Macular Pigment and Lutein Supplementation in ABCA4-Associated Retinal Degenerations

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PURPOSE. To determine macular pigment (MP) optical density (OD) in patients with ABCA4-associated retinal degenerations (ABCA4-RD) and the response of MP and vision to supplementation with lutein.

METHODS. Patients with Stargardt disease or cone–rod dystrophy and known or suspected disease-causing mutations in the ABCA4 gene were included. All patients had foveal fixation. MPOD profiles were measured with heterochromatic flicker photometry. Serum carotenoids, visual acuity, foveal sensitivity, and retinal thickness were quantified. Changes in MPOD and central vision were determined in a subset of patients receiving oral supplementation with lutein for 6 months.

RESULTS. MPOD in patients ranged from normal to markedly abnormal. As a group, patients with ABCA4-RD had reduced foveal MPOD, and there was a strong correlation with retinal thickness. Average foveal tissue concentration of MP, estimated by dividing MPOD by retinal thickness, was normal in patients, whereas serum concentration of lutein and zeaxanthin was significantly lower than normal. After oral lutein supplementation for 6 months, 91% of the patients showed significant increases in serum lutein, and 63% of the patients’ eyes showed a significant augmentation in MPOD. The retinal responders tended to be female and to have lower serum lutein and zeaxanthin, lower MPOD, and greater retinal thickness at baseline. Responding eyes had significantly lower baseline MP concentration than did non-responding eyes. Central vision was unchanged after the period of supplementation.

CONCLUSIONS. MP is strongly affected by the stage of ABCA4 disease leading to abnormal foveal architecture. MP could be augmented by supplemental lutein in some patients. There was no change in central vision after 6 months of lutein supplementation. Long-term influences of this supplement on the natural history of these macular degenerations require further study. (Invest Ophtalmol Vis Sci. 2007;48: 1319–1329) DOI:10.1167/iovs.06-0764

The ABCA4 gene encodes the ABCR protein, which localizes to the rims of rod and cone outer segments1,2 and accelerates removal of all-trans-retinal from light-exposed photoreceptors by transporting A2-PE, a retinoid adduct formed by all-trans-retinal and phosphatidylethanolamine.3–5 Mutations in the ABCA4 gene cause a major proportion of autosomal recessive retinal degenerations (RD) with macular involvement.6–12 Pathophysiology of ABCA4-RD involves trapping of A2-PE,3,13,14 within disc membranes of the photoreceptor outer segments (POS). Phagocytosis of the shed POS by adjacent retinal pigment epithelial (RPE) cells in ABCA4-deficient retinas results in excessive intracellular accumulation of lipofuscin, an aggregate of lipids, proteins, and fluorescent retinoids, including cytotoxic bis-retinoid A2E derived from the trapped A2-PE.3,15 In extramacular retinas of patients with known ABCA4 mutations, we have provided evidence supporting an abnormal increase in lipofuscin autofluorescence in the RPE preceding dysfunction and degeneration of the overlying retina.11 We have also shown that the parafoveal retina is relatively spared from retinal degeneration.12 Details of the macular disease sequence remain to be studied.

Macular degenerations, including those caused by ABCA4 mutations, commonly go through a counterintuitive stage during which foveal vision and structure are relatively preserved compared with the surrounding parafoveal region.3–11,16–18 It has been hypothesized that macular pigment (MP), a yellowish carotenoid mainly composed of lutein and zeaxanthin concentrated at the fovea, contributes to relative preservation of this retinal region in macular degenerations.19 The mechanism of MP protection may involve passive absorption of shorter wavelength light.20–25 Exposure to light not only causes A2E accumulation but also increases the potential toxicity of the accumulated A2E via photooxidation.26,27 Further, it has been proposed recently that lutein and zeaxanthin specifically protect A2-PE in photoreceptors and A2E in RPE cells from photooxidation, and thus MP may have a particularly important role in ABCA4 disease.28

To understand better the preserved foveas in ABCA4-RD, we explored the relationship between MP and systemic, ocular, and retinal features. Seeking ways to prevent loss of this remaining foveal vision in ABCA4-RD, we also performed a short-term open-label pilot study asking whether retinal MP could be modified with oral lutein supplementation.

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Informed consent was given by all subjects in this study. Patients and control subjects were recruited from an international clinical trial for lutein supplementation (Lutein Trial). A subset of the subjects (11 patients and 8 control subjects) underwent a pilot trial of supplementation and were included in this study (Table 1). All participants were in general good health. The study was approved by our institutional review board and was conducted in accordance with the tenets of the Declaration of Helsinki; informed consent was obtained from all subjects.

Subjects

Stargardt disease or cone-rod dystrophy patients (n = 17), most of whom had known ABCA4 gene mutations,\(^{11,12,29}\) were included in the study (Table 1). All participants were in general good health. The subjects, all with central retinal disease, were selected for participation because of their relatively spared foveal function in at least one eye. All subjects had a routine ocular examination and best corrected visual acuity determined by a Goldmann visual field perimeter (Humphrey Instruments; Carl Zeiss Meditec, Inc., Dublin, CA) and a red (650 nm) target (1.7° diameter, 200 ms duration) in the presence of a 6° diameter background field (1.5 log td, 470 nm) while the patients fixated centrally on a 5-minarc spot. Four different stimuli were used that consisted of two discs (0.34° and 1° diameter) and two annuli (2° and 4° diameter, 0.4° wide). We will assume that flicker perception is dominated by the edges,\(^{33}\) although other work has suggested that flicker may not be detected at the edge but is perceived by more central retinal eccentricities when using discoid stimuli.\(^{34}\)

Methods

Evaluation of the Macula: Macular Pigment and Central Retinal Function and Structure

Macular pigment optical density (MPOD) was measured using heterochromatic flicker photometry (HFP) with a LED-based MP densitometer (Macular Metrics Corp., Rehoboth, MA). This psychophysical technique compares flicker photometric sensitivity measured at and near the fovea with that obtained at a more peripheral retinal location.\(^{32,31}\) Sensitivity is determined by alternating a short wavelength test light that is maximally absorbed by MP in counterphase with a longer wavelength reference light that is not absorbed by MP. The intensity of the test light is adjusted until the perception of flicker is minimized or eliminated, at which point the two lights are equated in apparent brightness. The peripheral-to-foveal sensitivity ratio is used to determine the peak density of MP. Details of the methodology in patients with hereditary retinal degenerations have been provided.\(^{30,32}\) In brief, flickering stimuli (460 nm, test; 570 nm, reference, 1.7 log td) were centered on a 6° diameter background field (1.5 log td, 470 nm) while the patients fixated centrally on a 5-minarc spot. Four different stimuli were used that consisted of two discs (0.34° and 1° diameter) and two annuli (2° and 4° diameter, 0.4° wide). We will assume that flicker perception is dominated by the edges,\(^{35}\) although other work has suggested that flicker may not be detected at the edge but is perceived by more central retinal eccentricities when using discoid stimuli.\(^{34}\) Using the former assumption, these stimuli represent eccentricities of 0.17° and 0.5° (henceforth referred to as “foveal”) and 2° and 4° (henceforth referred to as “parafoveal”); 0.17° eccentric stimulus will be referred to as 0.2°. Peripheral sensitivities were determined with a 2° diameter disc centered on the background while subjects fixated on a small red LED situated 7° to the nasal side of the background field; the radiance setting of the 460-nm test light needed for a flicker null at this eccentricity was not significantly different between patients and normal subjects (171 ± 61 vs. 157 ± 18 counts; \(P = 0.17\)).

Foveal visual function was measured using a modified automated perimeter (Humphrey Instruments; Carl Zeiss Meditec, Inc., Dublin, CA) and a red (650 nm) target (1.7° diameter, 200 ms duration) in the dark-adapted state. Macular structure was quantified by OCT (Carl Zeiss Meditec, Inc.). The principles of OCT\(^{36}\) and our methodology\(^{11,37}\) have been published. Horizontal scans crossing the anatomic fovea were obtained in all subjects. Retinal thickness at the center of the fovea and at 0.5° of eccentricity was measured.\(^{37}\) Serum carotenoids (lutein, zeaxanthin, and \(\beta\)-carotene) were measured using high-performance liquid chromatography (HPLC; Craft Technologies, Inc., Wilson, NC). Dietary information was obtained with the Health Habits and History questionnaire (HHHQ) developed by the National Cancer Institute\(^{40}\); data were analyzed by using the HHHQ Diet System Analysis Software.\(^{39}\)
Supplementation with Lutein

A subset of patients \( n=11 \) participated in an open-label, 6-month pilot trial of oral lutein supplementation (Table 1). There was no placebo control group. After two baseline visits (separated by no more than 1 month; except patient 4, who had a single baseline visit), subjects supplemented their diets with a commercially available form of lutein at 20 mg per day (Twin Laboratories Inc., Hauppauge, NY). Subjects were instructed to take the lutein supplement with a meal with the most fat of the day, presuming this would enhance absorption. A further visit occurred 6 months after supplementation began. Baseline and follow-up visits included a clinical examination, fasting (overnight) venous blood sample for serum carotenoids, and measurements of MPOD and absolute dark-adapted sensitivity at the fovea with a 650-nm target.

Data Analysis

Statistical software (SAS, ver. 9.1; SAS, Cary, NC) was used to analyze data. Mean data from the two baseline visits were used to describe the study groups and calculate the change after lutein supplementation. \( t \)-Tests were performed to compare means and significance levels for correlation coefficients. Intersession variability was assessed with signed and absolute differences of measurements between the first and second baseline visits. Means of intersession differences and person-specific variables were compared with independent \( t \)-tests. Proportions were compared using \( \chi^2 \) tests with exact computation of the probabilities.

RESULTS

Patients had a clinical diagnosis of either Stargardt disease \( n=14 \) or cone–rod dystrophy \( n=3 \) and all but four had known mutations in the \( ABCA4 \) gene (Table 1). All patients had macular disease with foveal sparing in at least one eye. The macular appearance on infrared reflectance imaging ranged from a mottled pattern with scattered dark and light lesions, to dark areas surrounding a lighter foveal center and bordered by a granular-appearing annulus (Fig. 1A). Kinetic visual fields were full in peripheral extent in all but two patients (Table 1); small scotomas around fixation were frequently detected. The four examples of MPOD at different eccentricities displayed below the fundus images (Fig. 1B) indicate that diseased eyes could have results that are within normal limits or show abnormalities.

Low Macular Pigment in \( ABCA4 \)-RD

Eyes of patients had significantly lower MPOD than did those of normal subjects (Table 2). The distribution of MPOD in patients' eyes was shifted toward lower values than in normal eyes (Fig. 2A). At 0.5° eccentricity, 76% of the patients showed MPOD below 0.2, whereas only 7% of normal subjects had such low values. MPOD normally peaks near the center of the fovea and declines with eccentricity. The spatial distribution of MP was studied in a subset of 11 patients' eyes in which MPOD was measurable at all four eccentricities (Fig. 2B). On average, MPOD in these eyes was lower than normal at each eccentricity (Table 2, MPOD Profile). The distribution of MPOD profiles as estimated from the half-width at half-peak was narrower in these 11 patients than in the normal subjects (Table 2). The remaining six patients could not perform HFP at the parafoveal locations due to a lack of perception of the flicker. These eyes with an indeterminate spatial MPOD distribution corresponded to some of the lowest foveal MPOD (Fig. 2B, stars).

MP levels have been related to dietary, demographic, lifestyle, systemic, and ocular characteristics in studies of normal populations. Examination of some of these factors (Table 2) showed that the patients and normal subjects included in this study were well matched in age, body mass index (BMI), gender, smoking status, race, and color of irides. Mean dietary fat intake was higher in the patients than the normal subjects but the groups were not significantly different and the patients' levels were similar to those reported in other studies in.
normal subjects. Dietary lutein was similar in patients and normal subjects. The effect of factors contributing to low MPOD were further explored in patients by comparing subgroups in the first (MPOD < 0.08) and fourth (MPOD > 0.19) quartiles of the distribution of MP data in response to the conventional 0.5° eccentric stimulus. Females (60% vs. 40%, P = 0.36) and subjects with light-colored irides (60% vs. 20%, P = 0.52) were more commonly observed in the low MPOD group of patients; the only smoker in these two subsets was in the low MPOD group. These results are in agreement with our previous observations in patients with other hereditary retinal degenerations and in reports of normal subjects.

Low Serum Lutein in ABCA4-RD

Serum levels of lutein and zeaxanthin were significantly lower in the patients compared with those in the group of normal subjects (Table 2). Serum levels of xanthophylls in most patients also fell within the lower end of normal values reported by other investigators in large population-based studies. When the subset of subjects with low serum lutein (≤ 0.19 μmol/L, lowest quartile of our normal population) was considered, the patients had significantly lower zeaxanthin levels than did the normal subjects but could not be otherwise distinguished from them by other variables. Low serum xanthophyll levels were not associated with any one category of patients. For example, there were no significant differences

<table>
<thead>
<tr>
<th>Table 2. Baseline Group Summary Statistics*</th>
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<tbody>
<tr>
<td><strong>Patients</strong> (n = 17)</td>
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<tr>
<td><strong>General</strong></td>
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<tr>
<td>Age (y)</td>
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<tr>
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<tr>
<td>White (%)</td>
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<td>Light irides (%)</td>
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<tr>
<td><strong>Diet</strong></td>
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<td>Fat (g/day)</td>
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<tr>
<td><strong>Serum</strong></td>
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<tr>
<td>Lutein (μmol/L)</td>
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<td>Zeaxanthin (μmol/L)</td>
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<td>β-Carotene (μmol/L)</td>
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<td><strong>MPOD</strong></td>
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<tr>
<td>0.2°</td>
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<tr>
<td>0.5°</td>
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<tr>
<td><strong>Retinal thickness</strong></td>
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<td>0° (μm)</td>
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<tr>
<td>0.5° (μm)</td>
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<tr>
<td><strong>MP concentration</strong></td>
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<tr>
<td>0.2° (μm⁻¹)</td>
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<tr>
<td>0.5° (μm⁻¹)</td>
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<tr>
<td><strong>MPOD profile</strong></td>
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<tr>
<td>0.5°</td>
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<tr>
<td>1°</td>
</tr>
<tr>
<td>2°</td>
</tr>
<tr>
<td>Half-width at half-peak (deg)</td>
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</tbody>
</table>

* Noncategorical variables are specified as mean ± SD
† Not significant (NS) values correspond to P > 0.05.
‡ Determined in subset of nine patients with full spatial profiles.

![Figure 2. MPOD in patients compared with normal subjects. (A) Frequency-distribution histograms of MPOD measured at foveal locations (0.2° and 0.5°). Vertical dashed lines: median value of each distribution. (B) All individual MPOD values measured in patients and normal subjects. Circles: full profiles; stars: patient eyes in which MPOD could only be determined in a subset of the four eccentricities.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932941/)
between serum xanthophyll levels in patients based on gender, smoking status, and color of irides (data not shown). Examination of concurrent use of dietary supplements revealed that 8 of 17 patients were using multivitamins containing low doses of carotenoids before admission to the study. There were no differences, however, in any of the variables measured between those who had used supplements and the rest of the patients (data not shown).

Serum lutein was related to MPOD in patients and in normal subjects (Fig. 3A). Linear correlation coefficients between these variables were similar in patients ($r = 0.46; P = 0.05$) and normal subjects ($r = 0.44; P=0.002$) at 0.2° eccentricity. This relationship improved notably for the more eccentric 0.5° location in normal subjects ($r = 0.63; P < 0.001$), but remained unchanged in patients ($r = 0.47; P = 0.02$).

**Relationship of Foveal Structure to MPOD**

Cross-sectional images through the fovea in two patients (Fig. 3B, bottom) illustrate the types of abnormalities encountered. Patient 8 showed some photoreceptor layer thinning and localized disruption of the signal originating from the photoreceptor inner–outer segment interface. Patient 12, with more advanced disease, showed severe central retinal thinning and an adjacent ($\sim 1°$–3° eccentric) region with loss of the photoreceptor layer. MPOD in patient 8 was reduced but...
measurable at all eccentricities, but in patient, 12 MPOD was measurable only at the foveal locations (Fig 3B, top).

Average foveal thickness in patients was reduced to approximatly half of normal—a significant difference (Table 2). The relationship between foveal MPOD and retinal thickness was examined in the patients compared with normal subjects (Fig. 3C). Patients showed a positive correlation between MPOD at 0.2° and retinal thickness at the foveal center (Fig. 3C, r = 0.43, P = 0.05) and a robust relationship (r = 0.66, P < 0.001) at the 0.5° locus, consistent with previous observations.30,32,59

The relationship between retinal thickness and MPOD was much stronger when the patients and normal subjects were considered as a single group, under the assumption that patients’ foveas had normal thickness before the onset of retinal degeneration. The regression lines considering all subjects (Fig. 3C) had robust correlation coefficients (0.2° = 0.69 and 0.5° = 0.76, P < 0.0001); the intercepts were not significantly different from zero (0.2° = 0.10, P = 0.58; 0.5° = −0.06, P = 0.10).

To a first approximation, retinal tissue concentration of MP can be estimated by dividing MPOD by retinal thickness. Foveal MP concentration in the patients was not different from that in normal subjects at 0.2° but it was lower at 0.5° (Table 2). Serum lutein concentration in the patients did not correlate significantly with MP concentrations at 0.2° (r = 0.26, P = 0.44) and 0.5° (r = 0.36, P = 0.12) unlike the stronger relationship observed in the normal subjects (r = 0.28, P = 0.05 and r = 0.65, P < 0.001, for 0.2° and 0.5°, respectively).

The relationship between central visual function and MPOD was probed with visual acuity and dark-adapted sensitivity to a 650 nm stimulus. MPOD at 0.5° did not correlate significantly with visual acuity (r = 0.36, P=0.07) and dark-adapted foveal sensitivity (r = 0.33, P = 0.11).

**Effects of Lutein Supplementation**

The subset of patients (n = 11) and normal subjects (n = 8) who took part in the 6-month pilot trial of lutein supplementation (Table 1) were well matched in age, BMI, gender, smoking status, race, and color of irides (Table 3). All but one of the 11 patients responded with an increase in serum lutein. In the entire group of patients, the change in serum lutein with supplementation (postsupplementation level minus the mean of the two baseline values) was significantly greater than the mean absolute difference between the two baseline values (0.74 ± 0.44 vs. −0.01 ± 0.03 μmol/L; P < 0.001). After supplementation, there was no significant difference in serum lutein remaining between patients and normal subjects (Table 3). Serum zeaxanthin levels also showed an increase in both groups with supplementation, as expected from the small amounts of zeaxanthin contained in the marigold extracts, the main component of the supplementation capsule.32 However, serum zeaxanthin in the patients remained significantly lower than that in the normal subjects after supplementation (Table 3). There were no changes measured in serum β-carotene levels in either group (Table 3).

MPOD measurements before and after oral lutein supplementation are illustrated by using the data from two patients (Fig. 4A). Both patients had increases in serum lutein (patient 8: from 0.21 to 0.51 μmol/L; patient 6: from 0.08 to 0.64 μmol/L) after supplementation, but showed different retinal responses by MPOD. Patient 8 had increases in MPOD at each eccentricity when compared with the baseline levels; in contrast, the postsupplementation MPOD profile of patient 6 was very similar to the baseline profiles. The apparent differences in response to supplementation were not due to a lack of reliability in MPOD estimates. Intervisit reproducibility of the MPOD profiles was measured in two visits in 11 patients (13 eyes). Absolute differences between the MPODs obtained at each visit in the patients were not significantly different from those in the normal subjects (Fig. 4B). Previously published intervisit density differences21,31,47,60–63 showed similar ranges, suggesting that MPOD can be reproducibly obtained with HFP in eyes with maculopathy and foveal fixation.

Sixteen eyes of 10 patients (six bilateral, four unilateral measurements) were used for summary statistics of the MPOD response to lutein supplementation. Patient 4 showed no serum response to lutein supplementation and was not included in this analysis (she had no MPOD change). Mean foveal MPOD increased with supplementation at: 0.2°, from 0.17 ± 0.09 to 0.28 ± 0.14 (paired t-test, P < 0.001); at 0.5°, from 0.11 ± 0.06 to 0.18 ± 0.10 (P < 0.001). Parafoveal increases were not significant. Magnitude of MPOD changes with supplementation were larger in patients compared to normal subjects: at 0.2°, 0.12 ± 0.12 vs. 0.07 ± 0.06 (P = 0.28); at 0.5°, 0.06 ± 0.07 vs. 0.01 ± 0.04 (P = 0.02). These two foveal locations were used to assess changes in MP concentration with supplementation. In normal subjects, oral lutein led to significant increases in MP concentration at the foveal center but not at 0.5°. In patients, the retinal MP concentration increased at both foveal locations (Fig. 4C).

Retinal MPOD responders to lutein supplementation were then compared to nonresponders to seek explanations for their

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**Table 3. Supplemented Group Summary Statistics**

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 11)</th>
<th>Normal (n = 8)</th>
<th>P†</th>
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<tbody>
<tr>
<td><strong>General</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age (y)</td>
<td>30 ± 11</td>
<td>27 ± 8</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>23 ± 4</td>
<td>24 ± 4</td>
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<tr>
<td>Female gender (%)</td>
<td>36</td>
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<tr>
<td>Smoker (%)</td>
<td>27</td>
<td>13</td>
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<tr>
<td>White (%)</td>
<td>82</td>
<td>75</td>
<td>NS</td>
</tr>
<tr>
<td>Light irides (%)</td>
<td>55</td>
<td>38</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Serum (before supplementation)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lutein (μmol/L)</td>
<td>0.18 ± 0.08</td>
<td>0.34 ± 0.11</td>
<td>0.002</td>
</tr>
<tr>
<td>Zeaxanthin (μmol/L)</td>
<td>0.07 ± 0.03</td>
<td>0.14 ± 0.05</td>
<td>0.001</td>
</tr>
<tr>
<td>β-Carotene (μmol/L)</td>
<td>0.40 ± 0.51</td>
<td>0.44 ± 0.24</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Serum (after supplementation)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lutein (μmol/L)</td>
<td>0.84 ± 0.50</td>
<td>1.06 ± 0.41</td>
<td>NS</td>
</tr>
<tr>
<td>Zeaxanthin (μmol/L)</td>
<td>0.12 ± 0.04</td>
<td>0.21 ± 0.07</td>
<td>0.001</td>
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<tr>
<td>β-Carotene (μmol/L)</td>
<td>0.40 ± 0.48</td>
<td>0.44 ± 0.18</td>
<td>NS</td>
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</table>

* Noncategorical variables are specified as mean ± SD.
† Not significant (NS) values correspond to P > 0.05.
differences. For this analysis, responding was defined by the 95th percentile for differences between the two baseline MPODs (Fig. 4D). Over half of the eyes responded with a significant increase in MPOD (Fig. 4D, black squares). In most, the response occurred at both eccentricities, but in some eyes, the response was limited to one retinal location. Baseline serum levels of lutein and zeaxanthin in the responders were lower on average than those in the nonresponders, suggesting that changes in the retina are related to initial levels of serum xanthophylls. Although there was a small sample of subjects, we asked whether there was an association between the MPOD response and some of the general characteristics examined earlier. Of interest, the three female patients (5/5 eyes) who took supplements responded with an increase in MPOD, whereas responses in the male participants were less frequent (4/7 male patients; 5/11 eyes). Retinal responders and nonresponders did not differ significantly in age (36 ± 7 vs. 27 ± 12 years), frequency of lighter irides (60% vs. 50%), or smoking (both groups, 20%).

Were there eye-specific variables that could be related to the MPOD response? The responders tended to have lower MPOD at 0.2° and thicker foveas, but these differences did not reach statistical significance at this central foveal location (Fig. 4E) or at 0.5°. Baseline mean MP concentration on the other hand, was significantly lower in the responders than in the nonresponders (Fig. 4E), suggesting that MPOD changed in
those with the lowest initial MP concentrations. In terms of central visual function, the responders and nonresponders showed no differences in baseline visual acuity (0.19 ± 0.12 vs. 0.21 ± 0.10 logMAR) and foveal sensitivity (25.5 ± 4.5 vs. 28.8 ± 8.3 dB; Fig. 4E) group. An estimate of interocular differences in MPOD response to lutein supplementation was obtained in six patients with bilateral measurements. Three patients responded bilaterally, whereas one did not respond in either eye (Fig. 4D). Two patients showed unilateral responding; in both cases, eyes that responded had thicker retinas than did the eyes that did not respond.

After supplementation, foveal absolute sensitivity as a measure of central visual function was little changed from the mean baseline of 26.2 ± 6.5 to 26.0 ± 6.7 dB. Pre- and post-supplementation results correlated highly (r = 0.94, P < 0.001; Fig. 4F). The mean change in foveal sensitivity (−1.20 ± 2.5 dB) in eyes that responded with an increase in MPOD (Fig. 4F, black symbols) was not different from the mean change in nonresponding eyes (1.21 ± 2.7 dB; P > 0.05; Fig. 4F, gray symbols). Similarly, the mean change in logMAR acuity in responding eyes (−0.02 ± 0.03) was not different from the mean change in nonresponding eyes (−0.02 ± 0.06; P > 0.05).

**DISCUSSION**

The macular pigments, lutein and zeaxanthin, are highly concentrated at the fovea and are hypothesized to improve normal vision and protect photoreceptors and the RPE from oxidative damage.64 MPs originate from dietary consumption of lutein, and lutein-free diets in nonhuman primates can result in abnormalities of foveal photoreceptors and RPE.65-67 Epidemiologic studies have shown an association between lower dietary and serum levels of lutein and higher risk of age-related maculopathy,68,69 but questions about causality remain64 and await experimental clarification. Recently, one such specific antioxidant mechanism has been proposed. Lutein and zeaxanthin appear to protect visual cycle byproducts A2-PE and A2E from photooxidation.28 To extend the understanding of this new mechanism, we evaluated a cohort of patients with a shared prototypical lipofuscinopathy due to ABCA4 mutations and at a similar disease stage with relative preservation of the fovea compared with the surrounding parafoveal retina. Pathogenesis of human ABCA4 disease involves a dramatic increase in A2-PE and A2E.5,13,14 Consistent with earlier reports,70,71 we found foveal MPOD to be significantly lower than normal in these patients. Unexpectedly, we found MP concentration to be lower in the preserved foveal center in these patients reemphasizing the importance of measuring foveal structure when interpreting MPOD abnormalities.30,32 Our results lend support to the notion that MP has a role in ABCA4-RD and do not contradict the long-held hypothesis that protection afforded by MP contributes to foveal sparing.19 Future longitudinal studies could directly test whether the rate of foveal disease progression is related to MPOD and/or MP concentration.

Microdensitometry studies in primate retinas have shown that MP concentration is not uniform across the foveal depth; there is a major peak at the Henle fiber layer and relatively uniform lower concentration along photoreceptor nuclei and inner and outer segments.72 Our estimate of MP concentration derived by dividing the total MPOD by the total retinal thickness would not thus represent the true MP concentration in any given retinal layer. The estimate, however, may be a useful approximation of the average foveal MP concentration, under the assumption that cone outer segments and nuclei as well as cone axons in the Henle fiber layer thin proportionally in retinal degenerative disease. Future studies combining polarization-sensitive, OCT-based delineation of Henle fiber layer thickness and MP imaging42 may allow a better estimate of the maximum tissue concentration of MP and its relationship to foveal sparing in disease.

The psychophysical HFP technique used in the current work to estimate MPOD is the most common method; alternatives include retinal reflectance,70,72 lipofuscin fluorescence,73,74 Raman spectroscopy,75 and other psychophysical methods.76-79 All psychophysical methods, including HFP, require stable foveal fixation, and patients’ eyes were selected accordingly. HFP-based MPOD values in our patients were highly repeatable, with an intersession variability that was comparable to that in normal subjects in this study and other published work.51,44,60-62 Repeatability does not necessarily imply validity, and assumptions implicit in the use of the HFP technique to estimate MPOD were not explicitly proven in our study. It is assumed, for example, that the difference in M-cone mediated sensitivity to the blue and green stimuli is invariant across the measured central retinal locations.22,80 Theoretically, outer retinal degeneration could affect the relative abundance and/or photopigment density of L and M cones differentially across the regions tested. Our use of a molecularly homogeneous group of patients with ABCA4 lipofuscinopathy would be expected to minimize the potential for spatially variant degeneration of L and M cones. Further, reduced L/M-cone photopigment density previously reported in patients with retinal degenerations and/or maculopathies80-84 would be expected to diminish the spatial differences in cone pigment optical density and reduce the extent of MP density measurement error.

Selenium lutein and zeaxanthin levels of the patients at baseline as a group were about half of that observed in our normal subjects even though estimates of dietary lutein intake were not different between the two groups. Serum carotenoids in our patients were also at the low end of the distribution of values from large population-based studies.55-58 Patients with the lowest serum levels of lutein were not different from the rest of the patients or normal subjects in variables such as age, gender, diet, or BMI, although some showed concomitant low levels of zeaxanthin, possibly reflecting an overall low carotenoid intake or uptake not revealed by the dietary questionnaire. Analysis of patients with and without a previous history of multivitamin supplementation disclosed no differences in serum lutein and zeaxanthin concentrations, arguing against possible interactions with other carotenoids present in these preparations.85 It is tempting to consider a causal relationship between the serum lutein concentration and maculopathy. One possibility is that lower serum lutein levels predisposed this cohort of patients to more severe maculopathy, as has been hypothesized in age-related macular degeneration.86 Support for such a hypothesis is lacking, however, since the patients included in this study had relatively mild disease within the severity spectrum of ABCA4 lipofuscinopathy.11,12 An alternative hypothesis would be to consider the involvement of a systemic regulatory mechanism for lowering serum xanthophyll in response to reduced demand from degeneration of photoreceptors and RPE. Systemic signaling from the retina has been proposed to explain reduced blood levels of docosahexaenoic acid observed in many hereditary retinal degenerations.86

Augmentation of retinal MP concentration has been proposed not only to prevent age-related multifactorial degenerative diseases but also to prevent or delay retinal degeneration in Mendelian hereditary conditions.87,88 We supplemented the diets of our ABCA4 patients with lutein for 6 months to evaluate short-term effects as a prelude to longer-term studies. Serum lutein increased significantly in patients with supplemented diets, consistent with previous observations.30,32,63,89,90 Retinal MPOD and MP concentration also increased significantly in more than half of the eyes of patients, consistent with previous studies.30,32,63,89 Responders had a
tendency to be female and, at baseline, have lower serum lutein, greater retinal thickness and lower retinal MP concentrations. This suggests that low levels of baseline xanthophylls in serum and retina may help predict an increase of MPOD after supplementation. Whether other techniques of measuring MPOD would detect higher percentages of responders to supplementation or would be capable of detecting accumulation at cellular compartments such as the RPE24 not probed by the HFP method awaits further study.

Increasing knowledge about the molecular basis of genetic retinal degenerations has provided an opportunity to consider gene- or mechanism-specific therapeutic interventions in otherwise incurable diseases such as ABCA4-RD. In the present study we built on available knowledge and used clinically feasible techniques to evaluate molecularly identified patients at a specific disease stage. In this disease subset, we tried to understand whether there is vulnerability to a recently described antioxidant mechanism.28 The data were then used to perform a pilot trial of nutrient supplementation that could decrease vulnerability. Such strategic approaches in molecularly clarified retinopathies with specific consideration of baseline parameters that have high predictive value could reduce the variability of results and the length of clinical trials of these slowly progressive degenerative disorders.

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References


