences between the two groups in age, serum lutein and zeaxanthin, kinetic visual field extent, and foveal sensitivity. Comparison of MP in CME and non-CME eyes of four patients with unilateral CME showed that three of the four had slightly lower values in the eyes with macular edema. Mean MP of the four CME eyes was 0.097, whereas that of the non-CME eyes was 0.155. The results suggest that further complexity would probably have been introduced by including eyes with CME in our various analyses.

Effects of Lutein Supplementation

Figure 4 shows mean MP densities at four retinal eccentricities (0.17°, 0.5°, 1°, and 2°) at baseline and after 6 months of lutein supplementation in 8 normal subjects (Fig. 4A) and 21 patients with retinal degeneration (Fig. 4B). Individuals are represented as a single MP value (as in Fig. 2A). The mean MP at 0.17° increased by 0.07 in each group (normal subjects, $P = 0.04$; patients, $P = 0.02$). Patients showed statistically significant mean increases in MP of 0.07 at 0.5°, 0.08 at 1°, and 0.04 at 2° ($P = 0.001$, $P = 0.0004$, and $P = 0.01$, respectively). However, for normal subjects the mean increases were only 0.01 at 0.5°, 0.03 at 1°, and -0.003 at 2° and were not statistically significant ($P = 0.53$, $P = 0.11$, $P = 0.79$, respectively).

We then focused on the two central or peak measures in the patients and asked whether there was greater change in MP density than would be expected from intersession variability

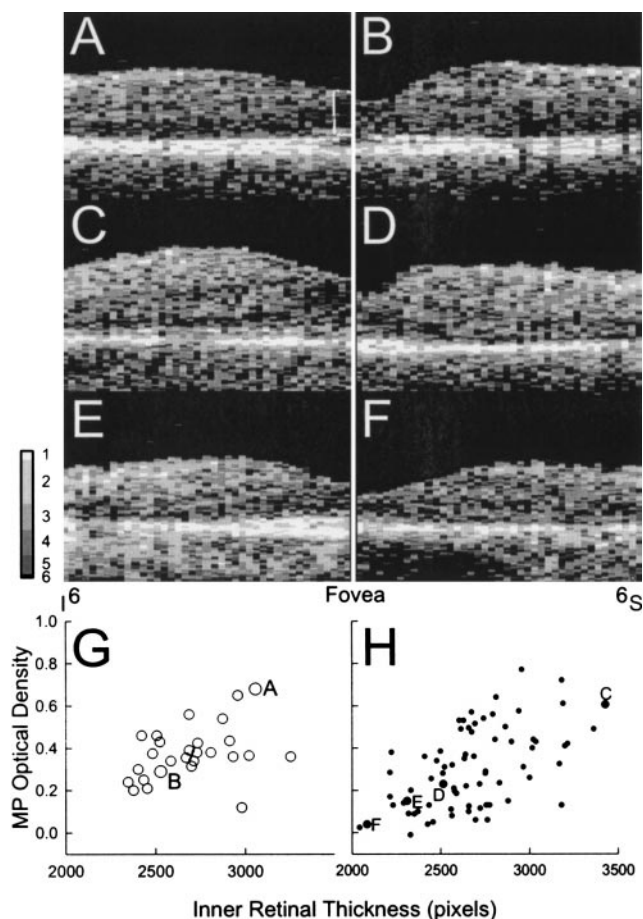


FIGURE 3. Relationship between MP and central retinal thickness. (A-F) Vertical OCT scans, from 6° inferior (*left*) to 6° superior retina (*right*) in two normal subjects (A, B) and four patients (C-F). Scans are split at the fovea to provide comparison of central thicknesses encountered in different subjects. OCT images are displayed with logarithm of reflectivity mapped to a gray scale (*lower left*). Numbers on the gray scale permit comparison of these OCT images with more commonly used pseudocolor displays (1, white; 2, red; 3, yellow; 4, green; 5, blue; and 6, black).^{38,40} The central 1° of scan used to quantify thickness is outlined in white in the split image for a normal subject (A). MP optical density at 0.5° eccentricity, as a function of retinal thickness, measured as OCT gray-scale image pixels, in normal subjects (G) and patients (H). Letters near symbols specify subjects whose images are shown in (A-F).

(Figs. 4C, 4D). The distribution of change between the two baseline values for each patient eye was compared with the distribution of change between baseline and 6 months after supplementation. For both central measures, there was overlap between the distributions of MP differences at baseline versus postsupplementation, but the latter was definitely shifted toward higher MP density ($P \leq 0.001$ for each target) and showed a wider spread in values. In a comparison of baseline and postsupplementation serum lutein levels, a pronounced shift of the distribution toward a range of higher levels was evident (Fig. 4E). In summary, all patients showed increased serum levels after supplementation but not all showed an increase of MP density.

Seeking to define further the response to lutein supplementation in patients and normal subjects, we plotted the differences in MP density in each eye between average baseline and after supplementation at 0.17° versus 0.5°. This was prompted by inspection of individual spatial profiles that showed some eyes changing at only one central target, whereas others

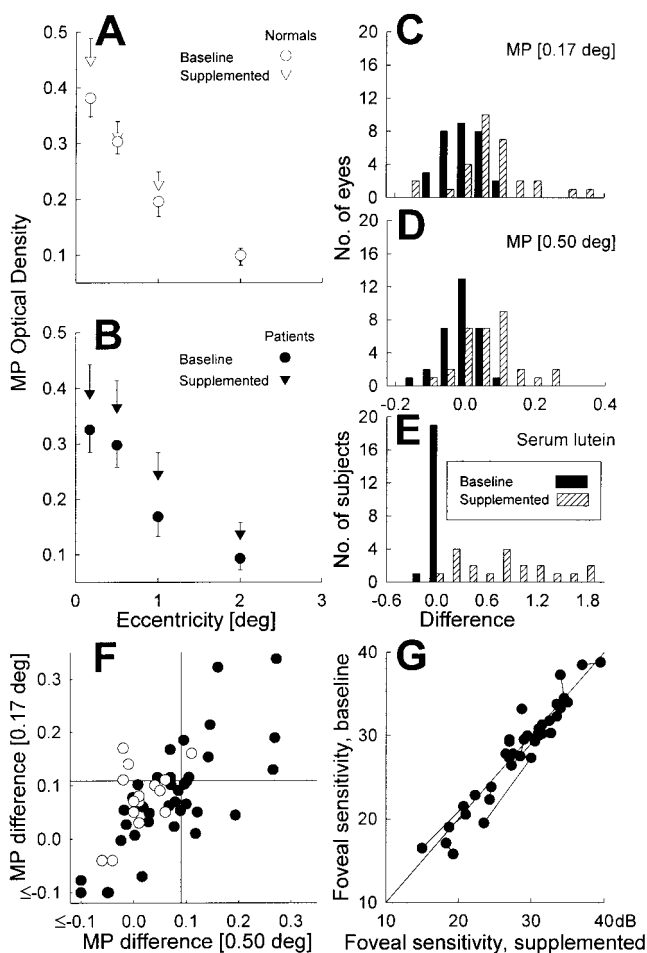


FIGURE 4. Effect of lutein supplementation on MP, serum lutein and foveal sensitivity. (A, B) Comparison of normal and patient data at baseline and after 6 months of lutein supplementation. Error bars, SEM. (C–E) Differences in MP optical density (C, D) and serum lutein (E) in patients between baseline visits and between mean baseline and 6 months after supplementation. (F) Change in MP, measured with 0.17° and 0.50° stimuli, after 6 months of supplementation. Horizontal and vertical lines represent 95% confidence limits of baseline intercession variability. Open symbols: normal subjects; solid symbols: patients. (G) Dark-adapted foveal sensitivities of patients at baseline versus 6 months after supplementation. Diagonal line represents no change. Lines connect the symbols for the two eyes of the same patient.

changed at both 0.17° and 0.5° (Fig. 4F). Selective response was unanticipated. At baseline, MP density for the 0.17° target, although generally higher than that for 0.5°, correlated strongly with MP at 0.5° (Spearman, $r = 0.95$). In Figure 4F, vertical and horizontal lines define the 95th percentile for differences within patients between the two baseline values. Of the normal eyes, two thirds (10/15) showed no change in MP with supplementation at either central target (Fig. 4F, lower left quadrant). When there was a response (5/15), it was most often an increase in MP density at 0.17° (4/15; Fig. 4F, upper left quadrant). In only one normal subject did MP density increase at both central loci (Fig. 4F, upper right quadrant). The patient data were not exactly the same as those of normal subjects. Approximately half (20/37) showed no increase in MP with either target. When there was a response (17/37), it was more often a response in MP for both targets (8/37). Smaller numbers of patients showed an increase in MP with only the 0.17° (3/37) or the 0.5° (6/37) target. A practical implication for future work is that the definition of a retinal responder versus a nonresponder to lutein supplementation

with this methodology may depend on which target is used in the measurement.

With the goal of identifying factors that may predict which patients would be responders to lutein supplementation, we used a generous criterion for responder that included statistically significant increases in MP for one or both targets—that is, 17 of 37 (46%) eyes were considered to have responded. Certain ocular and systemic factors of nonresponders and responders were then compared, even though we recognize that our relatively small numbers limited our power to detect differences. When factors were considered for the entire individual, three patients (of the 16 bilaterally tested individuals) who crossed the lines of criterion (Fig. 4F) were not included in this analysis.

We asked whether baseline serum lutein or the amount of change in serum lutein with supplementation influences whether a patient is a nonresponder or a responder. Baseline serum lutein values were identical in both groups (mean, $0.13 \pm 0.05 \mu\text{g/ml}$). Mean serum lutein increased fourfold (488% in nonresponders versus 442% in responders) after supplementation in each group. MP densities at baseline were somewhat lower for nonresponders at each eccentricity (0.29 vs. 0.38 at 0.17°, $P = 0.33$; 0.25 vs. 0.37 at 0.5°, $P = 0.17$), but not to a statistically significant degree. Nonresponder and responder groups showed no major differences in age (38 vs. 31 years), gender (60% vs. 50% female), or current smoking (10% vs. 0%) but among the nonresponders, there was a higher percentage of light-colored irides (50% vs. 25%, $P = 0.37$). In our measures of central disease severity, there were no differences between nonresponders and responders in mean baseline foveal absolute sensitivity (29.1 vs. 27.7 dB), logMAR visual acuity (0.13 in each group), or inner retinal thickness by OCT (2675 vs. 2687 pixels). As for retina-wide measures of disease, the percentage within each group that had detectable ERGs at baseline (75% vs. 80%) were not different, but baseline kinetic visual field extent was smaller in nonresponders than in responders (31% vs. 53%, $P = 0.03$).

An important question to ask is whether there were any detectable central visual changes between visits at baseline and 6 months after supplementation. Figure 4G shows that foveal absolute sensitivity after supplementation was little changed from the baseline value ($r = 0.95$). On average, visual acuity improved by approximately one letter (mean, logMAR, 0.02 ± 0.07 ; $P = 0.11$). Mean foveal absolute sensitivity increased by $0.30 \pm 1.85 \text{ dB}$ ($P = 0.33$) from a baseline value of 28.41 dB. The mean change in foveal sensitivity (mean, $0.29 \pm 1.99 \text{ dB}$) in eyes that responded with an increase in MP density was nearly identical with the mean change in nonresponding eyes (mean, $0.30 \pm 1.78 \text{ dB}$). Similarly, the mean change in logMAR visual acuity in responding eyes (mean, 0.01 ± 0.06) was nearly identical with the mean change in nonresponding eyes (mean, 0.02 ± 0.08).

DISCUSSION

High expectations have accompanied the increasing indirect evidence that there may be clinical value in supplementing the non-vitamin A carotenoid, lutein, in retinal degenerative disease, especially age-related macular degeneration, but recently also in inherited retinopathies.^{2–5,8,9,26} At present, however, there are very few published data about MP levels or response of MP to lutein supplementation in the target patient populations.⁵⁰ The modest purposes of the present study were to understand whether there were any marked differences in pattern of MP optical density in patients with retinal degeneration compared with normal subjects, and whether a short-term pilot trial (neither masked nor placebo-controlled) of

lutein supplementation would lead to any measurable effects on MP and vision in a subset of these patients.

We chose to study a group of 58 patients with RP or Usher syndrome, clinically diagnosed but not molecularly defined, and we characterized their *in vivo* retinal carotenoid content using a feasible and available psychophysical method of measuring MP optical density.²³ This HFP method had already been field-tested in a large normal population.²⁴ Spatial profiles of MP density among the patients were like those in normal subjects. Intersession variability was comparable to normal. Group statistics showed no difference in the wide range of MP density levels found in these patients and normal subjects from this study and from other published work. It is worthy of note that validity of the assumptions associated with the HFP technique was not explicitly proven in our patients. It is assumed, for example, that the relative sensitivities to blue and green lights at the (foveal) test locus and the reference locus in the parafovea differ only by the absorption of the blue light by MP at the test position.^{30,51} Further, it is assumed that all sensitivities are mediated with the same chromatic detection mechanism: long (L)- and medium (M)-wavelength cones in the current work. Differences in L/M-cone photopigment densities between test and reference have been hypothesized to cause an underestimation of MP density measured under L/M-cone isolation in normal subjects.^{21,51} In some patients in this study, it is likely that the overall L/M-cone photopigment density was abnormally reduced.⁵² Of interest, the resultant reduction in spatial differences in cone pigment optical density would theoretically reduce the extent of MP density measurement error in these patients. Despite this and other possible complicating factors due to retinal degeneration and the problems of applying a rather difficult psychophysical task to a visually (and, in many cases, hearing-) impaired patient population,⁵³ it is encouraging to know that our results are concordant with those from an earlier study of MP in five patients with RP, in whom fundus reflectometry found no major differences from normal.⁴⁹

Factors that have been associated with lower MP in normal subjects^{4,8,24,45-47} were examined in the patients. Patients with lower MP showed a higher percentage of females, smokers, and eyes with light irides. Diet and serum levels of lutein were not strongly related to MP density level in the patients, which is in concert with some studies of normal subjects but not others.^{10,24,45,47,54,55} Foveal architecture has been postulated to influence measured MP levels,²⁰ but there have been no previous *in vivo* measurements in humans with any technique. We used OCT methods³⁸⁻⁴⁰ and found MP density to be related to foveal structure in the patients and to a lesser degree in normal subjects. Patients with reduced inner retinal thickness had lower MP levels, which suggests that the loss of inner retinal tissue known to occur in outer retinal degenerations⁵⁶ may impact the level of measured MP. That there was no similarly strong tendency in our normal subjects suggests there is a more complex microanatomic relationship when retinal tissue is not diseased.

The expectation from earlier work in normal subjects using HFP to measure MP optical density was that oral supplementation of lutein would increase serum levels but may not predictably lead to increased levels in the target organ, the retina, in everyone.^{44,54,57} Lutein supplementation increased serum lutein levels in all the patients with retinal degeneration. We then tested the hypothesis that oral lutein intake would not assure measurable increases in MP in our patients. Relatively conservative statistical criteria were defined for MP response, and the results indicated that some patients were definite retinal responders, whereas others could be considered nonresponders.⁴⁴ It is, of course, possible that other techniques of measuring MP may provide other results.^{21,58,59} Comparative

studies using different methods in the same subjects would be worth performing. The issue of nonresponders may eventually become a nonissue with more sensitive, or just different, detection methods, but in the interim, we must hold that at this dose of oral lutein, for this duration of supplementation, using this HFP methodology, and in the type of subjects we studied, an increase in measured MP optical density was not a predictable consequence of increased intake of lutein. Higher doses were not used in the present study, because short- or long-term safety issues for lutein have not been addressed formally.⁶ When we sought simple reasons (other than dosage) that some patients responded or did not, we were unable to find characteristic or major differences between groups. There was a hint from the data that disease stage may influence response, but this needs further study. What could help explain the variation in individual response is greater understanding, for example, of the bioavailability and metabolism of lutein, the complexities about tissue competition for lutein,^{8,54} and the molecular genetic causes of these retinopathies and exact disease pathogenesis.

A recent study of lutein supplementation in RP suggests visual benefit to some but not all patients. The trial was for a period similar to that of the present work, but higher doses (40 mg/d) were given for the first 2 months.²⁶ It is notable that there have been other reports (some dating back >50 years) suggesting visual benefit in RP from lutein-containing medications (reviewed in Ref. 60). Our measures of central vision in the patients did not change over the 6 months of lutein supplementation, whether or not the patients showed increases in MP density. We must conclude that lutein supplementation at this dosage for 6 months did not lead to major increases in the foveal vision parameters measured. Yet, there was no decline. No loss of visual acuity in this interval, however, would be consistent with results of natural history studies in RP.^{61,62} Longer treatment times with the supplement and the use of additional measures of visual function should determine whether the natural history is altered by this supplement.

Although there is a wealth of scientific information about the dietary-derived xanthophyll carotenoids lutein and zeaxanthin and extensive work has been performed on the identification, localization, and quantitation of MP density in humans, further details of biochemical mechanisms in the normal human eye are still needed.^{8,59,63} Pertinent to our specific interest would be investigations to determine why supplemental lutein may affect the pathogenesis of retinal degenerative disease. The role for *in vivo* macular carotenoids as protective optical filters is intuitively understandable, but the exact pathways by which carotenoids would prevent apoptotic cell death in photoreceptors and RPE (presumably from oxidative damage) need greater clarification in the laboratory.^{18,64-66}

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